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UNIVERSITÀ DEGLI STUDI DI TORINO

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Evaluation of CMV-specific cellular immune response by EliSPOT assay in kidney transplant patients.

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22 **ABSTRACT**

23 **Background.** Immunological monitoring for CMV can be useful in transplant patients; however,
24 few centers perform it on a routine basis.

25 **Objectives.** In this study, CMV-specific cellular response was evaluated in a population of kidney
26 transplant recipients and related to viral infection/reactivation and other demographic and clinical
27 features.

28 **Study design.** Three-hundred-twenty-eight patients were studied by EliSPOT assay: 201
29 prospectively monitored in the first year posttransplantation, 127 with a single determination at >1
30 year. Clinical features, including occurrence of CMV-DNAemia, CMV serostatus, anti-viral
31 strategies and immunosuppressive protocols, were evaluated.

32 **Results.** Overall, 66.5% of patients were CMV- responders at EliSPOT assay. No episode of
33 infection occurred at follow-up (mean 24.5 months) in 73.4% responders versus 55.5% non-
34 responders (p <0.005); CMV-free period was significantly longer in responders (p<0.001).
35 Although no significant difference of peak viral load was found, prevalence of CMV-DNAemia
36 values >10⁵ copies/mL was significantly higher in non-responders versus responders (8.2% and
37 2.3%, p<0.05). Non-responder status was significantly associated to CMV-seronegativity (p
38 <0.0001), anti-viral prophylaxis use (p <0.0001), and immunosuppression induction with
39 basiliximab (p <0.005). No significant association was found for other clinical features and
40 immunosuppressive protocols.

41 **Conclusions.** Immunological data for CMV could be used in the clinical evaluation and decision-
42 making process, in combination with virological monitoring, in kidney transplant recipients.

43 .

44 **Keywords:** cytomegalovirus; cellular immune response; EliSPOT assay; CMV-DNAemia; kidney
45 transplantation.

46

47 **Background**

48 In transplant patients, Cytomegalovirus (CMV) may reactivate from latency due to
49 immunosuppression or cause primary infection in seronegative recipients. In kidney transplantation
50 (KT), CMV infection and disease have been reported in 8%-32% and 8%, respectively [1];
51 moreover, CMV has been associated to indirect effects, including rejection, chronic nephropathy,
52 and other opportunistic infections [1-3]. As CMV-specific T-cell response has been associated to
53 decreased rates of infection/disease [4-11], its evaluation may be valuable in combination with
54 viral monitoring, according to the Updated International Consensus Guidelines on the Management
55 of Cytomegalovirus in Solid Organ Transplantation [12]. Assays for immunological evaluation
56 include intracellular cytokine staining, MHC multimer staining, QuantiFERON-CMV, and
57 EliSPOT. An ideal assay should evaluate CD4+ and CD8+ T-cell response, optimally by measuring
58 interferon (IFN)- γ , and should be simple, rapid, cost-effective, and reproducible. At moment, no
59 assay is standardized with the exception of the QuantiFERON-CMV that, however, only evaluates
60 CD8+ responses. Other limitations include the need for a flow cytometer (intracellular cytokine
61 staining, MHC multimer staining) and HLA restriction (MHC multimer staining). EliSPOT
62 enumerates IFN- γ -secreting mononuclear cells (both CD4+ and CD8+, without differentiating) in
63 response to stimulus with CMV peptides and seems to represent a reproducible tool for monitoring
64 T-cell activity *ex-vivo* [12]. Current evidence suggests that viro-immunological evaluation can predict
65 the risk of CMV viremia and disease in the postprophylaxis and preemptive context [4-11]. At
66 moment, very few Italian centres perform CMV-specific immunological evaluation and its
67 implications in the clinical decision-making process are poorly defined. The Turin Renal Transplant
68 Centre is the first in Italy for activity volume (>100 KT/year).

69

70 **Objectives**

71 To evaluate CMV-specific cellular immune status in KT patients on a routine basis and investigate
72 the association to viremia, demographic and clinical features.

73 **Study design**

74 Three-hundred-twenty-eight consecutive KT recipients (M/F, 218/110; mean age, 54.7±14.2 years;
75 range, 28-75) were investigated in a mixed prospective-cross sectional study: 201 prospectively
76 monitored in the first year posttransplantation and 127 at >1 year (up to eight). Main features of
77 study population are summarized in Table 1. Informed written consent was obtained from all
78 patients; the study was conducted in accordance with the ethical standards and Helsinki Declaration
79 and approved by the Institutional Review Board. According to our centre's practice, virological
80 monitoring was performed by quantification of CMV-DNAemia on whole blood (using a
81 commercially available real-time PCR assay [CMV-ELITE MGB® kit, ELITechGroup, Milan,
82 Italy]) twice weekly in the first month, twice monthly up to 3 months, every three months up to 1
83 year, and yearly thereafter. Further specimens were collected in the presence of CMV-DNAemia,
84 usually within 7 days. Immunological evaluation was scheduled at 30, 60, 90, 180, and 360 days in
85 the first year posttransplantation; and once at any time point at >1 year. No baseline immunological
86 evaluation was made and no further specimens were collected in the presence of CMV-DNAemia.
87 However, due to missing sending or specimen unsuitability (i.e. insufficient number of cells, invalid
88 positive or negative control; see below for details), only 705 samples were available from the 201
89 patients evaluated in the first year posttransplantation (mean, 3.5/patient), in addition to 127
90 specimens from as many patients at >1 year, accounting for an overall number of 832 specimens.
91 Data of CMV-DNAemia were available for all patients (median time of follow-up 24.5 months,
92 range 24-42). Anti-CMV prophylaxis was administered for 3 months in high risk patients (i.e.
93 donor/recipient seromatching, D+/R-, N=30)[13]. Pre-emptive treatment with ganciclovir or
94 valganciclovir was administered in case of CMV-DNAemia >10⁴ copies/mL or based on clinical
95 judgment.

96 EliSPOT was performed as described elsewhere [14]. Briefly, automated separation of T cells from
97 fresh blood samples was performed with the RoboSepR instrument (StemCell Technologies,
98 Vancouver, Canada) using the EasySepTM Whole Blood T Cell Enrichment kit for immunomagnetic
99 negative selection (StemCell Technologies), following the manufacturer's instruction. This system
100 isolates cells from HetaSepTM-treated (ratio 1:5; StemCell Technologies) whole blood by targeting
101 unwanted cells for removal with Tetrameric Antibody Complexes recognizing CD14, CD16, CD19,
102 CD20, CD33, CD36, CD41, CD56, CD66b, CD123, glycophorin A and dextran-coated magnetic
103 particles; the labeled cells are separated using the EasySepTM magnet, whereas desired cells are
104 poured off into a new tube. According to manufacturer's, this system allows for an enrichment in
105 CD3+ fraction (approximately from 11% to >96%), with recovery of also dendritic cells and a
106 minimal amount of other cells, such as macrophages and B lymphocytes, functioning as antigen
107 presenting cells. No further method to assess specimen purity was used. Separated cells were
108 resuspended in RPMI-1640 medium (supplemented with 1% L-glutammine and 10% fetal calf
109 serum). An aliquot of 2×10^5 CD3+ cells (100 μ L/well from a 2×10^6 /mL mix) was incubated for 20-
110 24 h on an anti-IFN- γ antibody-coated plate (EliSPOT Interferon- γ Basis Kit; Autoimmun
111 Diagnostika, Strassberg, Germany) with CMV-specific peptide mix (CMV-Spot ELSP5530,
112 including pp65 and IE-1; Autoimmun Diagnostika), medium alone (negative control) or
113 phytohemagglutinin (positive control). IFN- γ production was visualized by an enzyme-labeled
114 detection antibody, with each spot representing a single cell secreting IFN- γ . Results were analyzed
115 using a computer-assisted system (AID EliSPOT Reader System, Autoimmun Diagnostika).
116 Background was subtracted for all the results (sample minus negative control). Results were
117 expressed as spot forming units (SFU)/ 2×10^5 CD3+ cells. Specific immune response was evaluated
118 as previously described [15]: invalid assay >5 SFU for the negative control and <20 for the positive
119 control; absent/weak response <20, strong response ≥ 20 (accordingly, non-responders and
120 responders patients).

121 Demographic and clinical variables were evaluated by univariate analysis and subsequently
122 included in a multivariate logistic regression analysis. The t test was used for comparisons of
123 quantitative variables between groups. Time to development of CMV reactivation was evaluated by
124 Kaplan-Meier curve analysis. Evaluation of an intersection point with high specificity and
125 sensitivity to differentiate patients with and without occurrence of CMV viremia in the subsequent 3
126 months on the basis of SFU values was made by ROC curve analysis. A commercially available
127 software was used (IBM SPSS Statistics version 21). A p value <0.05 was considered statistically
128 significant.

129

130 **Results**

131 Overall, 218/328 (66.5%) patients were CMV-responders (median SFU/2x10⁵ CD3+ cells 131;
132 range, 20-500); in particular, 125/201 (62.2%) individuals evaluated in the first year
133 posttransplantation (in this subgroup of patients, immunological status was defined considering the
134 whole period of study for each individual), with restoration of immune response within 6 months,
135 and 86/127 (67.7%) at >1 year. In Figure 1, rate of responders and median SFU/2x10⁵ CD3+ cells
136 at different time points are reported. At least one episode of viremia occurred in 107/328 (32.6%)
137 patients at follow-up: 58/218 (26.6%) responders versus 49/110 (44.5%) non-responders (p=0.002);
138 with repeated episodes of infection occurring in 13/218 (6.0%) versus 18/110 (16.4%; p=0.005),
139 respectively (Figure 2a). Viral load (peak level) tended to be higher in non-responders, although
140 difference was not significant (mean±standard deviation: 1.1x10⁵±3.4x10⁵ and 3.8x10⁵±8.4x10⁵
141 copies/mL in responders and non-responders, respectively; p >0.3)(Figure 2b). However, a
142 significantly higher prevalence of DNAemia values >10⁵ copies/mL was found in non-responders
143 versus responders (9/110, 8.2% versus 5/218, 2.3%, respectively, p<0.05)(Figure 2c). Kaplan-Meier
144 analysis evidenced that CMV-free cumulative incidence was significantly higher in responders
145 versus non-responders (p<0.001; Figure 3). No case of CMV disease occurred at follow-up.

Subsequently, relation between demographic and clinical features and CMV-specific immune status and viremia was evaluated (Table 2). At univariate analysis, non-responder status was significantly associated to male gender ($p<0.05$), seronegative recipient status (R-) at transplantation ($p<0.0001$), antiviral prophylaxis ($p<0.0001$), and immunosuppression induction with basiliximab ($p<0.005$); whereas no association was found for other features, including age, time from transplantation, immunosuppressive induction with anti-thymocyte globuline and immunosuppressive protocols including calcineurin-inhibitors and mTOR-inhibitors.

In particular, 28/39 (71.8%) R- patients were non-responders irrespective of donor serostatus, whereas the remaining R- individuals developed a CMV-specific cellular immune response in the first year posttransplantation following primary infection. Among D-/R- patients, one subject developed a primary infection at 1 month (viral load, 2,071,000 CMV-DNA copies/mL). As expected, EliSPOT evidenced a non-responder status in the first months posttransplantation, with development of CMV-specific response at 6 months (20 SFU// 2×10^5 CD3+ cells). For the analysis, this patient was included among the responders, however this level of response was only partially maintained (subsequent EliSPOT values between 12 and 20). On the other hand, 207/289 (71.6%) R+ patients displayed a responder status ($p<0.001$).

High risk patients (D+/R-) on antiviral prophylaxis usually exhibited a non-responder status, as expected; while pre-emptive treatment was associated with recovery and presence of CMV-specific cellular immune response ($p<0.001$).

As regards immunosuppression, only induction with basiliximab was significantly associated to a non-responder status. It is to note that anti-thymocyte globulin was used only in six patients, five of which reconstituted CMV-specific cellular immune response within three months.

Multivariate analysis of demographic and clinical features in relation to CMV non-responder status, evidenced a significant association for R- serostatus ($p<0.0001$; hazard ratio [HR] 13.02, 95% confidence interval [CI] 5.21-32.5) and male gender ($p<0.005$; HR 2.40, 95% CI 1.36-4.24).

171 Among demographic and clinical features evaluated at univariate analysis for the occurrence of
172 CMV viremia (Table 2), a significance was found only for age >50 years and induction with
173 basiliximab. No factor resulted significantly associated at multivariate analysis.
174 Figure 4 illustrates ROC curve analysis for SFU/2x10⁵ CD3+ cells values in terms of occurrence of
175 CMV-DNAemia in the subsequent 3 months.

176

177 **Discussion**

178 In the present study, CMV immunological data were routinely investigated in KT recipients by
179 EliSPOT, in contrast to previous studies performed on selected or voluntarily recruited patients and
180 more often by QuantiFERON-CMV assay. The optimization of the method with automated
181 separation of total CD3+ cells could improve specificity of this application, as EliSPOT usually
182 enumerates IFN-γ secreting mononuclear cells without distinguishing between NK and T cells.
183 Although the lack of baseline immunological data should be considered, most of the patients
184 evidenced recovery of CMV-specific T-cell response within the first months posttransplantation:
185 53.1% (median, >70 SFU/2x10⁵ CD3+ cells) at 3 months up to approximately 65% (median, >100)
186 at 6 months. Response was persistent throughout the follow-up with no subsequent relevant
187 increase in the total rate of responders and level of response. At follow-up, recovery of response
188 was significantly associated to a lower incidence of viremia (26.6% versus 44.5% in responders and
189 non-responders, respectively). In responders, episodes of infection were characterized by low level
190 CMV-DNAemia (< or slightly >2x10³ copies/mL, limit of detection of the real-time PCR assay)
191 and short duration (negative on subsequent evaluation, in the absence of antiviral administration). In
192 non-responders, occurrence of CMV-DNAemia was significantly higher, in particular repeated
193 episodes of infection; the higher occurrence of values >10⁵ copies/mL suggests a potential impact
194 on uncontrolled replication. It is to note that antiviral administration was based on clinical judgment
195 and/or CMV-DNAemia values >10⁴ copies/mL. It has been hypothesized that a certain level of viral

196 replication is required to stimulate an adequate immune response: CMV-DNAemia from 7000
197 copies/mL in R- patients when prophylaxis was discontinued in a previous study [7]. On the other
198 hand, the prompt administration of antivirals could interfere with immunological boost by reducing
199 viral exposure. This could explain the significantly higher frequency of repeated episodes of
200 reactivation in non-responders, as no sufficient exposure was accomplished by administering the
201 antiviral agent for low viral loads.

202 Considering serological matching and antiviral strategy, prophylaxis-treated D+/R- patients did not
203 usually mount an adequate response (73.3% non-responders up to 1 year posttransplantation), as
204 previously reported [7]. Despite of the high effectiveness of prophylaxis, primary infection may
205 occur, thus probably determining the priming of CMV-specific cellular response. This is well
206 recognized in KT (38% of 13 seronegative patients in the study by Abate and coll. [7]), as well as in
207 lung transplantation [16,17]. Priming of response can also occur in D-/R- patients following a
208 community-acquired infection. Seronegative status at transplantation and prophylaxis were among
209 the few factors significantly associated to the lack of an adequate response at univariate analysis;
210 association to seronegativity was highly significant also at multivariate analysis.

211 Considering immunosuppression, induction with basiliximab was significantly associated to non-
212 responder status and viremia in our study, in contrast with previously reported data. Basiliximab is
213 an IL-2 receptor antagonist that intervenes in a critical pathway involved in allograft rejection, thus
214 impairing the immune response to antigenic challenges. Nevertheless, previous studies have
215 reported similar incidence of CMV infection in basiliximab-treated patients and controls (17.3%
216 versus 14.5%) [18]. It could be hypothesized that other factors such as serostatus, antiviral strategy
217 or prophylaxis, and immunosuppressive protocols (triple immunosuppression including calcineurin-
218 inhibitors) play a role [19]. Protocols without mTOR inhibitors appear to delay CMV-specific
219 immune response and contribute to the onset of infection/disease in KT patients [20]; this has been
220 reported for lymphocyte-depleting agents (i.e. antithymocyte globulin) and calcineurin-inhibitor-

221 based regimens. In the present study, antithymocyte globulin was used only in six patients,
222 therefore no conclusion can be drawn; whereas the association between non-responder status and
223 the commonly used protocols including a calcineurin-inhibitor was only marginally significant.
224 Previous studies have evidenced a reduced incidence of CMV events for everolimus-treated
225 patients, in particular in the absence of prophylaxis [21-25]. Among the proposed mechanisms for
226 anti-CMV activity of mTOR inhibitors, an action on antiviral CD8⁺ memory T-cell generation has
227 been hypothesized [21].

228 An interesting finding was the higher frequency of non-responder status in men; it could be
229 hypothesized that sex might have an effect on patterns of CMV-response *per se* or that this is due to
230 differences in clinical features and management strategies (e.g., seronegativity more frequent in
231 males, 13.8% versus 6.4%), as previously reported for some CMV indirect effects [26].

232 In conclusion, viro-immunological routine monitoring of CMV evidenced that restoration of
233 specific T-cell response is frequently and stably achieved within few months posttransplantation
234 and is associated to a favorable outcome in terms of reactivation risk. Levels of response of 20
235 SFU/2x10⁵ CD3⁺ cells could be regarded as useful in terms of sensitivity and specificity for
236 evaluating the risk of viral reactivation. A subgroup of KT can display a persistently non-responder
237 status that could be due to other host-related determinants.

238 Based on this and considering the need for optimizing economic resources, the Turin Renal
239 Transplant Centre has proposed the subsequent protocol for CMV immunological monitoring in
240 KT: first evaluation at one month posttransplantation, in responders no further controls unless
241 therapy modifications, rejection or CMV infection occur; in responders with the above mentioned
242 conditions or non-responders, immunological evaluation in parallel to CMV-DNA up to 6 months
243 or up to the development of a responder status with a re-control at 12 months. A single study using
244 the QuantiFERON-CMV assay has also proposed the evaluation of pretransplant CMV responder
245 status to stratify the risk of viral reactivation posttransplantation [27]. The usefulness of EliSPOT

246 assay in this context has not been yet investigated and specific investigations are needed. Further
247 studies evaluating prophylaxis and pre-emptive treatment (in particular, the potential for defining
248 cut-off levels for starting pre-emptive treatment also on the basis of immunological status) are
249 needed, taking into consideration that a definitive evidence that immunological monitoring may
250 guide successful clinical intervention or add value to virological monitoring is still lacking.
251 Similarly, also the impact of immunosuppressive protocols, and other patient's and viral
252 determinants in relation to CMV-specific immunological status should be further investigated in
253 order to optimize the management of KT recipients.

254

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258 **Ethical approval:** Internal Review Board (ethical committee).

259

260

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332

333 **Figure 1. Rate of responders at different time points posttransplantation.** For each time point,
334 the median number of spot forming unit (SFU)/ 10^5 CD3+ cells is reported. Empty spaces in the
335 graph are for time intervals. Number of patients tested as the individual time points: 169 at 30 days,
336 124 at 60, 108 at 90, 156 at 180, 148 at 360 (considering an overall number of 201 patients
337 prospectively studied in the first year posttransplantation) and 127 patients at >360.

338

339 **Figure 2. Occurrence of CMV-DNAemia in relation to CMV-specific cellular immune**
340 **response.** Percentages of patients with no episode, single episode or repeated episodes of CMV
341 infection at follow up (mean 24.5 months, range 24-42) in responder and non-responder groups
342 (Figure 2a). Mean viral load in the two groups; values are expressed as $\log_{10}$ copies/ml whole blood
343 (peak level in each patient)(Figure 2b). Percentages of patients with CMV-DNAemia levels higher
344 or lower than 10^5 copies/mL whole blood in the two groups (peak level in each patient)(Figure 2c).

345

346 **Figure 3. Kaplan-Meier curve showing the time to occurrence of CMV viremia in responders**
347 **(continuous line) versus non-responders (dotted line) who presented at least one episode of**
348 **infection within 24 months posttransplantation.**

349

350 **Figure 4. Evaluation of operating characteristics for spot forming units (SFU/ 2×10^5 CD3+**
351 **cells) values in terms of occurrence of CMV-DNA in the subsequent 3 months by ROC curve**
352 **analysis.** 5 SFU/ 2×10^5 CD3+ cells: 51.4% sensitivity (95% confidence interval [CI] 41.5-61.3), 71.0% specificity
353 (95% CI 66.6-75.1); 20 SFU/ 2×10^5 CD3+ cells, 71.4% sensitivity (95% CI 61.8-79.8), 60.0% specificity (95% CI 55.3-
354 64.5); 100 SFU/ 2×10^5 CD3+ cells, 89.5% sensitivity (95% CI 82.0-94.5), 38.5% specificity (95% 34.1-43.1).

355

356

357 **Table 1. Main features of study population.**

	Total N= 328
Age, mean $\pm$ SD (range), years	54.7 $\pm$ 14.2 (28-75)
Gender Male Female	218 (66.5%) 110 (33.5%)
CMV serological matching D+/R+ D-/R+ D+/R- D-/R-	259 (79.0%) 30 (9.1%) 30 (9.1%) 9 (2.8%)
Time from transplantation < 1 year > 1 year (up to 8)	201 (61.3%) 127 (38.7%)
Immunosuppression induction ATG Basiliximab	6 (1.8%) 238 (72.6%)
Immunosuppressive protocol <i>Including CNI</i> Tac, MMF, steroid Tac, steroid CyA, MMF, steroid CyA, steroid Others <i>Including mTOR inhibitors (everolimus, sirolimus)</i>	313 (95.4%) 177 (54.0%) 81 (24.7%) 17 (5.2%) 8 (2.4%) 30 (9.1%) 28 (8.5%)
Antiviral strategy Prophylaxis (D+/R-) Pre-emptive therapy (D+/R+, D-/R+) None (D-/R-)	30 (9.1%) 289 (88.1%) 9 (2.8%)

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360 N, number; SD, standard deviation; D, donor; R, recipient; ATG, antithymocyte globulin; CNI,
361 calcineurin inhibitors; Tac, tacrolimus; MMF, mycophenolate mofetil; CyA, cyclosporine A;
362 mTOR, mammalian target of rapamycin.

363 **Table 2. Relation between demographic and clinical features of study population and CMV-specific cellular immune response status and**
364 **viremia occurrence.**

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Feature	N	Non-responders N (%)	OR (95% CI)	p	CMV-viremia N (%)	OR (95% CI)	p
Sex							
M	218	82 (37.6)	0.5663	0.0286	71 (32.6)	0.9928	0.9769
F	110	28 (25.4)	(0.3404-0.9422)		36 (32.7)	(0.6090-1.6186)	
Age ≥ 50 years							
Yes	221	78 (35.3)	0.7822	0.3331	82 (37.1)	1.9350	0.0136
No	107	32 (29.9)	(0.4757-1.2863)		25 (23.4)	(1.1453-3.2690)	
Recipient CMV serostatus							
R+	289	82 (28.4)	6.4257	<0.0001	97 (33.6)	1.4651	0.3241
R-	39	28 (71.8)	(3.0570-13.5065)		10 (25.6)	(0.6858-3.1300)	
Anti-viral prophylaxis							
Yes (D+/R-)	30	22 (73.3)	0.1524	<0.0001	8 (26.7)	0.7309	0.4669
No (D+/R+, D-/R+, D-/R-)	298	88 (29.5)	(0.0654-0.3553)		99 (33.2)	(0.3142-1.7005)	
Time from transplantation							
< 1 year	201	69 (34.3)	0.9120	0.7024	75 (37.3)	0.8306	0.4657
> 1 year	127	41 (32.3)	(0.5687-1.4627)		32 (25.2)	(0.5044-1.3678)	
Immunosuppression induction with basiliximab							
Yes	235	90 (38.3)	0.4414	0.0042	101 (43.0)	10.9291	< 0.0001
No	93	20 (21.5)	(0.2521-0.7729)		6 (6.5)	(4.5940-26.0002)	
Immunosuppression induction with anti-thymocyte globuline							
Yes	6	1 (16.7)	2.5587	0.3938	2 (33.3)	1.0333	0.9701
No	322	109 (33.8)	(0.2952-22.1744)		105 (32.6)	(0.1863-5.7323)	
Immunosuppressive protocol including calcineurin-inhibitors (cyclosporine A, tacrolimus)							

Yes	313	110 (35.1)	0.0594	0.0502	105 (33.5)	3.2813	0.1223
No	15	0 (0)	(0.0035-1.0024)		2 (13.3)	(0.7270-14.8104)	
Immunosuppressive protocol including mTOR-inhibitors (everolimus, sirolimus)							
Yes	28	13 (46.4)	0.05513	0.1352	9 (32.1)	0.9764	0.9549
No	300	97 (32.2)	(0.2525-1.2041)		98 (32.7)	(0.4262-2.2369)	
Total	328	110 (33.5)			107 (32.6)		

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368 D, donor; R, recipient; OR, odds ratio; CI, confidence interval.

369 **Figure 1**

Rate of responders

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375 **Figure 2**

376 **2a**

p = 0.002 for overall episodes of infection

p = 0.005 for repeated episodes of infection

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379 2b

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387 2c

p < 0.05

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390 3

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393 4

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