Assessment of CMV-specific cell-mediated immunity for the prediction of CMV disease in kidney and liver transplant patients: a pilot study

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INTRODUCTION AND OBJECTIVES

Cell mediated immunity (CMI) plays an important role in the defense against CMV infection, a common complication after transplantation (Tx). Assays detecting CMV specific CMI may help the current management of CMV infection in solid-organ transplant (SOT) recipients, by allowing a better risk stratification and influencing the way antiviral therapy and prophylaxis are administered. Three assays were evaluated in this study: QuantiFERON CMV® (Qiagen), T-Track CMV® (Lophius) and T-SPOT. CMV® (Oxford Immunotec). The primary endpoint was determining a cutoff for the cellular immune response, which protects against CMV disease. Secondary endpoint was performance of the three tests in comparison with the CMV serology.

STUDY DESIGN

30 KTx and 25 LTx patients were stratified according to their CMV- IgG serostatus pre-Tx and divided into 2 groups: pre-emptive (D-/R+ & D+/R+ KTx) and prophylaxis (D+/R-, D-/R+ & D+/R+ LTx; D+/R- and D+/R+ & D-/R+ KTx receiving ATG or eculizumab). Patients were included in a prospective longitudinal observational study, conducted over a 3 to 6 months period post-Tx or end of prophylaxis. T-Track and T-SPOT.CMV (ELISpots detecting IFN γ producing CD4+ and CD8+ T cells in response to stimulation with IE-1 and pp65) were performed at 1 month post-Tx (pre-emptive) and end of prophylaxis and 1 month after prophylaxis. QuantiFERON-CMV (ELISA quantifying IFN γ after stimulation with 22 viral peptides) was performed every 2-4 weeks (pre-emptive) or monthly (prophylaxis), parallel to the CMV viral load (PCR).

1) Kotton CN et al. Transplantation. 2013. 2) Melendez D. et al. Expert Rev. Clin. Immunol. 2014. 3) Ruis MF et al. Clinical & Translational Immunology 2014.

RESULTS

1. Patient characteristics

Total (no., %)	p N=21	P N=34
Type of transplant: KTx LTx	21 (100)	9 (26) 25 (74)
Gender : Male Female	13 (61) 8 (39)	25 (74) 9 (26)
Median age (IQR) (yr)	55 (34-71)	53 (20-79)
CMV Serostatus: D-/R+ D+/R+ D+/R-	4 (19) 17 (81)	9 (26.5) 16 (47) 9 (26.5)
Immunosuppressive regimens CNI, MMF and steroids CNI, mTOR, steroids: switch from MMF to mTOR CNI, steroids CNI, MMF, steroids, other CNI, steroids, other	 17 (81) 4 (19) 3 (14)	 25 (74) 15 (44) 12 (35) 2 (6) 3 (9) 1 (3)
Induction therapy: ATG Basiliximab	 1 (4,8) 20 (95,2)	 2 (6) 7 (21)
Allograft rejection therapy	4 (19)	8 (24)

p-pre-emptive, P-prophylaxis; D-Donor, R-Recipient, CNI-calcineurin inhibitor, MMF-micophenolat mophetil, ATG-antithymoglobulin

Total (no., %)	N=21	N=34
Patients with CMV DNAemia post Tx: D-/R+ D+/R+ D+/R-	12 (57) 1 (8) 11 (92)	18 (53) 3 (17) 9 (50) 6 (33)
Patients with CMV disease	1 (4.8)	1 (3)
Patients who received antiviral therapy	12 (57)	18 (53)

(CMV DNAemia was defined as: PCR CMV > 500 copies/ml (p), PCR CMV > 40 copies/ml (P).

Statistical analysis was performed using GraphPad Prism v7 (San Diego, CA) and IBM SPSS Statistics 22 (New York, NY) software. Two-sided p-values <0.05 were considered significant.

2. Primary endpoint (p)

Test	AUC	Cutoff	Sens.	Spec.
QuantiFERON-CMV (IU/mL)	0.472	85.1	22 %	100 %
T-Track CMV IE1 (SFU)	0.960	18.5	60 %	100 %
T-Track CMV pp65 (SFU)	0.640	495	20 %	100 %

Table 2. ROC analysis for the QuantiFERON-CMV and T-Track CMV. The values used were performed one month after Tx in patients with both ELISpot and QuantiFERON results or the first obtained QuantiFERON value before reactivation, for patients included in the study later post-Tx. T-Track (10 values included). T-SPOT.CMV (4 values, not included). The outcome was protection against reactivation in the next 3 months.
SFU-spot forming units, AUC-area under curve, Sens.-sensitivity, Spec.-Specificity.

3. Primary endpoint (P)

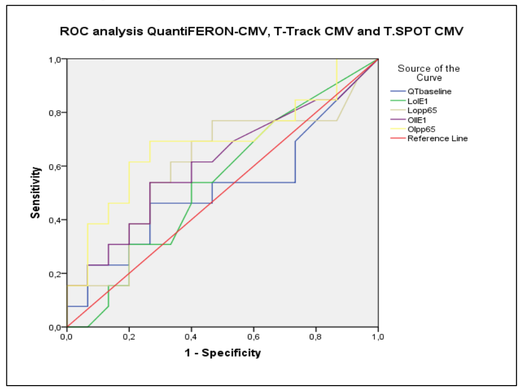

Figure 1. ROC analysis for the Quantiferon-CMV, T-Track CMV and T-SPOT.CMV. *The values used were performed at the end of prophylaxis. 28 patients included in the analysis.* The outcome was protection against reactivation in the next 3 months.
pp65 (T-SPOT.CMV) marker of protection in the prophylaxis group (AUC= 0.685, cutoff of 465 SFU Sensitivity 15.4 %, Specificity 100 %).
IE1 (T-SPOT.CMV) - AUC=0.613, cutoff 399 SFU Sensitivity 15.4 %, Specificity 100 %.
pp65 (T-Track CMV) - AUC=0.608 cutoff 342 SFU, Sensitivity 15.4 %, Specificity 100%.
QuantiFERON-CMV (AUC=0.518, cutoff 77 UI/mL) and IE1 T Track CMV (AUC= 0.541, cutoff 18.5 SFU).

4. Secondary endpoint: positive agreement between T-Track CMV, T-SPOT.CMV, QuantiFERON CMV and CMV IgG serology at baseline

Test	CMV positive serology	CMI +	CMI-	k	95 % CI
Quanti-FERON	48	41	7	0.599	0.345-0.853
T-Track	33	32	1	0.918	0.760-1.000
T-SPOT	27	27	0	1	

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