Possible nosocomial transmission of virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation

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Conflict-of-interest disclosure: The authors declare no conflict of interest.

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Abstract

Adenovirus (ADV) or BK virus (BKV)-associated hemorrhagic cystitis (HC) is a common complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Several risk factors have been previously reported; however, it is unclear whether virus-associated HC can be transmitted. To clarify this point, we performed a retrospective cohort study on 207 consecutive patients who underwent allo-HSCT at Kyoto University Hospital between 2012 and 2018. We evaluated the incidence and risk factors of virus-associated HC and performed a phylogenetic analysis of the ADV partial sequence. The median age at transplantation was 50 (range, 17–68) years. Fifty-eight patients (28%) developed HC. ADVs were detected in 18 cases, BKVs were detected in 51, both were detected in 12, and only John Cunningham virus (JCV) was detected in 1 case. No factor was significantly associated with HC. However, both ADV- and BKV-HC occurred intensively between April 2016 and September 2017, which suggested possible nosocomial transmission of ADV and BKV. Genome sequencing of the hexon, E3, and penton regions of detected ADVs identified 7 cases of ADV type 11, 2 cases of type 35, and 3 cases of a type 79-related strain. A sequence analysis revealed that these strains in each type were almost identical, except for one case of a type 79-related strain.

In conclusion, ADV-HCs with possible nosocomial transmission were described based on genotyping of the virus and partial sequencing of the viral genome. Although viral HC after allo-HSCT is thought to mainly be due to reactivation of a latent virus, nosocomial transmission of ADV or BKV should also be considered.

Key Word: allogeneic transplantation, hemorrhagic cystitis, adenovirus, BK virus, nosocomial transmission

Introduction

Virus-associated hemorrhagic cystitis (HC) is a common complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) , with an incidence of 10 to 30% [1-3]. The causative virus of HC is frequently adenovirus (ADV), BK virus (BKV), JC virus (JCV), and, in rare cases, cytomegalovirus (CMV) [4]. The clinical presentation of HC varies from microscopic hematuria to uncontrollable gross bleeding, urinary obstruction, and acute renal failure, and is often accompanied by pollakiuria and dysuria. In severe cases, prolonged anemia or bladder perforation can be fatal. It is often difficult to treat severe HC despite maintenance of urinary output by hydration, continuous bladder perfusion, bladder cauterization, reduction of immunosuppressive drugs, administration of ciprofloxacin, and/or intravesicular instillation or intravenous injection of cidofovir (CDV) [5-9], and a standard management of severe HC after allo-HSCT has not yet been established. Risk factors such as male, young patient, graft-versus-host disease (GVHD), unrelated donor, myeloablative conditioning and post-transplant mycophenolate were previously reported. Several studies suggested that virus-associated HC after allo HSCT is caused by reactivation of latent ADV or BKV [10, 11], but nosocomial transmission of ADV or BKV has rarely been reported. Two previous studies suggested the possibility that BKV-HC could develop due to nosocomial transmission [12, 13]. While there have been no reports of ADV-HC due to nosocomial transmission, nosocomial ADV pneumonia, conjunctivitis and enteritis have been previously reported [14, 15]. HC due to ADV or BKV nosocomial transmission is possible, because these non-enveloped viruses are relatively resistant to disinfection such as by alcohol or chlorhexidine in routine practice [16, 17]. If HC is caused not only by virus reactivation but also by de novo virus infection or nosocomial transmission, sufficient infection prevention measures would reduce the incidence of HC, and improve transplant outcomes and QOL of patients. At our institution, we have often encountered the frequent occurrence of HC over a short duration. To examine the possibility of nosocomial transmission, we performed a single-center

retrospective study of 207 patients who underwent allo-HSCT at Kyoto University Hospital between 2012 and 2018.

Methods

Patients

A total of 207 consecutive patients who received allo-HSCT from January 2012 to March 2018 at Kyoto University Hospital were included in this retrospective cohort study. Clinical data and laboratory results were reviewed and collected from medical records. This study was approved by the Institutional Review Board of Kyoto University Hospital in accordance with the Declaration of Helsinki, and informed consent was obtained from each participant.

Definition

HC was diagnosed by symptoms of bladder irritation and microscopic or macroscopic hematuria, and etiology was determined by polymerase chain reaction (PCR) for ADV, BKV, and JCV to prove the presence of virus in samples extracted from urine. PCR Screening of these viruses and screening for hematuria was not routinely performed before and after HCST, but was done when symptoms of bladder irritation, hematuria, or renal dysfunction appeared. In all HCST cases, CMV screening performed once a week with blood, but not with urine. Following previous reports, HC was graded as follows: grade 1, microscopic hematuria with symptoms of bladder irritation, grade 2, macroscopic hematuria, grade 3, macroscopic hematuria with clots, and grade 4, macroscopic hematuria with necessary intervention for clot evaluation and/or urinary retention [18]. We analyzed the incidence and risk factors for virus-associated HC and examined the possibility of nosocomial transmission based on the overlap of the hospitalization period between patients and partial sequencing of hexon,

E3, and penton regions with ADV-DNA detected in HC patients' urine. The ADV type was identified based on hexon and penton sequences.

Detection of ADV, BKV, and JCV

DNA was extracted from urine samples of HC cases (Qiamp MinElute Virus Spin kit, Qiagen, Hilden, Germany), and qualitative and quantitative tests were performed by PCR[19]. To examine the possibility of the nosocomial transmission of detected ADVs, hexon, E3 and penton coding regions were sequenced and analyzed using samples from patients who developed HC between April 2016 and September 2017.

Phylogenetic analysis of the partial sequence of the hexon, E3, and penton regions of ADV

Genome sequencing of detected adenoviruses was used to determine the adenovirus genotype. The partial sequences of the hexon, E3, and penton regions were compared with Ad11 (AF532578.1 and KF268121.1), Ad34 (AY737797.1), Ad35 (AY128640.2), Ad55 (FJ643676.1) and Ad79 (LC177352.1). The genome sequences were obtained from a public database (GenBank: [https://www.ncbi.nlm.nih.gov/nuccore]). Multiple alignments and phylogenetic tree analysis were performed using MEGA 7.0 software (https://www.megasoftware.net/). DNA sequences were aligned by ClustalW with an open gap penalty of 15, a gap extension penalty of 6.66, a transition weight of 0.5 with IUB DNA weight matrix and a delay divergent cutoff (%) of 30. The Neighbor-joining method was applied for the phylogenetic tree analysis, the reliability of which was assessed by bootstrap resampling (1000 pseudoreplicates). Genetic distances were calculated using Kimura’s 2-parameter method[20].

Statistical analysis

Categorical variables were compared using the chi-square test or Fishers' exact test, as appropriate. The probabilities of virus-associated hemorrhagic cystitis were estimated based on cumulative incidence curves. The competing event was death without hemorrhagic cystitis. Fine and Gray's proportional hazards model was used for the evaluation of risk factors for hemorrhagic cystitis. Factors with P-values of less than 0.20 in the univariate analysis were included in the multivariate analysis. P values of less than 0.05 were considered statistically significant. Statistical analyses were performed with Stata version 13.5 (Stata Corp, College Station, TX).

Results

Patient and transplant characteristics

Patient and transplant characteristics are listed in **Table 1**. Of the 207 patients, 130 were male and 77 were female. The median age at transplantation was 50 (range, 17-68) years. Underlying diagnoses were acute myeloid leukemia (39.1%), acute lymphoblastic leukemia (15.9%), myelodysplastic syndrome (16.9%), malignant lymphoma (15.9%), adult T-cell leukemia/lymphoma (5.3%), aplastic anemia (1.9%) and others (4.8%). Other underlying diagnoses included severe blastic plasmacytoid dendritic cell neoplasm, chronic myeloid leukemia, chronic lymphocytic leukemia, myelofibrosis, and plasmacytoma. Donor sources were related (6.8%) or unrelated (41.5%) bone marrow, related peripheral blood stem cells (14.0%), and unrelated cord blood (37.7%). A total of 91 patients (44.0%) received myeloablative preparative regimens and the remaining 116 (56.0%) received reduced-intensity conditioning. GVHD prophylaxis was based on tacrolimus or cyclosporine, and mycophenolate mofetil (MMF) and short-term methotrexate (MTX) were added as needed. Pretransplant conditioning and GVHD prophylaxis were determined at the discretion of the attending physician.

Incidence of HC

Among the 207 patients, 58 (28.0%) developed HC. The median date of onset was day 48 (day 0-1410) and the median duration of HC was 28 days (3-196 days) (**Table 2**). ADVs were detected in 18 cases, BKVs in 51 cases, both in 12 cases and only JCVs in 1 case by PCR on urine samples. Most cases of ADV-HC were grade 3 or 4, and the duration of ADV-HC was more than 2 weeks. ADV-HC tended to be more severe (P <0.001) and more prolonged (P = 0.021) than BKV-HC.

In the multivariate analysis, the number of HLA allele mismatches (4-6 mismatches vs. match, hazard ratio (HR) 1.90, P=0.080) and diagnosis (lymphoid vs. myeloid malignancies, HR 1.62, P=0.083) were marginally associated with the risk of HC. Myeloablative conditioning, patient age, and transplantation source did not affect the risk of HC (**Table 3**). The diagnosis was also marginally associated with the risk of severe HC (lymphoid vs. myeloid malignancies; HR 2.12, P=0.070) (**Supplemental Table 1**).

Phylogenetic analysis of the partial sequences of the hexon, E3, and penton regions of ADV

ADV-HC and BKV-HC were more frequent from the spring of 2016 to the summer of 2017 than in other periods (**Figure 1 and Supplemental Table 2**). This trend suggests the occurrence of HC epidemics. Between April 2016 and September 2017, 23 patients developed HC. ADV and BKV were detected in 12 and 18 cases, respectively. Therefore, we performed conventional typing of ADV and a phylogenetic analysis of the partial sequences of the hexon, E3, and penton regions of ADV. Genome sequencing of detected adenoviruses on the hexon, E3, and penton regions identified 7 cases of ADV type 11, 2 cases of type 35, and 3 cases of a type 79-related strain. The sequences of these strains were almost identical among all the cases of type 11 and type 35, and in 2 of the 3 cases of the type 79-related strain (i.e., cases 10 and 11) (**Figure 2**). The results of genome

sequencing and the overlap of hospitalization for each patient strongly suggested nosocomial transmission during this period (**Figure 3**).

Discussion

ADV- or BKV-associated HC is a common complication after allo-HSCT. It strongly affects the quality of life of patients and sometimes leads to a fatal complication. Several previous studies have analyzed the incidence of HC. While age at HSCT, unrelated donor, myeloablative conditioning and post-transplant mycophenolate mofetil administration have been reported as risk factors for HC [10, 11], these were not associated with HC in our study. Patients with lymphoid malignancies or HLA allele mismatches showed a tendency to develop HC, but this trend was not statistically significant. Repetitive and lymphosuppressive chemotherapy for lymphoid malignancies may have increased the risk of viral infection. HLA mismatches may have led to the risk of GVHD and subsequent viral infection. A weak association may be partly explained by the stronger impact of nosocomial transmission of HC at our center. The difference in patient and transplant backgrounds between the studies may also explain the different findings.

HC developed frequently during the short study period, and there were several cases of HC that had the potential for nosocomial transmission based on genotyping of the virus and partial sequencing of the viral genome. Since both ADV and BKV are non-enveloped viruses, which are resistant to disinfection such as by alcohol in routine medical care and benzalkonium chloride in environment cleaning[21], and an HC patient's urine contains enormous amounts of viral virions, it is possible that nosocomial transmission can occur via medical personnel, medical instruments or common toilets. The importance of environmental cleaning in the care of HC patients has not been stressed enough. Two previous studies suggested the possibility of nosocomial transmission based on a partial sequence of the BKV genome detected from BKV-HC patients after allo-HSCT[12, 13]. Sammons et al. suggested nosocomial transmission of ADV type 3 respiratory tract infection in the NICU based on the complete homology of the whole genome sequence of ADV [15]. In our 7 cases of ADV type

11 and 2 cases of ADV type 35, the partial sequences of the hexon, E3, and penton regions were almost identical, and the overlap of hospitalization (Figure 3) strongly suggested nosocomial transmission. In 2 of 3 cases of an ADV type 79-related strain, nosocomial transmission is thought to be possible, because of the identical partial sequences and overlapping hospitalization. Although sequencing was not performed for BKV, BKV HC frequently developed within a short period (Figure 3) and BKV nosocomial transmission was also suggested. In several studies, the causative ADV of HC was mainly ADV species B type 11, and sometimes type 34 and 35[22-24]. We analyzed the E3 and penton regions for 3 cases in which ADV type 34 was suspected by hexon analysis and judged that these cases were caused by an ADV type 79-related strain. The present study is the first to show that ADV-79 causes HC. ADV-79 was formerly detected only in sewage water in Japan[25]. Seven cases received transplants from family donors, and 16 did from unrelated donors (6 were adult unrelated donors and 10 were cord blood units). Although 13 of donors lived in Kinki region which contains Kyoto prefecture, geographical origins of donors and stem cell collection dates were diverse and similar to the non-HC population. National Institute of Infectious Diseases publishes Japanese annual ADV surveillance reports. In 2016 and 2017, adenovirus was detected in 2121 cases throughout Japan. Among them, ADV type 11 was detected in 5 cases, ADV type 35 was in 1 case, and ADV type 79 was in no case. In Kyoto Prefecture, there was no detection of these 3 viruses during the period. [Infectious Agents Surveillance Report: National Institute of Infectious Diseases (https://www.niid.go.jp/niid/ja/iasr)]

We should acknowledge that this study was not definitive because we did not sample the virus from the environment or check the whole genome sequence. Although the detailed transmission route is unknown, it could include direct transurethral infection from the external urethral meatus or spread from oral infection and viremia. Since both ADV and BKV are non-enveloped viruses that are

relatively resistant to disinfection in routine practice, nosocomial transmission can occur via medical personnel, medical instruments or common toilets. To prevent nosocomial transmission, private room management of HC patients, wearing of disposable gloves and aprons by staff when caring for HC patients, autoclaving of used medical instruments, and sufficient disinfection of toilets and common spaces with a chlorine-based disinfectant may be necessary. In order to obtain further confirmation of nosocomial transmission, viral screening of pre-hospital HCST patients, donors, medical staff and visitors, whole-genome analysis of the viruses, and comparison of detected ADV or BKV with strains that are prevalent in the region are future tasks.

In conclusion, HC developed frequently during a short period, and there were several cases of ADV-HCs that may have involved nosocomial transmission based on genotyping of the virus and partial sequencing of the viral genome. Although viral HC after allo-HSCT is mainly due to reactivation of a latent virus, nosocomial transmission of ADV or BKV should also be considered.

Acknowledgements

Mizuki Watanabe, Yasuyuki Arai, Masakatsu Hishizawa, Tadakazu Kondo and Kouhei Yamashita performed the research, Yoshiyuki Onda and Junya Kanda analysed the data and wrote the paper, Nozomu Hanaoka and Tsuguto Fujimoto performed viral analysis, and Akifumi Takaori-Kondo supervised the study.

This work was supported in part by the Fujiwara Memorial Foundation (J.K.), the Takeda Science Foundation (J.K.) and a Grant-in-Aid for Scientific Research (10110713) from the Ministry of Health, Labour, and Welfare.

Compliance with Ethical Standards:

Funding: This work was supported in part by the Fujiwara Memorial Foundation (J.K.), the Takeda Science Foundation (J.K.) and a Grant-in-Aid for Scientific Research (10110713) from the Ministry of Health, Labour, and Welfare.

Conflict of Interest: All author declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1 Patient characteristics

		Number of patients (n=207)	
Gender	Male	130	62.8%
	Female	77	37.2%
Patient age, years, median (range)	50	(17-68)	
	Age < 50	99	47.8%
	Age ≥ 50	108	52.2%
Underlying diagnosis	AML	81	39.1%
	ALL	33	15.9%
	MDS	35	16.9%
	ATL	11	5.3%
	Lymphoma	33	15.9%
	AA	4	1.9%
	Others	10	4.8%
Disease risk	Standard	107	51.7%
	High	100	48.3%
Stem cell source	Related		
	Bone marrow	14	6.8%
	Peripheral blood Stem cells	29	14.0%
	Unrelated		
	Bone marrow	86	41.5%
	Cord Blood	78	37.7%

Number of HLA allele	0	91	44.0%
mismatches	1	37	17.9%
(HLA-A, -B, -C, -DRB1)	2	20	9.7%
	3	26	12.6%
	4	22	10.6%
	5	7	3.4%
	6	2	1.0%
Conditioning intensity	MAC	91	44.0%
	RIC	116	56.0%
Conditioning regimen			
Cyclophosphamide	Yes	97	46.9%
	No	110	53.1%
Busulfan	Yes	44	21.3%
	No	163	78.7%
Total body irradiation	Yes	141	68.1%
	No	66	31.9%
GVHD prophylaxis			
Calcineurin inhibitor	Tacrolimus	192	92.8%
	Cyclosporine	15	7.3%
Methotrexate	Yes	129	61.8%
	No	78	36.7%
Mycophenolate mofetil	Yes	106	51.2%
	No	101	48.8%

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic

syndrome; ATL, adult T-cell leukemia/lymphoma; AA, aplastic anemia; HLA, human leukocyte antigen; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease

Table 2 Characteristics of virus-associated hemorrhagic cystitis

	Number		Number		Number		Number	
All grades of HC	58	28.0%						
Mild HC (G1, G2)	35	60.3%	[G1: 15 (25.8%), G2: 20 (34.5%)]					
Severe HC (G3, G4)	23	39.7%	[G3: 15 (25.8%), G4: 8 (13.8%)]					
Urine RT-PCR	All grades		Mild (G1, G2)		Severe (G3, G4)			
ADV +, BKV +	12	20.7%	1	1.7%	11	19.0%		
ADV +, BKV -	6	10.3%	1	1.7%	5	8.6%		
ADV -, BKV +	39	67.2%	32	55.2%	7	12.1%		
Onset date of HC	All HC		ADV(+)BKV(+)HC		ADV(+)BKV(-)HC		ADV(-)BKV(+)HC	
day 0-30	18	31.0%	3	25.0%	2	33.3%	13	33.3%
day 31-100	28	48.3%	4	33.3%	1	5.6%	22	56.4%
day 101-	12	20.7%	5	41.7%	3	50.0%	4	10.3%

Median onset date	day48 (range,0-1410)							
Duration of HC	All HC		ADV(+)BKV(+)HC		ADV(+)BKV(-)HC		ADV(-)BKV(+)HC	
≤ 2 weeks	17	29.3%	1	8.3%	0	0.0%	15	38.5%
> 2 weeks, ≤ 6 weeks	21	36.2%	6	50.0%	4	66.7%	11	28.2%
> 6 weeks	20	34.5%	5	41.7%	2	33.3%	13	33.3%
Median duration of HC		28 days (3-196)						

HC, hemorrhagic cystitis; RT-PCR, real-time polymerase chain reaction: ADV, adenovirus; BKV, BK virus; G, grade.

Table 3 Analysis of factors associated with virus-associated hemorrhagic cystitis

Variables		Univariate analysis			Multivariate analysis		
		HR	95% CI	P value	HR	95% CI	P value
Gender	Male	1.00		Reference			
	Female	0.99	0.58-1.71	0.981			
Age (y)	< 50	1.00		Reference	1.00		Reference
	≥ 50	0.68	0.41-1.15	0.152	0.71	0.42-1.22	0.216
Underlying diagnosis	Myeloid neoplasms	1.00		Reference	1.00		Reference
	Lymphoid neoplasms	1.68	0.99-2.84	0.055	1.62	0.94-2.79	0.083
	Benign diseases	2.41	0.68-8.54	0.172	2.03	0.53-7.84	0.303
Disease risk	Standard	1.00		Reference			
	High	1.03	0.61-1.73	0.912			
Stem cell source	Related						
	BM	1.00		Reference			
	PB	1.20	0.39-3.66	0.747			
	Unrelated						
	BM	0.85	0.31-2.33	0.753			
	CB	1.18	0.43-3.19	0.748			
HLA allele mismatch (HLA-A, -B, -C, -DRB1)	0	1.00		Reference	1.00		Reference
	1-3	0.94	0.52-1.71	0.845	0.99	0.55-1.80	0.985

	4-6	1.92	0.96-3.85	0.066	1.90	0.93-3.89	0.080
Conditioning	MAC	1.00		Reference			
	RIC	0.94	0.56-1.58	0.817			
Cyclophosphamide	Yes	1.00		Reference			
	No	1.17	0.69-1.96	0.562			
Busulfan	Yes	1.00		Reference			
	No	0.84	0.44-1.61	0.606			
TBI	Yes	1		Reference			
	No	1.39	0.78-2.49	0.263			
Calcineurin inhibitor	Tac	1.00		Reference			
	CsA	0.81	0.33-1.98	0.648			
MTX	Yes	1.00		Reference			
	No	1.00	0.58-1.72	0.991			
MMF	Yes	1.00		Reference			
	No	1.26	0.75-2.12	0.386			

BM, bone marrow; PB, peripheral blood stem cell; CB, cord blood; HLA, human leukocyte antigen; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; TBI, total body irradiation; Tac, tacrolimus; CsA, cyclosporine, MTX, methotrexate; MMF, mycophenolate mofetil; HR, hazard ratio; CI, confidence interval.

Figure legends

Figure 1 Number of cases of adenovirus or BK virus hemorrhagic cystitis

Solid black bars indicate adenovirus hemorrhagic cystitis (ADV HC) cases; hashed bars indicate BK virus hemorrhagic cystitis (BK HC) cases. A significant difference in the incidence of HC between Apr-Dec 2016 and other periods by Fisher's exact test is indicated by " * ".

Figure 2 Molecular Phylogenetic Analysis by the Neighbor-joining Method

Phylogenetic analysis of adenovirus strains detected in this study, compared to several other human adenovirus reference strains. Hexon (A), E3 (B) and penton (C) partial sequences were aligned using ClustalW in MEGA7 (https://www.megasoftware.net), and the evolutionary history was inferred by using the Neighbor-Joining method[26]. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches[27]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 20 nucleotide sequences. Codon positions included were 3rd (A), 1st (B) and 2nd (C). All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA7[28].

*The lengths of the partial sequences of the hexon, E3 and penton regions were as follows; 755bp, 581bp and 870bp.

Figure 3 Hospitalization periods and cases of hemorrhagic cystitis

Rectangles indicate hospitalization periods and triangles indicate the onset of hemorrhagic

cystitis. The possibility of nosocomial transmission cannot be denied on the basis of overlapping hospitalization periods for each patient. (A) adenovirus type 11 – hemorrhagic cystitis (ADV type 11), (B) adenovirus type 35 – hemorrhagic cystitis (ADV type 35), (C) adenovirus type 79 and 55 – hemorrhagic cystitis (ADV type 79 and ADV type 55), (D) BK virus – hemorrhagic cystitis (BKV-HC)

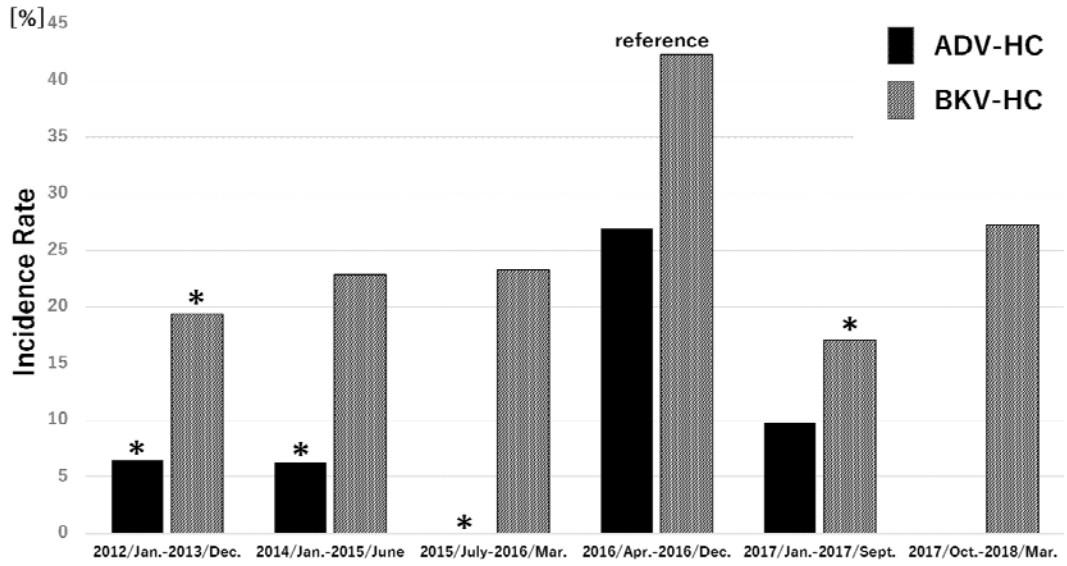

[%]
45
40
35
30
25
20
15
10
5
0
Incidence Rate
reference
ADV-HC
BKV-HC
*
*
*
*
*
2012/Jan.-2013/Dec.
2014/Jan.-2015/June
2015/July-2016/Mar.
2016/Apr.-2016/Dec.
2017/Jan.-2017/Sept.
2017/Oct.-2018/Mar.

Figure 1

Figure 2

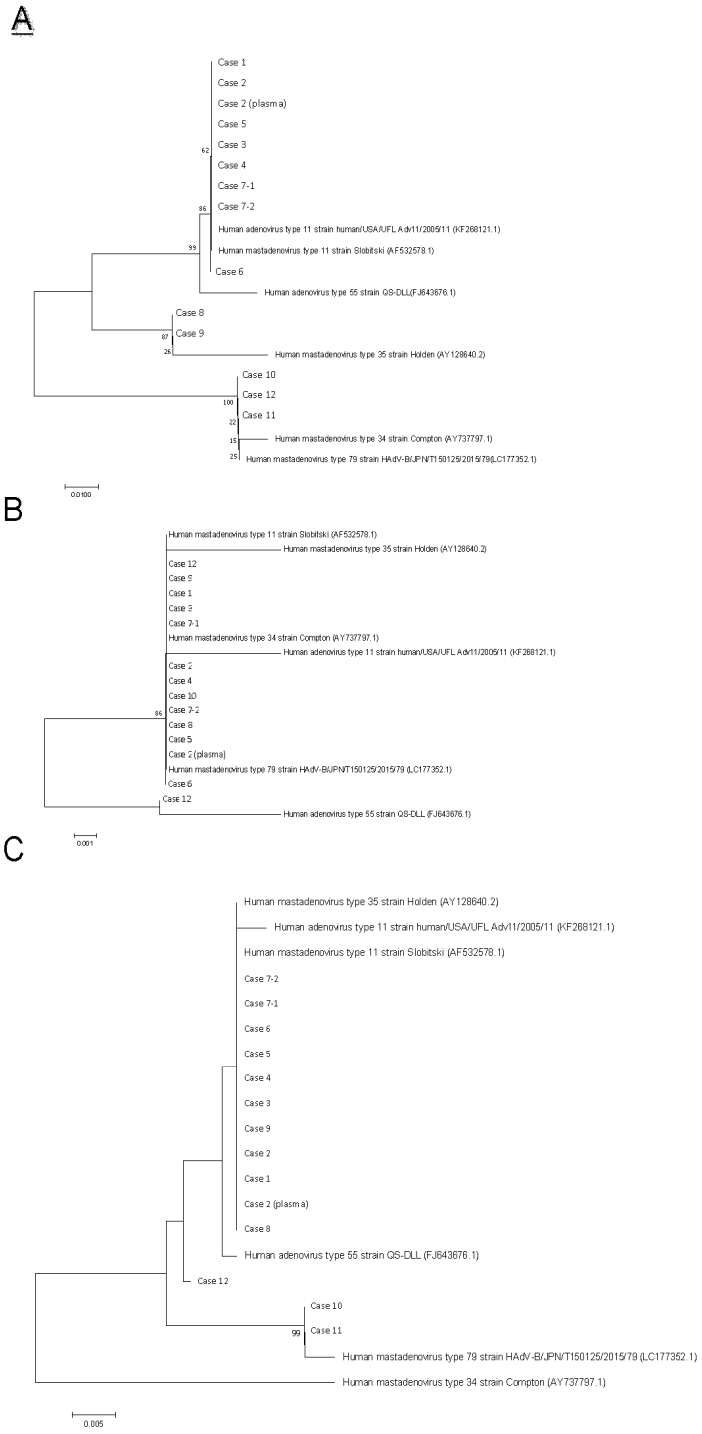
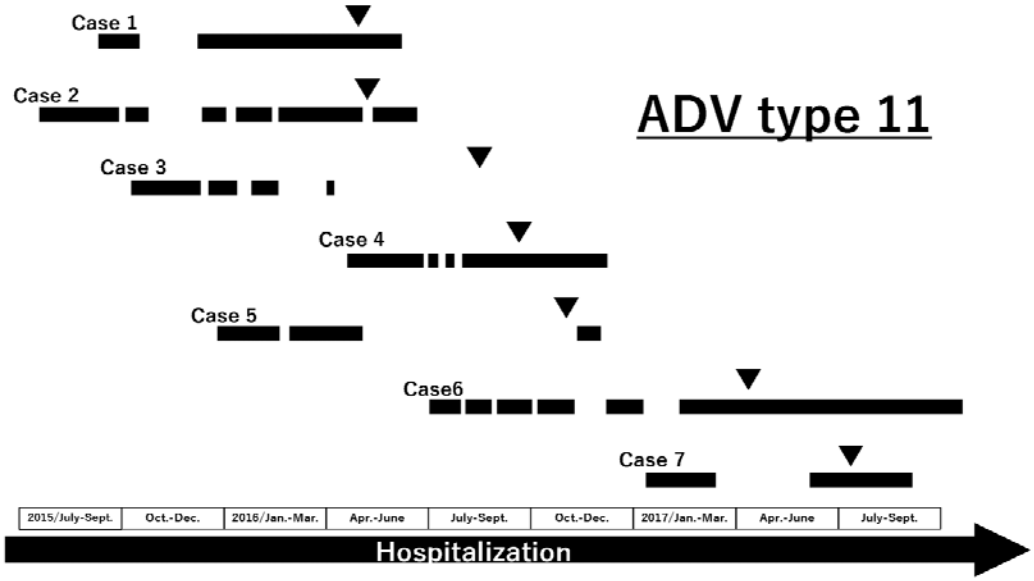
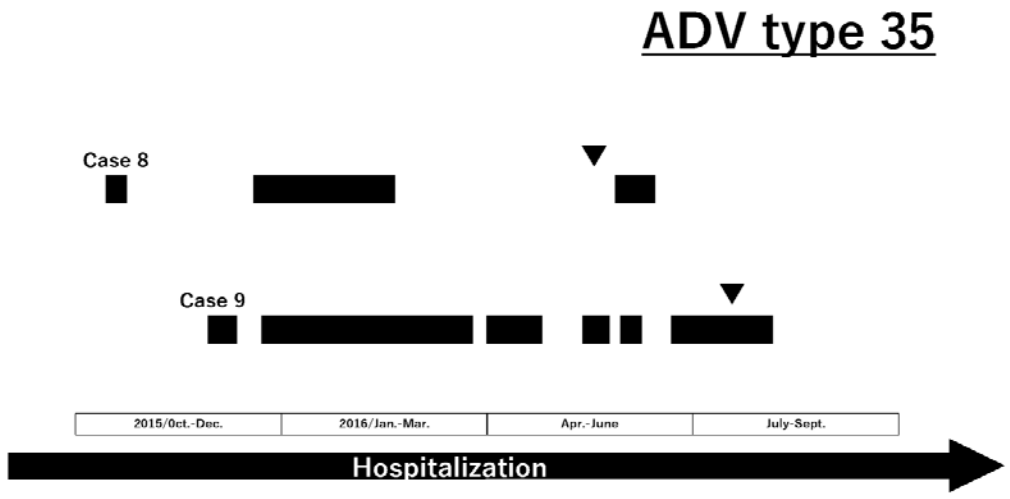


Figure 3

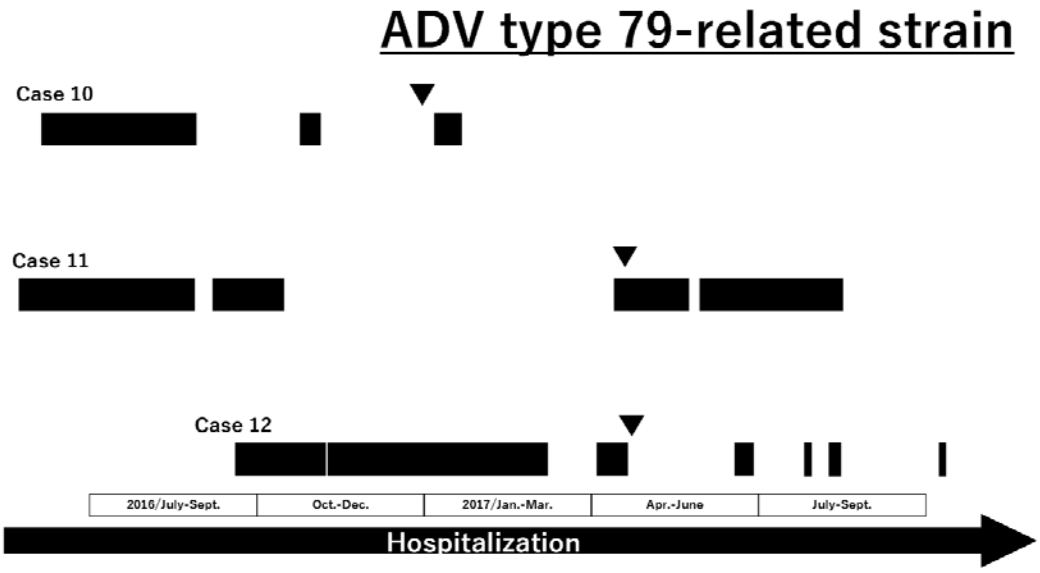
(A)


Case 1
Case 2
Case 3
Case 4
Case 5
Case6
Case 7
ADV type 11
2015/July-Sept.
Oct.-Dec.
2016/Jan.-Mar.
Apr.-June
July-Sept.
Oct.-Dec.
2017/Jan.-Mar.
Apr.-June
July-Sept.
Hospitalization

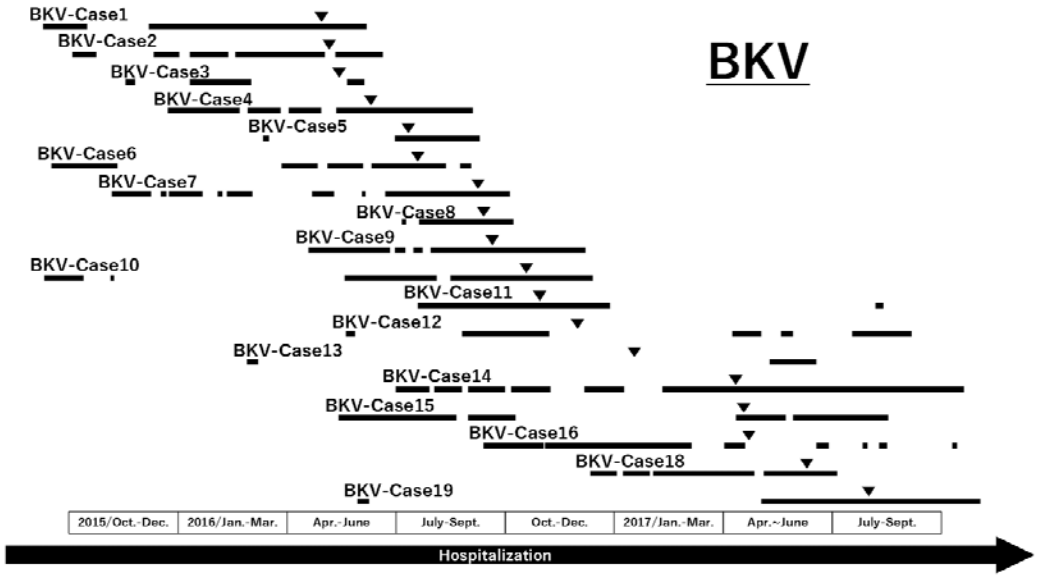
(B)


ADV type 35
Case 8
Case 9
2015/Oct.-Dec.
2016/Jan.-Mar.
Apr.-June
July-Sept.
Hospitalization

(C)


ADV type 79-related strain
Case 10
Case 11
Case 12
2016/July-Sept.
Oct.-Dec.
2017/Jan.-Mar.
Apr.-June
July-Sept.
Hospitalization

(D)


BKV
BKV-Case1
BKV-Case2
BKV-Case3
BKV-Case4
BKV-Case5
BKV-Case6
BKV-Case7
BKV-Case8
BKV-Case9
BKV-Case10
BKV-Case11
BKV-Case12
BKV-Case13
BKV-Case14
BKV-Case15
BKV-Case16
BKV-Case18
BKV-Case19
2015/Oct.-Dec.
2016/Jan.-Mar.
Apr.-June
July-Sept.
Oct.-Dec.
2017/Jan.-Mar.
Apr.-June
July-Sept.
Hospitalization