Università degli Studi di Genova

Dipartimento di Scienze della Salute

XXXV ciclo del Dottorato di ricerca in Scienze della Salute

Curriculum EPIDEMIOLOGIA, FISIOPATOLOGIA E GESTIONE DIAGNOSTICO-CLINICA DELLE MALATTIE INFETTIVE

Tesi di perfezionamento

Adenovirus infection in adult patients undergoing allogeneic hematopoietic stem cell transplant: incidence, clinical management and outcome.

Dottorando: Elisa Balletto

Relatore: Prof.ssa M. Mikulska

Coordinatore: Prof. A. Izzotti

INTRODUCTION

In the last decades Adenovirus infection (ADVi) has been increasingly recognized as a significant cause of morbidity and mortality among patients undergoing hematopoietic stem cell transplant (HSCT) (1). Recently large surveys reported an ADVi rate of 15-32% for children and 4-6% for adults. Most cases of ADVi are identified in the first 100 days post-transplantation (2, 3, 4).
To date the main reports on ADVi in HSCT are pediatrics, because of the higher incidence of viral in general and ADVi in this population. However, the impact of viral infectious complications on outcome is recognized to be as significant in adult as in pediatric patients undergoing allogeneic HSCT (allo-HSCT) (5). In fact, in case of ADV viremia (ADVv) reported mortality rate is up to 73% in adult (6) and 82% in children (7, 8, 9).

Available knowledge show that ADVi is related to severe suppression of T-cell function. Particularly T-cell depleted grafts (ex-vivo or serotherapy), severe graft-versus-host-disease (GVHD, grade >II), severe lymphopenia (< 200 cell/microlitre) and previous ADVi are identified as risk factors in children (10, 11). Similarly, in adults the recognized risk factors for ADVi are GVHD requiring immunosuppressive therapy, lymphopenia, use of alemtuzumab and younger age; with respect to donor type considering matched unrelated donor at standard risk, HLA-mismatched transplantation particularly mismatched unrelated donor and umbilical cord blood are at higher risk, on the contrary haploidentical donor using post-transplantation cyclophosphamide is reported at lower risk (6, 12, 13). Many recent studies found ADV related mortality associated to higher ADV viral load and ADV reactivation duration (14, 15, 16).

Despite the lack of literature data clearly supporting the proper management of ADVi in the adult patient undergoing allo-HSCT, current guidelines recommend: weekly ADV-DNA monitoring in peripheral blood using a sensitive quantitative PCR technique until recovery of CD3+ T- cells above 300/microlitre and/or cessation of immunosuppressive therapy and preemptive treatment, including antiviral therapy and tapering of immunosuppressive therapy whenever possible, in the presence of viraemia >10^3 ADV copies per milliliter (cp/mL) in lymphopenic hosts (17).

The primary aim of the study was to describe incidence of ADVi at day + 180 post-transplant in adult patients who underwent allo-HSCT in our Centre. Secondary objectives were: to describe timing, clinical presentation, management and outcome of ADVi particularly in case of ADVv and to identify

risk factors for ADVi and ADV-related mortality.

MATERIAL AND METHODS

Study population

All patients who underwent allo-HSCT at the Bone Marrow Transplant Centre of the San Martino Hospital in Genoa, Italy, from 1st Jan 2014 to 31 Dec 2019 were included into the study. The minimum follow-up time was 180 days after transplantation. Patients were censured in case of second allo-HSCT.

Data were collected through the hospital's computerized systems and individual patients' medical records.

The study was performed in accordance with the Declaration of Helsinki. The protocol of the study with retrospective ADV testing was approved by the Regional Ethics Committee (approval 509REG2014), all participants provided signed informed consent for data collection at HSCT, the need for specific study-related consent was waived.

ADV testing

ADVi episodes were searched through the virological database. Only the first case of ADVi was considered for each patient.

ADV-DNA testing in peripheral blood was performed predominantly on a symptoms-driven basis. Similarly, other samples were tested for ADV-DNA based on clinical presentation (stool, pharyngeal swab, bronchoalveolar lavage fluid, conjunctival swab, cerebrospinal fluid, bioptic samples). The transplant centre's diagnostic protocol included systematic detection of ADV-DNA using multiplex PCR diagnostic kits in all molecular pharyngeal swabs and bronchoalveolar lavage fluid samples.

ADV-DNA screening was gradually introduced following international recommendations (1, 17). The adherence to the ADV screening protocol was defined as systematic research of ADV-DNA in blood samples in asymptomatic patients at least twice monthly until day +30 and at least once monthly from day +31 until day +100 post-transplant procedure. After day + 100, patients often discontinue systematic follow-up at the Transplant Centre so that the screening strategy cannot longer be applied.

Quantitative detection of ADV-DNA was performed by real-time PCR Kit Real Cycler Adenovirus

(Progenie molecular, Valencia, Espana), which has a detection limit > 100 cp/mL.

Qualitative detection of ADV-DNA was performed on pharyngeal swab or bronchoalveolar lavage fluid with Seeplex RV12 ACE Detection PCR Kit procedure (Seegene, Seoul, South Korea). The multiplex PCR assay allowed the simultaneous detection of 12 respiratory viruses: influenza A/B, human adenovirus, respiratory syncytial virus (RSV) A/B, human metapneumovirus virus, human parainfluenza virus 1/2/3, human rhinovirus A/ B, human coronavirus 229E/NL63–OC43.

Collected data

The following data were collected for each patient: sex, age, transplant date, diagnosis, disease status at transplant, conditioning regimen, GVHD prophylaxis, ATG, type of transplant, acute GVHD (aGVHD), aGVHD grade (I - IV), chronic GVHD (cGVHD), cGVHD grade (mild, moderate, severe), relapse, second transplant, death. Moreover, we evaluated for patients with ADVi the immunodeficiency scoring index (ISI-score) developed to predict poor outcomes in HSCT recipients with respiratory syncytial virus (RSV) infection. The overall score equals the sum of the scores for the immunodeficiency indicators present at the time of diagnosis of viral infection: neutrophils count < 500/microL score 3, lymphocytes count < 200 microL score 3, Age ≥ 40 score 2, myeloablative conditioning regimen score 1, GVHD (acute or chronic) score 1, steroid score 1, recent or pre-engraftment allo-HSCT score 1 (overall score interpretation: low 0-2, intermediate 3-6, high 7-12) (18).

Moreover, the following ADVi-related data were collected: adherence to screening protocol from transplant to day + 100, time from transplant, ADV infection type (systemic/local), ADV disease, ADV antiviral therapy, ADV-related mortality.

Antiviral treatment, cidofovir or brincidofovir, was started in symptomatic patients with no alternative diagnosis when ADV-DNA was > 10^3 cp/mL in peripheral blood, accordingly to guidelines (1, 17). Data on immunosuppression tapering were not systematically collected.

Definitions

The definition of ADV infection and ADV disease conforms to ECIL criteria (1):

- Systemic infection/viremia (ADVv): positive ADV-DNA, virus isolation, or antigen detection in peripheral blood.
- Local infection (local ADV): positive ADV-DNA, virus isolation, or antigen detection in biopsy material or in body fluids other than peripheral blood.

- ADV disease (ADVd): ADV infection plus corresponding symptoms and signs, probable without histological confirmation, proven with histological confirmation of ADV in the appropriate location

In addition, we applied the following definitions:

- Late ADV infection: ADV local or systemic infection that develops more than 180 days after HSCT
- Blip: a single VL < 10^3 cp/mL with a subsequent negative or reduced value
- ADV related death: death during an episode of ADVi in the absence of other identifiable causes

Statistical analysis

Characteristics of the patients were reported as mean (SD) or median (IQR) for continuous variables and as N (%) for the categorical ones; t-test and Pearson chi square test or Fisher's exact test were performed to compare patients with and without ADVi.

Patients were followed until the end of the follow-up or until the date of the second transplant. In the analysis of the risk factors, GVHD was analyzed as a time-dependent variable. Risk factors for development of ADVi within 180 days were evaluated considering death as a competing event with respect to development of ADVi. Univariable competing-risks regression models according to the method of Fine and were performed and a multivariable model was run including all the variables showing a p-value<0.10 in the univariable analysis. Results were shown as Cumulative Incidence Curves taking into consideration the competing event death. To evaluate the proportion of screening practice over the years (2014-2017 vs 2018-2019) we performed the chi-square test. Restricting to the patients with ADVv, Univariable Cox proportional hazards models were performed to study the association between transplant characteristics and ADV-related mortality.

All tests were two-sided and P-values < 0.05 were considered statistically significant. All statistical analyses were performed using Stata version 16.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Patients' characteristics

During the study period 445 patients underwent allo-HSCT and were included into the study.

Their mean age was 49,14 years old (SD 14.30), 54% were males and acute myeloid leukemia/ myelodysplastic syndrome was the underlying malignancy in 50%. Most patients (76%) received transplant from an haploidentical donor with post-transplant cyclophosphamide as GVHD prophylaxis.

Patients with and without ADVi were compared and a statistically significant difference was found for the variables sex (p 0.025), underlying disease (p 0.045), aGVHD grade ≥ 2 (p 0.018). (Table 1).

Table 1. Characteristics of the study population divided into those with and without ADV infection. P-values refer to two-sample t test for age and to Pearson chi square test or Fisher's exact test for the categorical variables.

	Total N 445	**No ADV infection** n 394 (89%)	**Any ADV infection** n 51 (11%)	**p-value**
Sex, male (n, %)	240 (54%)	220 (56%) 92%	20 (39%) 8%	**0.025**
Mean age (SD)	49,14 (SD 14,30)	49,25 (SD 14,32)	48,27(SD 14,24)	0.645
Disease (n, %)				**0.045**
- **AML/MDS**	222 (50%)	206 (52%) 93%	16 (31%) 7%	
- **ALL**	70 (16%)	56 (14%) 80%	14 (27%) 20%	
- **CLL**	7 (2%)	6 (2%) 86%	1 (2%) 14%	
- **NHL**	32 (7%)	26 (7%) 81%	6 (12%) 19%	
- **HD**	31 (7%)	28 (7%) 90%	3 (6%) 10%	
- **MM**	19 (4%)	14 (4%) 74%	5 (10%) 26%	
- **MFI**	44 (10%)	40 (10%) 91%	4 (8%) 9%	
- **CML/CGL**	7 (2%)	6 (2%) 86%	1 (2%) 14%	
- **SAA**	11 (2%)	10 (3%) 91%	1 (2%) 9%	
- **Fanconi syndrome**	2 (0,5%)	2 (0,5%) 100%	0	
Donor				0.278
- **Haploidentical donor**	336 (76%)	296 (75%) 88%	40 (78%) 12%	

- **Matched related donor**	67 (15%)	63 (16%) 94%	4 (8%) 6%	
- **Matched unrelated donor**	40 (9%)	33 (8%) 83%	7 (14%) 17%	
- **Cord blood**	2 (0,5%)	2 (0,5%) 100%	0 (0%)	
Disease phase at allo-HSCT				0.858
- **Complete remission**	184 (41%)	163 (41%) 89%	21 (41%) 11%	
- **Partial remission**	162 (36%)	142 (36%) 88%	20 (39%) 12%	
- **Active disease**	99 (22%)	89 (23%) 90%	10 (20%) 10%	
Conditioning regimen				0.629
- **Myeloablative**	392 (88%)	347 (88%) 89%	45 (88%) 11%	
- **Non myeloablative**	35 (8%)	32 (8%) 91%	3 (6%) 9%	
- **Reduced intensity**	18 (4%)	15 (4%) 83%	3 (6%) 17%	
GVHD prophylaxis				0.608
- **Post transplant cyclofosfamide**	327 (73%)	288 (73%) 88%	39 (76%) 12%	
- **Methotrexate + Cyclosporine**	118 (27%)	106 (27%) 90%	12 (24%) 10%	
ATG, yes	42 (9%)	35 (9%) 83%	7 (14%) 17%	0.266
aGVHD, grade ≥ 2 (n, %)	85 (19%)	69 (18%) 81%	16 (31%) 19%	**0.018**
cGVHD, moderate or severe (n, %)	84 (19%)	74 (19%) 88%	10 (20%) 12%	0.887

ADV infection and disease

Among 445 patients, 51 (11%) developed ADVi: 18 (4%) only localized disease and 33 (7%) ADVv, with or without disease as shown in Figure 1.

The cumulative incidence of ADVi and ADVv and ADVv with VL max > 10^3 cp/mL and of death as a competing event are shown in Figure 2-4.

ADVi within 180 days from allo-HSCT was diagnosed in 39 patients (9%): local ADV in 15 patients (3%) and ADVv in 24 (5%), of which 12 (3%) reached a maximum viral load (VL max) > 10^3 cp/mL. At ADVi 10 patients had acute and 1 chronic GVHD grade ≥ 2. Median time (IQR) to ADVi within 180 days from HSCT was 65 (19; 94) days.

Late episodes of ADVi developed in additional 12 patients, 9 ADVv and 3 local ADV, all cases involving patients undergoing immunosuppressive therapy. Particularly, ADVv cases were diagnosed in 7 patients with chronic extensive cGVHD, 2 receiving steroid therapy for other causes. Median (IQR) time to late ADVi was 464 (264; 725) days.

Among patients with ADVv, antiviral treatments were 10: cidofovir in 8 cases and brincidofovir in 2.

Risk factors

The univariable analysis of risk factors for the development of any ADVi at 180 days post-transplant demonstrated a statistically significant association with lymphoproliferative disease (p 0.011) and aGVHD (p 0.021), whilst a trend towards significance was observed for cGVHD (p 0.071) and female sex (p 0.073) (Table 2). An association was confirmed for lymphoproliferative disease (p 0.009) in the multivariable analysis. No ADVi was observed in relapsed patients.

Tab. 2 The univariable analysis of risk factors for the development of any ADVi at 180 days post-transplant (n= 39) considering death as a competing event for ADVi (n=43); the multivariable analysis was performed including variables showing p-value<0.10 in the univariable analysis.

	Univariable		***Multivariable***	
	SHR(95% CI)	***p-value***	***SHR(95% CI)***	***p-value***

Age (10-unit)	0.95(0.77; 1.17)	0.607	---	---
Sex				
Male	1.00(ref)	---	1.00(ref)	---
Female	1.79 (0.95; 3.37)	0.073	1.82(0.97; 3.41)	0.063
Type of donor				
MUD	1.00(ref)	---	---	---
MRD	0.59 (0.15; 2.38)	0.460		
APLO+CB	0.95 (0.34; 2.69)	0.929	---	---
Diagnosis				
Myeloprolipherative,SAA	1.00(ref)	---	1.00(ref)	---
Lymphoprolipherative	2.25 (1.20; 4.22)	**0.011**	2.30(1.24; 4.29)	**0.009**
Disease phase				
Clinical remission	1.00(ref)	---	---	---
Active disease	0.88(0.41; 1.91)	0.752	---	---
Conditioning regimen				
Reduced intensity, Non myeloablative	1.00(ref)	---	---	---
Myeloablative	0.94(0.36; 2.41)	0.896	---	---
ATG				
No	1.00(ref)	---	---	---
Yes	1.08(0.38; 3.07)	0.886	---	---
GVHD prophylaxis				
1 MTX+Cya	1.00(ref)	---	---	---
2 CY	1.46(0.67; 3.20)	0.345	---	---
GVHD Acute				
No	1.00(ref)	---	1.00(ref)	---
Yes	2.46(1.15; 5.30)	**0.021**	2.09(0.96; 4.55)	0.062
GVHD Chronic				
No	1.00 (ref)	---	1.00(ref)	---
Yes	3.93 (0.89; 17.40)	0.071	3.03(0.68; 13.39)	0.144

Screening compared to symptoms-based ADVv testing

Overall, 338 (76%) allo-HSCT patients had at least one blood sample tested for ADV-DNA during the study period.

The ADV screening strategy was fully applied from transplant to day +100 in 33 patients during the study period, increasing from 4% (13/321) in the period 2014-2017 to 16% (20/124) in the period 2018-2019 ($p < 0.001$). The implementation of the screening strategy over time is shown in Figure 5.

When considering 33 patients who performed consistently the screening until day +100, 7 patients had positive ADVv (7/33 21%), 5 patients reached VL max > 10^5 cp/mL (5/7 71%), all 5 developed ADVd, received antiviral treatment and 2 died (2/7 29%). Among patients who did not undergo screening, on the other hand, there were 26 symptoms based ADVv diagnoses (26/412 6%), 7 had VL max > 10^5 (7/26 27%), all were diagnosed with ADVd and 4 died (4/26 15%).

The detailed characteristics of ADVv diagnoses divided by diagnostic strategy, viral load and outcome is shown in the supplementary material as Figure S1.

There were no statistically significant differences among 33 fully screened patients and the rest of population in terms of sex, age, diagnosis, disease status at transplant, conditioning regimen, GVHD prophylaxis, ATG, type of transplant, aGVHD, cGVHD (data shown as supplementary material Table S1).

Mortality

In our cohort non-relapse mortality respectively at day + 30, + 100, +180, +365 and last follow up was 2%, 4%, 8%, 12% and 17%.

ADV related mortality was observed only in case of ADVv with a mortality rate of 1.4% (6/445) in the whole cohort and 18% (6/33) among viraemic patients. Notably none of the patients with ADVv VL max <10^5 cp/mL died, while 6 among 12 (50%) patients with VL max > 10^5 cp/mL died despite antiviral treatment.

Considering the time of onset of ADVv, within +180 days post-transplant compared to late cases, the mortality rate was 13% (3/24) and 33% (3/9) respectively.

Among 6 patients who died with ADVi, 2 cases were diagnosed during screening and 4 in symptomatic patients. All 6 patients had ADVd and ADV VL max > 10^5 cp/mL. The underlying disease was lymphoproliferative in 4/6 cases (2 NHL, 1 ALL, 1 MM), 3/6 patients received transplant from MUD donor with MTX+Cya and ATG as GVHD prophylaxis, 3/6 patients had a diagnosis of aGVHD and 2 of these had also cGVHD at ADVi, in 5/6 cases steroid therapy was ongoing.

The analysis of risk factors for ADVv related mortality at any time after transplant shown statistically significant association with active disease at transplant (p. 0.039), MRD/MUD/CB donor (p. 0.001), MTX + Cya GVHD prophylaxis (p. 0.004) and ATG prophylaxis (p. 0.004) (Table 3).

Table 3. The univariable analysis of risk factors for ADVv related mortality (n=6) at any time after transplant among patients with any ADVv (n=33)

	HR (95% CI)	p-value
Age (10-unit)	1.39(0.75; 2.58)	0.295
Sex		
Male	1.00(ref)	---
Female	0.59(0.12; 2.91)	0.514
Type of donor		
MUD	1.00 (ref)	---
MRD	---	---
APLO+CB	0.05 (0.01; 0.30)	**0.001**
Diagnosis		

Myeloprolipherative,SAA	1.00 (ref)	---
Lymphoprolipherative	1.66 (0.30; 9.05)	0.561
Disease phase		
Clinical remission	1.00 (ref)	---
Active disease	5.43 (1.09; 27.13)	**0.039**
ATG		
No	1.00(ref)	---
Yes	10.90 (2.17; 54.79)	**0.004**
GVHD prophylaxis		
1 MTX+CsA (MMF?)	1.00 (ref)	---
2 CY	0.08 (0.01; 0.45)	**0.004**
GVHD Acute		
No	1.00(ref)	---
Yes	1.78(0.36; 8.876)	0.480
GVHD Chronic		
No	1.00 (ref)	---
Yes	1.59(0.27; 9.98)	0.611
ADVv within day + 180		
no	1.00(ref)	---
yes	0.50 (0.10; 2.49)	0.395
Disseminated ADV disease		
no	1.00(ref)	---
yes	1.99 (0.40; 9.86)	0.402
Lymphocytes < 200/mm3		
no	1.00(ref)	---
yes	1.62 (0.32; 8.07)	0.560
Steroid therapy		
no	1.00(ref)	---
yes	2.12 (0.25; 18.07)	0.494
ISI SCORE		
low+intermediate	1.00 (ref)	---
high	1.61 (0.32;8.07)	0.560

DISCUSSION

ADVi is reported in literature as a not common complication after allo-HSCT in adults but related to high mortality in case of viremia and disseminated disease (6).
With regard to the infection rate, the results of our study shown an incidence of ADVi in adult patients within day +180 after allo-HSCT of 9%, of which 5% were ADVv and 3% ADVv with a VL max ≥ 10^3 cp/mL, higher than previously reported. In fact, data from The AdVance study, a recent multicentric European retrospective study, which revised clinical records of 1736 children and 2540 adults who underwent allo-HSCT between 2013 and 2015 (2), shown in adult patients an ADVi rate of 6%, an ADVv rate of 3% and a ADVv VL max ≥ 10^3 cp/mL in 2% of patients. Similar infection rates were reported in adults by Papanicolaou et al. from a survey in the United States (3): ADV infection, ADV viremia and ADVv VL max ≥ 10^3 copies/mL within 6 months after allo-HSCT were respectively 5%, 3% and 2% in adults, and from Cesaro et al. with an ADVi rate of 4.1% (4). At our centre were most patients received allo-HSCT with post-transplant cyclophosphamide as GvHD prophylaxis (76% Vs 11% in previously mentioned studies) an higher incidence compared to literature data was unexpected. In fact, MUD and not haploidentical HLA-mismatched transplant had previously been identified as being associated with ADVi (6, 10, 11, 12). However in recent study the role of haploidentical donors as risk factor for ADVi is controversial: in a retrospective study which included 59 post-transplant cyclofosfamide (PTCY) haploidentical HSCT (haploHSCT) and 68 recipients of MUD transplant with ATG as GvHD prophylaxis, from years 2010 – 2018, at day +180 the rate of ADV was slightly higher in haploHSCT (6.8% vs. 1.5%), without reaching statistical significance (19). On the contrary, lower rate of ADVi was reported in PTCY haploHSCT compared to other alternative donors: 6% vs 10% (13). Recently Metha et al. observed similar rate of ADVi in PTCY and tacrolimus/MTX/ATG groups (20). In our study the analysis of risk factors did not identify the type of transplant as being significantly associated with the development of ADVi. Conversely confirmed risk factors at the univariate analysis for the development of ADVi were lymphoproliferative disease (p 0.011), confirmed in the multivariable analysis, and aGVHD (p 0.021).

With regard to the outcome of ADVi in our study this was favourable for local ADV with spontaneous resolution in all cases. Otherwise for ADVv the mortality rate was 18% in the whole cohort, increasing for higher viral loads (50% for VL max ≥ 10^5 cp/mL). Despite the low number of patients (6 ADV related deaths/ 33 ADVv), significative risk factors for ADV-related mortality were in our cohort active disease at transplantation (p 0.039) and MUD/MRD/CB donor with MTX+Cya and

ATG as GVHD prophylaxis (0.001, 0.004, 0.004). The low number of cases compared to controls did not allow to perform a multivariate analysis of risk factors for ADV-related mortality.

The reported mortality rates are in accordance with the ones shown by the most recent surveys in adult patients: 19% for patients with ADVv, 23% for patients with ADVv VL max ≥ 10^3 cp/mL (3), 30% for disseminated disease (21). Considering the time of onset of ADVv, within +180 days post-transplant compared to late cases, the mortality rate was 13% (3/24) and 33% (3/9) respectively, so that although late cases are rarer, they have a high mortality rate, mostly in patients who are severely immunosuppressed due to transplant complications (e.g. cGVHD).

About the antiviral treatment of ADVi, among 18 patients with ADVv and VL max > 10^3 cp/mL, 10 were treated with antivirals and 6 died despite treatment. Among 8 for whom clinical decision was made not to provide antiviral treatment, none died with/for ADVi and all cases resolved spontaneously. Despite bias related to the retrospective design of the study, antiviral treatment did not show a survival benefit in our experience (ADV-related mortality 0% (0/10) in untreated patients and 58% (7/11) in treated patients), probably because delayed treatments when VL max was > 10^5 cp/mL. Moreover, we did not assess in which cases viremia fell spontaneously as opposed to cases in which there was a response to reduction of immunosuppressive therapy as a therapeutic strategy.

In adults there is no evidence for intestinal ADV persistence and the main source of virus reactivation remains unknown (23). No data are available on the role of ADV screening in stools and screening on peripheral blood is recommended on the basis of low-grade evidence (17). During the observation period at our Center, a growing attention was observed towards ADV infection after allo-HSCT, with a significative increase in the application of the ADV screening strategy overtime ($p < 0.001$). However, only 33 patients were screened continuously throughout the whole risk period from transplant to day + 100 resulting in a lower rate than reported in the literature. In fact, a recent large EBMT survey over the period 2013-2016 which involved 20% of the partner Centres (41 adult, 57 pediatric and 9 mixed Centers), reported a screening application rate of 70% in pediatric centers and 57% in adult centers, while in 19% of centers screening was only applied in patients at risk and in 14% ADV was searched on a symptoms-based strategy (4).

Furthermore, we observed 9 late cases from day + 180 up to 3 years post-transplant for which a screening strategy is not considered and nevertheless in which high mortality has been observed (40%).

The ADV screening strategy compared to symptoms-based testing in our Centre led to the diagnosis of 21% (7/33) vs. 6% (26/412) cases of ADVv respectively with an higher mortality in the screening group (29% vs. 15%).
Thus, contrary to expectation, screening strategy led to an high rate of diagnosis (21% of screened patients had ADVi) but did not allow outcome improvement in our experience. In fact, screened patients had an high rate of progression to ADVd (71%) and ADV-related mortality (29%). This could be explained by a selection bias between the groups of screened and unscreened patients, with patients included in the screening group at a higher a priori risk of developing ADVi. However, no statistically significant differences were found between the two groups, probably due to the low numerosity of the former group compared to the latter.

The main limitations of our study are the retrospective design, the limited number of ADVi compared to controls which did not allow a consistent statistical analysis and the lack of informations about the reduction of immunosuppression as a therapeutic strategy in case of ADVi.

CONCLUSIONS

In our study, ADVi was found to have a higher incidence than expected in a transplant Centre were most patients received PTCY haploHSCT.
Moreover, while local ADV has a good prognois, ADVv was confirmed to be associated with high mortality especially in case of ADVv VL max > 10^5 cp/mL.
The screening strategy should be broadly applied in the period of highest risk by day + 100 post-transplant or until resolution of immunosuppression and a high level of suspicion should be maintained in patients with post-transplant complication requiring immunosuppressive therapies (eg. GVHD). Furthermore, prompt antiviral therapy should be started when confirmed ADVv VL > 10^3 cp/mL is detected because of high mortality related to higher viral loads.

Further studies are needed to confirm the characteristics of the subgroup of adult allo-HSCT patients at risk for ADVi and ADV-related mortality with the aim to elaborate a specific risk score to target the screening strategy and to evaluate the real impact of different therapeutic strategies (reduction of immunosuppression, antivirals, T-cell targeted therapy) on patient's outcome.

BIBLIOGRAPHY

1. Matthes-Martin S, Feuchtinger T, Shaw PJ, Engelhard D, Hirsch HH, Cordonnier C, et al. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). Transpl Infect Dis. 2012;14(6):555-63.
2. Sedlacek P, Petterson T, Robin M, Sivaprakasam P, Vainorius E, Brundage T, et al. Incidence of Adenovirus Infection in Hematopoietic Stem Cell Transplantation Recipients: Findings from the AdVance Study. Biol Blood Marrow Transplant. 2019;25(4):810-8.
3. Papanicolaou GA, Dvorak CC, Dadwal S, Maron G, Prasad VK, Giller R, et al. Practice patterns and incidence of adenovirus infection in allogeneic hematopoietic cell transplant recipients: Multicenter survey of transplant centers in the United States. Transpl Infect Dis. 2020;22(4):e13283.
4. Cesaro S, Berger M, Tridello G, Mikulska M, Ward KN, Ljungman P, et al. A survey on incidence and management of adenovirus infection after allogeneic HSCT. Bone Marrow Transplant. 2019;54(8):1275-80.
5. Czyzewski K, Styczynski J, Giebel S, Fraczkiewicz J, Salamonowicz M, Zajac-Spychala O, et al. Age-dependent determinants of infectious complications profile in children and adults after hematopoietic cell transplantation: lesson from the nationwide study. Ann Hematol. 2019;98(9):2197-211.
6. La Rosa AM, Champlin RE, Mirza N, Gajewski J, Giralt S, Rolston KV, et al. Adenovirus infections in adult recipients of blood and marrow transplants. Clin Infect Dis. 2001;32(6):871-6.
7. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. 2014;27(3):441-62.
8. Bil-Lula I, Ussowicz M, Rybka B, Wendycz-Domalewska D, Ryczan R, Gorczynska E, et al. PCR diagnostics and monitoring of adenoviral infections in hematopoietic stem cell transplantation recipients. Arch Virol. 2010;155(12):2007-15.
9. Kampmann B, Cubitt D, Walls T, Naik P, Depala M, Samarasinghe S, et al. Improved outcome for children with disseminated adenoviral infection following allogeneic stem cell transplantation. Br J Haematol. 2005;130(4):595-603.
10. Feghoul L, Chevret S, Cuinet A, Dalle JH, Ouachee M, Yacouben K, et al. Adenovirus infection and disease in paediatric haematopoietic stem cell transplant patients: clues for antiviral pre-emptive treatment. Clin Microbiol Infect. 2015;21(7):701-9.
11. Hiwarkar P, Gaspar HB, Gilmour K, Jagani M, Chiesa R, Bennett-Rees N, et al. Impact of viral reactivations in the era of pre-emptive antiviral drug therapy following allogeneic haematopoietic SCT in paediatric recipients. Bone Marrow Transplant. 2013;48(6):803-8.
12. Chakrabarti S, Mautner V, Osman H, Collingham KE, Fegan CD, Klapper PE, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. Blood. 2002;100(5):1619-27.
13. Hubmann M, Fritsch S, Zoellner AK, Prevalsek D, Engel N, Bucklein V, et al. Occurrence, risk factors and outcome of adenovirus infection in adult recipients of allogeneic hematopoietic stem cell transplantation. J Clin Virol. 2016;82:33-40.
14. Ehlert K, Schulte JH, Kuhl JS, Lang P, Eggert A, Voigt S. Efficacy of Brincidofovir in Pediatric Stem Cell Transplant Recipients With Adenovirus Infections. J Pediatric Infect Dis Soc. 2021.
15. Wiriyachai T, Chaya W, Anurathapan U, Rattanasiri S, Boonsathorn S, Chaisavaneeyakorn S, et al. Association between adenovirus infection and mortality outcome among pediatric patients after hematopoietic stem cell transplant. Transpl Infect Dis. 2021;23(6):e13742.

16. Peker BO, Tuysuz Kintrup G, Saglik I, Can Sarinoglu R, Guler E, Mutlu D, et al. Follow-up of human adenovirus viral load in pediatric hematopoietic stem cell transplant recipients. Clin Transplant. 2021;35(3):e14209.
17. Hiwarkar P, Kosulin K, Cesaro S, Mikulska M, Styczynski J, Wynn R, et al. Management of adenovirus infection in patients after haematopoietic stem cell transplantation: State-of-the-art and real-life current approach: A position statement on behalf of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation. Rev Med Virol. 2018;28(3):e1980.
18. Shah DP, Ghantoji SS, Ariza-Heredia EJ, Shah JN, El Taoum KK, Shah PK, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. Blood. 2014;123(21):3263-8.
19. Kerbauy MN, Ribeiro AAF, Arcuri LJ, Kerbauy LN, da Silva CC, Camargo LFA, et al. Clinical impact of multiple DNA virus infections in nondepleted haploidentical and unrelated allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis. 2021;23(4):e13626.
20. Mehta RS, Saliba RM, Rondon G, Al-Atrash G, Bashir Q, Hosing CM, et al. Post-Transplantation Cyclophosphamide Versus Tacrolimus and Methotrexate Graft-Versus-Host Disease Prophylaxis for HLA-Matched Donor Transplantation. Transplant Cell Ther. 2022;28(10):695 e1- e10.
21. Ramsay ID, Attwood C, Irish D, Griffiths PD, Kyriakou C, Lowe DM. Disseminated adenovirus infection after allogeneic stem cell transplant and the potential role of brincidofovir - Case series and 10 year experience of management in an adult transplant cohort. J Clin Virol. 2017;96:73-9.
22. Takamatsu A, Tagashira Y, Hasegawa S, Honda H. Disseminated adenovirus infection in a patient with a hematologic malignancy: a case report and literature review. Future Sci OA. 2019;5(8):FSO412.
23. Lion T. Adenovirus persistence, reactivation, and clinical management. FEBS Lett. 2019;593(24):3571-82.

Figure 1. ADV disease

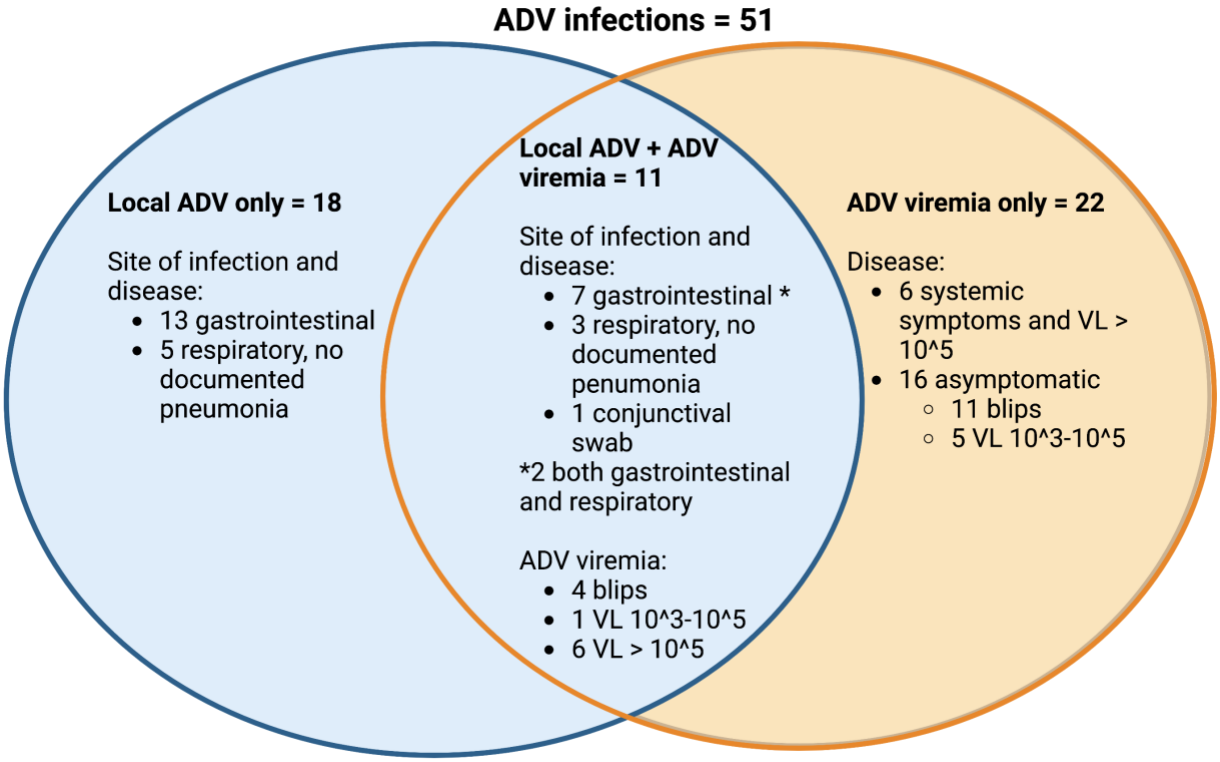

Figure 2. Cumulative incidences of any ADV infection

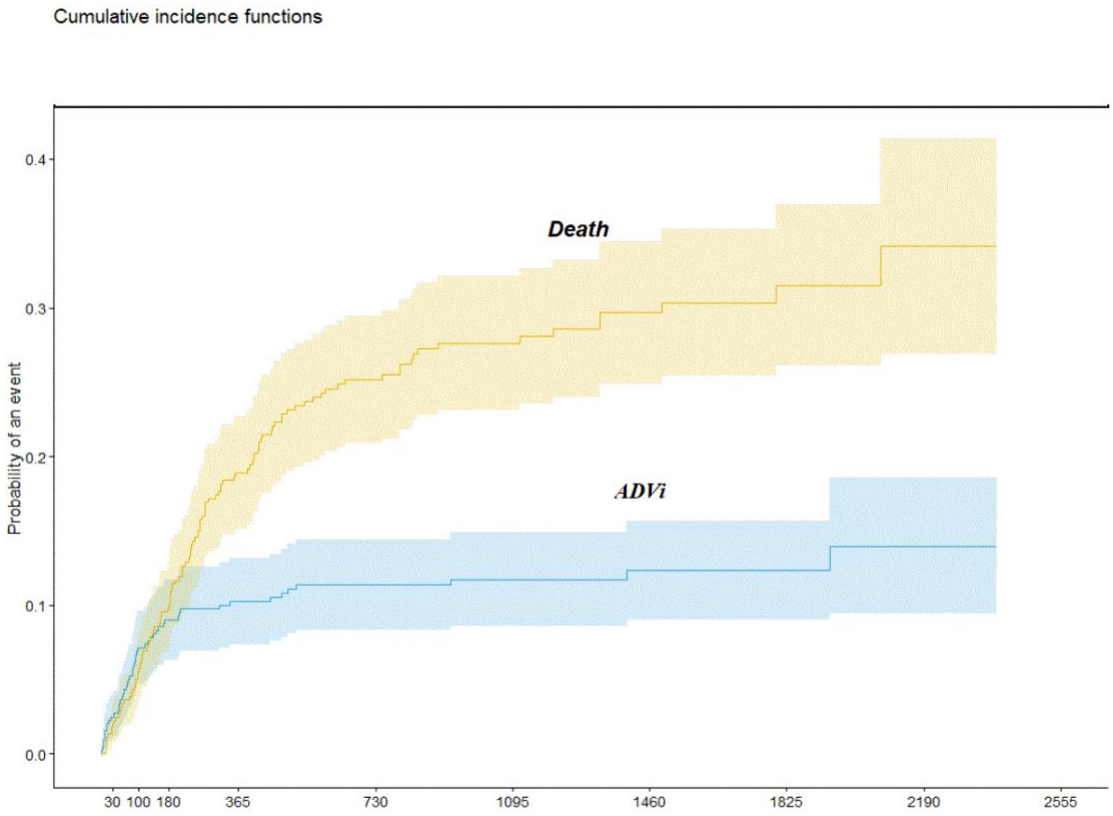

Figure 3. Cumulative incidences of ADV viremia

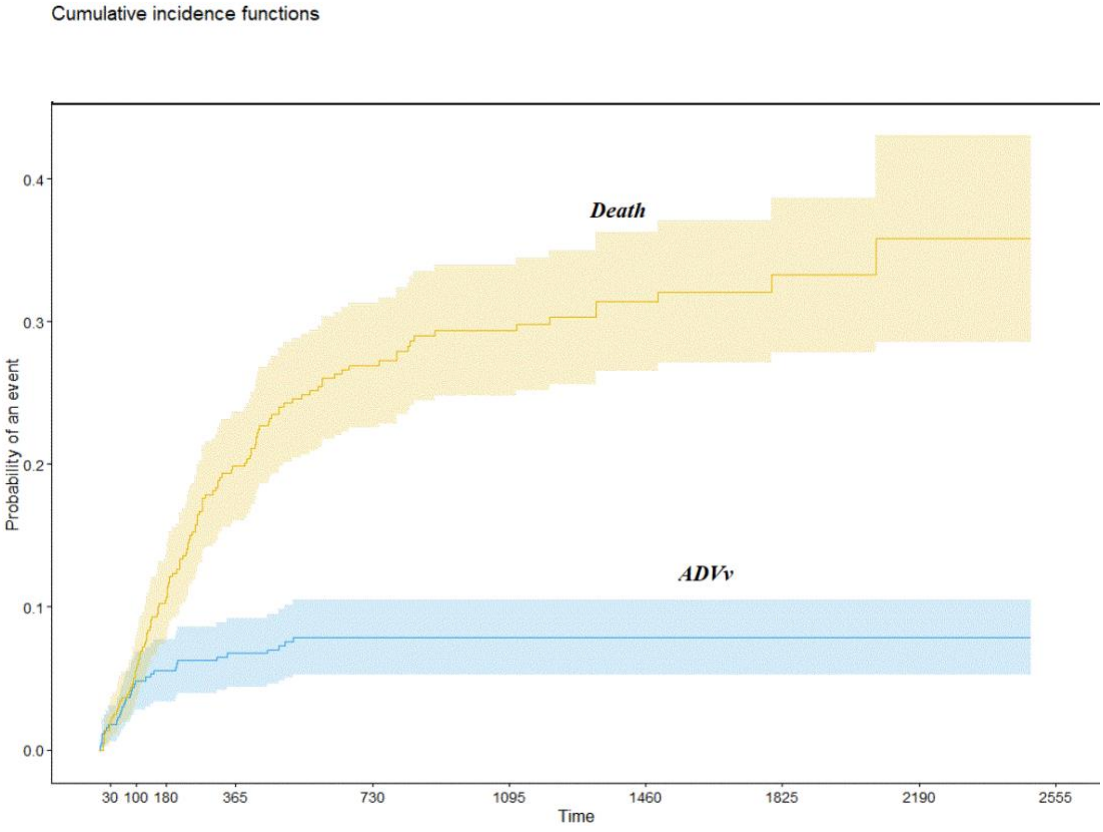

Figure 4. Cumulative incidences of ADV viremia with VL max > 10^3 cp/ml

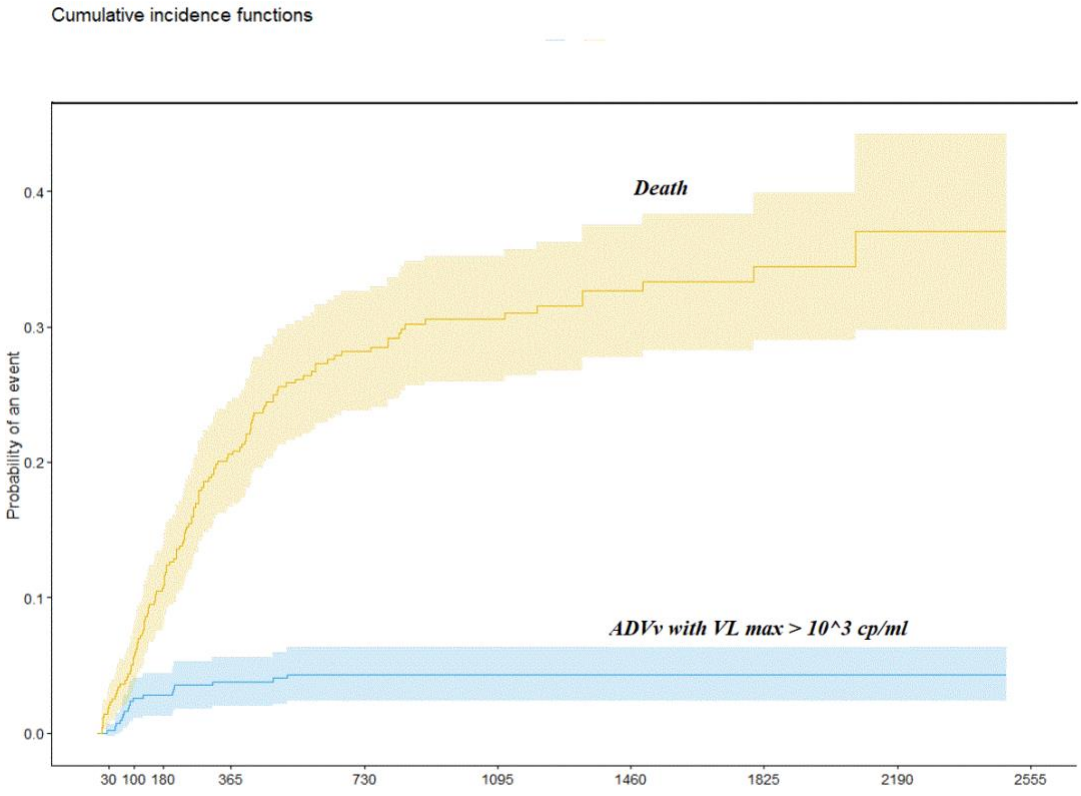

Figure 5. Number of patients undergoing ADV screening strategy by year

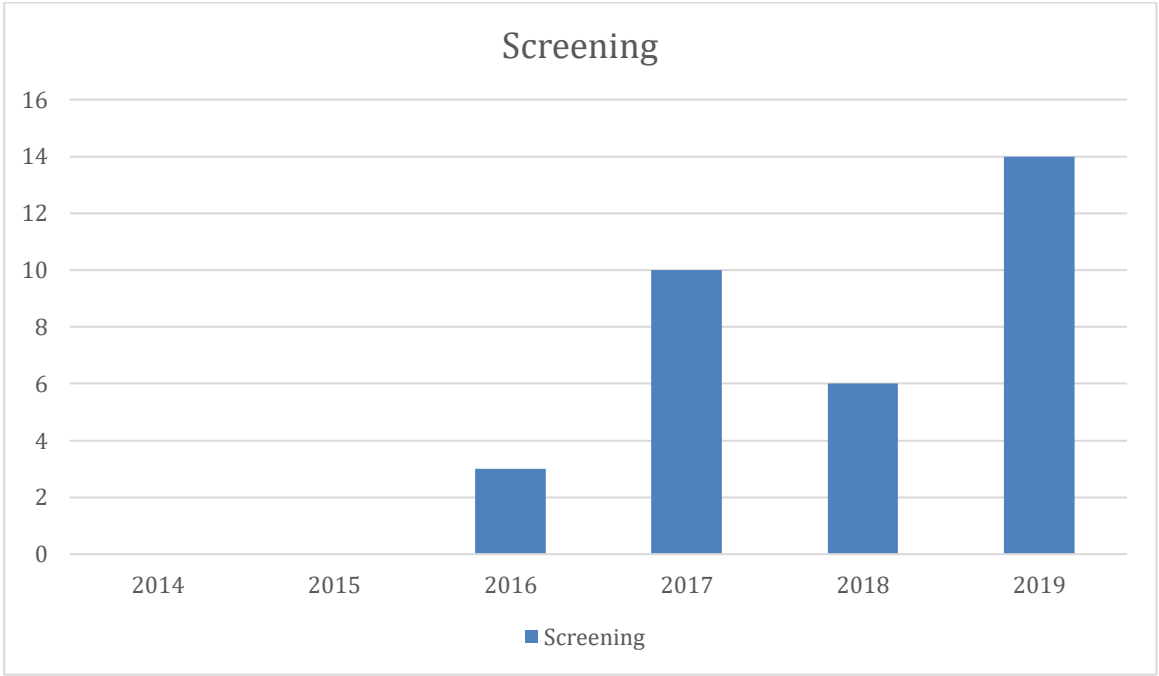

Table S1. Characteristics of screened versus not-screened patients

		Total N=445	0 N=412	1 N=33	p-value
Age		49 (14)	49 (14)	49 (14)	0.93
Sex: 1 male, 2 female	1	240 (53.9%)	223 (54.1%)	17 (51.5%)	0.77
	2	205 (46.1%)	189 (45.9%)	16 (48.5%)	
Diagnosis: 1 myeloprolipherative, 2 lymphoprolipherative	1	293 (65.8%)	270 (65.5%)	23 (69.7%)	0.63
	2	152 (34.2%)	142 (34.5%)	10 (30.3%)	
Type of donor: 0 MUD, 1 MRD, 2 haplo+CB	0	40 (9.0%)	35 (8.5%)	5 (15.2%)	0.18
	1	67 (15.1%)	65 (15.8%)	2 (6.1%)	
	2	338 (76.0%)	312 (75.7%)	26 (78.8%)	
Disease phase: 1 clinical remissione, 3 active disease	1	346 (77.8%)	321 (77.9%)	25 (75.8%)	0.77
	3	99 (22.2%)	91 (22.1%)	8 (24.2%)	
Conditioning regimen: 1 reduced intensity/non myeloablative, 2 myeloablative	1	392 (88.1%)	363 (88.1%)	29 (87.9%)	0.97
	2	53 (11.9%)	49 (11.9%)	4 (12.1%)	
GVHD prophylaxis: 1 MTX+Cya, 2 PTCY	1	118 (26.5%)	109 (26.5%)	9 (27.3%)	0.92
	2	327 (73.5%)	303 (73.5%)	24 (72.7%)	
ATG: 0 no, 1 yes	0	403 (90.6%)	375 (91.0%)	28 (84.8%)	0.24
	1	42 (9.4%)	37 (9.0%)	5 (15.2%)	
Acute GVHD: 0 no, 1 yes	0	360 (80.9%)	330 (80.1%)	30 (90.9%)	0.13
	1	85 (19.1%)	82 (19.9%)	3 (9.1%)	
Cronic GVHD: 0 no, 1 yes	0	361 (81.1%)	335 (81.3%)	26 (78.8%)	0.72
	1	84 (18.9%)	77 (18.7%)	7 (21.2%)	

Figure S1. Comparison of ADV viral load trend, disease and outcome between screened and not-screened patients