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1 **Monitoring of CMV specific cell-mediated immunity in kidney transplant recipients with a high risk**
2 **of CMV disease (D+/R-): A case series**

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1 **Abstract**

2 Cytomegalovirus (CMV) is the most common viral pathogen in kidney transplant recipients (KTR) and
3 CMV disease impacts patient and graft survivals. CMV-specific CD8 T-cell mediated immunity (CMI)
4 may help to assess the risk of CMV disease and to adapt preventive treatment strategies.

5 High risk KTR with CMV seropositive donors/seronegative recipients (D+/R-) were prospectively
6 monitored after CMV prophylaxis discontinuation and during the first year post-transplant for CMV
7 viremia (WHO standardization) and CMI (QuantiFERON®-CMV). We analyzed the ability of CMI-test
8 to predict either subsequent spontaneous viral clearance or CMV disease after prophylaxis discontinuation
9 in patients with asymptomatic viremia.

10 We enrolled 12 consecutive (D+/R-) KTR. Eleven patients developed a viremia during follow-up, but
11 seven of them (64 %) exhibited a spontaneous viral clearance. At viremia onset, six of 11 patients (55 %)
12 had a positive CMI-test, and all of them (6 of 6, 100%) had subsequent spontaneous viral clearance,
13 compared with only one of five (20%) patients displaying a nonreactive CMI ($p = 0.02$). This latter patient
14 exhibited a positive CMI-test 15 days after viremia onset. Four of the 11 patients (36 %) developed a
15 CMV disease, and their CMI either remained nonreactive or became positive only after anti-viral
16 treatment.

17 We conclude that D+/R- KTR with asymptomatic viremia after prophylaxis discontinuation may benefit
18 from QuantiFERON®-CMV to predict when positive the spontaneous viral clearance or when persistently
19 negative the development of a CMV disease.

20

21 **Introduction**

22 Human cytomegalovirus (CMV) a member of the human herpes virus family can remain latent (i.e. non-
23 replicating) for years although transcription may still occur, and can start replicating especially in
24 immunocompromised patients such as transplant recipients. CMV replication may remain asymptomatic
25 or become symptomatic (CMV disease). In high-risk patients (i.e. seropositive donor/seronegative
26 recipient, D+/R-), CMV viremia occurs in about 37 % of KTR during the first year of follow-up, of whom
27 43 % will develop a CMV disease (1). The CMV disease is independently associated with the occurrence
28 of chronic allograft rejection, cardiac complications and mortality.

29 Strategies to prevent CMV diseases have been proposed such as CMV pre-emptive strategies and antiviral
30 prophylactic treatments. In pre-emptive strategies, patients are monitored with CMV viral load, and those
31 displaying viremia over a previously determined threshold are treated before symptoms occurrence. For
32 D+/R- high-risk patients, prophylaxis might have some advantages over pre-emptive strategies (2).
33 Indeed, prophylaxis results in a significant reduction of CMV diseases and all-cause mortality primarily
34 due to reduced mortality from CMV diseases (3). However, widespread use of antiviral prophylaxis leads
35 to an increased prevalence of late-onset CMV diseases (4,5) after the discontinuation of antiviral
36 prophylaxis, in the first year post transplantation in most cases (6). Late-onset CMV diseases may be less
37 severe than early diseases but may increase mortality within the first year post-transplantation (7).

38 Observational studies have shown that higher blood viral load values correlate with an increased risk for
39 disease development (8). However, spontaneous viral clearance does occur in about 30 to 80 % of solid
40 organ recipients (9,10) after viremia. Therefore the treatment of asymptomatic viremia remains
41 controversial. Early accurate markers of viral and clinical progression are yet warranted.

42 Functional mechanisms leading to the control of CMV replication have been investigated (11–13). CD8 +
43 cytotoxic T cells specifically recognize and kill CMV-infected cells, with CD4 + T helper cells providing
44 the necessary stimulatory signals. Post-transplant functional impairment of CD8 + T cells may contribute
45 to CMV replication after solid-organ transplantation (14–16). Therefore, measuring individual's CMI

46 response to CMV might be a useful predictor of spontaneous viral clearance versus viral and clinical
47 progression.

48 The QuantiFERON®-CMV assay (Cellestis GmbH, a QIAGEN company, Darmstadt, Germany) is the
49 only commercially available and standardized kit for monitoring the response of CMV-specific CD8 +
50 cytotoxic T cells (17). This assay is based on the quantification of interferon- γ production by CMV
51 specific CD8 + T cells after ex-vivo stimulation with various HLA class I restricted CMV T-cell epitopes
52 (18), and may help to stratify the risk for subsequent late-onset CMV disease after a standard course of
53 antiviral prophylaxis in solid organ transplant recipients (19,20). Other tests like ELISPOT Assays based
54 on interferon- γ production are also available.

55 We hypothesized that, in case of viremia after prophylaxis discontinuation, the progression of CMV
56 viremia to spontaneous viral clearance versus CMV disease might be related to the degree of CMV
57 replication control by cytotoxic CD8+ T cells. Therefore, we analysed intra individual kinetics of CMV-
58 specific lymphocytes IFN- γ responses in a homogenous cohort of D+/R- KTR, with asymptomatic
59 viremia after prophylaxis discontinuation during the first year post transplantation.

60 **Materials and Methods**

61 **Patients enrolment**

62 Adult KTR were eligible if they had a pre-transplant D⁺/R⁻ CMV serostatus and were scheduled to
63 receive antiviral prophylaxis with either ganciclovir or valganciclovir for 6 months, adapted to estimated
64 GFR:

- 65 - eGFR < 10 ml/mn/1.73m²: ganciclovir 1.25 mg/kg /day
- 66 - eGFR between 10-25 ml/mn/1.73m²: ganciclovir 2.5 mg/kg/day
- 67 - eGFR between 25-40 ml/mn/1.73m² valganciclovir PO: 450 mg 1/2day
- 68 - eGFR > 40 ml/mn/1.73m² valganciclovir PO 450 mg 1/day
- 69 - eGFR > 60 ml/mn/1.73m² valganciclovir PO 450 mg x2/day.

70 Following prophylaxis, patients were monthly examined at our out-patient clinic and monitored for viral
71 load for 6 months. Immunosuppression protocols were unaltered after the inclusion of patients.

72 Enrolments were prospectively carried out between March 2013 and March 2014. Informed written
73 consents were obtained from every patient and the study was conducted in accordance with the declaration
74 of Helsinki. Cell-mediated immunity was measured at 8 time-points: at the time of prophylaxis
75 discontinuation (6 months post-transplantation), at 15 and 30 days post prophylaxis discontinuation and
76 then monthly up to 6 months post-prophylaxis discontinuation. No additional invasive procedures or
77 appointments to the usual management were requested from patients thus approval of ethics committee
78 was not sought. Transplant physicians (L.A, E.C, A.J) who followed these patients and the principal
79 investigator (M.A) had access to patient identifying informations. Patients were thus followed for the
80 development of CMV disease for up to 12 months post-transplantation and treated according to
81 international recommendations (2,21) in case of disease onset independently of the CMI assay results.

82 Definitions

83 Spontaneous viral clearance of CMV was defined as a non-significant viral load < 62.4 IU/ml (< 40
84 copies/mL) without any CMV-disease symptoms and without any specific antiviral treatment. CMV
85 disease was defined if the patient developed symptoms consistent with the definition of the American
86 Society of Transplantation (22), and was therefore treated with antiviral treatment (intravenous ganciclovir
87 or oral valganciclovir).

88 Cell-Mediated Immunity Testing

89 QuantiFERON®-CMV assay (Cellestis GmbH, QIAGEN, Darmstadt, Germany)

90 Cell mediated immunity was determined using the QuantiFERON®-CMV assay. The QuantiFERON®-
91 CMV assay is an *in vitro* diagnostic test that uses HLA-restricted CMV antigens to stimulate CD8+ T cells
92 to produce interferon- γ measured by enzyme-linked immunosorbent assay (ELISA). One-millilitre
93 aliquots of whole blood were collected during routine monitoring into 3 heparinized tubes. One tube
94 contained a mix of 22 CMV CD8+ T-cell synthetic epitopes (CMV tube); one tube contained
95 phytohemagglutinin (PHA positive control); and the third tube contained only heparin (negative control). .
96 According to previous studies in D+/R- solid organ transplants, a cut-off of 0.1 IU/mL of IFN- γ (IFN- γ
97 level in the CMV tube minus IFN- γ level in the negative control tube) was used to define positivity of the
98 assay(18,19). If the level was <0.1 IU/mL and the PHA positive control was positive (IFN- γ in the PHA
99 tube minus IFN- γ in the negative control tube \geq 0.5 IU/mL), the test was considered as negative. If the
100 level was <0.1 IU/mL and the PHA positive control was negative (IFN- γ in the PHA tube - IFN- γ in the
101 negative control tube < 0.5 IU/mL), the result was reported as indeterminate. Negative and indeterminate
102 results were classified together as being nonreactive. The result of the assay was not given to the physician
103 in charge of the patient.

104 Viral monitoring

105 Quantitative real-time PCR assay (Abbott Molecular Inc., Des Plaines, IL, USA).

106 Quantification of CMV in whole blood was carried out with the Abbott RealTime CMV on the m2000
107 RealTime platform that includes the m2000sp instrument for automated extraction of DNA and the
108 m2000rt instrument for real-time PCR in batches of 24 or 48 tests. Extraction of DNA was done with the
109 Abbott mSample Preparation System. The Abbott RealTime CMV assay uses three reagent kits, the
110 amplification reagent kit for the amplification, the control kit for positive and negative controls, and the
111 calibrator kit for the standard curve. The amplification targets two highly conserved regions, within UL34
112 and UL80.5 genes. An internal control was also supplied to check the overall process, including DNA
113 extraction and possible PCR inhibition. Automated DNA extraction was performed from 600 µl whole
114 blood (elution in 150 µl) in the Abbott m2000sp instrument, followed by automated addition onto the PCR
115 plate of the master mixture and DNA extracts. The sealed PCR plate was loaded on the m2000rt
116 instrument for quantification of viral CMV DNA. Two controls (one positive and one negative) provided
117 by the manufacturer were included in each run. Two calibrators (A and B) analysed in triplicate were used
118 to establish the standard curve and calculate the CMV DNA concentrations in samples. The results were
119 expressed as follows: no replication, no detection of amplification signal; low level replication, detection
120 of amplification signal with a value of < 62.4 IU/ml (< 40 copies/ml); active replication, absolute values
121 for quantification between 62.4 IU/ml and 8.19 log IU/ml. The conversion factor of copies/ml to IU/ml
122 was determined by the manufacturer.

123 **Primary end-point**

124 The primary outcome of the study was to investigate the ability of the QuantiFERON®-CMV assay to
125 predict spontaneous viral clearance in D+/R- KTR with CMV viremia after the end of prophylaxis.

126 **Statistical analysis**

127 Graph Pad Prism 6 (GraphPad Software, Inc., La Jolla, CA) was used for statistical analysis and graph
128 generation. Fisher's exact test was used to compare categorical variables, and Mann-Whitney U test to
129 compare continuous variables. Spearman's test was used to assess the correlation between two continuous
130 variables. Receiver operator characteristics curve analysis was used to evaluate the performance of CMV-

131 specific interferon- γ production for the prediction of spontaneous viral clearance. A p value less than 0.05
132 was considered statistically significant (two-tailed).

133 **Results**

134 **Study population**

135 Twelve patients were included in the study. Only one patient did not develop CMV viremia (Fig 1). The
136 baseline characteristics of the remaining 11 patients are shown in Table. Initial nephropathies were
137 distributed as follows: undetermined (n=5), diabetic (n=2), hypertensive nephrosclerosis (n=1), focal
138 segmental glomerulosclerosis (n=1), autosomal dominant polycystic kidney disease (n=1) and
139 membranoproliferative glomerulonephritis (n=1). Three patients were pre-emptively transplanted. The
140 remaining eight patients were transplanted after hemodialysis (n=7) or peritoneal dialysis (n=1) after a
141 median dialysis duration of 26.5 months. Eight donors were extended criteria donors and two recipients
142 experienced delayed graft function. The median cold ischemia time was 1040 minutes. As per protocol, all
143 patients received 6 months post-transplant anti-CMV prophylaxis.

144 During the first year following kidney transplantation, four of 11 (36 %) patients developed late-onset
145 CMV disease at a median of 8 months post-transplantation and seven (64 %) experienced spontaneous
146 viral clearance. The median of the first viral load was 1100 UI/mL in patients who subsequently exhibited
147 a spontaneous viral clearance and 335 UI/mL in patients who developed a CMV disease (p=0.45). There
148 was no difference in induction treatment and long-term immunosuppression between patients with and
149 without CMV diseases (Table) and no patient experienced graft rejection requiring immunosuppressive
150 treatment intensification during follow-up.

151 **CMV specific CMI evolution during follow-up**

152 At the end of the 6-month prophylaxis, the QuantiFERON®-CMV assay was positive in four of 11
153 patients (36 %), and nonreactive in seven patients (64 %): negative in four and indeterminate in three
154 patients. None of the induction and maintenance immunosuppressive therapies were associated with
155 baseline results of CMI assays at the time of prophylaxis discontinuation.

156 The kinetics of QuantiFERON®-CMV assay positivity and viremia is shown in Figure 1. All patients who
157 became positive for CMV-specific CMI maintained a positive response during follow-up. The CMI was
158 non-reactive in seven patients at the time of prophylaxis discontinuation, but became positive in two
159 patients at time of viremia onset and in one additional patient 15 days after viremia onset. One patient had
160 negative CMI at viremia onset, together with a CMV disease and refused the subsequent protocol follow-
161 up. Only three patients exhibited sustained non-reactive CMI (n=1) or late CMI positivity (n=2) only after
162 anti-CMV treatments (range 1 to 3 months after viremia onset) (Fig 1).

163 **CMV specific CMI and viremia dynamics**

164 At viremia onset, the QuantiFERON®-CMV® assay was positive in 6 of 11 patients (55 %), and
165 nonreactive in 5 of 11 patients (45 %). In patients with a positive CMI, the incidence of subsequent
166 spontaneous viral clearance (free of CMV disease) was 6 of 6 (100%) compared with 1 of 5 (20%) in
167 patients with a nonreactive CMI at viremia onset ($p = 0.02$). In fact, this latter patient who subsequently
168 exhibited spontaneous viral clearance developed a positive CMI only 15 days after viremia onset. The four
169 remaining patients developed a CMV disease and exhibited either no or late CMI positivity only after anti-
170 viral treatments.

171 In contrast, the QuantiFERON®-CMV assay result at time of prophylaxis discontinuation was not
172 predictive of spontaneous viral clearance: 4/4 spontaneous viral clearance (100%) in the CMI positive
173 group versus 3/7 (43 %) in the CMI nonreactive group ($p=0.19$).

174 **Interferon- γ production**

175 Interferon- γ production in the QuantiFERON®-CMV assay was also analysed as a continuous variable.
176 We compared CMV-specific IFN- γ production in patients with and without CMV spontaneous viral
177 clearance: IFN- γ levels were higher at viremia onset in patients with spontaneous viral clearance. Median
178 IFN- γ levels at viremia onset were 0.95 IU/mL (IQR, 0.28–2.16) in patients with spontaneous viral
179 clearance and 0.00 IU/mL (IQR, 0–0.015) in patients with subsequent CMV disease ($p=0.006$). The

180 clinical evolutions of some representative patients with various kinetics of QuantiFERON®-CMV assay
181 positivity are shown in Figure 2.

182 **CMV-disease recurrence**

183 At the time of viremia onset, 4 of 5 (80%) patients with a non-reactive CMI-test developed a CMV-
184 disease. The only patient who did not developed a CMV disease exhibited a positive CMI-test at the
185 following sampling, 15 days later, and subsequently developed a spontaneous viral clearance. The four
186 other patients were treated as recommended by international recommendations. Two of them showed a
187 positive CMI 1 and 3 months respectively after starting the curative treatment and did not relapse despite
188 discontinuation of the treatment. One patient who remained negative for CMI-test despite anti-viral
189 treatment experienced a disease recurrence requiring a second treatment. The last patient refused the
190 protocol follow-up.

191 **Lymphocyte count**

192 There was no significant difference in lymphocyte counts between patients with a positive CMI and
193 patients with non-reactive CMI at viremia onset (median CD8+ lymphocyte count: 677.5/ μ L in patients
194 with positive CMI versus 199/ μ L in patients with a non-reactive CMI, $p=0.16$). CMV-specific IFN- γ
195 production was not correlated with CD8+ lymphocyte counts (Spearman's rho 0.61, $p=0.052$). There was,
196 however, a significantly higher total lymphocyte count in the spontaneous viral clearance group compared
197 to the CMV disease group as shown in Table 1. We wanted to test if this difference had a consequence on
198 PHA induced IFN- γ production between patients with or without spontaneous viral clearance. There was
199 no significant difference in median IFN- γ levels in the positive control PHA tube at viremia onset between
200 patients with or without spontaneous viral clearance. Median IFN- γ levels at viremia onset were 4.91
201 IU/mL (IQR, 1.13–6.29) in patients with spontaneous viral clearance and 0.81 IU/mL (IQR, 0.18–7.84) in
202 patients with subsequent CMV diseases ($p=0.56$).

203

204 Discussion

205 In this study with small sample size, we studied the benefit of QuantiFERON®-CMV in high-risk KTR
206 patients. We here observe that D+/R- KTR with asymptomatic viremia after prophylaxis discontinuation
207 may benefit from QuantiFERON®-CMV to predict when positive the spontaneous viral clearance or when
208 persistently negative the development of a CMV disease and the need for specific antiviral treatments.
209 Furthermore, our study confirms that patients who develop a positive CMI after anti-viral treatments may
210 not relapse a CMV disease after treatment discontinuation (23). To our knowledge, this is the first
211 prospective study about QuantiFERON®-CMV clinical utility in predicting spontaneous viral clearance in
212 a homogeneous prospective cohort of D+/R- kidney transplant recipients. Furthermore, we obtained the
213 CMI assay on the day of viremia onset and we extended CMI-monitoring for up to one-year post-
214 transplantation (compared with only 2 months post-prophylaxis discontinuation for previous studies).
215 Finally, it is the second study to express CMV viral load in IU/mL as recommended by the « World
216 Health Organization (WHO) Expert Committee on Biological Standardization” to standardize results and
217 overcome the variability between laboratories (24,25). Manuel *et al.* used either antigenemia or
218 polymerase chain reaction (PCR) assay while Lisboa *et al.* used an in-house real-time PCR assay (20,26).
219 The QuantiFERON®-CMV assay is a standardized commercial assay, readily available for routine clinical
220 practice, approved for in vitro diagnostics in Europe, Australia, and Korea and for investigational use only
221 in the United States and Canada. Most of the studies have used a cut-off for positivity of > 0.2 IU/mL,
222 although in D+/R- patients, a cut-off of > 0.1 IU/mL increases the sensitivity of the test (18–20). In our
223 study, a receiver operator characteristics curve analysis was performed for prediction of spontaneous viral
224 clearance of viremia based on CMV-specific interferon- γ levels at viremia onset. Cut-off values of INF- γ
225 levels up to 0.0350 IU/mL were able to conserve a positive predictive value of 100% to predict
226 spontaneous viral clearance (data not shown). In contrast, Abate *et al.*(27) studied 20 D+/R- KTR after
227 prophylaxis discontinuation and suggested that values of >1 to 6 IU/mL of INF- γ levels were protective
228 against the development of CMV viremia but did not correlate to the risk of symptomatic CMV disease or

229 spontaneous clearance which could be achievable with a lower CMI.

230 However, the use of the QuantiFERON®-CMV assay requires some precautions in interpreting results.

231 The QuantiFERON®-CMV is HLA restricted, such that patients with uncommon HLA subtypes may not

232 be represented in the peptide pool used for stimulation(28). This could be a cause for false-negative tests.

233 However, the peptides used are known to provide HLA coverage for greater than 95% of any given racial

234 population. Indeed, we retrospectively determined the HLA genotypes of each of our patients and found

235 that none of them had a non-represented genotype. In our patients, absolute CD8 lymphocyte counts were

236 also predictive of spontaneous viral clearance, as previously described (29). However, despite a low CD8

237 lymphocyte count in patients who developed a CMV-disease, the majority of patients (3/4, 75%) had

238 lymphocytes that were still able to respond to PHA. This indicates that the interferon production deficit in

239 the QuantiFERON®-CMV assay is a specific functional deficit of cellular immunity against CMV and is

240 not only related to the absolute value of T-lymphocytes.

241 We found no difference in immunosuppression between the patients with spontaneous viral clearance and

242 those who developed a CMV disease. This is probably due to insufficient statistical power since most

243 previous studies (30–32) showed that strong immunosuppressive regimens, and particularly induction

244 therapy with antilymphocyte globuline, increase the risk of CMV diseases.

245 In contrast to the studies of Kumar and Manuel(19,20), CMI at prophylaxis discontinuation was not

246 predictive of a spontaneous viral clearance in our study. The viral load at viremia onset was not predictive

247 either of CMV disease in our cohort, probably because of a lack of statistical power (8). These results

248 further suggest that the kinetics of CMI-test positivity after viremia onset are indeed the most powerful

249 predictor of spontaneous or treatment induced viral clearance. Cantisan *et al.*(33) recently showed that

250 positive QF CMV-CMI patients had significantly higher percentages of late-differentiated effector

251 memory CD8+ T cells (CD45RA+ CD27-) and highly experienced late-differentiated effector memory

252 CD8+ T cells (loss of CD28 or gain of CD57) that could explain better protection. The main limit of this

253 study is the low number of patients evaluated, the absence of a validation cohort and the impossibility to
254 predict CMV disease at the time of prophylaxis cessation but at viremia.

255
256 Incidence of viremia was higher in our study (92 %) than in the IMPACT study(1) after 200 days of
257 prophylaxis (37.4%) while patients' profile (D+/R- KTR), prophylaxis strategy and monitoring rates were
258 identical. However, different methods were used in the IMPACT study to assess CMV viral load such as
259 PCR or antigenemia, and induction with anti-lymphocyte antibodies was less used in the IMPACT study
260 (33 %) than in our study (64 %). Nevertheless, the rates of progression to CMV-diseases after CMV-
261 viremia were comparable in the two studies (36 % in our cases and 43 % in IMPACT).

262

263 **Conclusion**

264 This study using the QuantiFERON®-CMV assay that prospectively assessed the clinical utility of CMV-
265 specific IFN-gamma response dynamics to predict spontaneous viral clearance versus CMV disease in
266 homogeneous CMV D+/R- KTR at time of CMV viremia. Patients with a positive CMV-CMI assay at the
267 time of viremia onset could be less frequently monitored hoping for spontaneous viral clearance. In
268 contrast, patients with a nonreactive CMV-CMI assay should be closely monitored for signs of CMV
269 diseases to start timely antiviral treatment and reduce the incidence of CMV-disease complications.
270 Furthermore, QuantiFERON®-CMV might also guide the duration of CMV-disease treatment, with
271 discontinuation as soon as the CMI-test becomes positive and need to be confirmed in a validation cohort.

272

273 **Highlights**

274 • Patients with a positive CMV-CMI assay at the time of viremia onset could be less frequently

275 monitored hoping for spontaneous viral clearance

276 • Patients with a nonreactive CMV-CMI assay should be closely monitored for signs of CMV
277 diseases to start timely antiviral treatment and reduce the incidence of CMV-disease
278 complications

279 • QuantiFERON®-CMV might also guide the duration of CMV-disease treatment, with
280 discontinuation as soon as the CMI-test becomes positive

281 The authors declare no financial disclosure.

282

283

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389 **Figure legend**

390 **Fig. 1 Flowchart of the inclusion and outcome during the study.**

391 *D+/R-*, Seronegative kidney transplant recipients for CMV receiving a graft from seropositive donors.

392 *PCR*, Polymerase chain reaction for CMV viral load quantification

393 *CMI*, CMV specific cell mediated immunity

394 *CMV*, Cytomegalovirus

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396 **Fig. 2 CMV-specific IFN- γ responses in representative patients.**

397 (A) Patient with a CMV specific IFN- γ response detected at time of prophylaxis discontinuation before

398 CMV viremia and who had a subsequent spontaneous viral clearance. (B) Patient with concomitant CMV

399 viremia and CMV-specific IFN- γ response who had a subsequent spontaneous viral clearance. (C) Patient

400 with CMV viremia and undetectable CMV-specific IFN- γ response who developed a CMV disease, but

401 exhibited a positive CMV-specific IFN- γ response after treatment. (D) Patient with CMV viremia and

402 undetectable CMV-specific IFN- γ response throughout follow-up: he developed a CMV disease and

403 relapsed after treatment discontinuation.

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408 Table . Characteristics of study patients and viremia episodes.

	Spontaneous viral clearance (n=7)	CMV disease (n=4)	p value
Gender, n (%)			
Male	5 (71.4)	3 (75)	1
Female	2 (28.6)	1 (25)	1
Age (yr), median	58 [49; 67]	71.5 [60; 80]	0.11
Induction therapy, n (%)			
Basiliximab	3 (42.9)	1 (25)	1
Antilymphocyte globulin	4 (57.1)	3 (75)	1
Immunosuppression			
Tacrolimus, n (%)	6 (85.7)	4 (100)	1
Everolimus, n (%)	1 (14.3)	0 (0)	1
Residual Tac blood level at viremia ($\mu\text{g/L}$), median	7.3 [5.9; 8.2]	7.4 [5.0; 7.8]	0.61
Viremia episode, median			
Time posttransplant (m)	9 [6; 11]	7.5 [6; 9]	0.74
Time to viral clearance (m)	2 [1; 3]	3.5 [2; 4]	0.01
First viral load (IU/ml)	1100[200;15000]	335 [100;165143]	0.45
Peak viral load (IU/ml)	7300[530;15000]	139000[24250;1330000]	0.09
Lymphocytes count, median			
Total CD4+ lymphocytes at viremia episode (μL)	270 [118. 452]	70 [65. 435]	0.22
Total CD8+ lymphocytes at viremia episode (μL)	996 [359; 1390]	133 [60; 240]	0.02

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410 *CMV, cytomegalovirus*

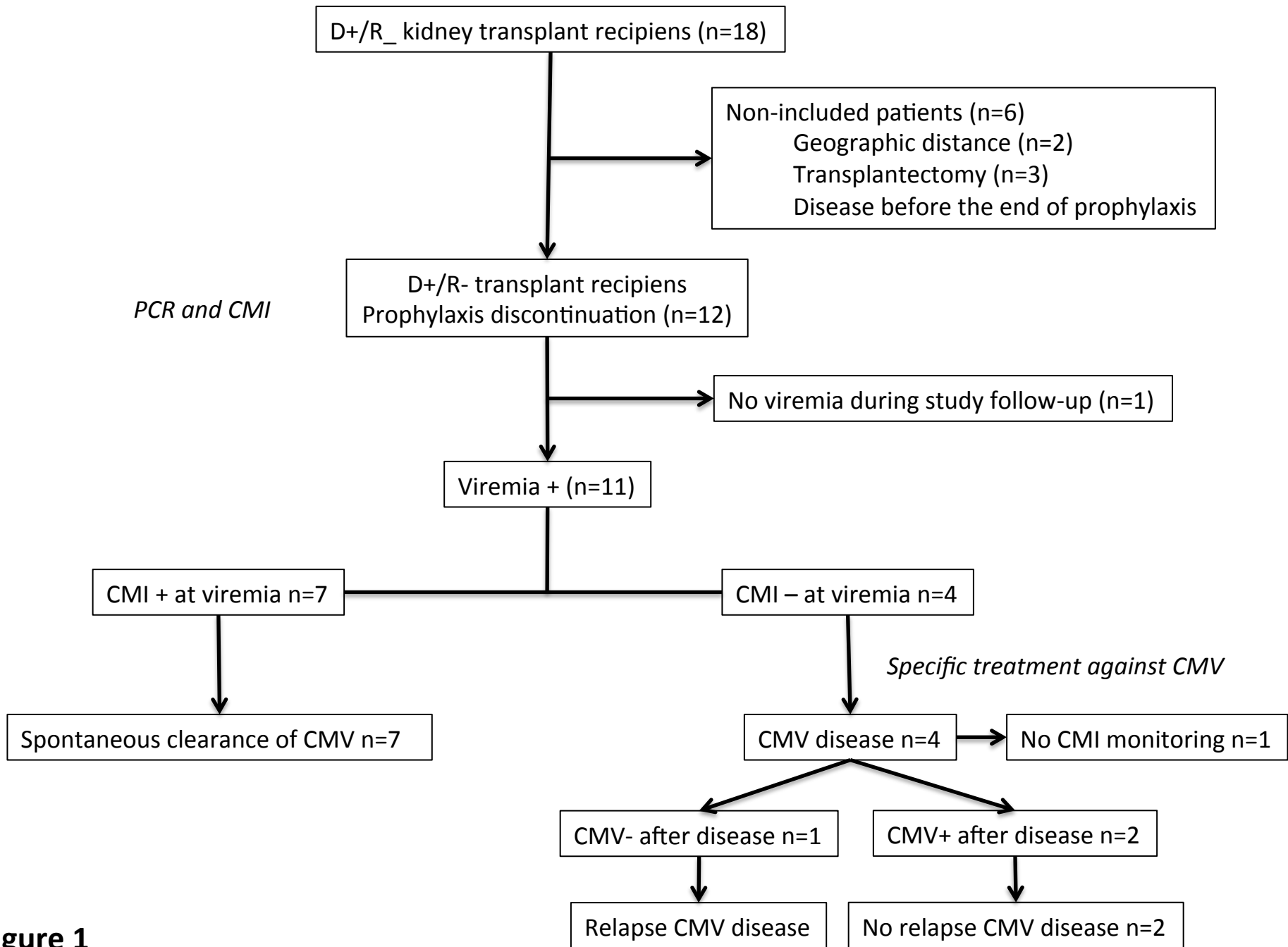


Figure 1

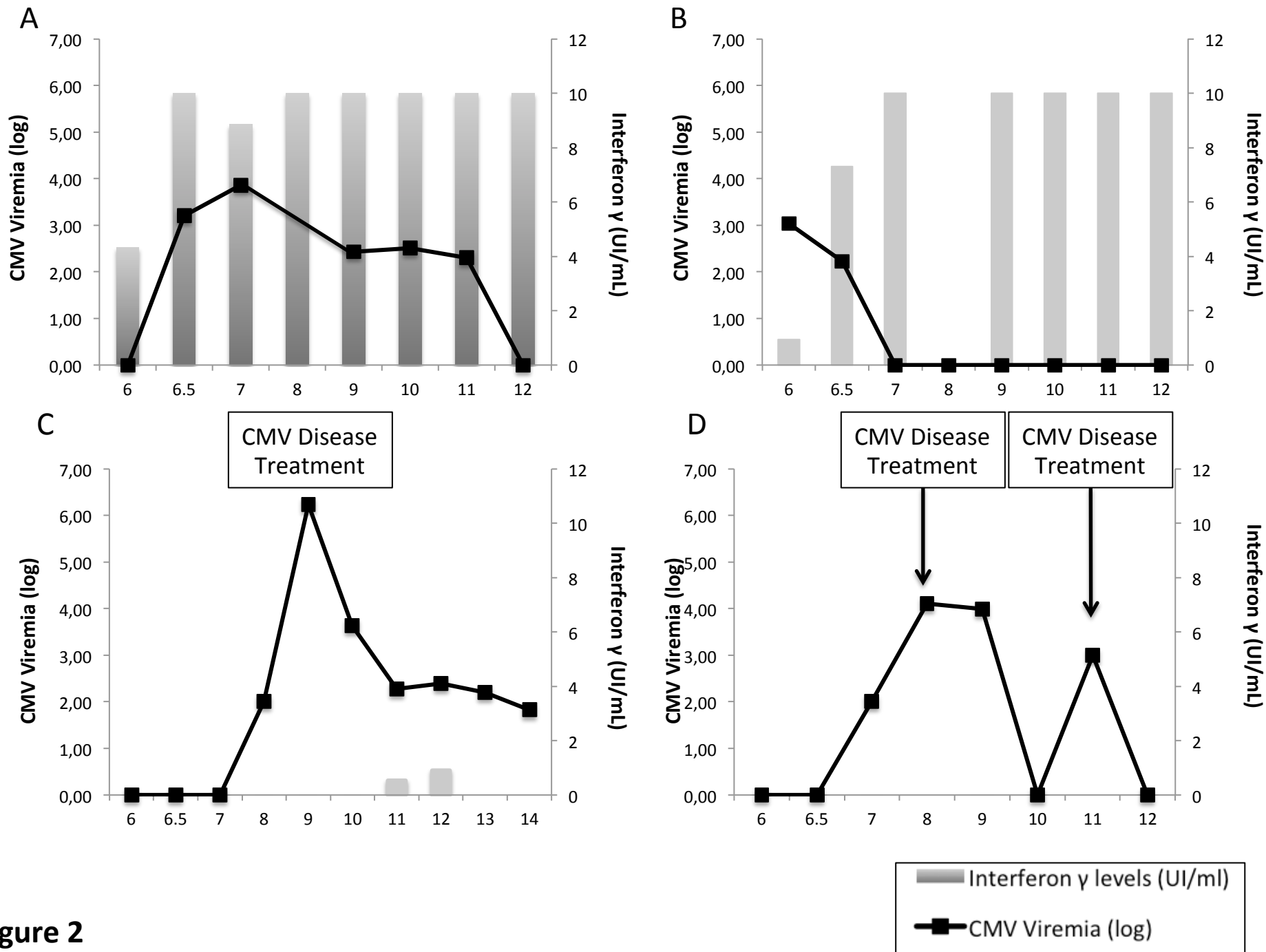


Figure 2