Poster No. 1584

IDWeek 2018

Development and Dynamics of Cytomegalovirus UL97 Ganciclovir Resistance Mutations in Transplant Recipients Detected by Next Generation Sequencing

I.P. Lodding[1], M. Jørgensen[1], M. Bennedbæk[1], N. Kirkby[2], K. Naegele[3], F. Gustafsson[4], M. Perch[5], A. Rasmussen[6], H. Sengeløv[7], S. S. Sørensen[8], H. H. Hirsch[3, 9], J. D. Lundgren[1]

[1]Centre of Excellence for Health, Immunity and Infections (CHIP), Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [2]Department of Clinical Microbiology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [3]Transplantation & Clinical Virology Department of Biomedicine, University of Basel, Basel, Switzerland; [4]Department of Cardiology, University of Copenhagen, Copenhagen, Denmark; [5]Department of Cardiology, Section for Lung Transplantation, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [6]Department of Abdominal Surgery, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [7]Department of Haematology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [8]Department of Nephrology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [9]Infectious Diseases & Hospital Epidemiology, University Hospital Basel, Basel, Switzerland.

MATCH Management of Post-Transplant Infections in Collaborating Hospitals

BACKGROUND

(Val)ganciclovir resistance mutations (GRMs) in CMV UL97 (UL97-GCV-R) complicate prophylaxis and therapy of solid organ transplant (SOT) and hematopoietic (stem) cell transplant (HCT) recipients[1], but data on prevalence and dynamics are scarce. We investigated UL97-GCV-R using next generation sequencing (NGS) in transplant recipients with refractory CMV DNAemia[2] episodes and a control group.

METHODS

Between Jan 1, 2010 - July 16, 2016, 385 transplant recipients were screened for plasma CMV DNAemia. 87 patients (54 SOT, 33 HCT) with available plasma samples had refractory CMV replication at viral loads ≥910 IU/mL and were analysed by NGS (**Table 1**). If UL97-GCV-R were detected in >10% of the NGS reads, all earlier plasma samples were also analysed by NGS. For comparison, this approach was also performed in a control group of 21 patients (14 SOT, 7 HSCT) with DNAemia episodes resolving under antiviral therapy. UL97-targeted NGS was performed using Illumina MiSeq and analysed by LoFreq for variant calling.

RESULTS

A total of 108 transplant recipients were included in the study (Table 1). Of the 87 recipients with refractory CMV replication, 19 (22%) had ≥1 UL97-GCV-R detected by NGS (**Table 2, Figure 1**), in comparison to 0/21 (0%) of the controls (p=0.02). Amongst the resistant cases, 14/19 cases (corresponding to a total of 20 induced mutations) had NGS performed < 4 weeks from onset of CMV replication. In this sample, the mutation was either not detected, detected as minority, or detected as the dominating variant for 11, 7 and 2 mutations respectively. In the majority of recipients one dominant mutant was induced (68%); ≥2 mutations were detected in the remaining recipients (**Table 2**).

Table 1
Baseline demographics of 108 included transplant recipients, stratified by clinical presentation and overall type of transplantation

Demographics	Resistant cases (n=19)		Refractory cases (n=68)		Control cases (n=21)		Total (n=108)	
	SOT (n=13)	HCT (n=6)	SOT (n=41)	HCT (n=27)	SOT (n=14)	HCT (n=7)	SOT (n=68)	HCT (n=40)
Age and gender								
Median (IQR) age at transplantation	47.0 (38.0-51.0)	42.5 (4.0-45.0)	53.0 (42.0-64.0)	48.0 (25.0-62.0)	55.0 (43.0-63.0)	15.0 (8.0-40.0)	51.5 (40.5-61.0)	43.0 (16-54.5)
% male	9/13	5/6	24/41	13/27	8/14	3/7	41/68	21/40
Type of transplantation								
SOT								
Kidney	8/13	NA	24/41	NA	6/14	NA	38/68	NA
Liver	3/13	NA	7/41	NA	4/14	NA	14/68	NA
Lung	2/13	NA	7/41	NA	3/14	NA	12/68	NA
Heart	0/13	NA	3/41	NA	1/14	NA	4/68	NA
HCT								
MAC	NA	5/6	NA	13/27	NA	5/7	NA	23/40
NMA	NA	1/6	NA	11/27	NA	2/7	NA	14/40
UCB	NA	0/6	NA	3/27	NA	0/7	NA	3/40
Donor(D)/Recipient (R) CMV IgG								
D+/R+	1/13	0/6	10/41	12/27	7/14	3/7	18/68	15/40
D+/R-	12/13	0/6	25/41	1/27	6/14	0/7	43/68	1/40
D-/R+	0/13	4/6	3/41	13/27	0/14	4/7	3/68	21/40
D-/R-	0/13	1/6	1/41	0/27	0/14	0/7	1/68	1/40
Unknown	0/13	1/6	2/41	1/27	1/14	0/7	3/68	2/40

Abbreviations: HCT; haematopoietic stem cell transplantation, IQR; inter quartile range, MAC; myeloablative conditioning regimen, NMA; non-myeloablative conditioning regimen SOT; solid organ transplantation, UCB; umbilical cord blood

Table 2
Details on genotypic features of 19 transplant recipients with induced* ganciclovir resistance mutations in the CMV UL97 gene

Patients	Transplant type	Codon in amino acid							No. of GRMs induced/ patient
		460 I/V	520 Q	594 V/G	595 S/F	596 G	603 W	607 Y/F	
1	MAC							++**	2
2	MAC						++		1
3	MAC	++				++			2
4	MAC			++	++				2
5	MAC			++					1
6	NMA			++					1
7	Liver		++				++		2
8	Liver						++		1
9	Liver						++		1
10	Lung				++				1
11	Lung				++				1
12	Kidney			++			++	++	3
13	Kidney				++				1
14	Kidney		++	++			++		3
15	Kidney			++**	++				3
16	Kidney				++				1
17	Kidney				++				1
18	Kidney	++							1
19	Kidney				++				1
No. of patients with mutation		2/19	2/19	6/19	8/19	1/19	6/19	2/19	
% with mutation induced		10,5	10,5	31,6	42,1	5,3	31,6	10,5	

*Induced mutations defined as mutations with a maximum frequency ≥25%, and a minimum increase of 10% in the subsequent sample
**Both induced mutation within same codon
Abbreviations: GRM; ganciclovir resistance mutation, MAC; myeloablative conditioning regimen, NMA; non-myeloablative conditioning regimen, UCB; umbilical cord blood

RESULTS (CONTINUED)

Most frequent UL97-GCV-R affected codon-595 (42%), -594 (32%) or -603 (32%)[1]. The frequency of C592G was low in in all episodes (<15%) without changing during the course (**Figure 1**).There was a trend towards higher frequencies of donor (D)/recipient (R) CMV high-risk mismatch, CMV disease and prior failure to valganciclovir prophylaxis (SOT) among the cases with UL97-GCV-R (**Figure 2**).

DISCUSSION

In this study, the prevalence of UL97-GCV-R in a large cohort of SOT and HCT recipients was investigated using NGS. In patients experiencing refractory CMV replication, approximately 22% also have at least one induced UL97-GCV-R, and important clinical factors such as CMV IgG D/R mismatch and CMV disease tended to be more common amongst these resistant cases; especially amongst the HCT recipients. Furthermore, prior failure of valganciclovir prophylaxis explained a significant proportion of the resistant SOT cases, emphasising the importance of successful viral suppression when administering (val)ganciclovir.
The C592G mutation was present at low frequencies in all patients suggesting that this mutation may be part of the quasispecies, but not selected by ganciclovir resistance. Thus, our data indicate that C592G could be considered a normal variation and not associated with treatment resistance.

CONCLUSION

UL97-GCV-R remains a challenge in the post-transplant course. A high level of clinical suspicion is warranted in transplant recipients presenting with refractory CMV replication. Further investigations on this potentially serious outcome is warranted, preferably in the setting of larger, prospective and multicentre collaborations.

Figure 1
Sequential NGS data for 19 cases with known UL97 resistant mutations

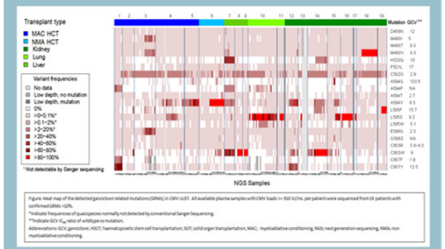
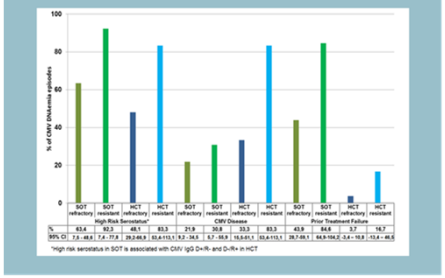


Figure 2
Comparison of clinical factors of CMV DNAemia between included refractory and resistant cases



Download poster at: www.chip.dk

References:
1. Kotton, CN. et al, Transplantation. 102(6):900–931, JUN 2018
2. Chemaly RF. et al, Clin Infect Dis. 2018 Aug 22. doi: 10.1093/cid/ciy696. [Epub ahead of print]

Isabelle Paula Lodding
Blegdamsvej 9,
2100 Copenhagen, Denmark
Tel: +45 35455754
Fax: +45 35455757
paula.isabelle.lodding@regionh.dk

chip Centre of Excellence for Health, Immunity and Infections
Rigshospitalet
PERSIMUNE CENTRE OF EXCELLENCE FOR PERSONALISED MEDICINE OF INFECTIOUS COMPLICATIONS IN IMMUNE DEFICIENCY
Danmarks Grundforskningsfond Danish National Research Foundation