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A New Prognostic Score Based on Cell-Mediated Immunity for Cytomegalovirus Infection After Transplantation

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Introduction: The interferon gamma (IFN-γ) enzyme-linked immunosorbent spot is a highly sensitive immune assay that enables the assessment of cytomegalovirus (CMV)-specific cell-mediated immunity (CMI) and can identify at-risk transplant patients of CMV infection; however, its clinical implementation remains elusive.

Methods: We developed a novel CMV-CMI risk-score based on the standardized T-SPOT.CMV assay against 2 CMV antigens (immediate-early protein 1 [IE-1] and 65 kDa phosphoprotein [pp65]), a biomarker predicting CMV infection, both high viral replication, and disease by performing a pooled analysis of 570 kidney transplants participating in different clinical trials and subsequently validating it in 146 consecutives solid organ transplants (SOT) in an interventional trial. By incorporating clinical variables into the CMV-CMI risk-score, we built an integrative prognostic system quantifying the risk of CMV infection

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(CMV-PrognosTIC score) using elastic net penalized regression analysis.

Results: In the pooled derivation cohort, whereas specific IE-1/pp65-specific CMV-CMI frequencies independently correlated with high risk of CMV infection (areas under the curve [AUCs]: 0.694, $P < 0.0001$; 0.719, $P < 0.0001$, respectively), by combining both responses, 3 CMV-CMI risk-scores appeared, accurately discriminating low-risk (LR) from intermediate-risk (IR) and high-risk (HR) patients (98.7% negative predictive value [NPV], 97.2% sensitivity). Its prospective implementation guiding decision-making in an independent SOT cohort confirmed the very high NPV and sensitivity identifying LR patients. By integrating type of preventive therapy, patient age, and donor (D) and recipient (R) CMV-serostatus to the CMV-CMI risk-score, we generated a global risk-prognostic model showing accurate discrimination and calibration in both derivation (AUC: 0.807) and validation cohorts (AUC: 0.719).

Conclusion: We developed a robust CMV-PrognosTIC score to quantify the risk of CMV infection in SOT, which may be readily implemented in clinical transplantation to personalize CMV preventive therapies.

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Despite the implementation of highly effective antiviral therapies and sensitive molecular diagnostic assays, CMV infection remains a major complication after SOT.[1] Current immune-risk stratification of CMV infection in SOT relies on donor and recipient serological status, together with clinical variables such as types of immunosuppressants and of SOT,[2] and are far from being precise. Furthermore, though main CMV preventive strategies have allowed a significant reduction of CMV infection rates, especially end-organ disease,[2] important caveats are still present and related to decision-making, especially in terms of individualizing the type and duration of these therapies.[3,4]

In the past decade, a large body of evidence has highlighted the importance of CMV-CMI controlling viral replication in SOT.[5-7] With the development and refinement of different immune tests that can measure CMV-specific IFN-γ-producing T cells, a close association between weaker CMV-CMI and higher risk of CMV infection has been reported, evaluating different transplant settings (either to decide initiation of preventive therapy or for safe prophylaxis/treatment withdrawal).[6,8-10] However, the different methodological nature of these assays, together with the relatively weak predictive values to identify SOT at low risk of CMV infection,[11-15] makes it difficult to establish robust conclusions on how to implement these technologies at the individual patient level.[3] Among the different commercially available CMV-CMI assays, the enzyme-linked immunosorbent spot–based T-SPOT.CMV assay has shown the highest accuracy in measuring CMV-specific CMI[16,17] and discriminating SOT and hematopoietic stem cell transplant at distinct risks of CMV infection.[18-22] Despite such important correlations with main clinical outcomes, its implementation still remains elusive, because the interpretation of the assay read-out and most optimal thresholds, the precise clinical scenarios to be used and its added value besides main clinical, demographic, and other immunological factors are not well-defined.

The aims of this study were to develop a standardized CMV-CMI risk-stratification score for CMV infection, including high level viral replication and disease, using the T-SPOT.CMV assay in different, multicenter cohorts of transplant patients and prospectively validate it in an interventional clinical trial. Finally, we integrated key clinical factors associated with CMV infection into this new CMV-CMI risk-score to build a more accurate and readily implementable model quantifying the risk of CMV infection after SOT.

METHODS

Study Design and Patients

We first performed a pooled analysis of 4 different international studies using the same CMV-CMI assay in kidney transplant recipients (KTRs). Gathering together these patients, we built a derivation cohort to assess and develop an accurate CMV-CMI cut-off discriminating patients at different risks of CMV infection (CMV-CMI risk score), and then assessed its clinical value in a new SOT cohort participating in an interventional trial guiding the type and duration of preventive therapy based on the CMV-CMI risk score (Figure 1).

Derivation Cohorts

The derivation cohort consisted of 570 adult KTRs from 4 multicenter studies measuring CMV-CMI with the T-SPOT.CMV assay[11,15,19] (NCT03652402), including diverse serostatus (CMV IgG seropositive donor-CMV IgG seronegative recipient [D+/R−] and CMV IgG seropositive recipient [R+]) and clinical settings (early CMV-CMI measurement at 15 days after transplant or later at prophylaxis withdrawal at 3 or 6 months after

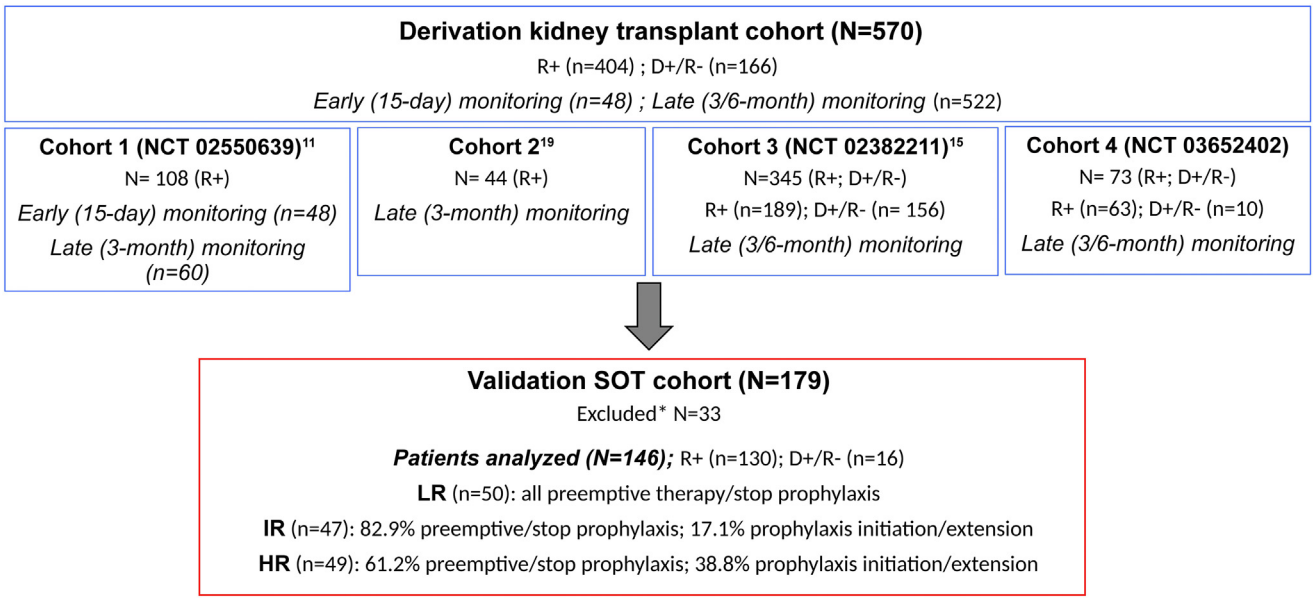


Figure 1. Flow-chart of the study. D+, CMV IgG seropositive donor; HR, high-risk CMI; IR, intermediate-risk CMI; LR, low-risk CMI; R−, CMV IgG seronegative recipient; R+, CMV IgG seropositive-recipient; SOT, solid organ transplants. *Patients excluded from validation cohort because of loss of follow-up ($n = 13$), use of antirejection therapy ($n = 7$), unavailable blood samples ($n = 7$), and prophylaxis initiation before the CMV-CMI test ($n = 6$).

transplantation) (Table 1). In all of these cohorts, clinicians were not aware of the immune assay result.

The first cohort comprised 108 R+ KTRs participating in a prospective, randomized, interventional clinical trial (NCT02550639) performed among 4 different transplant centers in Spain.[11] Here, the T-SPOT.CMV was carried out either for early immune monitoring on day 15 in patients receiving preemptive therapy or late monitoring at prophylaxis withdrawal (3 months). A second cohort was made of 44 consecutives R+ KTRs from a prospective, single center observational study, all receiving prophylaxis.[19] The T-SPOT.CMV assay was done at a late time point at 3-month prophylaxis withdrawal. A third cohort consisted of 345 KTRs from distinct transplant centers in the USA, UK, and Canada, participating in a prospective, observational trial (NCT 02382211).[15] All patients received 3- or 6-month antiviral prophylaxis if R+ or D+/R−, respectively. All patients with a T-SPOT.CMV test performed at prophylaxis withdrawal and who had not developed any CMV event before the assay had been performed, were included in the analysis (n = 345, 189 R+ and 156 D+/R−). The fourth cohort comprised a new independent set of 73 KTRs (63 R+ and 10 D+/R−) participating in the European, multicenter, cohort study EU-TRAIN (NCT03652402). All patients received either a 3-month (R+) or 6-month (D+/R−) course antiviral prophylaxis and the immune assay was done at the time of prophylaxis withdrawal.

Patients receiving antirejection rescue therapies during the study follow-up, and adjunction induction immunosuppression such as anti-CD20 mAb, plasmapheresis or immunoadsorption, and i.v. Ig were excluded from the study.

Validation Cohort

Aiming at assessing the clinical usefulness of implementing the CMV-CMI risk-score in real clinical transplant practice, we designed a prospective interventional study considering SOT patients transplanted in 1 center (Bellvitge University Hospital, Barcelona, Spain) in whom peripheral blood samples could be obtained to perform the T-SPOT.CMV assay either on day 15 after transplantation in R+ not receiving T-cell depletion or between 2 to 3 months after transplantation in those R+ having received T-cell depletion and between 5 to 6 months in D+/R− SOT. The decision of the type of preventive therapy was made by transplant clinicians after being informed of the CMV-CMI risk-score result. The main objective of the study was to describe the incidence of CMV infection events between different risk categories given by the CMV-CMI assay and; as secondary end point, to evaluate the impact of the type of antiviral preventive therapy in each risk group. The sample size was based on a 95% confidence interval (CI) with a 5% margin of error; thus, the total number of patients required for the study was 180, including 15% of drop-out rates. The validation cohort comprised 179 consecutive heart, liver and kidney transplant patients transplanted at Bellvitge University Hospital (Barcelona, Spain) who underwent transplantation between January 2020 and December 2021 (Figure 1). Thirty-three patients were excluded from the study due to loss of follow-up ($n = 13$), use of antirejection therapy ($n = 7$),

Table 1. Main clinical, demographic and immunological characteristics of the derivation cohorts

Characteristics	All patients ($N = 570$)	Early CMV-CMI R+ ($n = 48$)	Late CMV-CMI R+ ($n = 356$)	Late CMV-CMI D+/R- ($n = 166$)	*P*-value
Recipient gender (male) (*n*; %)	371 (65.1%)	32 (66.7%)	220 (61.8%)	119 (71.7%)	0.085
Recipient age (median; IQR)	55.2 (45.0–64.9)	63.0 (49.7–71.9)	55.0 (46.0–64.0)	53.0 (43.0–62.0)	< 0.001
Induction treatment (*n*; %)					< 0.001
T cell depletion	343 (60.2%)	0 (0.0%)	241 (67.7%)	102 (61.4%)	
No T cell depletion	227 (39.8%)	48 (100.0%)	115 (32.3%)	64 (38.6%)	
CMV serological risk (*n*; %)					< 0.001
Intermediate risk (R+)	404 (70.9%)	48 (100.0%)	356 (100.0%)	0 (0.0%)	
High risk (D+/R−)	166 (29.1%)	0 (0.0%)	0 (0.0 %)	166 (100.0%)	
CMV prophylaxis (*n*; %)	522 (91.6%)	0 (0.0%)	356 (100.0%)	166 (100.0%)	
Time to T-SPOT.CMV (d) (median; IQR)	91.0 (90.0–160.5)	15.0 (14.0–21.0)	90.0 (90.0–98.0)	178.0 (106–190)	< 0.001
T-SPOT.CMV (median; IQR)					
IE-1 (IFN-γ spots/250.10^5 PBMC)	7.0 (0.0–86.2)	23.3 (2.7–110.6)	27.0 (4.0–148.5)	0.0 (0.0–1.0)	< 0.001
pp65 (IFN-γ spots/250.10^5 PBMC)	73.5 (2.0–294.7)	141.3 (44.0–287.3)	164.9 (42.1–349.3)	0.0 (0.0–2.0)	< 0.001
CMV infection					
Any replication (*n*; %)	115 (20.2%)	25 (52.1%)	47 (13.2%)	43 (25.9%)	< 0.001
Clinically significant infection (*n*; %)	71 (12.5%)	15 (31.3%)	20 (5.6%)	36 (21.7%)	< 0.001
Disease (*n*; %)	16 (2.8%)	6 (12.5%)	5 (1.4%)	5 (3.0%)	< 0.001

CMI, cell-mediated immunity; CMV, cytomegalovirus; D+, CMV IgG seropositive donor; IE-1, immediate-early protein 1; IFN-γ, interferon gamma; IQR interquartile range; PBMC, peripheral blood mononuclear cell; pp65, 65 kDa phosphoprotein; R−, CMV IgG seronegative recipient; R+, CMV IgG seropositive-recipient.

unavailable blood samples ($n = 7$), and prophylaxis initiation before the CMV-CMI test ($n = 6$); thus, a total of 146 SOT were analyzed (81 kidney, 40 liver, and 25 heart transplants). The patients were further stratified into 2 groups according to the use or not of T-cell depletion and D/R serological mismatch (Table 2) as follows: (i) group A: ($n = 75$) R+ patients without T-cell depletion, in whom the CMV-CMI risk-score was assessed on day 15 after transplantation (median 11 days; interquartile range: 9–17) to decide whether to start preemptive therapy or antiviral prophylaxis; and (ii) group B ($n = 71$), composed of D+/R− and R+ patients receiving T-cell depletion in whom an initial antiviral prophylaxis therapy was indicated, and in whom the CMV-CMI risk-score was assessed at later time points (median: 102 days; 88–133) to decide whether to stop or continue with a one-time 4 week prophylaxis extension. The test was done once in each patient. The study was approved by local institutional review board (PR302/13).

The clinical and research activities being reported are consistent with the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

CMV-CMI

In all study subjects, CMV-CMI was evaluated using the T-SPOT.CMV (Oxford Immunotec, Inc, Oxford, UK) against 2 main CMV antigens (IE-1 and pp65), following the manufacturer recommendations (Supplementary Methods).[15]

Definition of Main CMV Outcomes

The main outcomes of the study were the advent of CMV infection during first year posttransplantation.[2] The definitions of the different CMV events were harmonized across all studies following the most updated consensus definitions of CMV infection and disease in transplant patients.[23] "Any CMV replication" refers to any CMV DNA detection in whole blood or plasma. "CMV disease" was defined as CMV replication with attributable symptoms (viral syndrome and/or invasive tissue infection). In addition, we analyzed the outcome clinically significant CMV infection (CSI) as defined as any CMV infection leading to a change in antiviral therapy, either at a site-determined viremia threshold considered as clinically relevant, or because of CMV-related symptoms (CMV disease). CMV infection outcomes were considered either as early or late-onset when CMV events occurred during a preemptive strategy or after prophylaxis withdrawal, respectively. We focused on CSI as the main clinical infection outcome to develop the CMV-CMI risk score as well as the main end point of the validation study, as a combined clinically relevant infection variable due to its clinical relevance and high correlation with CMV-CMI and avoid unspecific, low-level viral replication events that spontaneously clear from peripheral blood.

Development of the CMV-CMI Risk Score

The best threshold for each CMV-CMI antigen result to predict CSI was determined by using receiver operating characteristic curve analysis, using the Youden index. Quantitative IFN-γ–producing T-cell frequencies against each CMV antigen were used to obtain the most sensitive and specific risk-stratification score predicting CSI. Three qualitative risk categories were observed when combining the 2 CMV antigens CMI

Table 2. Main clinical, demographic, and immunological variables of the validation cohort

Validation cohort	Whole cohort $N = 146$	Group A Early CMV CMI $n = 75$	Group B Late CMV CMI entire cohort $n = 71$	Group B	
				Group B Late CMV CMI R+ T-cell depletion ($n = 55$)	Group B Late CMV CMI D+/R− ($n = 16$)
Recipient gender, (male, *n*; %)	88 (60%)	54 (72.0 %)	34 (47.9 %)	22 (40.0 %)	12 (75.0%)
Recipient age (mean +/- SD), yrs	55 +/- 11	56 +/-9	55 +/- 13	57 +/- 12	50+/−13
Transplanted organ (*n*; %)					
Liver	40 (27.4 %)	40 (53.3 %)			
Heart	25 (17.1 %)	25 (33.3 %)			
Kidney	81 (55.5 %)	10 (13.3 %)	71 (100.0 %)	55 (100.0 %)	16 (100.0 %)
Previous transplant (*n*, %), No	118 (71.3 %)	71 (94.7 %)	47 (66.2 %)	33 (60.0 %)	14 (87.5 %)
Type of donor (*n*, %)					
Living donor	15 (10.3 %)	1 (1.3 %)	14 (19.7 %)	7 (12.7 %)	7 (43.8 %)
Type of deceased donors (*n*; % of deceased donors)					
Donation after brain-death	111 (84.7 %)	67 (90.5 %)	44 (77.2 %)	39 (81.3 %)	5 (55.5 %)
T-cell depletion induction (*n*, %)	57 (39.0%)	0 (0.0 %)	57 (80.3 %)	54 (98.2 %)[a]	3 (18.8 %)
Maintenance IS (*n*, %)					
TAC + MMF	143 (98.0 %)	74 (98.7 %)	69 (97.2 %)	54 (98.2 %)	15 (93.8 %)
TAC	2 (1.4 %)	1 (1.3 %)	1 (1.4 %)	1 (1.8 %)	
TAC + mTORi	1 (0.7 %)	0 (0.0 %)	1 (1.4 %)	0 (0.0 %)	1 (6.2 %)
CMV serological risk (*n*; %)					
R+	130 (89.0%)	75 (100.0%)	55 (77.5%)	55 (100.0%)	0 (0.0%)
D+/R−	16 (11.0 %)	0 (0.0 %)	16 (22.5 %)	0 (0.0 %)	16 (100.0 %)
Time to T-SPOT-CMV (median; IQR), d	48 (11–104)	11 (9–17)	102 (88–133)	97 (84–116)	166 (107–188)
CMV-CMI risk-score (*n*; %)					
Low risk	50 (34.2%)	23 (30.6%)	27 (38.0%)	25 (45.5%)	2 (12.5%)
Intermediate risk	37 (25.3%)	27 (36%)	20 (28.2%)	17 (30.9%)	3 (18.8%)
High risk	49 (33.6%)	25 (33.3%)	24 (33.8%)	13 (23.6%)	11 (68.8%)
Prophylaxis use (initiation (group A)/ prolongation (group B) (*n*; %)	27 (18.5%)	15 (20.0%)	12 (16.9%)	7 (12.7%)	5 (31.3%)
Prophylaxis use in each group of CMV-CMI risk score (*n*; % of patients in this group)					
Low risk	0 (0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate risk	8 (21.6 %)	5 (18.5%)	3 (15.0%)	2 (11.8%)	1 (33.3%)
High risk	19 (38.8%)	10 (40.0%)	9 (37.5%)	5 (38.5%)	4 (44.4%)
CMV infection (*n*; %)					
CMV replication	35 (23.9%)	14 (18.7%)	21 (29.5%)	11 (20.0%)	10 (62.5%)
CMV-CSI	18 (12.3%)	6 (8.0%)	12 (16.9%)	3 (5.4%)	9 (56.3%)
CMV disease	8 (5.4%)	3 (4.0%)	5 (7.0%)	0 (0.0%)	5 (31.3%)

ABMR, antibody mediated rejection; CMI, cell-mediated immunity; CMV, cytomegalovirus; D+, CMV IgG seropositive donor; IQR interquartile range; IS, immunosuppression; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitor; pp65, 65 kDa phosphoprotein; R−-, CMV IgG seronegative recipient; R+, CMV IgG seropositive-recipient; TAC, tacrolimus.
[a]One patient with basiliximab induction, early ABMR treated with plasma exchange and i.v. Ig.

(CMV-CMI risk-score) as follows: (i) HR (both responses below their respective threshold), (ii) IR (1 of the 2 responses below the respective threshold), and (iii) LR (both responses above their respective thresholds).

Types of CMV Preventive Therapies

In the derivation cohort, patients received either antiviral prophylaxis with oral valganciclovir 900 mg/d (adjusted to estimated glomerular filtration rate) during the first 90 days in R+ receiving T-cell depletion or 180 days in D+/R−, or preemptive therapy based on weekly CMV quantitative nucleic acid testing on whole blood or plasma during the first month, every 2 weeks until month 3, monthly until months 6, and every 2 months until month 12 after transplantation in R+ patients not receiving T-cell depletion.[2]

In the validation cohort (Supplementary Figure S1), decision to start or not prophylaxis in R+ not receiving T-cell depletion (early CMV-CMI monitoring) and to stop or extend prophylaxis after usual prophylaxis duration in R+ patients receiving prophylaxis (3 months) and D+/R− patients (6 months) was made by the clinician, being aware of the result of the CMV-CMI risk score. In all patients displaying an LR CMV-CMI risk-score, physicians followed a preemptive therapy (either initially after transplantation in group A, or after prophylaxis withdrawal in group B). In 82.9% and 61.2% of patients with an IR and HR CMV-CMI risk-scores, respectively, clinicians also followed a preemptive strategy; whereas in 17.1% and 38.8% of IR and HR patients, respectively, physicians initiated (group A) or prolonged (group B) antiviral prophylaxis.

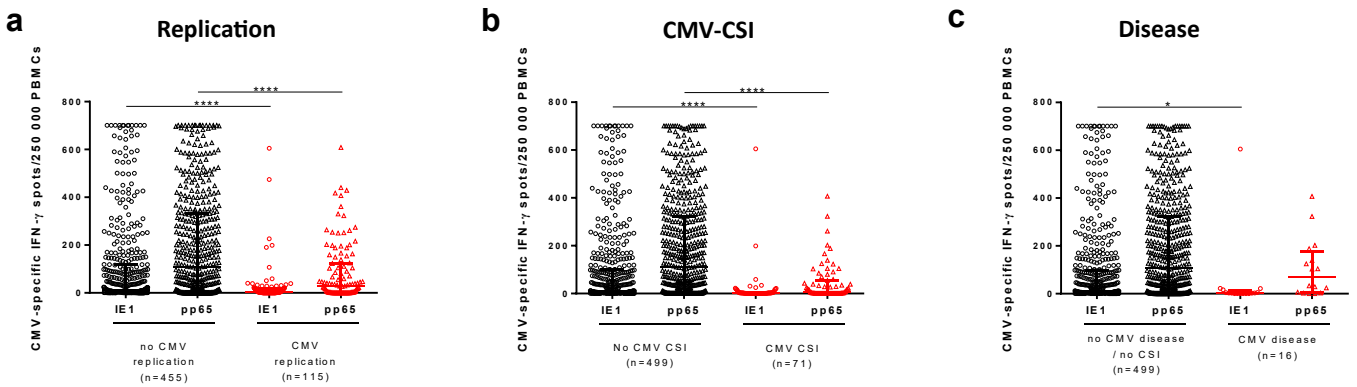


Figure 2. CMV-CMI results in the derivation cohort. CMV-CMI for patients developing (a) CMV replication, (b) CMV-CSI, and (c) CMV disease. (a) Any CMV replication versus no replication among all patients: 2.0 (0.0–16.0) versus 10.0 (0.0–118.0) median IE-1-specific IFN-γ spots/2.5 × 10^5 PBMC respectively, $P < 0.0001$, and 27.0 (1.0–123.0) vs. 109.0 (4.0–332.0) median pp65-specific IFN-γ spots/2.5 × 10^5 PBMC, respectively; $P < 0.0001$. (b) CMV-CSI versus no CMV-CSI among all patients: 1.0 (0.0–4.0) versus 10.0 (0.0–99.0) median IE-1-specific IFN-γ spots/2.5 × 10^5 PBMC respectively; $P < 0.0001$, and 6.0 (0.0–55.0) versus 109.0 (6.0–323.0) versus median pp65-specific IFN-γ spots/2.5 × 10^5 PBMC respectively; $P < 0.0001$. (c) CMV disease versus no CMV disease among all patients: 2.5 (0.0–13.3) versus 10.0 (0.0–99.0) median IE-1-specific IFN-γ spots/2.5 × 10^5 PBMC, respectively; $P = 0.0424$, and 68.2 (3.8–175.8.0) versus 109.0 (6.0–323.0) median pp65-specific IFN-γ spots/2.5 × 10^5 PBMC respectively, $P = 0.2903$). CMI, cell-mediated immunity; CMV, cytomegalovirus; CSI, clinically significant infection; D+, CMV IgG seropositive donor; IE-1, immediate-early protein 1; IFN-γ, interferon gamma; PBMC, peripheral blood mononuclear cell; pp65, 65 kDa phosphoprotein; R–, CMV IgG seronegative recipient; R+, CMV IgG seropositive-recipient.

To ensure the safety and accuracy of the CMV-CMI risk-score, patients undergoing prophylaxis withdrawal were followed-up with polymerase chain reaction monitoring during the following 3 (R+) and 6 months (D+/R−).

Statistical Analysis

Continuous data of CMV-specific T-SPOT.CMV against each antigen (IE-1 and pp65) are presented as median and interquartile range because of a nonnormal distribution. Categorical data are presented as number and percentage of patients. Groups were compared using nonparametric Mann-Whitney U test for nonnormally distributed continuous variables and χ^2 test for categorical variables. The incidence of CMV events was compared between groups with Kaplan Meier curves using log-rank test. The evaluation of the potential variation of the results between cohorts due to a batch-to-batch effect was ruled out by performing a Kruskal-Wallis test, followed by a *post hoc* Dunn test (data not shown).

The statistical significance level was defined as 2-tailed P-value < 0.05. Statistical analyses were performed with IBM SPSS statistics (version 23, IMB Corp., Amonk, NY) and GraphPad Prism (version 6.0, GraphPad Software, San Diego, CA).

The CMV-CMI risk score predicting CMV infection was done using receiver operating characteristic curve analyses to obtain the most accurate thresholds for each CMV-CMI antigen. Logistic regression analyses were performed to determine the independent correlation between variables and infection. The results were expressed as odds ratios with 95% CIs. The development of a CMV-PrognosTIC risk model to quantify the risk of infection was built using elastic net penalized regression with the glmnet R package[24,25] using the complete derivation cohort, and subsequently assessed in the prospective validation cohort. A thorough explanation and key TRIPOD items are presented in the Supplementary Methods.

RESULTS

Characteristics of the Derivation Cohort

The derivation cohort included 570 kidney transplant patients (Figure 1 and Table 1), including 166 D+/R− and 404 R+ patients. Early CMV-CMI monitoring was performed in 48 R+ patients (8.4%) who did not receive T-cell depletion and on preemptive therapy at a median time of 15 days posttransplant. All other patients were monitored later at the time of prophylaxis withdrawal ($n = 522$ [91.6%], 356 R+ and 166 D+/R−, median time to CMV-CMI testing 90 (90–98) days and 178 (106–198) days, respectively). In late CMV-CMI monitoring groups, 241 of the 356 R+ patients (67.7%) and 102 of the 166 D+/R− patients (61.4%) received T cell depletion (3–4.5 mg/kg total dose).

Frequencies of CMV-CMI, Absolute Lymphocyte Counts and CMV Infection Rates in the Derivation Cohort

Median pp65 CMV-CMI frequencies were significantly higher than IE-1-specific CMI; both being significantly higher in R+ than D+/R− (Supplementary Figure S2A and B). Patients who did not develop any viral

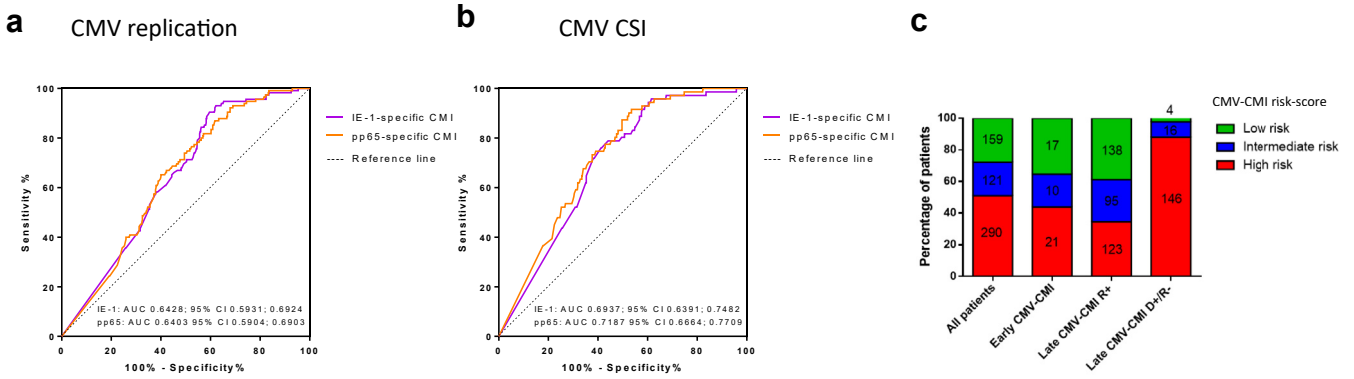


Figure 3. Risk-score classification with IE-1 and pp65-specific CMV-CMI. (a) ROC curves for the risk of CMV replication. (b) ROC curves for the risk of CMV-CSI. (c) Classification of patients in 3 distinct risk categories considering the combination of both IE-1 and pp65-specific CMI (CMV-CMI risk-score): low-risk (LR), intermediate-risk (IR), and high-risk (HR). CMI, cell-mediated immunity; CMV, cytomegalovirus; CSI, clinically significant infection; IE-1, immediate-early protein 1; pp65, 65 kDa phosphoprotein; ROC, receiver operating characteristics.

replication or CSI displayed higher IE-1 and pp65-specific CMV-CMI frequencies than patients who did (Figure 2a and b). Patients who developed CMV disease showed lower IE-1-specific CMV-CMI (Figure 2c). These differences were similar if assessed at either early or late time points (Supplementary Figures S3 and S4). There were no statistically significant differences, albeit numerically, between groups when only studying D+/R− patients (Supplementary Figure S5). No association was observed between absolute lymphocyte counts and subsequent CMV infection, or with either IE-1 or pp65-specific CMI (Supplementary Figure S6).

Development of a CMV-CMI Risk-Score for Immune Stratification

CMV-CMI AUCs for CSI significantly outperformed those for any viral replication (Figure 3a and b). Most sensitive or specific thresholds were 34 and 126 IFN-γ spots/2.5×10^5 peripheral blood mononuclear cells (PBMC) for IE-1 and pp65, respectively. Using these CMV-CMI thresholds, patients were classified based on each individual antigen into either HR (CMV-CMI responses below the threshold) or LR (CMV-CMI responses above the threshold), which resulted in good sensitivity and NPV for CSI. However, when we combined the 2 CMV-CMI antigen responses, patients were reclassified into 3 risk categories: HR (low responses against both antigens), LR (high responses against both antigens), or IR (combined low and high response against one of the 2 CMV antigens), improving their predictive accuracy (Supplementary Table S1). As shown in Figure 3c, whereas approximately 50% of HR patients were observed at the early monitoring assessment and within D+/R− patients (87%), IR patients were similarly represented in all clinical settings. Notably, almost 40% of R+ patients (36% R+ without T-cell depletion assessed early after transplant and 39% at 3-month posttransplant in R+ receiving T-cell depletion) showed an LR score.

Performance of the CMV-CMI Risk-Score in Distinct Clinical Settings and Derivation Cohorts

Cumulative infection rates (any replication, CSI, and disease) when considering CMV-CMI for each individual CMV antigen were significantly higher among HR patients (Figure 4). When patients were classified into the 3-group risk categories by combining both CMV antigens (using the CMV-CMI risk score), several patients considered as LR either by the IE-1 or pp65 CMV-CMI response were reclassified as IR and developed higher rates of CMV replication and CSI. Although CSI occurred in 3 of 195 LR (1.5%) and in 68 of 375 HR patients (18.1%) when considering IE-1 CMV-CMI, and in 7 of 244 LR (2.9%) and 64 of 326 HR patients (19.6%) when using pp65 CMV-CMI (Log-rank test $P < 0.0001$), with the CMV-CMI risk-score, 2 of 159 LR patients (1.3 %), 6 of 121 IR (5.0 %), and 62 of 290 HR patients (21.7%) developed CSI (Log-rank test $P < 0.0001$).

The same differences were observed when we assessed the impact of the distinct immune-risk scores on the rates of CMV replication, CSI, and disease when CMV-CMI was assessed early after transplantation (Supplementary Figure S7) and later at prophylaxis withdrawal among R+ (Supplementary Figure S8) and D+/R− patients (Supplementary Figure S9). The composition of the IR group was fundamentally made up of patients with preserved reactivity against pp65 but not against IE-1 (Supplementary Figure S10).

Prospective Validation of the CMV-CMI Risk-Score

The incidence of CSI and disease in the whole validation cohort was 12.3% and 5.4%, respectively (Table 2). None of the patients with LR developed CSI,

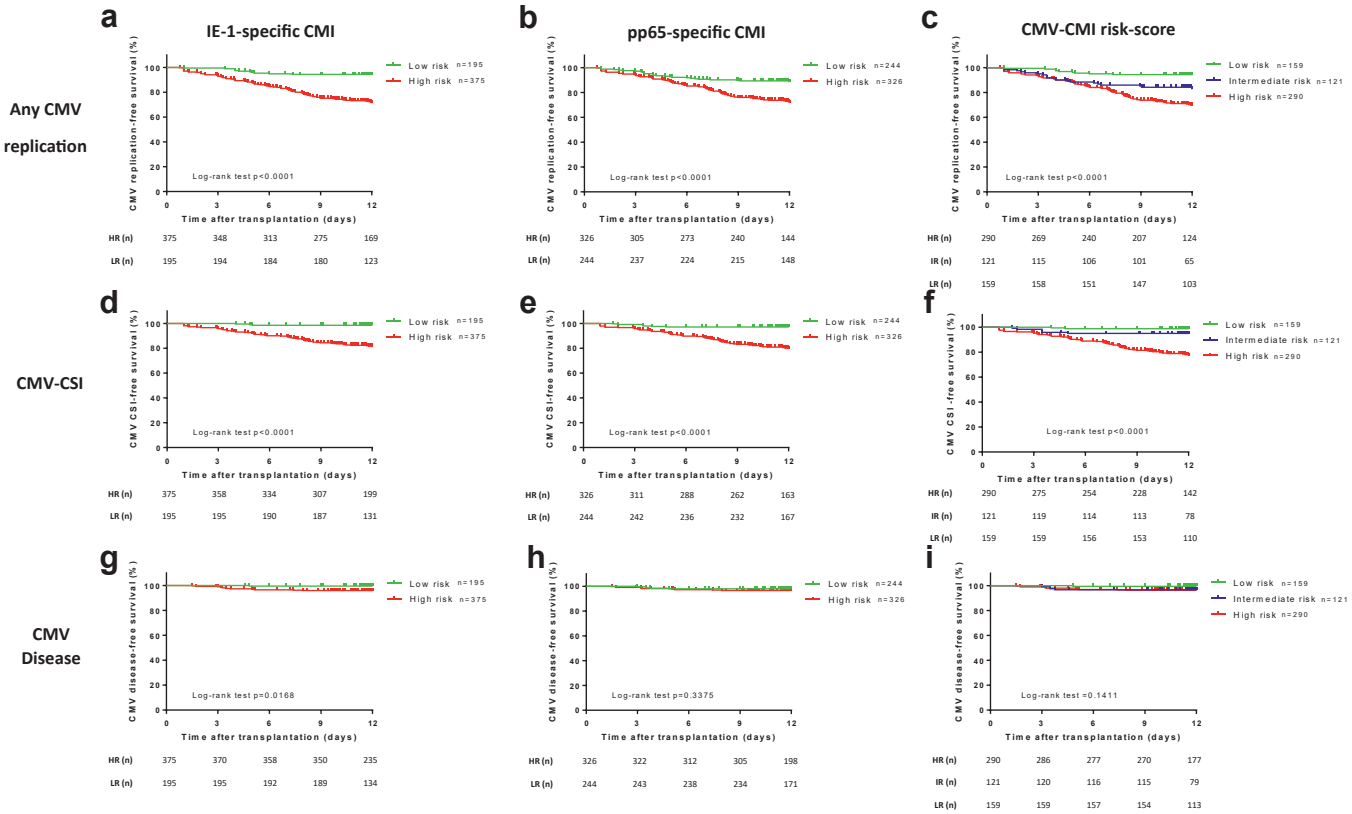


Figure 4. CMV infection rates according to CMV-CMI risk stratification in the derivation cohort. Kaplan-Meier CMV infection-free survival curves for (a, b, c) any CMV replication, (c, d, e) CMV-CSI, and (g, h, i) CMV disease in all patients of the derivation cohort according to (a, d, g) IE-1-specific CMV-CMI, (c, f, i) pp65-specific CMV-CMI and for the combined IE-1 and pp65-specific CMV-CMI risk-score. CMI, cell-mediated immunity; CMV, cytomegalovirus; CSI, clinically significant infection; IE-1, immediate-early protein 1; pp65, 65 kDa phosphoprotein.

which only occurred among IR and particularly among HR SOT (12.8%, odds ratio: 18.82, 95% CI: 0.86–289.3, $P = 0.0091$ and 24.5%, odds ratio: 33.65, 95% CI: 1.93–587, $P = 0.0002$, respectively) (Figure 5 and Table 3). In this cohort, the NPV of the CMV-CMI risk score for CMI (considering LR vs. IR/HR) was 100%. When we stratified the groups according to the type of preventive strategy used, IR and HR patients following a preemptive therapy (both in those following preemptive therapy initiation early after transplantation in group A, and those with prophylaxis withdrawal at the time of the test in group B) developed significantly higher CSI than those receiving antiviral prophylaxis (both in those with early prophylaxis initiation in

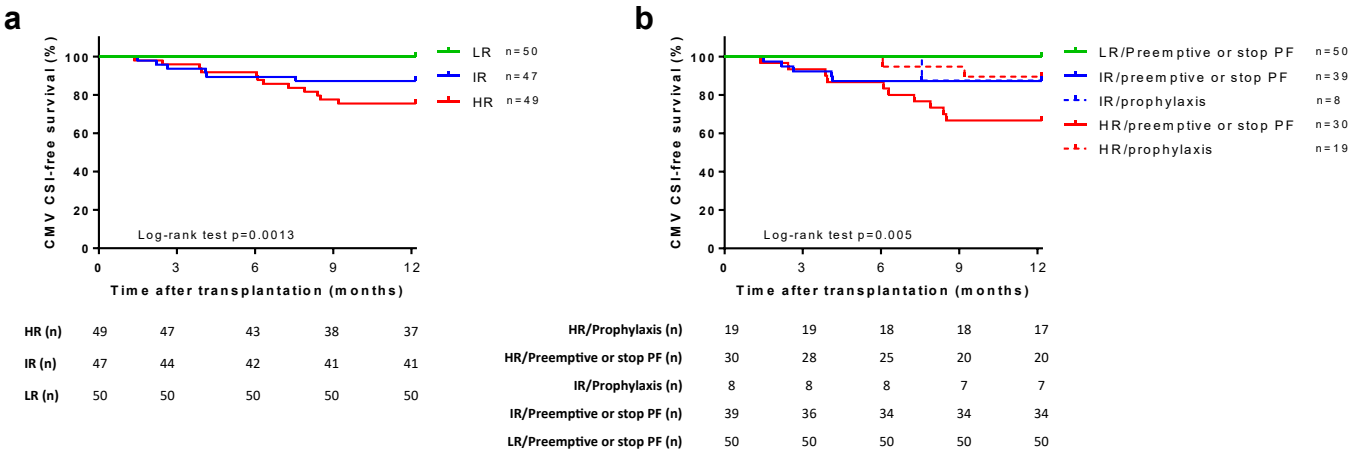


Figure 5. Kaplan-Meier CMV CSI-free survival curves in the validation cohort. (a) KM CMV-CSI-free survival curves between different CMI-CMV risk-scores and (b) when stratified according to the prevention strategies used. CMI, cell-mediated immunity; CMV, cytomegalovirus; CSI, clinically significant infection; HR, high-risk CMI; IR, intermediate-risk CMI; LR, low-risk CMI.

Table 3. Efficacy end points of different CMV-CMI risk-scores for CMV clinically significant infection in the validation cohort

	CMV-CMI risk-score			IR vs. LR				HR vs. LR				HR vs. IR			
End point	LR CMI $n = 50$	IR CMI $n = 47$	HR CMI $n = 49$	Difference (%)	OR	95% CI	*P*-value	Difference (%)	OR	95% CI	*P*-value	Difference (%)	OR	95% CI	*P*-value
CMV-CSI	0 (0%)	6 (12.8%)	12 (24.5%)	12.8	15.82	0.86–289.3	0.0091	24.5	33.67	1.93–587.2	0.0002	11.7	2.22	0.76–6.50	0.1412
	LR CMI $n = 50$	IR preemptive/Stop PF $n = 39$		IR prophylaxis $n = 8$				IR preemptive/Stop PF vs. LR				IR prophylaxis vs. LR			
CMV-CSI	0 (0%)	5 (12.8%)		1 (12.5 %)		12.8	16.10	0.86–300.9	0.0092	12.5	20.20	0.75–543.3	0.0117		
	LR CMI $n = 50$	HR preemptive/Stop PF $n = 30$		HR prophylaxis $n = 19$				HR preemptive/Stop PF vs. LR				HR prophylaxis vs. LR			
CMV-CSI	0 (0%)	10 (33.3%)		2 (10.5%)		33.3	51.73	2.89–925.1	< 0.0001	10.5	14.43	0.66–315.6	0.0199		

CI, confidence interval; CMV, cytomegalovirus; CMV-CMI, cytomegalovirus cell–mediated immunity; CMV-CSI, cytomegalovirus clinically significant infection; HR, high-risk; IR, intermediate-risk; LR, low-risk; OR, odds ratio; PF, prophylaxis.
Groups were compared using the χ^2 test as both outcomes, and variables are categorical variables.

group A, and those following 4 additional weeks of prophylaxis in group B) (Supplementary Tables 2 and 3 and Supplementary Figure S11). Interestingly, none of the IR and HR patients starting on prophylaxis within the early-monitoring group (group A) developed CMV-CSI, whereas those following preemptive therapy did develop CSI. Conversely, among the late monitoring group (group B), IR and HR patients in whom an extension period of prophylaxis was added, displayed a significant delay of CSI as compared with those stopping prophylaxis therapy. Notably, those IR and HR who developed CSI after an extension period of prophylaxis were all high serological risk (D+/R−) (Supplementary Figure S11). The CMV-CMI risk-score stratified by type of SOT revealed that heart and liver transplants display higher HR CMV-CMI risk-score than kidney transplant patients, only at early time point posttransplantation (Supplementary Figure S12).

Integrative Prognostic Model for the Risk of CSI

We finally analyzed major clinical and immunological variables associated with CMV infection in the derivation cohort. As shown in Table 4, the type of preventive therapy, recipient's age, and CMV serostatus together with CMV-CMI were independent correlates of CSI; and the combination of all of them best predicted CSI than each variable individually (AUC: 0.808) (Figure 6). We generated a prognostic score system (CMV-PrognosTIC score) to quantify the risk of infection. The performance of the model in the derivation cohort showed an AUC of 0.807 (95% CI: 0.7581–0.856) with a sensitivity of 0.7647, specificity of 0.7597, positive predictive value of 31.5%, and NPV of 95.9% (Figure 7a); and AUC of 0.7193 (95% CI: 0.6233–0.8153), sensitivity of 1, specificity of 0.5133, positive predictive value of 24.7%, and NPV of 100.0% in the validation cohort (Figure 7b and Supplementary Figure S13). The calibration of the model was optimal in all the risk percentiles, with certain overdiagnosis of HR of CSI (Figure 7c). A density plot was built combining the different risk estimates with the 3 CMV-CMI risk strata in both derivation (Figure 7d) and validation cohorts (Figure 7e), observing a clear differentiation between patients across the 3 risk groups.

DISCUSSION

In this study, we developed a new CMV-CMI risk-score that can stratify the risk of CSIs, and built a risk prognostic algorithm, the CMV-PrognosTIC, which combines major clinical parameters with the CMV-CMI risk-score allowing an accurate quantification of the relative risk of infection in different clinical scenarios and SOT. By performing a pooled analysis in a large multicenter kidney transplant cohort, we have been

Table 4. Univariate and multivariate logistic regression analysis of main clinical, demographic and immunological variables predicting CMV-CSI

	Univariate analysis			Multivariate analysis		
Variables predicting CMV-CSI	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Type of CMV preventive therapy (Prophylaxis vs. preemptive)	0.264	0.135–0.517	< 0.0001	0.171	0.064–0.456	< 0.0001
Sex (Male)	1.058	0.626–1.788	0.834			
Age (yrs)	1.030	1.009–1.052	0.004	1.034	1.011–1.058	0.004
Induction (T-cell depletion)	0.603	0.366–0.993	0.047	1.215	0.646–2.282	0.546
Serological risk (D+/R− vs R+)	2.920	1.760–4.844	< 0.0001	2.222	1.173–4.210	0.014
IE-1 HR vs. LR	14.176	4.399–45.686	< 0.0001	5.721	1.565–20.913	0.008
Pp65 HR vs. LR	8.270	3.717–18.402	< 0.0001	4.198	1.635–10.780	0.003
Absolute lymphocyte counts (Giga/L)	1.204	0.858–1.691	0.283			

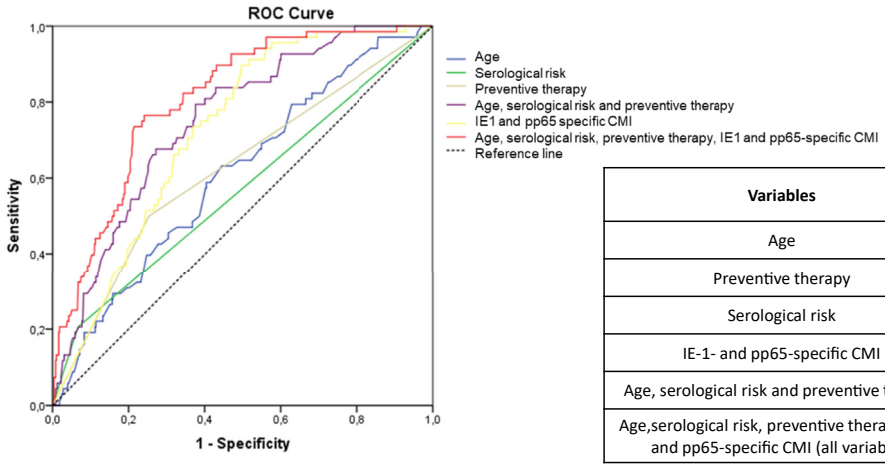
CI, confidence interval; CMI, cell-mediated immunity; CMV, cytomegalovirus; CSI, clinically significant infection; D+, CMV IgG seropositive donor; HR, high-risk CMI; IE-1, immediate-early protein 1; LR, low-risk CMI; OR, odds ratio; pp65, 65 kDa phosphoprotein; R−-, CMV IgG seronegative recipient; R+, CMV IgG seropositive-recipient.

able to describe robust CMV-CMI cut-offs against 2 main CMV antigens (IE-1 and pp65), which stratify patients into 3 different risk layers. Most importantly, we show the high NPV of this CMV-CMI risk-score, identifying patients at very LR of developing CSIs, both asymptomatic high viral replication and disease among different patient phenotypes and clinical scenarios; and thus, may help to safely stop or avoid stringent preventive therapies. Interestingly, patients classified as HR by the CMV-CMI risk-score in whom prophylaxis therapy was initiated or prolonged had lower incidences of infections than those undergoing routine monitoring on preemptive therapy, because they were reduced by almost 50%. These results open the field for interventional trials to investigate the best prevention strategy and duration in IR and HR patients.

Although fewer D+/R− patients developed detectable CMV-CMI over time, the CMV-CMI risk-score could also detect patients at very LR and in whom prophylaxis was safely withdrawn in this group of patients. The majority of D+/R− patients showed an HR profile with a low positive predictive value of the test (having a negative test), which strongly suggests that other immune cell subset counterparts may play a key role protecting from CMV infection among these patients.

Unlike other IFN-γ-release assays such as the enzyme-linked immunosorbent assay–based QuantiFERON assay, main advantages of this CMV-CMI risk-score based on the T-SPOT.CMV are that it provides quantifiable CMV-specific T-cell frequencies and informs of the CMI response against each of the 2 main immunogenic CMV antigens, which seem to confer differential immune protection than the assessment of a single antigenic response.[11,18,19,22,26]

Notably, though the CMV-CMI risk-score identifies 3 risk groups, their quantifiable relative risk may differ according to distinct clinical variables and scenarios. Therefore, we incorporated key clinical and immunological predictors of CMV infection such as type of preventive therapy, patient age, and serostatus mismatch to the CMV-CMI risk-score to build a novel CMV-PrognosTIC Score system, to better contextualize



Variables	AUC	CI 95 %	P-value vs AUC all variables
Age	0.610	0.540; 0.679	<0.0001
Preventive therapy	0.573	0.496; 0.649	<0.0001
Serological risk	0.623	0.551; 0.696	<0.0001
IE-1- and pp65-specific CMI	0.728	0.676; 0.779	0.0027
Age, serological risk and preventive therapy	0.751	0.696; 0.778	0.03
Age,serological risk, preventive therapy, IE-1- and pp65-specific CMI (all variables)	0.808	0.759; 0.857	-

Figure 6. ROC curves of each variable and combination of variables for the risk of CMV-CSI, with respective AUC. AUC, area under the curve; CMV-CSI, cytomegalovirus clinically significant infection; ROC, receiver operating characteristics. ROC curves were compared using the DeLong method.

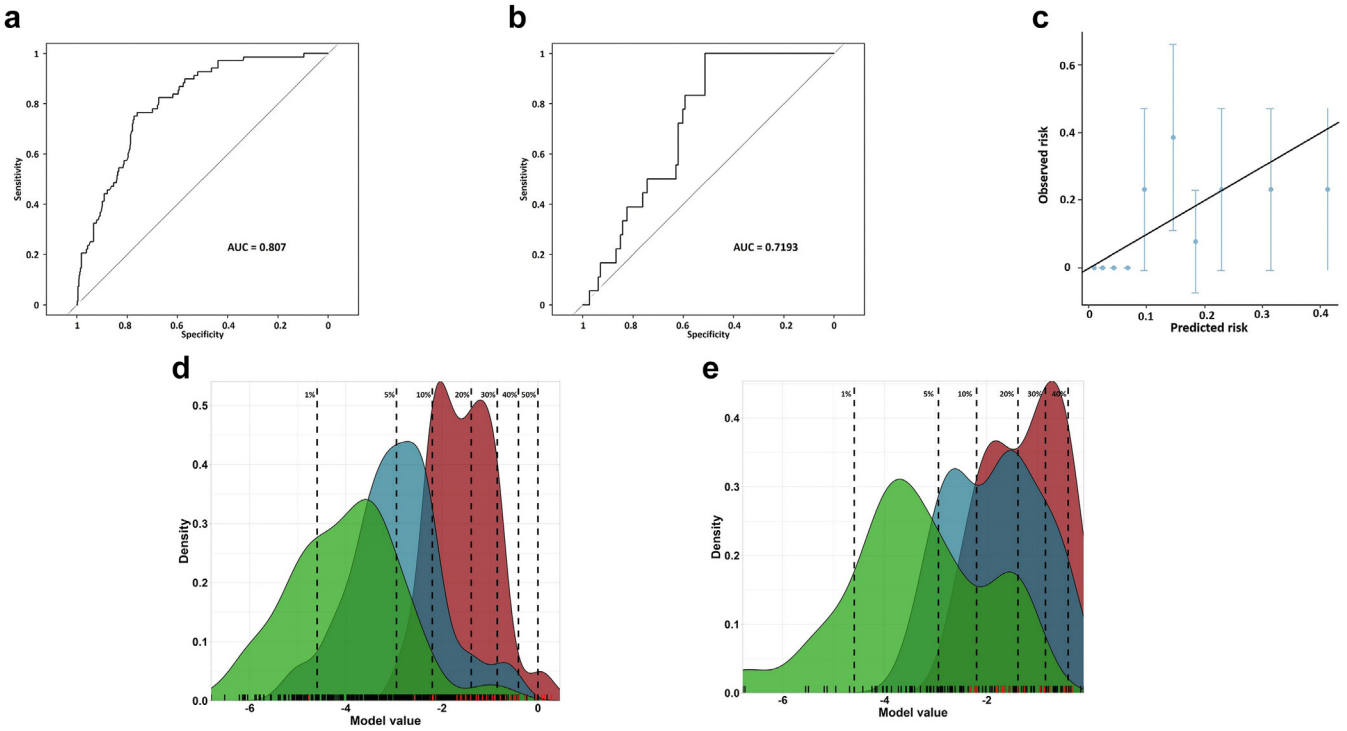


Figure 7. CMV-PrognosTIC score performances. ROC curves of the CMV-PrognosTIC score in the (a) derivation and (b) validation cohorts. (c) Calibration plot of the CMV-PrognosTIC score for the validation cohort. The graph represents the ratio of positive cases and the standard error of the observed risk of CMV-CSI (Y axis) according to each centile of CMV-CSI risk predicted by the CMV-CMI PrognosTIC score (x axis), illustrating the fit between expected and observed risk. Perfect calibration is represented by the line. We observe good predictions along all the risk percentiles, with certain overdiagnosis of high-risk CMV-CMI for CSI (patient with a lower observed risk than the predicted risk when the predicted risk is high). Density plot of the patients from the (d) derivation cohort and (e) validation cohort. Each model value (x axis) is associated with a risk of CMV-CSI (dash lines representing different values of CMV-CSI risk). Density of patients for each model value is represented on the graph, stratifying patients in 3 categories according to their CMV-CMI risk-score: LR (green), IR (blue) and HR (red). Furthermore, individual patient's model value given by the CMV-Prognostic score is represented on the x-axis, characterizing each patient by the occurrence (red) or not (black) of a CMV-CSI during follow-up. CMI, cell-mediated immunity; CMV, cytomegalovirus; CSI, clinically significant infection; HR, high-risk CMI; IE-1, immediate-early protein 1; IR, intermediate-risk CMI; LR, low-risk CMI; pp65, 65 kDa phosphoprotein.

the global risk of infection and help decision-making regarding the type and duration of preventive therapy. As illustrated in Figure 6, the CMV-PrognosTIC Score may provide some overlap between the 3 CMV-CMI risk categories, which describe the influence of the distinct clinical variables on the CMV-CMI risk-score.

Our findings outperform data reported in recent studies, including prospective, interventional, randomized trials using different commercially available assays.[12-14,27] Although all of them showed the potential usefulness of these CMV-CMI assays for early prophylaxis withdrawal to avoid side effects and costs of current antiviral therapies, they all showed a relatively poor capacity of predicting CMV events, thus exposing patients to unpredictable CMV replication after earlier prophylaxis withdrawal. Although the ideal diagnostic biomarker would be that with the highest specificity and positive predictive value,[28] in the setting of preventive management of CMV infection, a sensitive assay with high NPV accurately excluding the risk of CMV-CSI is a successful achievement, because almost 40% of R+ patients may safely benefit from reducing rigorous preventive strategies, while reducing overall related health care costs.[29] In addition, with the advent of novel antiviral agents for preventing CMV infection,[30,31] this new CMV risk prognostic tool may also help to better individualize their use.

Our study has some limitations. First, both derivation cohorts pooled from different studies and validation cohorts with the inclusion of distinct SOT may introduce a certain risk of selection bias. Moreover, the number of patients assessed in the different clinical scenarios is not balanced and fewer patients were assessed early after transplantation as compared with later time points. Nonetheless, the strong validation performance of the assay in the validation study in which we reproduced the same outcomes, strongly supports the high similarities and value of this

predictive system across different SOT patients and clinical settings. However, the value of this CMV-CMI risk-score has not been evaluated in lung and hematopoietic stem cell transplant recipients and thus, would need to be confirmed.[12,22] Finally, because all patients of the study receiving T-cell depletion received universal prophylaxis, we could not assess whether the use of T-cell depletion was an independent variable predicting CMV-CSI.

In conclusion, we have developed and validated a CMV-CMI risk-score using the T-SPOT.CMV assay that accurately identifies SOT at different risks of developing clinically relevant CMV infections. We have shown its reproducibility across multiple patient cohorts and within a prospective interventional study. By integrating key clinical risk factors into the CMV-CMI risk-score, we have built a robust prognostic CMV risk-score system, the CMV-PrognosTIC Score, which allows for a precise quantification of CMV infection risk at the individual patient level that may be readily implementable in clinical transplantation and ultimately improve the safety and clinical outcomes of SOT recipients.

DISCLOSURE

OB has received speaker, consulting, and travel fees from Oxford Immunotec related to this work. All the other authors declared no competing interests.

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We are grateful to all patients and families for their kind acceptance to participate in this research study. The studies included in the derivation cohort were registered as mentioned for each study in the methods section. Validation cohort study was not registered. Patients and public were not involved during study design, conduct, reporting, interpretation of the study. Patients were involved in the dissemination (patients associations).

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DATA AVAILABILITY STATEMENT

Data are available upon request to the corresponding author.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Detailed flow chart of the validation cohort.

Figure S2. CMV-CMI results in the derivation cohort (A, $n = 570$) and stratified between seropositive (R+) and mismatch patients (D+/R−) recipients.

Figure S3. CMV-CMI data for patients developing (A) CMV replication, (B) CMV-CSI infection, and (C) a CMV disease for early CMV-CMI monitoring ($n = 48$) in the derivation cohort.

Figure S4. CMV-CMI data for patients developing (A) CMV replication, (B) CMV-CSI infection, and (C) a CMV disease for R+ patients with late CMV-CMI monitoring ($n = 356$) in the derivation cohort.

Figure S5. CMV-CMI data for patients developing (A) CMV replication, (B) CMV-CSI infection, and (C) a CMV disease for D+/R− patients with late CMV-CMI monitoring ($n = 166$) in the derivation cohort.

Figure S6. Absolute lymphocyte counts (ALC), CMV-CMI results and CMV events. ALC was compared between patients developing CMV-CSI and those that did not, among all patients with available ALC.

Figure S7. Kaplan-Meier analysis of CMV infection-free survival when CMV-CMI was assessed early after transplantation ($n = 48$).

Figure S8. Kaplan-Meier analysis of CMV infection-free survival when CMV-CMI was assessed late after transplantation at prophylaxis withdrawal among R+ patients ($n = 356$).

Figure S9. Kaplan-Meier analysis of CMV infection-free survival when CMV-CMI was assessed late after transplantation at prophylaxis withdrawal among D+/R− patients ($n = 166$).

Figure S10. Patient stratification with the CMV-CMI risk score in the derivation cohort, identifying the IR CMI category in IE1+/pp65− and IE-1-/pp65+.

Figure S11. Kaplan-Meier CMV-CSI-free survival curves in each group of the validation cohort.

Figure S12. CMV-CMI data from the validation cohort, stratified by type of SOT.

Figure S13. AUC histograms of the CMV-PrognosTIC model for CMV-CSI in the derivation cohort.

Table S1. Predictive values of CMV-CMI for each CMV antigen or when combined for CSI.

Table S2. Efficacy end points of different CMV-CMI risk-scores for CMV clinically significant infection in the validation cohort, group A (R+ early CMI monitoring).

Table S3. Efficacy end points of different CMV-CMI risk-scores for CMV clinically significant infection in the

validation cohort, group B (R+ rATG and D+R−, late CMI monitoring).
TRIPOD Checklist.

REFERENCES

1. Raval AD, Kistler KD, Tang Y, Murata Y, Snydman DR. Epidemiology, risk factors, and outcomes associated with cytomegalovirus in adult kidney transplant recipients: a systematic literature review of real-world evidence. *Transpl Infect Dis*. 2021;23:e13483. https://doi.org/10.1111/tid.13483
2. Kotton CN, Kumar D, Caliendo AM, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation*. 2018;102:900–931. https://doi.org/10.1097/TP.0000000000002191
3. Bestard O, Kaminski H, Couzi L, Fernández-Ruiz M, Manuel O. Cytomegalovirus cell-mediated immunity: ready for routine use? *Transpl Int*. 2023;36:11963. https://doi.org/10.3389/ti.2023.11963
4. Lúcia M, Crespo E, Cruzado JM, Grinyó JM, Bestard O. Human CMV-specific T-cell responses in kidney transplantation; toward changing current risk-stratification paradigm. *Transpl Int*. 2014;27:643–656. https://doi.org/10.1111/tri.12318
5. Griffiths P, Reeves M. Pathogenesis of human cytomegalovirus in the immunocompromised host. *Nat Rev Microbiol*. 2021;19:759–773. https://doi.org/10.1038/s41579-021-00582-z
6. Kumar D, Chernenko S, Moussa G, et al. Cell-mediated immunity to predict cytomegalovirus disease in high-risk solid organ transplant recipients. *Am J Transplant*. 2009;9:1214–1222. https://doi.org/10.1111/j.1600-6143.2009.02618.x
7. Donadeu L, Jouve T, Bin S, et al. High-dimensional mass cytometry identified circulating natural killer T-cell subsets associated with protection from cytomegalovirus infection in kidney transplant recipients. *Kidney Int*. 2024;106:482–495. https://doi.org/10.1016/j.kint.2024.03.027
8. Manuel O, Husain S, Kumar D, et al. Assessment of cytomegalovirus-specific cell-mediated immunity for the prediction of cytomegalovirus disease in high-risk solid-organ transplant recipients: a multicenter cohort study. *Clin Infect Dis*. 2013;56:817–824. https://doi.org/10.1093/cid/cis993
9. Cantisán S, Lara R, Montejo M, et al. Pretransplant interferon-γ secretion by CMV-specific CD8+ T cells informs the risk of CMV replication after transplantation. *Am J Transplant*. 2013;13:738–745. https://doi.org/10.1111/ajt.12049
10. Lúcia M, Crespo E, Melilli E, et al. Preformed frequencies of cytomegalovirus (CMV)–specific memory T and B cells identify protected CMV-sensitized individuals among seronegative kidney transplant recipients. *Clin Infect Dis*. 2014;59:1537–1545. https://doi.org/10.1093/cid/ciu589
11. Jarque M, Crespo E, Melilli E, et al. Cellular immunity to predict the risk of cytomegalovirus infection in kidney transplantation: a prospective, interventional, multicenter clinical trial. *Clin Infect Dis*. 2020;71:2375–2385. https://doi.org/10.1093/cid/ciz1209
12. Westall GP, Cristiano Y, Levvey BJ, et al. A randomized study of QuantiFERON CMV-directed versus fixed-duration valganciclovir prophylaxis to reduce late CMV after lung transplantation. *Transplantation*. 2019;103:1005–1013. https://doi.org/10.1097/TP.0000000000002454
13. Páez-Vega A, Gutiérrez-Gutiérrez B, Agüera ML, et al. Immunoguided discontinuation of prophylaxis for cytomegalovirus disease in kidney transplant recipients treated with antithymocyte globulin: a randomized clinical trial. *Clin Infect Dis*. 2022;74:757–765. https://doi.org/10.1093/cid/ciab574
14. Manuel O, Laager M, Hirzel C, et al. Immune monitoring-guided versus fixed duration of antiviral prophylaxis against cytomegalovirus in solid-organ transplant recipients: a multicenter, randomized clinical trial. *Clin Infect Dis*. 2024;78:312–323. https://doi.org/10.1093/cid/ciad575
15. Kumar D, Chin-Hong P, Kayler L, et al. A prospective multicenter observational study of cell-mediated immunity as a predictor for cytomegalovirus infection in kidney transplant recipients. *Am J Transplant*. 2019;19:2505–2516. https://doi.org/10.1111/ajt.15315
16. Ruan Y, Guo W, Liang S, Xu Z, Niu T. Diagnostic performance of cytomegalovirus (CMV) immune monitoring with ELISpot and QuantiFERON-CMV assay in kidney transplantation: a PRISMA-compliant article. *Medicine (Baltimore)*. 2019;98:e15228. https://doi.org/10.1097/MD.0000000000015228
17. Gliga S, Fiedler M, Dornieden T, et al. Comparison of three cellular assays to predict the course of CMV infection in liver transplant recipients. *Vaccines (Basel)*. 2021;9:88. https://doi.org/10.3390/vaccines9020088
18. Bestard O, Lucia M, Crespo E, et al. Pretransplant immediately Early-1-Specific T cell responses provide protection for CMV infection after kidney transplantation. *Am J Transplant*. 2013;13:1793–1805. https://doi.org/10.1111/ajt.12256
19. Jarque M, Melilli E, Crespo E, et al. CMV-specific cell-mediated immunity at 3-month prophylaxis withdrawal discriminates D+/R+ kidney transplants at risk of late-onset CMV infection regardless the type of induction therapy. *Transplantation*. 2018;102:e472–e480. https://doi.org/10.1097/TP.0000000000002421
20. Chanouzas D, Small A, Borrows R, Ball S. Assessment of the T-SPOTCMV interferon-γ release assay in renal transplant recipients: a single center cohort study. *PLoS One*. 2018;13(3):e0193968. https://doi.org/10.1371/journal.pone.0193968
21. Donadeu L, Revilla-López E, Jarque M, et al. CMV-specific cell-mediated immunity predicts a high level of CMV replication after prophylaxis withdrawal in lung transplant recipients. *J Infect Dis*. 2021;224:526–531. https://doi.org/10.1093/infdis/jiaa727
22. Chemaly RF, El Haddad L, Winston DJ, et al. Cytomegalovirus (CMV) cell-mediated immunity and CMV infection after allogeneic hematopoietic cell transplantation: the REACT study. *Clin Infect Dis*. 2020;71:2365–2374. https://doi.org/10.1093/cid/ciz1210
23. Ljungman P, Chemaly RF, Khawaya F, et al. Forum, consensus definitions of cytomegalovirus (CMV) infection and disease in transplant patients including resistant and refractory CMV for use in clinical trials: 2024 update from the transplant associated virus infections forum. *Clin Infect Dis*. 2024;79:787–794. https://doi.org/10.1093/cid/ciae321
24. Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. *J Stat Softw*. 2010;33:1–22. https://doi.org/10.18637/jss.v033.i01
25. Hastie T, Tibshirani R, Friedman J. *The Elements of Statistical Learning* (*Springer Series in Statistics*). Springer. 2009.

Accessed November 7, 2023. http://link.springer.com/10.1007/978-0-387-84858-7

26. Kotton CN, Torre-Cisneros J, Yakoub-Agha I, CMV International Symposium Faculty. Slaying the "Troll of Transplantation"-new frontiers in cytomegalovirus management. A report from the CMV International Symposium 2023. 2024;9:e14183. https://doi.org/10.1111/tid.14183
27. Reusing JO Jr, Agena F, Kotton CN, Campana G, Pierrotti LC, David-Neto E. QuantiFERON-CMV as a predictor of CMV events during preemptive therapy in CMV-seropositive kidney transplant recipients. *Transplantation*. 2023;108:985–995. https://doi.org/10.1097/TP.0000000000004870
28. Naesens M, Anglicheau D. Precision transplant medicine: biomarkers to the rescue. *J Am Soc Nephrol*. 2018;29:24–34. https://doi.org/10.1681/ASN.2017010004
29. Khoury JA, Storch GA, Bohl DL, et al. Prophylactic versus preemptive oral valganciclovir for the management of cytomegalovirus infection in adult renal transplant recipients. *Am J Transplant*. 2006;6:2134–2143. https://doi.org/10.1111/j.1600-6143.2006.01413.x
30. Avery RK, Alain S, Alexander BD, et al. Maribavir for refractory cytomegalovirus infections with or without resistance post-transplant: results from a Phase 3 randomized clinical trial. *Clin Infect Dis*. 2022;75:690–701. https://doi.org/10.1093/cid/ciab988
31. Limaye AP, Budde K, Humar A, et al. Letermovir vs valganciclovir for prophylaxis of cytomegalovirus in high-risk kidney transplant recipients: a randomized clinical trial. *JAMA*. 2023;330:33–42. https://doi.org/10.1001/jama.2023.9106