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ORIGINAL ARTICLE

Kinetics of peripheral blood lymphocyte subpopulations predicts the occurrence of opportunistic infection after kidney transplantation

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cytomegalovirus, kidney transplantation, lymphocytopenia, monitoring, opportunistic infection, peripheral blood lymphocyte subpopulations.

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Conflicts of interest
All the authors declare no conflict of interest.

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Summary

Serial monitoring of peripheral blood lymphocyte subpopulations (PBLSs) counts might be useful in predicting post-transplant opportunistic infection (OI) after kidney transplantation (KT). PBLSs were prospectively measured in 304 KT recipients at baseline and post-transplant months 1 and 6. Areas under receiver operating characteristic curves were used to evaluate the accuracy of different subpopulations in predicting the occurrence of overall OI and, specifically, cytomegalovirus (CMV) disease. We separately analyzed patients not receiving (n = 164) or receiving (n = 140) antithymocyte globulin (ATG) as induction therapy. In the non-ATG group, a $CD8^+$ T-cell count at month 1 $<0.100 \times 10^3$ cells/μl had negative predictive values of 0.84 and 0.86 for the subsequent occurrence of overall OI and CMV disease, respectively. In the multivariate Cox model, a $CD8^+$ T-cell count $<0.100 \times 10^3$ cells/μl was an independent risk factor for OI (adjusted hazard ratio: 3.55; P-value = 0.002). In the ATG group, a $CD4^+$ T-cell count at month 1 $<0.050 \times 10^3$ cells/μl showed negative predictive values of 0.92 for the subsequent occurrence of overall OI and CMV disease. PBLSs monitoring effectively identify KT recipients at low risk of OI, providing an opportunity for individualizing post-transplant prophylaxis practices.

Introduction

Infection remains one of the main causes of morbidity and mortality after kidney transplantation (KT) [1,2]. Current immunosuppressive regimens are mostly targeted against the adaptive arm of the immune system [3]. Monitoring of cell-mediated immunity (CMI) has been proposed as a promising strategy to reduce the incidence of post-transplant infection by individualizing immunosuppressive therapy. This monitoring may rely on *in vitro* functional measures after nonspecific (i.e., phytohemagglutinin) or specific antigen stimulation [i.e., cytomegalovirus (CMV) viral peptides] [4–6]. In addition, an alternative approach to the CMI status could be based on quantitative surrogate parameters, such as the counts of total lymphocyte and peripheral blood lymphocyte subpopulations (PBLSs). Kinetics of PBLSs could be able to identify recipients at risk of post-transplant infection in a similar way than that of other immunocompromised hosts, such as those infected with human immunodeficiency virus (HIV) [7,8].

Others and we have analyzed the performance of monitoring of PBLSs in predicting the occurrence of infection in different transplant populations [9–14]. Nevertheless, previous studies focused on KT are old, retrospective in design, comprised small sample sizes or included only HIV-infected recipients [11–13]. On the other hand, it is well recognized the long-lasting and profound dose-dependent T-cell depletion induced by polyclonal antithymocyte preparations [3,15–17]. Therefore, the assessment of the predictive capacity of PBLSs for post-transplant infection should take into account the type of induction therapy used. Notwithstanding this rationale, some of the previous studies did not separately control for the effect of such a variable [11].

This study was aimed at analyzing the association between total lymphocyte and PBLS counts—as surrogate markers of post-transplant CMI status—and the occurrence of opportunistic infection in a cohort of KT recipients stratified by use of antithymocyte globulin (ATG) as induction therapy.

Patients and methods

Study population

Beginning in November 2008, all consecutive adult patients who underwent KT at the University Hospital "12 de Octubre" (Madrid, Spain) are being enrolled in a prospective immune status assessment that includes measurements of total lymphocyte and PBLS counts, serum immunoglobulin levels, and serum complement levels at different time points, as detailed below and elsewhere [18,19]. Patients with known pretransplant primary immunodeficiencies or HIV infection and those who died or developed graft loss within the first week after transplantation are excluded. Our Clinical Research Ethics Committee approved the study protocol, and written informed consent is obtained from all participants prior to their inclusion. This study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki (2008 version) and the Declaration of Istanbul.

Immune status assessment

Whole-blood samples were collected just before transplantation (baseline) and at post-transplant months 1 and 6 and analyzed within 18 h at the Department of Immunology. Whole blood (50 μl) was stained with 10 μl of BD Multitest 6-color TBNK reagent in Trucount tubes for 15 min. Red blood cells were lysed using fluorescence-activated cell sorting lysing solution. Determination of PBLSs was performed with a FACSCanto II flow cytometer, and data analyzed by FACSCanto clinical software (BD Biosciences, San Jose, CA, USA) [20].

Study design

Patients were enrolled at the time of transplantation and followed for at least 1 year, unless death or graft loss occurred earlier. We divided the post-transplant follow-up period in three intervals: early (first month), intermediate (months 1–6), and late (>6 months). All the patients were seen regularly in our outpatient transplant clinic. The *primary study outcomes* were the occurrence of overall opportunistic infection (including CMV disease, either viral syndrome or end-organ disease) as defined below and, independently, the occurrence of CMV disease during each post-transplant period. All the episodes of infection were prospectively collected by an infectious disease specialist who was unaware of the patient's immunological status. We pre-established two different subgroups of patients: those who received ATG as induction (ATG group) and those who received anti-CD25 monoclonal antibodies or no induction therapy (non-ATG group).

Immunosuppression and prophylaxis regimens

In an attempt to minimize the risk of calcineurin inhibitor (CNI)-related nephrotoxicity, all recipients of organs from donors after circulatory death (DCD) underwent induction with intravenous (IV) rabbit ATG (ATG-Fresenius, 1.00 mg/kg daily for 5–7 days), with the delayed introduction of the CNI from day 6. Recipients at high immunological risk—peak panel-reactive antibody >50%, second kidney transplant in case the first graft was lost to rejection within 2 years, or those receiving a third or fourth kidney

graft—also received ATG induction for 1–3 days with the early initiation of a CNI from day 0. Basiliximab induction (20 mg on days 0 and 4) was used in patients at high risk of CNI-related nephrotoxicity due to advanced age or pre-transplant comorbidities, with the delayed introduction of the CNI from day 5. Maintenance immunosuppression consisted of tacrolimus (0.1 mg/kg daily, adjusted to a target level of 10–15 ng/ml for the first month and 5–10 ng/ml for maintenance); mycophenolate mofetil (1000 mg twice daily) or mycophenolic acid (360 mg twice daily); and prednisone (1 mg/kg daily with progressive tapering).

All patients received a single dose of IV cefazolin preoperatively. Prophylaxis for *Pneumocystis jiroveci* pneumonia with trimethoprim–sulfamethoxazole (160/800 mg three times weekly) was administered for 9 months. In those patients at high risk for CMV disease (serology mismatch [donor positive (D+) and recipient negative (R−)] or induction with ATG), either IV ganciclovir (5 mg/kg daily) or oral valganciclovir (900 mg daily) was administered for 3 months. As neither systematic CMV viremia monitoring nor pre-emptive therapy was performed during the study period in the intermediate-risk group (R+ patients not receiving ATG), we did not include the occurrence of asymptomatic CMV viremia within the analysis of opportunistic infection.

Definitions

Opportunist infections were defined as those due to predominantly intracellular bacteria (mycobacteria, *Nocardia* spp., *Legionella* spp. and *Listeria monocytogenes*), herpesviruses [CMV, herpes simplex virus (HSV) and varicella-zoster virus (VZV) and Epstein-Barr virus-related post-transplant lymphoproliferative disease], polyomaviruses [polyomavirus BK-associated nephropathy (PyVAN)], yeasts (*Candida* spp. and *Cryptococcus* spp.), molds, *Pneumocystis jiroveci*, and parasites (*Toxoplasma gondii* and *Leishmania* spp.) [13,21,22]. Bloodstream, intra-abdominal, surgical site, and urinary tract infections due to Candida spp. were excluded as these episodes are usually related to previous surgery or indwelling catheters rather than to the CMI status. Tuberculosis was diagnosed if *Mycobacterium tuberculosis* was isolated by culture or if *M. tuberculosis* DNA was identified by polymerase chain reaction (PCR) assay from a representative clinical sample; patients for whom tuberculosis was demonstrated histopathologically were also accepted [23]. CMV disease included viral syndrome (defined by the demonstration of CMV by pp65 antigenemia or a PCR-based assays plus one or more of the following: fever; new onset malaise; leukopenia; atypical lymphocytosis; thrombocytopenia; or elevation of ALT or AST higher than two times the upper limit of normal) or end-organ disease [24]. Invasive fungal infection was defined as per the criteria proposed by the European Organization on Research and Treatment in Cancer and the Mycoses Study Group [25]. Only proven or probable cases were included. Pretransplant immunosuppressive therapy was defined as the use of corticosteroids (prednisone ≥5 mg daily for >2 weeks) or other immunosuppressive drugs (i.e., rituximab or cyclophosphamide) within 6 months before transplantation. Delayed graft function denotes the need for dialysis within the first week after transplantation. Acute graft rejection was suspected in case of an elevation of the serum creatinine and diagnosed by histological examination if possible [26]. Graft loss was defined as permanent return to dialysis or retransplantation.

Statistical analysis

Both cumulative incidences and incidence rates for each post-transplant period (early, intermediate or late) were calculated using as denominators the number of patients with available samples for PBLSs measurement at the beginning of each period. Quantitative data were shown as the mean ± standard deviation (SD) or the median with interquartile range (IQR). Qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the chi-squared test, whereas Student's T-test or U Mann–Whitney test was applied for continuous variables. Areas under receiver operating characteristic curves (auROC) were employed to assess the diagnostic accuracy of total lymphocyte and each PBLS in predicting the occurrence of the primary study outcomes during the different post-transplant periods in both ATG and non-ATG groups. The best cut-off values were then assessed through the calculations of sensitivity and specificity. Survival curves to first episode of opportunistic infection or CMV disease were plotted by the Kaplan–Meier method, and differences between groups were compared with the log-rank test. Univariate and multivariate (backward conditional selection) Cox regression models were used to evaluate the association between the most predictive parameters identified by auROC analysis and the primary study outcomes. Some variables not achieving statistical significance in the univariate analyses were forced into the multivariate models due to its clinical relevance. Results were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). Correlations between recipient age and clinically relevant PBLS counts were assessed by Pearson's correlation coefficients (r). All the significance tests were two-tailed. Statistical analysis was performed using SPSS v. 15.0 (Statistical Package for Social Sciences, Inc., Chicago, IL, USA) and EPIDAT v. 3.1 (Conselleria de Sanidade, Xunta de Galicia, Spain).

Results

Baseline characteristics

We included 304 patients (164 in the non-ATG group and 140 in the ATG group) from November 2008 to July 2011, whose clinical characteristics are summarized in Table 1. Patients in the ATG group were younger, more likely to have received a graft from a DCD, and exhibited lower cold ischemia time and higher rates of retransplantation and delayed graft function. All patients had measurements of total lymphocyte and PBLS counts at baseline. Samples at months 1 and 6 were available for 266 and 211 of these patients, respectively (87.8% and 75.0% of those alive and with functional grafts at each point). There were no significant differences in baseline characteristics between patients with immune assessment at months 1 and 6, and those from whom no whole-blood specimens could be obtained. The median interval between baseline blood sampling and the onset of the first episode of opportunistic infection during the early period (first month) was 27 days (IQR, 21.7–29 days). The median interval between sampling at month 1 and the onset of the first episode of infection during the intermediate period (months 1–6) was 48 days (IQR, 12–107 days). Finally, the median interval between sampling at month 6 and the onset of the first episode of late infection (>6 months) was 102 days (IQR, 21–240 days). The dynamics of major PBLS counts in both the non-ATG and ATG groups during the monitoring period are depicted in the Fig. 1.

Post-transplant outcomes

The median follow-up was 476.0 days (IQR: 407.2–707.2 days), with 263 patients (86.5%) reaching ≥12 months. Death-censored graft survival rates at 1 and 3 years were 95% and 93%, respectively. All-cause mortality was 6.3% [19 patients died at a median interval from transplantation of 144 days (IQR: 55–364 days)]. The most common causes of death were infection and cardiovascular disease (12 and 3 patients, respectively). One- and 3-year survival rates were 95% and 92%.

Overall, 78 patients (25.6%) had 104 episodes of opportunistic infection, with no significant difference between non-ATG and ATG groups (incidence rates: 0.71 and 0.51 episodes per 1000 transplant-days, respectively; *P*-value = 0.14). CMV was the most common agent, with 71 episodes diagnosed in 62 patients. Other agents included HSV (12 episodes), VZV (7 episodes), *L. donovani* complex (3 episodes), *M. tuberculosis* (3 episodes), *Nocardia* spp. (2 episodes), *Aspergillus fumigatus* (2 episodes), PyVAN (2 episodes), *C. albicans,* and mucorales (one episode each). The incidence of each type of opportunistic infection according to the post-transplant month and the precise distribution of the clinical syndromes are detailed in Fig. 2 and Table S1. The cumulative incidences of CMV disease at months 6 and 12 after transplant according to the D/R CMV serostatus are shown in Table S2.

Predictive role of total lymphocyte and PBLS counts in the non-ATG group

There were no significant differences in the PBLS counts at baseline between patients with or without opportunistic infection during the early period (data not shown). On the opposite, total lymphocyte, $CD3^+$, $CD4^+$ and $CD8^+$ T cells, and NK-cell counts at month 1 were significantly decreased in those patients who subsequently developed an opportunistic infection during the intermediate period (Fig. 3a). At month 6, we also found significant differences in $CD8^+$ T-cell counts between patients with and without late infection (Fig. 3b).

When performing auROC analyses, the $CD8^+$ T-cell count at month 1 was found to be the most predictive parameter for the subsequent occurrence of overall opportunistic infection (auROC: 0.739; *P*-value <0.001) and CMV disease (auROC: 0.685; *P*-value = 0.004) during the intermediate period. At month 6, $CD8^+$ T-cell count was also the most predictive parameter for both late overall opportunistic infection (auROC: 0.738; *P*-value = 0.03) and late CMV disease (auROC: 0.756; *P*-value = 0.05) (Table S3). There was a statistically significant negative correlation between recipient age and the $CD8^+$ T-cell count at month 1 after transplantation (*r*: −0.326; *P*-value <0.0001), but not at month 6.

As shown in Table 2, patients with a $CD8^+$ T-cell count $<0.100 \times 10^3$ cells/μl at month 1 had higher cumulative incidences of overall opportunistic infection (*P*-value <0.001), CMV disease (*P*-value = 0.007) and CMV end-organ disease (*P*-value = 0.02) at the end of the intermediate period. The incidence rates of overall opportunistic infection and, specifically, CMV disease throughout such period were also significantly higher (*P*-values <0.001 for both comparisons). These associations remained essentially unchanged in sensitivity analyses stratified by the use of basiliximab induction or the receipt of antirejection therapy during the early post-transplant period (Tables S4 and S5). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of this cut-off value for predicting subsequent infection are detailed in Table 3.

With regards to the late period, patients with a $CD8^+$ T-cell count $<0.600 \times 10^3$ cells/μl at month 6 had higher cumulative incidences of overall opportunistic infection (*P*-value = 0.003) and CMV disease (*P*-value = 0.03), as well as significantly higher incidence rates of both events (Table 2). The performance of this cut-off value for predicting late infection is shown in Table 3.

Table 1. Clinical characteristics and post-transplant outcome in the study cohort.

Variable	Overall (n = 304)	Non-ATG group (n = 164)	ATG group (n = 140)	P-value*
Age of recipient, years (mean ± SD)	55.0 ± 14.9	59.4 ± 15.3	49.9 ± 12.7	<0.001
Gender (male) [n (%)]	191 (62.8)	110 (67.1)	81 (57.9)	0.097
Pretransplant chronic comorbidities [n (%)]				
Diabetes mellitus	76 (25.0)	43 (26.2)	33 (23.6)	0.595
Heart disease	72 (23.7)	39 (23.8)	33 (23.6)	0.966
Chronic lung disease	41 (13.5)	28 (17.1)	13 (9.3)	0.048
Peripheral arterial disease	28 (9.2)	18 (11.0)	10 (7.1)	0.249
Chronic liver disease	16 (5.3)	12 (7.3)	4 (2.9)	0.083
Pretransplant immunosuppressive therapy [n (%)]	38 (12.5)	18 (11.0)	20 (14.3)	0.395
Previous solid organ transplantation [n (%)]	66 (21.7)	27 (16.5)	39 (27.9)	0.016
≥2 previous transplants	16 (5.3)	2 (1.2)	14 (10.0)	0.001
Etiology of underlying ESRD [n (%)]				
Glomerulonephritis	68 (22.4)	36 (22.0)	32 (22.9)	0.550
Diabetic nephropathy	54 (17.8)	29 (17.7)	25 (17.9)	
Nephroangiosclerosis	42 (13.8)	25 (15.2)	17 (12.1)	
Policystosis	36 (11.8)	18 (11.0)	18 (12.9)	
Chronic interstitial nephropathy	25 (8.2)	15 (9.1)	10 (7.1)	
Reflux nephropathy	13 (4.3)	6 (3.7)	7 (5.0)	
Unknown	23 (7.6)	13 (7.9)	10 (7.1)	
Other	43 (14.1)	22 (13.4)	21 (15.0)	
Baseline serostatus [n (%)]				
Hepatitis C virus	31 (10.2)	15 (9.1)	16 (11.4)	0.512
Hepatitis B virus	4 (1.3)	3 (1.8)	1 (0.7)	0.373
CMV status D+/R−	23 (7.6)	11 (6.7)	12 (8.6)	0.540
CMV status D−/R−	4 (1.3)	2 (1.2)	2 (1.4)	0.627
Pretransplant renal replacement therapy [n (%)]				
Hemodialysis	248 (81.6)	130 (79.3)	118 (84.3)	0.299
Continuous ambulatory peritoneal dialysis	35 (11.5)	15 (9.1)	20 (14.3)	0.207
Age of donor, years (mean ± SD)	53.3 ± 16.8	59.7 ± 17.1	45.8 ± 12.8	<0.001
Type of donor [n (%)]				
DBD donor	204 (67.1)	146 (89.0)	58 (41.4)	<0.001
DCD donor	87 (28.6)	5 (3.0)	82 (58.6)	
Living donor	13 (4.3)	13 (7.9)	0 (0.0)	
Number of HLA mismatches [median (IQR)]	4.0 (4.0–5.0)	4.0 (3.0–5.0)	4.5 (4.0–5.0)	0.031
Cold ischemia time, hours (mean ± SD)	16.7 ± 6.8	18.5 ± 7.1	14.7 ± 5.7	<0.001
Induction therapy [n (%)]				
None	53 (17.4)	53 (32.3)	–	–
Basiliximab	111 (36.5)	111 (67.7)	–	
ATG	140 (46.1)	–	140 (100.0)	
Primary immunosuppression scheme [n (%)]				
Tacrolimus, mycophenolate mofetil, and steroids	268 (88.9)	147 (89.6)	125 (89.3)	0.767
Tacrolimus, azathioprine, and steroids	31 (10.2)	16 (9.8)	15 (10.7)	0.783
Post-transplant complications [n (%)]				
Delayed graft function	182 (59.9)	79 (48.2)	103 (73.6)	<0.001
Requirement of surgical reintervention†	40 (13.2)	18 (11.0)	22 (15.7)	0.223
Renal artery stenosis	56 (18.4)	31 (18.9)	25 (17.9)	0.815
De novo post-transplant diabetes mellitus	42 (13.8)	22 (13.4)	20 (14.3)	0.766
≥1 episode of acute graft rejection	67 (22.0)	40 (24.4)	27 (19.3)	0.285
2 episodes	7 (2.3)	5 (3.0)	2 (1.4)	0.294
Overall patient mortality [n (%)]	19 (6.3)	12 (7.3)	7 (5.0)	0.405
Infection-related mortality	12/19 (63.1)	8/12 (66.7)	4/7 (57.1)	0.367
Graft loss [n (%)]	18 (5.9)	8 (4.9)	10 (7.1)	0.404

ATG, antithymocyte globulin; CMV, cytomegalovirus; D, donor; DBD, donation after brain death; DCD, donation after circulatory death; ESRD, end-stage renal disease; HLA, human leukocyte antigen; IQR, interquartile range; KT, kidney transplant; R, recipient; SD, standard deviation.
*Comparison between non-ATG and ATG groups.
†Within the first post-transplant month.

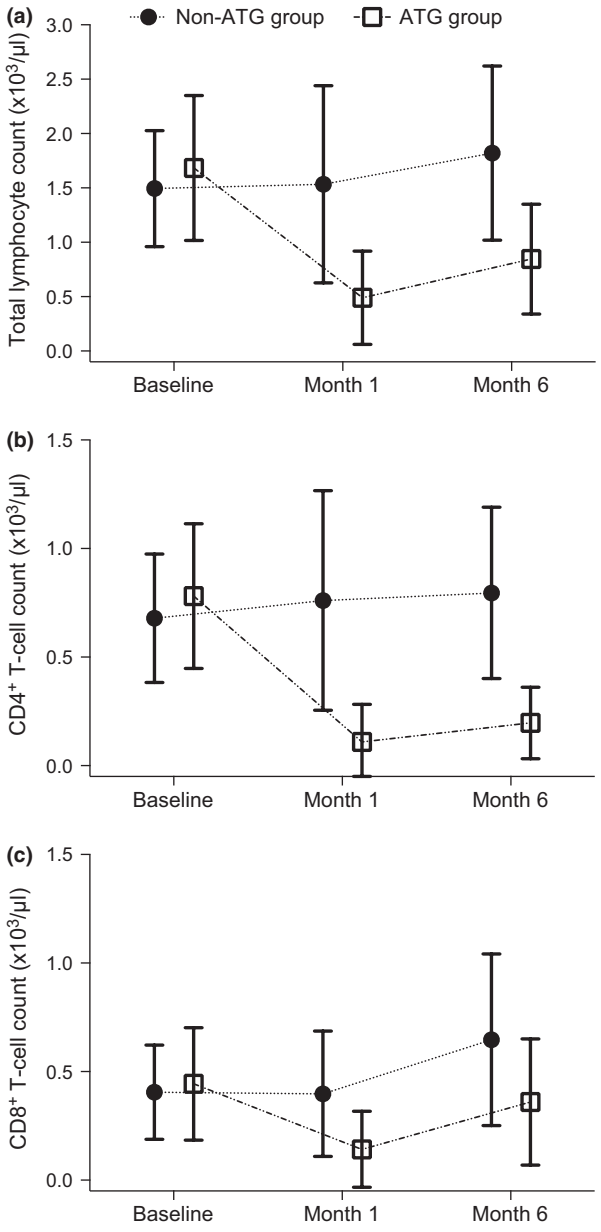

Figure 1 Dynamics at different time points of total lymphocyte (a), $CD4^+$ (b) and $CD8^+$ T-cell counts (c) in the non-ATG and ATG groups (ATG, antithymocyte globulin).

In the multivariate Cox regression models, a $CD8^+$ T-cell count $<0.100 \times 10^3$ cells/µl at month 1 was identified as an independent risk factor for overall opportunistic infection (HR: 3.55; 95% CI: 1.56–8.06; *P*-value = 0.002) and, separately, for CMV disease (HR: 4.19; 95% CI: 1.79–9.77; *P*-value = 0.001) during the intermediate period (Table S6). The low number of events beyond the month 6 prevented us from performing a multivariate Cox model for the late period.

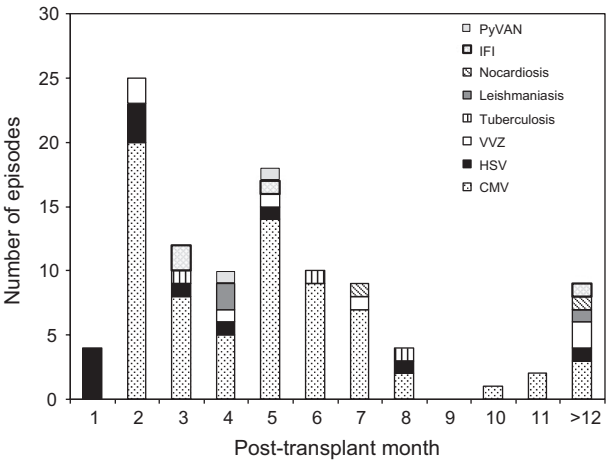

Figure 2 Incidence of different types of opportunistic infection according to the post-transplant month (CMV, cytomegalovirus; HSV, herpes simplex virus; IFI, invasive fungal infection; PyVAN, polyomavirus BK-associated nephropathy; VZV, varicella-zoster virus).

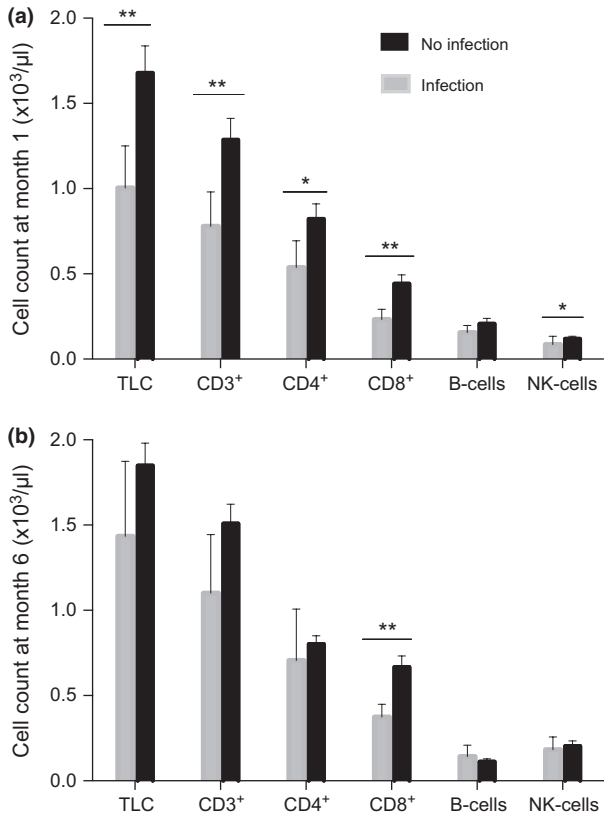

Figure 3 Non-ATG group: Mean values of total lymphocyte and PBLS counts at months 1 and 6 according to the occurrence of opportunistic infection during the intermediate (a) and late (b) post-transplant periods, respectively. Whiskers indicate 95% confidence interval. **P*-value = 0.001; ***P*-value <0.001 (ATG, antithymocyte globulin; NK, natural killer; TLC, total lymphocyte count).

Table 2. Overall opportunistic infection and CMV disease in the non-ATG group during the intermediate (months 1–6) and late periods (>6 months) according to $CD8^+$ T-cell counts (unless otherwise specified, cumulative incidence rates are shown).

	$CD8^+$ T-cell count at month 1		
Infection in the intermediate period (months 1–6)	$<0.100 \times 10^3$ cells/μl (n = 16)	$\geq 0.100 \times 10^3$ cells/μl (n = 125)	P-value
Overall opportunistic infection*	10 (62.5)	21 (16.8)	<0.001
Incidence rate (episodes per 1000 transplant-days)	6.77	1.55	<0.001
CMV disease	8 (50.0)	17 (13.6)	0.007
Incidence rate (episodes per 1000 transplant-days)	4.96	1.05	<0.001
CMV end-organ disease	3 (18.8)	3 (2.4)	0.02
Nonviral opportunistic infection	2 (12.5)	2 (1.6)	0.06
	$CD8^+$ T-cell count at month 6		
Infection in the late period (>6 months)	$<0.600 \times 10^3$ cells/μl (n = 58)	$\geq 0.600 \times 10^3$ cells/μl (n = 57)	P-value
Overall opportunistic infection†	8 (13.8)	0 (0.0)	0.003
Incidence rate (episodes per 1000 transplant-days)	0.95	0.00	<0.001
CMV disease	5 (8.6)	0 (0.0)	0.03
Incidence rate (episodes per 1000 transplant-days)	0.59	0.00	0.02
CMV end-organ disease	2 (3.4)	0 (0.0)	0.3
Nonviral opportunistic infection	2 (3.4)	0 (0.0)	0.3

ATG, antithymocyte globulin; CMV, cytomegalovirus.
*Data on the $CD8^+$ T-cell count at month 1 were absent in 5 patients.
†Data on the $CD8^+$ T-cell count at month 6 were absent in 4 patients.

Table 3. Performance of $CD8^+$ T-cell counts at months 1 and 6 for predicting the occurrence of overall opportunistic infection and, specifically, CMV disease in the non-ATG group.

Cut-off value	Predicted event	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
$CD8^+$ T-cell count at month 1 <0.100×10^3 cells/μl	Opportunistic infection in months 1–6	0.32 (0.16–0.49)	0.95 (0.90–0.99)	0.63 (0.39–0.86)	0.83 (0.77–0.90)
	CMV disease in months 1–6	0.32 (0.14–0.50)	0.93 (0.88–0.98)	0.50 (0.26–0.75)	0.86 (0.80–0.90)
$CD8^+$ T-cell count at month 6 <0.600×10^3 cells/μl	Opportunistic infection beyond month 6	1.00 (1.00–1.00)	0.53 (0.44–0.63)	0.14 (0.05–0.23)	1.00 (1.00–1.00)
	CMV disease beyond month 6	1.00 (1.00–1.00)	0.52 (0.42–0.61)	0.09 (0.01–0.16)	1.00 (1.00–1.00)

ATG, antithymocyte globulin; CI, confidence interval; CMV, cytomegalovirus; NPV, negative predictive value; PPV, positive predictive value.

Predictive role of total lymphocyte and PBLS counts in the ATG group

We found no significant differences in the PBLS counts at baseline between patients with or without opportunistic infection during the early period (data not shown). Total lymphocyte, $CD3^+$, $CD4^+$ and $CD8^+$ T-cell counts at month 1 were significantly lower in those patients with subsequent opportunistic infection during the intermediate period (Fig. 4a). At month 6, total lymphocyte, $CD3^+$ and $CD4^+$ T cells, and B cells and NK-cell counts were also significantly lower in patients with opportunistic infection during the late period (Fig. 4b).

The $CD4^+$ T-cell count at month 1 was the most predictive parameter for the subsequent occurrence of overall opportunistic infection (auROC: 0.668; P-value = 0.02) and, specifically, CMV disease (auROC: 0.634; P-value = 0.08). Again, there was a statistically significant negative correlation between recipient age and the $CD8^+$ T-cell count at month 1 after transplantation (r: −0.361; P-value <0.0001). At month 6, total lymphocyte count emerged as the most predictive parameter for overall opportunistic infection (auROC: 0.820; P-value = 0.005) and CMV disease (auROC: 0.837; P-value = 0.006) during the late period (Table S3).

Patients in the ATG group with a $CD4^+$ T-cell count $<0.050 \times 10^3$ cells/μl at month 1 had higher cumulative incidences of overall opportunistic infection (P-value = 0.003), CMV disease (P-value = 0.03), and nonviral opportunistic infection (P-value = 0.05) at the end of the intermediate period (Table 4). The pattern of these associations remained similar in a sensitivity analysis stratified by the receipt of antirejection therapy during the early period (Table S7). The sensitivity, specificity, PPV, and NPV of this cut-off value are shown in Table 5.

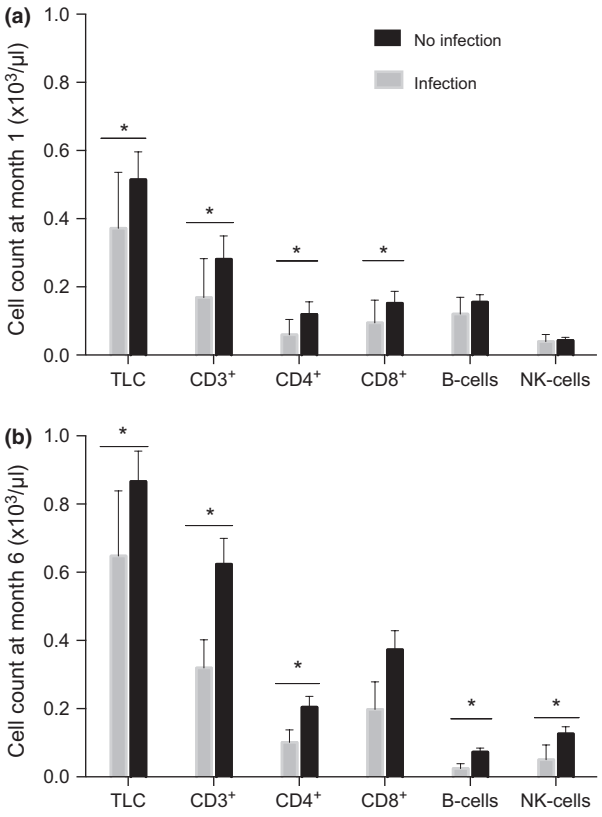

Figure 4 ATG group: Mean values of total lymphocyte and PBLS counts at months 1 and 6 according to the occurrence of opportunistic infection during the intermediate (a) and late (b) post-transplant periods, respectively. Whiskers indicate 95% confidence interval. **P*-value <0.005 (ATG, antithymocyte globulin; NK, natural killer; TLC, total lymphocyte count).

Throughout the late period, patients with a total lymphocyte count <0.750 × 10^3 cells/µl at month 6 had higher cumulative incidences of overall opportunistic infection and CMV disease (*P*-values = 0.02 for both). The incidence rates of overall opportunistic infection (*P*-value = 0.004) and CMV disease (*P*-value = 0.02) were also significantly increased (Table 4). The performance of this cut-off value for predicting late infection is shown in Table 5.

In the final multivariate Cox regression model for overall opportunistic infection during the intermediate period, the $CD4^+$ T-cell count <0.050 × 10^3 cells/µl at month 1 was retained without reaching statistical significance (HR: 2.53; 95% CI: 0.88–7.26; *P*-value = 0.08) (Table S8). The low number of late events precluded multivariate analysis for the late post-transplant period.

Discussion

In the present study, we demonstrate that an affordable approach to the post-transplant CMI status based on the scheduled monitoring of total lymphocytes and selected PBLSs may have a role in predicting opportunistic infection in KT recipients. We also found that stratifying by the use of ATG induction permits to separate two different subgroups with respect to the most predictive parameter. In patients not receiving ATG, a low $CD8^+$ T-cell count at month 1 was associated with a 3.5-fold increase in the risk of opportunistic infection and, specifically, a 4.2-fold increase in the risk of CMV disease. In the group receiving ATG induction, a low $CD4^+$ T-cell count at month 1 was associated with a nearly significant 2.5-fold increase in the risk of overall opportunistic infection. Interestingly, the selected cut-offs at each time point exhibited excellent NPVs for the subsequent occurrence of infection. Through the identification of those patients at a very low risk of infection in which prophylaxis and/or viral monitoring could be safely discontinued, this strategy provides an opportunity for individualizing and optimizing post-transplant practices.

There are few studies on the usefulness of monitoring of lymphocyte counts to predict immunosuppression-related adverse events after KT [11–13,27–29]. Nonetheless, the increased risk of infection carried by patients receiving T-cell-depleting antibodies (ATG, OKT3 or alemtuzumab) has been extensively documented for CMV [30,31] and other agents [32–35]. Various studies have reported that KT recipients with *P. jiroveci* pneumonia have significantly lower lymphocyte counts than controls [36–40], and it has been proposed that the kinetics of the $CD4^+$ T-cell subset may help to determine the duration of certain prophylaxis regimens [37,41].

In the ATG group, we found that a cut-off value in $CD4^+$ T-cell count at month 1 of 0.050 × 10^3 cells/µl had good sensitivity (76%) and excellent NPV (92%) for the occurrence of infection during the intermediate period. At month 6, a total lymphocyte count of 0.750 × 10^3 cells/µl exhibited even better sensitivity (83%) and NPV (97%) for late infection. Thus, these surrogate markers of CMI accurately stratified the risk of infection within a subgroup of KT recipients at high risk *per se* for infectious complications.

Notably, we were able to reproduce these findings in the absence of previous induction with ATG. As these patients are not subject to an obvious cause of T-cell depletion, most clinicians would not have probably considered post-transplant lymphocytopenia as a predictable complication. A cut-off value in $CD8^+$ T-cell count at month 1 of 0.100 × 10^3 cells/µl showed a very good NPV for predicting subsequent opportunistic infection (83%) during the intermediate period, whereas a cut-off of 0.600 × 10^3 cells/µl at month 6 had a NPV of 100% for late infection. Although the PPVs at months 1 and 6 were suboptimal (63% and 15%, respectively), the appeal of monitoring $CD8^+$ T-cell counts lies in the ability to effectively discriminate low-risk

Table 4. Overall opportunistic infection and CMV disease in the ATG group during the intermediate (months 1–6) and late periods (>6 months) according to total lymphocyte and $CD4^+$ T-cell counts (unless otherwise specified, cumulative incidence rates are shown).

	$CD4^+$ T-cell count at month 1		
Infection in the intermediate period (months 1–6)	$<0.050 \times 10^3$ cells/μl ($n = 58$)	$\geq 0.050 \times 10^3$ cells/μl ($n = 66$)	*P*-value
Overall opportunistic infection*	16 (27.6)	5 (7.6)	0.003
Incidence rate (episodes per 1000 transplant-days)	1.91	0.52	0.01
CMV disease	12 (20.7)	5 (7.6)	0.03
Incidence rate (episodes per 1000 transplant-days)	1.43	0.52	0.08
CMV end-organ disease	3 (5.2)	1 (1.5)	0.3
Nonviral opportunistic infection	4 (6.9)	0 (0.0)	0.05
	Total lymphocyte count at month 6		
Infection in the late period (>6 months)	$<0.750 \times 10^3$ cells/μl ($n = 68$)	$\geq 0.750 \times 10^3$ cells/μl ($n = 63$)	*P*-value
Overall opportunistic infection	10 (14.7)	2 (3.2)	0.02
Incidence rate (episodes per 1000 transplant-days)	1.22	0.16	0.004
CMV disease	8 (11.8)	1 (1.6)	0.02
Incidence rate (episodes per 1000 transplant-days)	0.81	0.08	0.02
CMV end-organ disease	1 (1.5)	0 (0.0)	0.5
Nonviral opportunistic infection	3 (4.4)	1 (1.6)	0.3

ATG, antithymocyte globulin; CMV, cytomegalovirus.
*Data on the $CD4^+$ T-cell count at month 1 were absent in 3 patients.

Table 5. Performances of $CD4^+$ T-cell count at month 1 and total lymphocyte count at month 6 for predicting the occurrence of overall opportunistic infection and, specifically, CMV disease in the ATG group.

Cut-off value	Predicted event	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
$CD4^+$ T-cell count at month 1 $<0.050 \times 10^3$ cells/μl	Opportunistic infection in months 1–6	0.76 (0.58–0.94)	0.59 (0.50–0.69)	0.28 (0.16–0.39)	0.92 (0.86–0.99)
	CMV disease in months 1–6	0.71 (0.49–0.92)	0.57 (0.48–0.66)	0.21 (0.10–0.31)	0.92 (0.86–0.99)
Total lymphocyte count at month 6 $<0.750 \times 10^3$ cells/μl	Opportunistic infection beyond month 6	0.83 (0.62–1.04)	0.51 (0.42–0.60)	0.15 (0.06–0.23)	0.97 (0.92–1.01)
	CMV disease beyond month 6	0.91 (0.74–1.08)	0.51 (0.42–0.60)	0.14 (0.06–0.22)	0.98 (0.95–1.01)

ATG, antithymocyte globulin; CI, confidence interval; CMV, cytomegalovirus; NPV, negative predictive value; PPV, positive predictive value.

patients, even by assuming that the actual odds of opportunistic infection in the group below the cut-off value might turn out to be relatively low. Overall, these findings could be applied in the clinical practice to reduce the length of prophylaxis with trimethoprim–sulfamethoxazole or acyclovir or the frequency of clinical follow-up in patients with PBLS counts over the protective threshold.

The administration of ATG exerted a differential impact on the kinetics of some PBLSs. As shown in the Fig. 1b, the absolute count of $CD4^+$ T-cell lymphocytes in patients receiving ATG showed a dramatic drop from baseline to month 1, with only a slight recovery at month 6. Conversely, in the non-ATG group, the kinetics experienced a moderate but steady increase from baseline to month 6. This notion that there is no "one-size-fits-all" when seeking the best predictive PBLSs among different induction therapy groups is further supported by a recent study with a design similar to ours, in which the prognostic accuracy of $CD4^+$ and $CD8^+$ T-cell subpopulations were retrospectively analyzed in 48 heart and 42 KT recipients [13]. The authors found that the $CD4^+$ T-cell counts were associated with the risk of opportunistic infection only in heart transplant recipients, whereas the $CD8^+$ T-cell subset performed better in KT recipients. Of note, 90% of patients in the former group had undergone ATG induction, whereas most in the latter group had received basiliximab [13]. We also found a strong negative correlation between recipient age at transplantation and either $CD8^+$ or $CD4^+$ T-cell counts at month 1 in the non-ATG and ATG groups, respectively. As recently demonstrated, reduced thymic output seems to play a role in the age-related decrease in T-cell numbers [40].

One of the potential contributions of our study may lie on the utility of monitoring of PBLSs to stratify the risk of CMV disease. Growing interest has been focused in the past years to measure individual's CMV-specific CMI response by a number of approaches [5,6,42,43]. Nevertheless, these techniques are not widespread because of the lack of standardized cut-off values, their labor-intensive nature, and the need of specialized equipment [6]. The ATG group was formed by recipients deemed at increased risk of CMV disease regardless their D/R serostatus and, in accordance to the current guidelines [31,44], received antiviral prophylaxis for 3 months. Using the aforementioned cut-off values for $CD4^+$ T-cell and total lymphocyte counts, we obtained excellent NPVs for predicting subsequent CMV disease. This approach could identify those recipients that will not likely benefit from the extended use of anti-CMV agents beyond the standard prophylaxis regimen of 3 months in view of the very low risk of subsequent CMV disease. Conversely, it could be reasonable to continue valganciclovir in those with persistent lymphocyte depletion. The non-ATG was more heterogeneous in terms of CMV infection risk, as it comprised high- (D+/R−), intermediate- (R+), and low-risk patients (D−/R−) on the basis of their serology status. Notwithstanding this fact, the selected cut-off values for $CD8^+$ T-cell count at months 1 and 6 also showed excellent NPVs. Furthermore, in the Cox regression model, the impact of $CD8^+$ T-cell count at month 1 remained even after adjusting for CMV mismatch and use of antiviral prophylaxis.

This study has some limitations. Firstly, and despite the large sample size, the incidence of late infection was relatively low and prevented us from performing further multivariate analyses. The high NPVs obtained for PBLS counts may be influenced by this circumstance. As previously discussed, the selected cut-offs exhibited only moderate PPVs and, in some cases, poor sensitivity values (i.e., $CD8^+$ T-cell count at month 1 in the non-ATG group). CMV disease accounted for most of the observed episodes, thus limiting our capacity to assess the accuracy of the proposed strategy for predicting non-CMV infection. On the other hand, the potential feasibility of monitoring of PBLSs to guide CMV prophylaxis should be taken with caution as different serology risk categories were jointly analyzed and remain to be validated in separate cohorts. Moreover, we did not systematically monitor CMV antigenemia in intermediate-risk patients. Finally, our approach to the CMI status was based solely on quantitative parameters measured at two post-transplant time points: We did not perform any functional assay [4–6], nor break down the $CD4^+$ or $CD8^+$ T-cell pools into their different subsets. Perhaps, it might be worth considering the convenience of intensifying this schedule of testing to get better detail into the dynamics of immune recovery throughout the post-transplant period. Nevertheless, we do consider that the strength of our findings lies on the very affordability of the monitoring strategy that we propose, thus facilitating its application in day-to-day practice. Enumeration of PBLSs may be performed in a fully automated way, with reliable and reproducible results [20]. Although previous studies had analyzed the impact of PBLSs kinetics on the occurrence of various outcomes after KT [11–13,27,28], ours is unique in terms of prospective design, large sample size, and detailed assessment of infectious events.

To conclude, monitoring of PBLS counts may be a useful tool to predict the occurrence of opportunistic infection in KT recipients. In particular, patients not receiving ATG induction with $CD8^+$ T-cell counts above 0.100×10^3 and 0.600×10^3 cells/μl at months 1 and 6, respectively, had a very low risk of developing subsequent infection. The same would apply to patients with a $CD4^+$ T cell above 0.050×10^3 cells/μl at month 1 after induction therapy with ATG. We have previously demonstrated that post-transplant hypogammaglobulinemia acts as an independent risk factor for bacterial infection, namely acute pyelonephritis and bloodstream infection [18]. Therefore, our findings overall offer support for tailoring of immunosuppressive and prophylactic regimens according to individual's risk profile and pave the way for future intervention studies based on these simple approaches to the infection risk assessment after KT.

Authorship

MFR, FLM, EPA and JMA: designed research. MFR, FLM, LMA, AA, RSJ, CL and JMM: performed research. MFR and AGR: collected data. MFR, FLM and LMA: analyzed data. MFR and FLM: wrote the paper. AA, JMM, EPA and JMA: revised and completed the final draft of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Description of the clinical syndromes and causative agents involved in the 104 episodes of post-transplant opportunistic infection.

Table S2. Cumulative incidences of CMV disease at months 6 and 12 after transplantation according to the donor/recipient CMV serostatus.

Table S3. auROC analysis of total lymphocyte and PBLS counts at months 1 and 6 for predicting the subsequent occurrence of opportunistic infection.

Table S4. Sensitivity analysis stratified by the use of basiliximab induction in the non-ATG group.

Table S5. Sensitivity analysis stratified by the receipt of antirejection therapy during the early post-transplant period (month 1) in the non-ATG group.

Table S6. Cox regression models for overall opportunistic infection and, specifically, CMV disease in the non-ATG group during the intermediate post-transplant period (months 1–6).

Table S7. Sensitivity analysis stratified by the receipt of antirejection therapy during the early post-transplant period (month 1) in the ATG group.

Table S8. Cox regression models for overall opportunistic infection and, specifically, CMV disease in the ATG group during the intermediate post-transplant period (months 1–6).

References

1. Linares L, Cofán F, Cervera C, *et al.* Infection-related mortality in a large cohort of renal transplant recipients. *Transplant Proc* 2007; **39**: 2225.
2. Legendre C, Canaud G, Martinez F. Factors influencing long-term outcome after kidney transplantation. *Transpl Int* 2014; **27**: 19.
3. Khurana A, Brennan DC. Current concepts of immunosuppression and side effects. In: Liapis H, Wang HL, eds. *Pathology of Solid Organ Transplantation*. Berlin, Germany: Springer-Verlag, 2011: 11–30.
4. Ling X, Xiong J, Liang W, *et al.* Can immune cell function assay identify patients at risk of infection or rejection? A meta-analysis *Transplantation* 2012; **93**: 737.
5. 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.
6. Egli A, Humar A, Kumar D. State-of-the-art on monitoring of cytomegalovirus-specific cell mediated immunity after organ transplant: a primer for the clinician. *Clin Infect Dis* 2012; **55**: 1678.
7. Kaplan JE, Benson C, Holmes KH, *et al.* Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009; **58**: 1.
8. Zonios DI, Falloon J, Bennett JE, *et al.* Idiopathic CD4+ lymphocytopenia: natural history and prognostic factors. *Blood* 2008; **112**: 287.
9. Carbone J, Micheloud D, Salcedo M, *et al.* Humoral and cellular immune monitoring might be useful to identify liver transplant recipients at risk for development of infection. *Transpl Infect Dis* 2008; **10**: 396.
10. Fernández-Ruiz M, López-Medrano F, Romo EM, *et al.* Pretransplant lymphocyte count predicts the incidence of infection during the first two years after liver transplantation. *Liver Transpl* 2009; **15**: 1209.
11. Ducloux D, Carron PL, Racadot E, *et al.* CD4 lymphocytopenia in long-term renal transplant recipients. *Transplant Proc* 1998; **30**: 2859.
12. Carter JT, Melcher ML, Carlson LL, Roland ME, Stock PG. Thymoglobulin-associated Cd4+ T-cell depletion and infection risk in HIV-infected renal transplant recipients. *Am J Transplant* 2006; **6**: 753.
13. Calarota SA, Zelini P, De Silvestri A, *et al.* Kinetics of T-lymphocyte subsets and posttransplant opportunistic infections in heart and kidney transplant recipients. *Transplantation* 2012; **93**: 112.
14. Sarmiento E, Del Pozo N, Gallego A, *et al.* Decreased levels of serum complement C3 and natural killer cells add to the predictive value of total immunoglobulin G for severe infection in heart transplant recipients. *Transpl Infect Dis* 2012; **14**: 526.
15. Müller TF, Grebe SO, Neumann MC, *et al.* Persistent long-term changes in lymphocyte subsets induced by polyclonal antibodies. *Transplantation* 1997; **64**: 1432.
16. Bas J, Mestre M, Grinyó JM, *et al.* Peripheral blood lymphoid subsets and long-term clinical course of kidney recipients: a longitudinal study. *Cytometry* 1998; **34**: 103.
17. Martins L, Ventura A, Dias L, Henriques A, Sarmento A, Guimarães S. Long-term effects of ATG therapy on lymphocyte subsets. *Transplant Proc* 2001; **33**: 2186.
18. Fernández-Ruiz M, López-Medrano F, Varela-Peña P, *et al.* Monitoring of immunoglobulin levels identifies kidney transplant recipients at high risk of infection. *Am J Transplant* 2012; **12**: 2763.
19. Fernández-Ruiz M, López-Medrano F, Varela-Peña P, *et al.* Hypocomplementemia in kidney transplant recipients: impact on the risk of infectious complications. *Am J Transplant* 2013; **13**: 685.
20. Ruiz P, Borowitz MJ, Tilahun H, *et al.* BD Biosciences Application Note. Productivity and efficiency of 6-color BD Multitest and BD Trucount technologies. 2007. Available at: http://www.bd.com/resource.aspx?IDX=17742. (last review 1 November 2013).

21. Humar A, Kumar D, Caliendo AM, *et al.* Clinical impact of human herpesvirus 6 infection after liver transplantation. *Transplantation* 2002; **73**: 599.
22. Garrido RS, Aguado JM, Díaz-Pedroche C, *et al.* A review of critical periods for opportunistic infection in the new transplantation era. *Transplantation* 2006; **82**: 1457.
23. Torre-Cisneros J, Doblas A, Aguado JM, *et al.* Tuberculosis after solid-organ transplant: incidence, risk factors, and clinical characteristics in the RESITRA (Spanish Network of Infection in Transplantation) cohort. *Clin Infect Dis* 2009; **48**: 1657.
24. Humar A, Michaels M, AST ID Working Group on Infectious Disease Monitoring. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. *Am J Transplant* 2006; **6**: 262.
25. De Pauw B, Walsh TJ, Donnelly JP, *et al.* Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; **46**: 1813.
26. European Expert Group on Renal Transplantation (EBPG), European Renal Association (ERA-EDTA), European Society for Organ Transplantation (ESOT). European Best Practice Guidelines for Renal Transplantation (part 1). *Nephrol Dial Transplant* 2000; **15**(Suppl 7): S1.
27. Ducloux D, Carron PL, Motte G, *et al.* Lymphocyte subsets and assessment of cancer risk in renal transplant recipients. *Transpl Int* 2002; **15**: 393.
28. Thibaudin D, Alamartine E, Mariat C, Absi L, Berthoux F. Long-term kinetic of T-lymphocyte subsets in kidney-transplant recipients: influence of anti-T-cell antibodies and association with posttransplant malignancies. *Transplantation* 2005; **80**: 1514.
29. Ducloux D, Courivaud C, Bamoulid J, *et al.* Prolonged CD4 T cell lymphopenia increases morbidity and mortality after renal transplantation. *J Am Soc Nephrol* 2010; **21**: 868.
30. Issa NC, Fishman JA. Infectious complications of antilymphocyte therapies in solid organ transplantation. *Clin Infect Dis* 2009; **48**: 772.
31. de la Torre-Cisneros J, Fariñas MC, Castón JJ, *et al.* GESITRA-SEIMC/REIPI recommendations for the management of cytomegalovirus infection in solid-organ transplant patients. *Enferm Infecc Microbiol Clin* 2011; **29**: 735.
32. Yu X, Han F, Wu J, *et al. Nocardia* infection in kidney transplant recipients: case report and analysis of 66 published cases. *Transpl Infect Dis* 2011; **13**: 385.
33. Martina MN, Cervera C, Esforzado N, *et al. Toxoplasma gondii* primary infection in renal transplant recipients. Two case reports and literature review. *Transpl Int* 2011; **24**: e6.
34. Oh CS, Stratta RJ, Fox BC, Sollinger HW, Belzer FO, Maki DG. Increased infections associated with the use of OKT3 for treatment of steroid-resistant rejection in renal transplantation. *Transplantation* 1988; **45**: 68.
35. Peleg AY, Husain S, Kwak EJ, *et al.* Opportunistic infections in 547 organ transplant recipients receiving alemtuzumab, a humanized monoclonal CD-52 antibody. *Clin Infect Dis* 2007; **44**: 204.
36. De Castro N, Xu F, Porcher R, Pavie J, Molina JM, Peraldi MN. *Pneumocystis jirovecii* pneumonia in renal transplant recipients occurring after discontinuation of prophylaxis: a case-control study. *Clin Microbiol Infect* 2010; **16**: 1375.
37. Struijk GH, Gijsen AF, Yong SL, *et al.* Risk of *Pneumocystis jiroveci* pneumonia in patients long after renal transplantation. *Nephrol Dial Transplant* 2011; **26**: 3391.
38. Fily F, Lachkar S, Thiberville L, Favennec L, Caron F. Risk of *Pneumocystis jiroveci* pneumonia in patients long after renal transplantation. *Med Mal Infect* 2011; **41**: 526.
39. Brunot V, Pernin V, Chartier C, *et al.* An epidemic of *Pneumocystis jiroveci* pneumonia in a renal transplantation center: role of T-cell lymphopenia. *Transplant Proc* 2012; **44**: 2818.
40. Schürmann M, Schürmann D, Schindler R, *et al.* Impaired thymic function and CD4+ T lymphopenia, but not mannose-binding lectin deficiency, are risk factors for *Pneumocystis jirovecii* pneumonia in kidney transplant recipients. *Transpl Immunol* 2013; **28**: 159.
41. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Prevention and Treatment of Cancer-Related Infections (version 1.2012). Available at: http://www.nccn.org/professionals/physician_gls/pdf/infections.pdf. (last review 30 October 2013).
42. Sund F, Lidehäll AK, Claesson K, *et al.* CMV-specific T-cell immunity, viral load, and clinical outcome in seropositive renal transplant recipients: a pilot study. *Clin Transplant* 2010; **24**: 401.
43. Tormo N, Solano C, Benet I, *et al.* Reconstitution of CMV pp65 and IE-1-specific IFN-γ CD8(+) and CD4(+) T-cell responses affording protection from CMV DNAemia following allogeneic hematopoietic SCT. *Bone Marrow Transplant* 2011; **46**: 1437.
44. Kotton CN, Kumar D, Caliendo AM, *et al.* International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010; **89**: 779.