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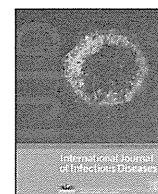
Association of viral isolates from stool samples with intussusception in children

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SUMMARY

Background: Intussusception is the most common cause of intestinal obstruction in young children. The pathogenesis of intussusception is still not well understood. In this study the pathogens from stool specimens were investigated in children with intussusception.
Methods: Patients diagnosed with primary idiopathic intussusception were enrolled. Pathogenic bacteria and viruses were detected in the stool samples by routine culture, cell culture, polymerase chain reaction, reverse transcriptase-polymerase chain reaction, enzyme immunoassay, and electron microscopy examinations.
Results: A total of 71 samples were analyzed during the 2-year study period. The patients ranged in age from 4 to 47 months. Viruses were detected in 56 of the 71 stool samples (78.9%). Adenovirus was found in 19 of 35 cases aged <2 years, whereas it was found in 17 of 21 cases aged ≥2 years. The majority of adenovirus isolates were non-enteric organisms generally associated with respiratory tract symptoms.
Conclusions: These results suggest a casual association of viral infections in children with intussusception. Adenovirus infection, especially with the primary non-enteric types, is a significant risk factor for developing intussusception in children, particularly those aged over 2 years.
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1. Introduction

Intussusception is the most common cause of intestinal obstruction in young children. The majority of cases occur in children under the age of 24 months.[1,2] Several studies have demonstrated that the incidence of intussusception varies among countries and also over time. A recent report prospectively compared the incidence of intussusception between Vietnam and Australia, and showed that the incidence in Vietnam was more than four-fold higher than that in Australia.[3] The incidence of intussusception observed in Vietnam is also higher than that in any other country for which incidence data are available, including recent reports from the USA and Latin America.[4–7] Studies from China also suggest a high incidence,[8] however, those reported in the USA, Denmark, and Australia have significantly declined over the past decade.[2,4,9] These results may reflect the presence of an environmental risk factor and/or infectious etiology in developing intussusception.

The underlying cause of intussusception in children remains unknown, but it has been associated with several pathogens,

including adenovirus. Previous studies have demonstrated adenoviruses in from 30% to 50% of stool specimens, and in intestinal or lymphoid tissue specimens from children with intussusception.[3,10–16] Murphy et al. reported an increased risk of intussusception after the administration of tetravalent rotavirus vaccine composed of rhesus rotavirus (RRV) and three human RRV reassortant strains, RRV-TV.[17] These results suggest an association between viral infection and the development of intussusception in young children.

The present study analyzed the association of infectious pathogens with the development of intussusception in children by a detailed examination of stool specimens during hospitalization. Results showed a high prevalence of viral isolates and a strong association with adenoviruses in patients with intussusception, particularly those over 2 years of age.

2. Materials and methods

2.1. Patients

The study was conducted at Hiroshima City Funairi Hospital, Hiroshima, Japan, over a 2-year period (August 2006 through July 2008). The study complied with the Declaration of Helsinki. The research protocol was approved by the ethics committee, and

informed consent was obtained from the patients' guardians. Children with signs and symptoms suggestive of intussusception and seeking care for this problem were considered for enrollment into the study. The diagnosis of primary idiopathic intussusception was made according to the clinical history, physical findings, abdominal radiograph, ultrasounds, and finally an air and/or contrast enema, using the case definition of the Brighton Collaboration Intussusception Working Group.[18] All patients with intussusception were admitted to the hospital. During the same 2 years, all in-patients with uncomplicated gastroenteritis aged <4 years who were examined for the presence of viral isolates in stool specimens in order to identify the cause, were enrolled in the study.

2.2. Assessment of infectious pathogens

Stool samples were collected from patients with intussusception during hospitalization. All samples were collected within 24 h of hospitalization and stored at 4 °C. Routine culture media were used to assay for common bacterial pathogens. Screening for enterovirus, poliovirus, echovirus, and adenovirus was performed using routine cell culture, HE, Hep-2, RD-18S and Vero cells, to second-generation of serial passage, and immunofluorescence detection.[19] Calicivirus detection was conducted using reverse transcriptase-polymerase chain reaction (RT-PCR) assays.[20] Rotavirus was tested for using an enzyme-linked immunosorbent assay (ELISA) and RT-PCR assays.[21] All patients positive by culture were examined by neutralization tests for enterovirus, poliovirus, echovirus, and adenovirus. In those patients negative by cell culture and by the other tests described above, additional examinations were performed for the detection of viral inclusion by electron microscope and using PCR assays for adenovirus, parechovirus, sapovirus, astrovirus, and aichivirus.[22,23]

2.3. Statistical analysis

Statistical significance was determined using the Chi-square test, Fisher's exact test, or Ryan's test according to the StatView software program (version 5.0I; SAS Institute, Inc., Cary, NC, USA). These tests were used to assess differences in stool findings between age groups and differences in the clinical symptoms between patients with the detection of adenovirus and those with virus other than adenovirus and negative isolation. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Study participants

During the 2-year study period, 83 children with primary idiopathic intussusception were diagnosed and admitted to the hospital. Twelve patients were excluded from the analysis because the stool specimens from these patients were not obtained during their hospitalization. Therefore, 71 patients were enrolled in the study. There was no discernible seasonal variation in the incidence of intussusception, nor was there any significant variation in the annual presentation rate over the 10-year period 1998–2008 (data not shown).

3.2. Infectious pathogens

Figure 1 shows the results of viral detection assays and age distribution in the patients. The 71 patients ranged in age from 4 to 47 months; 23 were aged <12 months (32.4%), 25 were aged 12–24 months (35.2%), and 23 were aged >24 months (32.4%). The male to female ratio of patients was approximately 2.6:1 throughout the age groups (data not shown). Fifty-six of the 71 patients (78.9%) had stool specimens positive for viruses. The rate of viral detection gradually increased with age: 15 of 23 (65.2%) aged <12 months, 20 of 25 (80%) aged 12–24 months, and 21 of 23 (91.3%) aged >24 months. Similarly, the prevalence of adenovirus isolation increased significantly with older age. Of those patients with virus detected, adenovirus was found in seven of 15 (46.7%) aged under 12 months, 12 of 20 (60%) aged 12–24 months, and 17 of 21 (81.0%) aged over 24 months. The detection of adenovirus from stool specimens of intussusception patients gradually increased with older age. Table 1 shows the number of patients with adenovirus, as well as other viral pathogens, namely rotavirus, enterovirus, parechovirus, norovirus, and poliovirus, detected in the stool specimens. Multiple viral infections occurred in one patient. Viruses causing enteritis were more frequently detected in patients under 2 years of age. A bacterial pathogen, *Campylobacter jejuni*, was simultaneously cultured in one patient (22 months of age) whose stool was positive for adenovirus type 1 (data not shown). No other bacterial pathogens were cultured from the patient stool specimens.

During the same 2 years, stool specimens from 82 patients with uncomplicated gastroenteritis aged under 48 months were screened for viral isolates (Table 2). The rate of patients with
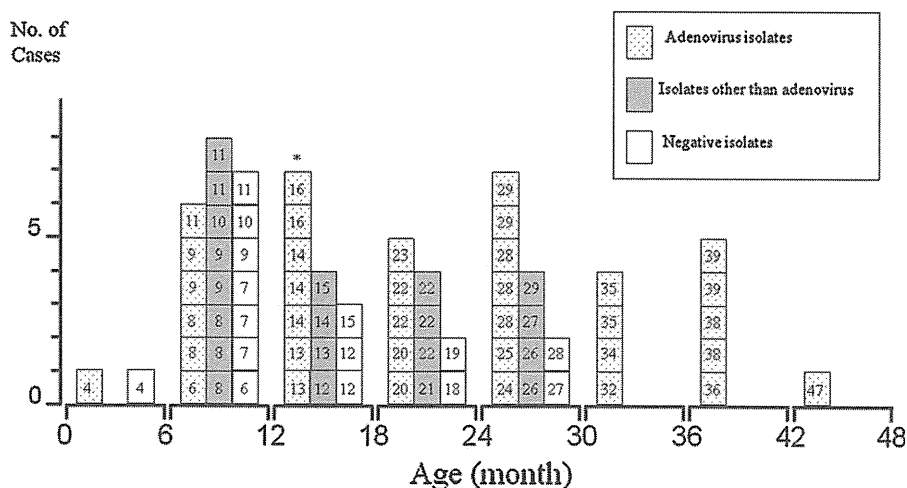
Figure 1. The number of cases with intussusception by age and viral isolate. Data represent the number of cases with adenovirus isolates (n = 36), isolates other than adenovirus (n = 20), and no isolates (n = 15) according to age. The numeral in the square represents the accurate age (in months) for each individual. The figure includes one case of multiple viral infections (*): a 14-month-old girl in whom both adenovirus type 5 and norovirus were detected.

Table 1
Viral isolates in children with intussusception

Virus	No. of patients with viral detections		
	Overall (n = 57)[a]	<2 years (n = 36)	≥2 years (n = 21)
Adenovirus	36	19[c]	17[c]
Poliovirus	5	5	0
Enterovirus	5	4	1
Parechovirus	4	3	1
Norovirus	4	3	1
Rotavirus	1	0	1
ND[b]	2	2	0

[a] Viruses were detected in 56 patients. This table includes a 14-month-old girl in whom adenovirus type 5 and norovirus were both detected. Three cases were detected to have adenovirus (AdV-31, 1 case; AdV41, 2 cases) and a case of parechovirus was identified by PCR.

[b] ND = viruses were present in the viral culture, but were not identified.

[c] p = 0.03 comparing age groups, determined by the Chi-square test.

positive viral isolates was 68.3%. Norovirus was identified in the majority of cases (78.6%), while others were adenovirus (7.1%), enterovirus (5.4%), parechovirus (5.4%), and rotavirus (3.6%). The detection of adenovirus in intussusception patients was significantly higher than that in patients with uncomplicated gastroenteritis ($p < 0.0001$).

3.3. Analysis of adenovirus types

Human adenoviruses are classified into 51 serotypes within six subgenera (A to F). Serotyping of 36 adenovirus isolates obtained from stool specimens was performed using various anti-adenovirus sera and PCR methods. As shown in Table 3, adenovirus serotype 1 (AdV-1) and AdV-5 were frequently detected in patients aged <2 years of age, whereas AdV-2 and AdV-3 were frequently

found in those aged ≥2 years. AdV-5 was most frequently isolated in patients under 2 years of age, while AdV-3 was prominent in those over 2 years of age. The isolation of enteric types of adenovirus (AdV-31 and AdV-40/41) was found in five patients: three aged <2 years and two aged ≥2 years. The majority of adenovirus (subgenera B and C) isolates were non-enteric organisms generally associated with respiratory tract symptoms.[24–28]

3.4. Clinical manifestations

The classical clinical manifestations of intussusception, i.e., abdominal pain, vomiting, rectal bleeding/bloody stool, abdominal mass, and lethargy/irritability, were analyzed based on the age distribution and the presence of viral detections (Table 4). Abdominal pain was the most frequent presentation in patients ≥2 years of age. In contrast, lethargy/irritability and rectal bleeding/bloody stool were less frequent symptoms in older patients with intussusception. Rectal bleeding/bloody stool was a more frequent presentation in patients <2 years of age (77.1%) than in those ≥2 years of age (26.1%). No differences in the frequencies of vomiting and abdominal mass were noted between the age groups. Furthermore, there were significant differences in the primary symptoms among patients positive for adenovirus, positive for virus other than adenovirus, and negative for pathogens (Table 4). The symptom of abdominal pain was frequently seen among patients positive for adenovirus, but rectal bleeding/bloody stool was seen in less than half of these patients. However, no difference in the frequency of typical clinical manifestations was noted between those positive and negative for adenovirus isolates in each age group (data not shown).

Most patients sought medical attention and were diagnosed within 24 h after the onset of symptoms. Forty-five of 48 patients

Table 2
The number of cases with uncomplicated gastroenteritis by age and viral isolate

	No. of cases					
	Overall (%)	<1 year	≥1 to <2 years	≥2 to <3 years	≥3 to <4 years	≥4 to <5 years
Positive	56 (68.3)	16	26	8	4	2
Adenovirus	4 (4.9)	1	2	1	0	0
Type 3				1		
Type 5		1	1			
Type 31			1			
Enterovirus	3 (3.7)	2	1	0	0	0
Parechovirus	3 (3.7)	3	0	0	0	0
Norovirus	44 (53.7)	10	23	6	4	1
Rotavirus	2 (2.4)	0	0	1	0	1
Negative	26 (31.7)	13	6	2	2	3

Note. Viral isolates from stool specimens were identified in 82 patients aged under 48 months with uncomplicated gastroenteritis. The rate of patients with positive viral isolates was 68.3%; norovirus was identified in the majority of cases (78.6%), while others were adenovirus (7.1%), enterovirus (5.4%), parechovirus (5.4%), and rotavirus (3.6%).

Table 3
Types of adenovirus isolates from stool samples

Adenovirus serotype (subgenus)	No. of cases		
	Overall (n = 36)	<2 years (n = 19)	≥2 years (n = 17)
1 (C)	5	4	1
2 (C)	5	2	3
3 (B)	10	3	7
5 (C)	9	7	2
6 (C)	1	0	1
7 (B)	1	0	1
31 (A)	2	1	1
40/41 (F)	3	2	1

Note. Table 3 shows subgenus C is a dominant adenovirus species among intussusception cases. Subgenus C is a non-enteric organism generally associated with fever and upper respiratory tract symptoms. Subgenera A and F are enteric adenoviruses. With regard to age, adenovirus types 1 and 5 were dominant in cases <2 years of age, but type 3 was more frequently detected in cases ≥2 years of age.

Table 4
Clinical manifestations of intussusception cases by age and viral isolate

Symptom	No. of cases (%)[a]			No. of cases (%)[b]		
	Overall (n = 71)	<2 years (n = 48)	≥2 years (n = 23)	Adenovirus (n = 35)	Other than adenovirus (n = 20)	Negative isolation (n = 15)
Abdominal pain	36 (51)	14[c] (29)	22[c] (96)	24[d] (69)	9 (45)	2[d] (13)
Vomiting	41 (58)	31 (65)	10 (43)	18 (51)	13 (65)	9 (60)
Rectal bleeding/bloody stool	45 (63)	38[c] (79)	7[c] (23)	17[e] (49)	16[e] (80)	12[e] (80)
Lethargy/irritability	53 (75)	43[c] (90)	10[c] (43)	22 (63)	17 (85)	13 (87)
Abdominal mass	27 (38)	17 (35)	10 (43)	13 (37)	10 (50)	4 (27)

[a] Data represent the number of cases presenting the symptoms. Data show that in cases under 2 years of age, there was a high frequency of lethargy/irritability and rectal bleeding/bloody stool; in cases over 2 years of age, there was a high frequency of abdominal pain.

[b] Data represent the number of cases presenting the symptoms, except for one case of multiple viral infections. The symptom abdominal pain was frequently seen in cases where adenovirus was detected, but rectal bleeding/bloody stool was observed in less than half of them.

[c] $p < 0.0001$ comparing age groups, determined by Chi-square test.

[d] $p < 0.01$.

[e] $p < 0.05$, determined by the Chi-square test and Ryan's test, when comparing adenovirus cases with those in whom a virus other than adenovirus was detected and those in whom virus was not detected.

aged <2 years and 18 of 23 patients aged ≥2 years were diagnosed in less than 24 h. None of the patients enrolled in this study required surgical intervention.

4. Discussion

The underlying cause of intussusception in young children is unknown. However, it has been associated with several pathogens, including adenovirus and rotavirus.[3,10–16,28–32] In the current study various viruses from stool specimens were isolated in 78.9% of patients with intussusception during their hospitalization. In addition, 63.2% of the pathogens were adenovirus and the others were various pathogens commonly observed in patients aged under 2 years. The isolation of adenovirus was significantly increased to 73.9% in patients over 2 years of age. Among the patients with uncomplicated gastroenteritis, adenovirus was detected in only four cases, thus suggesting that adenovirus infection had not reached epidemic proportions.

In this report, we compared the rate of virus isolation from stool specimens between patients with intussusception and those with uncomplicated gastroenteritis. A study of viral isolates from healthy children is required to conclusively determine the involvement of viral infections, including adenovirus infections, in intussusception. However, we were unable to examine the viral isolates in stool specimens from healthy subjects (children) as a control group because of the difficulty in obtaining informed consent from such individuals. Nevertheless, our results show an apparent high number of adenovirus isolates. It would also be helpful to identify other studies that have searched for such adenoviruses in healthy controls.

The causative incidence of adenovirus isolation ranges from 30% to 50%.[3,10–16] Adenovirus is frequently seen in the mucosa and hyperplasic lymph nodes at the lead point and in the appendices of intussusception patients.[30,33] In the current study the detection rate of adenovirus (36 of 71, 50.7%) was remarkably high in comparison to previous reports. A recent study comparing Vietnam and Australia showed that 34% of patients were positive for adenovirus in stool in Vietnam and 40% in Australia. All patients were aged ≤25 months. Bines et al. reported that the significantly increased isolation rate in comparison to that of healthy controls in each country strongly supports an association.[3] Similarly in a recent study from Taiwan, 44% of patients with intussusception shed adenovirus in the throat or rectal specimens in comparison to only 3.8% of healthy controls. Furthermore, in another study, acute primary viral infections were identified in 65% of intussusception patients for whom paired sera were available (39.5% adenovirus, 9.3% human herpes virus 6, 11.6% human herpes virus 7, 4.7%

Epstein–Barr virus).[14] The adenovirus genome was detected in four of nine mesenteric lymph nodes; 75% of the patients in whom a primary adenovirus infection was confirmed by seroconversion were aged >1 year.[14] These reports reduce the likelihood that shedding of adenovirus in intussusception patients is coincidental. The frequency of adenovirus isolates in this study was almost comparable to the data from Vietnam and Australia in patients <2 years of age. Therefore, almost 40% of the patients with intussusception aged below 2 years may have adenovirus infections.

The majority of patients with intussusception are under 1 year of age.[1] The present study in Hiroshima shows a different age distribution in patients with intussusception in comparison to other studies: 32.4% were aged <1 year, 35.2% were aged 1–2 years, and 32.4% were aged >2 years (Figure 1). Furthermore, another remarkable finding was the increased frequency of adenovirus isolates in those patients aged over 2 years. No precise evidence for the prevalence of adenovirus infection in the city of Hiroshima was noted during the study period. There were no significant variations in the annual presentation rate and the age distribution of intussusception patients over the 10-year period (data not shown). Therefore, it appears that the reason for the difference in age distribution of intussusception patients in Hiroshima is not due to an epidemic of adenovirus infection over the last 2 years. Alternatively, adenovirus infection may be a major risk factor for intussusception in children over 2 years of age.

Adenoviruses cause acute respiratory disease in children plus a wide array of other syndromes, including pharyngoconjunctival fever, epidemic keratoconjunctivitis, myocarditis, hemorrhagic cystitis, acute diarrhea, intussusception, and encephalomyelitis.[25] Respiratory tract infections are the most common manifestation of adenovirus infections in children and are caused by the respiratory types: AdV-1, 2, 3, 5, and 6 (subgenera B and C).[24,26] AdV-31 and AdV-40/41 (subgenera A and F), which are the important serotypes causing enteritis,[24,27] were detected by PCR in the stool specimens of only five patients. The current study showed AdV-1 and AdV-5 (species C) to be dominant in the age group <2 years, and AdV-3 and AdV-7 (species B) to be dominant in the age group ≥2 years. Schmitz et al. reported an age predilection for adenovirus.[25] The highly significant age predilections were subgenus A (AdV-12, 18, 31) in infants and subgenus C (AdV-1, 2, 5, 6) in infants and small children. Regarding subgenus B, AdV-3 is common in school children, AdV-7 is common in school children and adults, and AdV-4 and AdV-8 and other species of subgenera B and D are common in adults. The current results show an age distribution of adenovirus isolates similar to the above age predilections of adenovirus. Salvaraj et al. reported the intussusception-associated adenovirus

isolates to be similar to the circulating non-intussusception-associated strains, and the host immune response may be the key determinant of the clinical course of infection after adenovirus colonization.[32] In the current study, the common adenovirus types causing respiratory tract infection were detected at a high frequency associated with the development of intussusception in children. An evaluation of host responses to adenovirus, together with an exploration of adenovirus virulence determinants remains a priority area of investigation into the most common cause of acute bowel obstruction in young children.

Recently, Blanch et al. reported that the classic picture of intussusception might frequently not be present in the majority of current cases.[34] The incidences of the typical clinical manifestations in the cases in this study were also less frequent (Table 4) and the presence of the classic triad of vomiting, abdominal pain, and rectal bleeding/bloody stool was rare (data not shown). Furthermore, it was found in the present study that the primary clinical manifestations were different in the two different age groups (Table 4). Particularly, the presence of rectal bleeding/bloody stool and lethargy/irritability was frequently observed in cases <2 years of age. Lethargy/irritability was particularly frequent in the cases <2 years of age; in contrast abdominal pain was frequent in the cases ≥2 years of age. Furthermore, there were significant differences in the primary symptoms among cases positive and negative for adenovirus (Table 4). No difference in the frequency of typical clinical manifestations was noted in patients positive and negative for adenovirus isolates in each age group (data not shown). It appears that the age factor rather than adenovirus infection may result in the differences in primary symptoms. Blanch et al.[34] suggested that the early diagnosis of intussusception may decrease the frequency of the classical clinical presentations. In this study, the fact that no cases required surgical treatment may support the possibility of early diagnosis of intussusception.

In this study a high prevalence of viral isolates was found in the stools of patients with intussusception during early childhood. Adenovirus was significantly isolated in patients with intussusception aged ≥2 years, thus suggesting that adenovirus is an important risk factor for the development of intussusception in young children, particularly those ≥2 years of age.

Conflict of interest: None of the authors have any financial support or any conflict of interest.

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Institutional report - Thoracic oncologic

Repeat resection of pulmonary metastasis is beneficial for patients with osteosarcoma of the extremities

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Abstract

Pulmonary metastasectomy in osteosarcoma can lead to long-term survival, but the role for repeat pulmonary metastasectomy is undefined. To confirm the value of repeat pulmonary resection of recurrent pulmonary metastases, we herein reviewed our institutional experience. Between 1989 and 2007, 25 patients with pulmonary metastases from osteosarcomas of the extremities underwent pulmonary resection, and 14 patients underwent repeat pulmonary metastasectomy. Ten of 14 patients underwent complete resection. Various perioperative variables were investigated retrospectively in these patients to confirm a role for repeat metastasectomy and analyze prognostic factors for overall survival (OS) after repeat pulmonary metastasectomy. OS rate after repeat pulmonary metastasectomy was 43% at two years and 19% at five years. On multivariate analysis, patients with complete resection presented significantly favorable OS (P=0.02). Interestingly enough, survival curve of patients with complete resection after the first pulmonary metastasectomy was almost the same as that of patients with complete resection after the second pulmonary metastasectomy. In conclusion, patients with complete resection for recurrent pulmonary metastasis show a significantly better prognosis after repeat pulmonary metastasectomy. Our data imply that repeat pulmonary metastasectomy might be beneficial because it can salvage a subset of patients with osteosarcoma who retain favorable prognostic determinants.
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Keywords: Lung; Metastasectomy; Osteosarcoma; Recurrence; Repeat resection

1. Introduction

Surgical resection has been consistently shown to prolong survival among patients with pulmonary metastases [1], and similar results are obtained for lung metastases from osteosarcoma [2–7]. The unique characteristics of osteosarcoma, including metastatic affinity for the lung, make pulmonary metastasectomy a central component of therapy for this disease [1]. Approximately 50% of patients with osteosarcoma develop synchronous or metachronous metastatic lung diseases, and only 20–40% of those found to have pulmonary metastases will survive five years [2–7]. Although the prognosis for patients with osteosarcoma has recently improved dramatically, those who develop metastatic pulmonary disease still continue to pose a particularly difficult challenge.

There are several reports about the survival and the prognostic factors for patients with pulmonary metastasectomy for osteosarcoma [2–7], but there are few data in the literature on patients who have undergone repeat pulmonary metastasectomy for osteosarcoma [8, 9]. Thus,

we reviewed the clinical data of patients with osteosarcoma treated with pulmonary metastasectomy in our hospital to determine the long-term results and the prognostic predictors of survival in this subset of patients.

2. Patients and methods

From December 1989 to November 2007, according to our medical records, 25 patients had received pulmonary resection for the first time due to metastases of osteosarcoma of the extremities. Twenty-three of them underwent complete resection. Furthermore, 17 patients presented recurrence after the first metastasectomy and 14 patients underwent repeat pulmonary metastasectomy (Fig. 1). The patients were 10 males and 4 females with a median age of 22 years (range, 10–54 years) at the time of repeat pulmonary metastasectomy. Multimodality treatment consisting of surgery and chemotherapy were conducted in all cases. Diagnosis of metastatic pulmonary nodules was made by X-rays and computed tomography that was routinely examined at several months interval after the first diagnosis of primary tumor. Further examinations were also performed to exclude extrapulmonary metastases.

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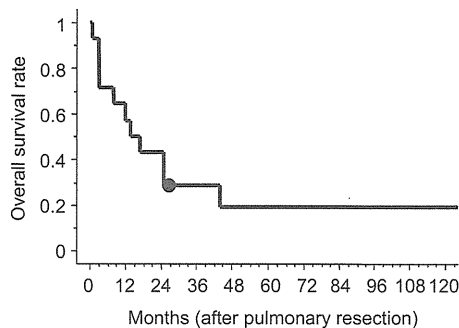E-mail address: hdate@kuhp.kyoto-u.ac.jp (H. Date).

Fig. 1. Overall survival of patients following repeat pulmonary metastasectomy ($n=14$).

All patients who underwent resection of their pulmonary metastases at any time met the following criteria: (1) pulmonary lesions were deemed resectable not only by radiological examinations but also by the patients' general conditions, (2) metastatic disease was limited to the lungs, and (3) locoregional control of their primary cancer was obtained or obtainable. Complete resection was defined as no tumor cell at the surgical margin of the resected lung that was examined macroscopically and histologically. All the visible and palpable nodules were resected during the surgery and subjected to histological examination for confirmation of the diagnosis of metastases. The surgical approach was chosen according to the location and number of pulmonary nodules.

Therapeutic control for patients with lung metastases was based on the surgery in combination with pre- and/or postoperative multiagent chemotherapy. As to the treatment of osteosarcoma in the original site, all the patients undertook the surgery in combination with neoadjuvant and/or adjuvant chemotherapy consisting of multiagents. The detailed regimens of chemotherapy were different among patients; however, cisplatin, adriamycin, etoposide, methotrexate, and isofamide were used as antitumor drugs for chemotherapy pre- and postoperatively.

All patients were retrospectively analyzed for age, gender, detection of the first pulmonary metastasis, recurrence of primary tumors before pulmonary metastases, disease-free interval (DFI), number of pulmonary metastases, location of metastases, complete resection, and number of metastasectomy for pulmonary recurrence, regarding long-term survival. The endpoint was overall survival (OS) after the repeat pulmonary metastasectomy. DFI-1 was defined as the duration from the resection of the primary tumor to the initial diagnosis of the metastatic pulmonary tumor. DFI-2 was defined as the duration from the first pulmonary metastasectomy to the diagnosis of the recurrent pulmonary metastasis. OS was defined as the time between the repeat resection of pulmonary metastasis and the date of the last follow-up or death.

2.1. Statistical analysis

Statistical analysis was performed using the StatView (version 4.5) software package (Abacus Concepts, Berkeley, CA). The postoperative survival rate was analyzed by the Kaplan–Meier method. The prognostic influence of varia-

bles on survival was analyzed using the log-rank test for univariate analyses and the Cox's proportional hazards model for multivariate analyses. Differences were considered significant when $P<0.05$.

3. Results

Fourteen patients with recurrent pulmonary metastases from osteosarcomas underwent a thoracotomy and resection. The sites of primary tumors were the femur in eight cases, the tibia in five cases, and the humerus in one case (Table 1). In all cases, the primary tumors were resected completely. DFI-1 varied from 0 month to 9 years, including one case (7%) with simultaneous detection of the first pulmonary metastasis and the primary tumor. DFI-2 varied from 3 months to 8 years. In four patients (29%), lung metastases were found to be bilateral, while 10 patients (71%) had a solitary pulmonary metastasis. Ten of 14 patients underwent complete surgical resection. As of four patients with incomplete resection, two patients had bilateral metastatic lesions and were planned to undergo a staged bilateral thoracotomy; however, in all cases the

Table 1
Patient characteristics

Age	10–54 years (median 22 years)
Gender	
Male	10
Female	4
Location of primary tumors	
Femur	8
Tibia	5
Humerus	1
Detection of the first lung metastasis	
At initial presentation	1
During chemotherapy	3
After treatment	10
Recurrence of primary tumors ahead of lung metastases	
Yes	3
No	11
DFI-1	0–108 months (median 22 months)
DFI-2	3–95 months (median 7 months)
Number of recurrent pulmonary metastases	
<5	11
≥5	3
Location of recurrent metastases	
Unilateral	10
Bilateral	4
Surgical operation	
Wedge resection	11
Lobectomy	2
Pneumonectomy	1
Complete resection	
Yes	10
No	4
Number of surgery	
2	8
3	3
4	1
5	2

DFI (disease-free interval)-1: the duration from the resection of the primary tumor to the initial diagnosis of the metastatic pulmonary tumor. DFI-2: the duration from the first pulmonary metastasectomy to the diagnosis of the recurrent pulmonary metastasis.

Table 2
Univariate analysis for overall survival

Variables	Number of patients	2-year OS (%)	5-year OS (%)	Univariate analysis P-value
Age				
<20 years	6	33.3	16.7	0.74
≥20 years	8	50.0	12.5	
Gender				
Male	10	50.0	20.0	0.30
Female	4	25.0	0.0	
Pulmonary metastases identified during chemotherapy				
Yes	3	0.0	0.0	0.0036
No	11	54.5	18.2	
Recurrence of primary tumors ahead of lung metastases				
Yes	3	0.0	0.0	0.017
No	11	54.5	24.2	
DFI-1				
<1 year	6	16.7	0.0	0.029
≥1 year	8	62.5	25.0	
DFI-2				
<1 year	11	45.5	18.2	0.65
≥1 year	3	33.3	0.0	
Number of pulmonary metastases				
<5	11	54.5	18.2	0.25
≥5	3	0.0	0.0	
Location of metastases				
Unilateral	10	50.0	20.0	0.46
Bilateral	4	25.0	0.0	
Complete resection				
Yes	10	60.0	20.0	0.0058
No	4	0.0	0.0	
Number of metastasectomies for pulmonary recurrence				
2	8	37.5	12.5	0.36
≥3	6	50.0	16.7	

OS (overall survival): the time between the repeat resection of pulmonary metastasis and the date of the last follow-up or death. DFI (disease-free interval)-1: the duration from the resection of the primary tumor to the initial diagnosis of the metastatic pulmonary tumor. DFI-2: the duration from the first pulmonary metastasectomy to the diagnosis of the recurrent pulmonary metastasis.

disease exacerbated after unilateral metastasectomy. The remaining two patients had unilateral metastatic lesions, but one of them found pleural dissemination at the time of thoracotomy, and the other presented a new extrathoracic metastatic lesion at the perioperative time. Ten patients with unilateral tumors underwent a unilateral thoracotomy for resection of their metastases. One of four patients with bilateral tumors underwent simultaneous
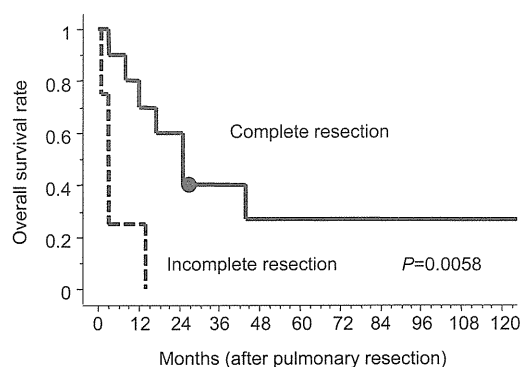

Fig. 2. Overall survival for patients concerning completeness of resection. A better overall survival was observed for patients with complete resection of recurrent pulmonary metastases ($P=0.0058$).

bilateral thoracotomy, whereas three patients had planned a staged bilateral thoracotomy for bilateral lesions; however, two patients were forced to abandon the contralateral surgery as described before. As for the number of surgeries, staged bilateral thoracotomies were counted as one operation. An attempt was made to conserve as much lung tissue as possible. This was reflected by the fact that wedge resections were the most common procedures performed. No patients died directly of surgery.

The median time at follow-up examination was 16 months (range 1–208 months). Of 10 patients with complete resection, three patients (30%) remained free of disease after repeat pulmonary metastasectomy, and seven patients (70%) developed recurrences, the majority of which were located in the chest. Seven of eight recurrent patients (88%) underwent a third metastasectomy for the re-recurrent pulmonary metastases. Three and two patients underwent fourth and fifth metastasectomy, respectively.

The OS rate was 42.9% at two years and 19.0% at five years after pulmonary resection (Fig. 1). Three (21%) patients are currently alive without evidence of disease and no patient is alive with disease. Eleven patients (79%) died of disease, and none died of other causes. Univariate analysis showed that a better OS was observed for patients without lung metastases identified during chemotherapy ($P=0.0036$), without recurrence of primary tumors ahead of lung metastases ($P=0.017$), with DFI-1 <1 year ($P=0.029$), and with complete resection ($P=0.0058$) (Table 2, and Fig. 2). However, on multivariate analysis, patients with complete resection presented significantly favorable OS ($P=0.021$) (Table 3). Interestingly enough, survival curve of patients with complete resection after the first pulmonary metastasectomy was almost the same as that of patients with complete resection after the second pulmonary metastasectomy (Fig. 3).

4. Discussion

Pulmonary metastasectomy has become the standard therapy for various metastatic malignancies to the lungs and pulmonary metastasectomy in osteosarcoma is also thought to lead to long-term survival [1, 2]. Unlike epithelial cancers, around 50–60% of patients with osteosarcoma relapse only in the lung, making pulmonary metastasectomy a viable option for treatment [8]. Furthermore, 40% of patients who relapse after pulmonary resection again

Table 3
Multivariate analyses for overall survival

Variables	Hazard ratio	95% CI	P-value
Pulmonary metastases identified during chemotherapy			
Yes	20.84	0.81–537.12	0.067
DFI-1			
<1 year	1.54	0.21–11.29	0.67
Recurrence of primary tumors ahead of lung metastases			
Yes	2.40	0.29–20.28	0.42
Complete resection			
Incomplete	16.64	1.53–180.60	0.021

CI, confidence interval. DFI (disease-free interval)-1: the duration from the resection of the primary tumor to the initial diagnosis of the metastatic pulmonary tumor.
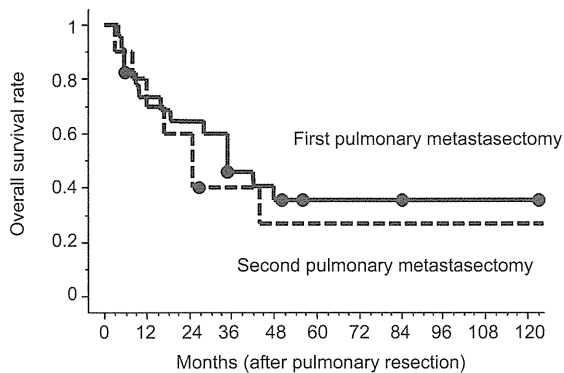
Fig. 3. Comparison with overall survival after the first pulmonary metastasectomy with complete resection and that after the second pulmonary metastasectomy with complete resection.

exhibit recurrence in the lung [10]. There are several encouraging studies about repeated surgical interventions for recurrent metastases from various primaries [11–13]; however, few data are available in the literature on patients who have undergone repeat pulmonary metastasectomy for osteosarcoma [8, 9]. Therefore, we decided to report our experience with repeat pulmonary metastasectomy in patients with osteosarcomas and to evaluate its role in their treatment focusing on OS. The 5-year OS rate for patients who underwent first pulmonary metastasectomy for osteosarcomas was up to 40% in several reports [3–7]. Since Fig. 3 showed that the 5-year OS rate for patients who underwent first pulmonary metastasectomy was 36%, our attitude toward the first pulmonary metastasectomy for osteosarcomas appeared to be acceptable. Biccoli et al. showed that patients who have a second metastasectomy have the same probability of disease-free survival as those operated upon the first time [8]. Bielack et al. stated in their most recent and largest series that five-year OS for the second recurrence was 16%, while it went up to 32% when a renewed surgical remission was achieved [9]. On the other hand, we demonstrated that patients who have a second metastasectomy with complete resection have the same probability of OS as those who have a first metastasectomy with complete resection.

To date, various parameters, such as DFI, completeness of the resection, timing of metastases, number of metastases, tumor size, and laterality of metastases have been reported as prognostic factors for the first metastasectomy [2–7], but there are few reports about the repeat pulmonary metastasectomy for osteosarcoma [9]. Bielack et al. described several parameters, such as shorter interval for recurrence, multiple lesions, failure to achieve a macroscopically complete surgical remission, and no chemotherapy administered for recurrence, as adverse prognostic factors for OS after the second recurrence on multivariate analysis [9]. In this study, several prognostic factors for the repeat pulmonary metastasectomy were found on univariate analysis, but a better OS was observed for patients with complete resection on multivariate analysis. Complete resection has been reported to be a better prognostic factor for OS after the first pulmonary metastasectomy in many studies [3, 6, 14]. Furthermore, complete resection has also been shown as a favorable prognostic factor for

reoperative pulmonary metastasectomy for osteosarcoma and sarcomatous pediatric histologies [9, 15]; however, we should keep in mind that our study consisted of a small number of patients, so the accumulation of cases is necessary to evaluate a prognostic factor properly and to determine the selection criteria for resection. It is interesting that whether complete resection is performed or not is only determined after the surgical intervention is done. In this sense, our results support the idea that survival benefit will be obtained if pulmonary metastasectomy is performed aggressively and repetitively [2, 4, 8, 9].

There are several limitations to our analysis. The retrospective design is the most practical way of addressing our question because of the incidence of osteosarcoma, but the results should be interpreted carefully. Chemotherapeutic regimens have evolved substantially and rapidly over the last three decades and continue to be highly individualized based on unique patient and tumor characteristics, but we could not analyze our patients in this study because of a lack of complete data. It could be hypothesized that long-term survivors have biologically different and less aggressive tumors. In addition, since our results were based on the small number of patients in one institution, we needed a long study period to increase the number of patients in our study; however, several factors related to this could affect the results, such as the fact that a diagnostic modality had been changed due to the introduction of PET scan. Therefore, a prospective, large-scale study with multiple institutions would be inevitable in the future to reconfirm the current results.

In conclusion, patients with complete resection for recurrent pulmonary metastasis show a significantly better prognosis after repeat pulmonary metastasectomy. Our data imply that repeat pulmonary metastasectomy might be beneficial because it can salvage a subset of patients with osteosarcoma who retain favorable prognostic determinants.

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