# Pretransplant Levels of C-Reactive Protein, Soluble TNF Receptor-1, and CD38+HLADR+ CD8 T Cells Predict Risk of Allograft Rejection in HIV+ Kidney Transplant Recipients

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**Introduction:** HIV-positive (HIV+) kidney transplant recipients exhibit a 2- to 3-fold increased risk of allograft rejection. Dysregulated immune activation in HIV infection persists despite successful antiretroviral therapy and is associated with non-AIDS morbidity, including renal disease. We hypothesized that the pathological levels of inflammation and immune activation associated with chronic HIV infection could have clinical utility in the prediction of rejection in HIV+ kidney recipients.

**Methods:** Prospective cohort study of 22 HIV-negative (HIV−; donor) to HIV+ (recipient) kidney transplant recipients who underwent biomarker assessment pretransplant and were subsequently followed for development of acute rejection. Plasma levels of markers of inflammation (soluble tumor necrosis factor receptor 1 [sTNF-R1] and C-reactive protein [CRP]) and microbial translocation (soluble CD14 and lipopolysaccharide) were measured by enzyme-linked immunosorbent assay or chromogenic endpoint assay. Levels of activated (CD38+HLADR+) CD4+ and CD8+ T cells, and T regulatory cells (CD4+CD25highFoxP3+) were measured by flow cytometry.

**Results:** Among the biomarkers evaluated, only the pretransplant levels of sTNF-R1, CRP, and frequencies of CD38+HLADR+ CD8 T cells, were found to be at significantly higher levels among patients who experienced biopsy-proven acute rejection. Confirming our hypothesis, patients with high pretransplant levels of sTNF-R1 or activated CD8+ T cells had a significantly increased 200-day cumulative incidence of biopsy-proven acute rejection (0 vs. 38% for both; $P = 0.01$). Similarly, pretransplant CRP levels higher than 5 μg/ml were associated with increased risk of acute rejection within the first 6 months post-transplant (0 vs. 43%; $P = 0.01$).

**Conclusion:** Biomarker-based identification of HIV+ recipients at increased risk for rejection might facilitate individualized induction immunosuppression regimens in this vulnerable patient population.

HIV+ individuals are at increased risk for kidney disease, including HIV-associated nephropathy, noncollapsing focal segmental glomerulosclerosis, immune-complex kidney disease, and comorbid kidney disease, as well as kidney injury resulting from prolonged exposure to antiretroviral therapy (ART) or from opportunistic infections.[1] Kidney function is abnormal in up to 30% of HIV+ patients, and HIV infection represents the third most common cause of end-stage renal disease (ESRD) among African American individuals aged <65 years, with approximately 900 HIV-infected patients per year starting dialysis in the United States.[2–5]

Kidney transplant has become a viable alternative for individuals with ESRD because it is associated with better quality of life, fewer medical complications, lower cost, and longer survival than chronic dialysis

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treatment,[6,7] even among HIV+ recipients.[4,8] Once considered a contraindication for solid organ transplantation, the feasibility of kidney transplantation in HIV+ individuals is now well-established.[4,8–19] In the absence of hepatitis C virus (HCV) coinfection, transplant outcomes and patient/graft survival rates resemble those of HIV− controls.[11,14] Even more, favorable outcomes in HIV+/HCV+ kidney recipients have been recently reported in the direct-acting antiviral era for HCV therapy.[20,21] In a study of the Scientific Registry of Transplant Recipients involving more than 1400 HIV+ kidney transplant candidates, compared with chronic dialysis therapy, the mortality at 5 years was nearly 80% lower after kidney transplant for HIV+ recipients and >90% lower among HIV+/HCV+ patients.[8]

Despite the benefits of kidney transplantation in HIV+ individuals, some major challenges remain. HIV+ patients have higher mortality on the transplant waitlist than their HIV− counterparts.[8,22] HIV-infected candidates are also less likely to be placed on the transplant waitlist.[22,23] Another major challenge is the 2- to 3-fold increased risk of allograft rejection.[4,10–12,17,19,24] The National Institutes of Health–sponsored multicenter trial of kidney transplantation in HIV+ recipients ($n = 150$) reported 1-year rejection rates as high as 31%.[17] Based on data from the Scientific Registry of Transplant Recipients (2002–2011; $n = 510$), HIV+ patients were more likely to develop acute rejection compared with the general HIV− population (17.8% vs. 8.8%, $P < 0.001$).[11] Preliminary results from the HIV Organ Policy Equity in Action clinical trial showed high incidence of rejection among HIV+ to HIV+ kidney transplant recipients (Durand C, et al. Abstract# 175, American Transplant Congress, 2018).

The mechanisms for increased immunological risk remain poorly understood, but it might include the following: complex drug-drug interactions leading to less exposure to immunosuppressants[17,25,26]; the fact that HIV+ recipients are less likely to receive T-cell depletion during induction[11]; and that HIV can infect the allograft after transplantation despite undetectable viremia, a phenomenon that occurs in up to 68% of HIV+ kidney recipients.[5]

Another potential, yet underexplored, mechanism of increased immunological risk is the dysregulated immune activation that is characteristic of chronic HIV infection.[27–34] Even among HIV+ individuals with evidence of viral load suppression and normalization of the CD4 count (>900 cells/μl) in response to ART, the frequency of activated effector memory T cells remains elevated compared with healthy subjects.[27] We have previously shown that compared with HIV− counterparts, HIV+ kidney transplant candidates with

well-controlled HIV infection exhibit abnormal levels of immune activation at the time of transplantation, and the levels of T-cell activation are not reduced by induction/maintenance immunosuppressive therapy.[32] It is increasingly recognized that the levels of immune activation in HIV+ individuals correlate with the incidence of non-AIDS morbidity and mortality.[30] Thus, we hypothesized that the baseline levels of immune activation in HIV+ candidates would correlate with their risk of acute rejection following kidney transplantation. Confirming this hypothesis, we observed that elevated pretransplant circulating levels of CRP, sTNF-R1, and activated (CD38+HLADR+) CD8+ T cells are associated with increased risk of biopsy-proven acute rejection in HIV+ kidney transplant recipients.

## METHODS

### Study Subjects

This single-center prospective cohort study of 22 consecutive HIV− (donor) to HIV+ (recipient) adult, first-time kidney transplants was performed between August 2015 and May 2017 at the Jackson Memorial Hospital–Miami Transplant Institute. One patient received a combined kidney/pancreas transplant. All HIV+ recipients had an undetectable viral load and a CD4+ T-cell count >200 cells/mm$^3$ at the time of transplantation. Twenty-seven healthy individuals and 9 male, predominantly of African American descent, HIV− ESRD transplant candidates enrolled during clinic visit encounter for pretransplant evaluation served as a control groups in selected analyses. The study was approved by the local institutional review board (#20150614), and was conducted consistent with principles embodied in the Declaration of Helsinki.

### Study Procedures

Blood samples were collected in a prospective fashion on the day of admission for transplantation (day 0), within 12 hours before transfer to the operating room and before administration of any immunosuppressive therapy. In a subset of patients ($n = 12$), follow-up blood samples were collected on day +90 following kidney transplantation. Peripheral blood mononuclear cells were prepared by density gradient centrifugation (Ficoll-Paque Plus; GE Healthcare, Little Chalfont, UK) and cryopreserved in liquid N$_2$ and plasma samples were stored at −80 °C until experimental analysis. The plasma levels of the inflammatory markers sTNF-R1 and CRP, and the levels of soluble CD14 (sCD14; a marker of monocyte activation) were measured by immunoassay; plasma lipopolysaccharide (LPS; the major cell wall component of gram-negative bacteria, ligand of CD14, and a marker of microbial

translocation) levels were measured by chromogenic endpoint assay. The levels of activated (CD38+HLADR+) CD4+ and CD8+ T cells, and T regulatory cells (Tregs; CD4+CD25highFoxP3+) were measured by flow cytometry. All samples were processed the same day to avoid technical variability. Patients underwent routine clinical follow-up after kidney transplantation for the development of biopsy-proven rejection. Only patients with suspected rejection (e.g., unexplained rising serum creatinine) underwent kidney biopsy.

## Immunosuppression Protocol and Prophylaxis

All transplant patients received induction immunosuppression with anti-thymocyte globulin (ATG; 1 mg/kg i.v. × 3 doses) plus anti-CD25 monoclonal antibody × 2 doses and methylprednisolone (500 mg i.v. daily × 3 doses). Two additional doses of ATG were given to the kidney-pancreas recipient and those with delayed graft function. Maintenance immunosuppression consisted of tacrolimus, mycophenolate, and prednisone. At our center, tacrolimus is typically started on postoperative day 1 or 2 with a target level of 6 to 8 ng/ml during the first 3 months and 5 to 7 ng/ml thereafter. Higher levels are targeted for highly sensitized patients. All patients received cytomegalovirus (valganciclovir 900 mg daily, renally dosed as needed) and *Pneumocystis jirovecii* pneumonia (trimethoprim-sulfamethoxazole 1 double-strength tablet 3 times a week, or dapsone 100 mg daily if sulfa allergy) prophylaxis per local protocol.

## Flow Cytometry

Cryopreserved peripheral blood mononuclear cells were thawed and rested overnight in Roswell Park Memorial Institute medium including 10% fetal bovine serum, then washed and stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham, MA) before labeling for cell surface and intracellular protein expression using commercially available fluorescent marker–conjugated monoclonal antibodies. After staining, cells were analyzed using a BD Fortessa instrument (BD Biosciences, San Jose, CA) using FlowJo (Ashland, OR) V10 software.[35]

## Measurement of Inflammatory and Microbial Translocation Biomarkers

Plasma levels of sCD14, sTNF-R1, and CRP molecules were determined by the use of Human Quantikine Immunoassay (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. LPS levels were measured in plasma samples by the use of the Limulus amebocyte lysate chromogenic endpoint assay

(Lonza Group Ltd, Allendale, NJ) according to the manufacturer's recommendations.[35,36]

## Statistics

Demographic, medical, and treatment characteristics were summarized using descriptive statistics. The Kaplan-Meier plots with a log-rank test, Fisher's exact test, Wilcoxon matched-pairs signed ranked test, and Mann-Whitney tests were used where appropriate. Optimal biomarker cutoffs were identified by using minimum $P$-value approach.[37] The primary outcome was biopsy-proven rejection. We also conducted similar analyses using a composite outcome consisting of delayed graft function (defined as need for hemodialysis during the first week after transplantation), biopsy-proven acute rejection, graft loss (defined as permanent return to dialysis), serious infection (defined as infections requiring admission to the intensive care unit during initial transplant hospitalization or readmission to the hospital after discharge), or death.[10] All tests were 2-sided and $P < 0.05$ was considered as statistically significant. Statistical analyses were performed using GraphPad Prism Software, Inc., version 7.03 (La Jolla, CA).

## RESULTS

### Patient Characteristics

A total of 22 HIV+ patients were enrolled in the study. General characteristics of study subjects are presented in Table 1. Most patients were men, predominantly of African American descent. All patients had long-standing HIV infection, with a median time of HIV diagnosis to transplantation of 17 years, and all had received chronic dialysis therapy for a median of 7 years before transplantation. HIV infection was well controlled with median pretransplant CD4 count >450 cells/mm$^3$ and ART-induced HIV viral load suppression (<50 copies/ml) documented in all study subjects. Post-transplant nadir CD4 count was 252 cells/mm$^3$ (interquartile range, 198–396). HIV infection remained well controlled in the post-transplant period with documented viral load suppression for all patients. Protease inhibitor–based ART regimens were the most common pretransplantation ($n = 14$); 12 of these patients underwent ART switch to an integrase inhibitor–based regimen to avoid drug-drug interactions with calcineurin inhibitors. Median plasma tacrolimus levels at day 30 post-transplantation were 6.8 ng/ml (interquartile range, 6.0–7.7).

### Transplant Outcomes

Transplant outcomes were excellent, with 12-month graft survival and patient survival of 91% and 100%, respectively. After a median follow-up of 689

**Table 1.** Baseline characteristics of study participants

| Variable | All patients n = 22 (%) | Rejection Yes n = 3 (%) | Rejection No n = 19 (%) | Composite outcome[a] Yes n = 8 (%) | Composite outcome[a] No n = 14 (%) |
|---|---|---|---|---|---|
| Demographics | | | | | |
| Age, yr, median (IQR) | 47 (40–61) | 50 (48–58) | 45 (40–61) | 49 (44–56) | 44 (39–62) |
| Male sex | 16 (72) | 3 (100) | 13 (68) | 7 (88) | 9 (64) |
| African American | 20 (91) | 3 (100) | 17 (89) | 7 (88) | 13 (93) |
| Time on dialysis before transplantation, yr, median (IQR) | 7 (2–9) | 8 (7–18) | 7 (2–9) | 8 (7–13) | 7 (2–9) |
| HIV infection | | | | | |
| Pretransplant CD4 count, cells/mm$^3$, median (IQR) | 453 (336–716) | 429 (349–467) | 475 (298–741) | 452 (369–656) | 457 (293–762) |
| Pretransplant HIV viral load, <50 copies/ml | 22 (100) | 3 (100) | 19 (100) | 8 (100) | 14 (100) |
| Time from HIV diagnosis, yr, median (IQR) | 17 (10–23) | 26 (13–28) | 14 (9–23) | 20 (13–25) | 13 (9–23) |
| Protease inhibitor–containing regimen, pretransplant | 14 (64) | 3 (100) | 11 (58) | **8 (100)** | **6 (43)** |
| Protease inhibitor–containing regimen, post-transplant | 2 (9) | 0 | 2 (11) | 1 (13) | 1 (7) |
| Comorbidities | | | | | |
| Hepatitis C | 6 (27) | 0 | 6 (32) | 0 | 6 (43) |
| Diabetes mellitus | 6 (27) | 1 (33) | 5 (26) | 3 (38) | 3 (21) |
| Hypertension | 12 (55) | 3 (100) | 9 (47) | 4 (50) | 8 (57) |
| HIVAN[b] | 19 (86) | 3 (100) | 16 (84) | 7 (88) | 12 (86) |
| Overweight (BMI >25) | 9 (41) | 1 (33) | 8 (42) | 4 (50) | 5 (36) |
| Immunosuppression[c] | | | | | |
| Prednisone | 22 (100) | 3 (100) | 19 (100) | 8 (100) | 14 (100) |
| i.v. Ig | 1 (5) | 0 | 1 (5) | 0 | 1 (7) |
| Rituximab | 2 (9) | 1 (33) | 1 (5) | 1 (13) | 1 (7) |
| Tacrolimus | 22 (100) | 3 (100) | 19 (100) | 8 (100) | 14 (100) |
| MMF | 22 (100) | 3 (100) | 19 (100) | 8 (100) | 14 (100) |
| Everolimus | 2 (9) | 0 | 2 (11) | 1 (13) | 1 (7) |
| Kidney allograft | | | | | |
| Post-transplant follow-up, d, median (IQR)[d] | 689 (511–844) | 331 (99–1076) | 736 (527–856) | 520 (340–711) | 779 (532–886) |
| Living donor[e] | 1 (5) | 0 | 0 | 1 (13) | 0 |
| Donor age, yr, median (IQR) | 36 (22–46) | 47 (45–50) | 32 (21–42) | 45 (19–50) | 35 (23–42) |
| Donor terminal creatinine, mg/dl, median (IQR) | 0.8 (0.73–0.98) | n/a[f] | n/a | 0.8 (0.67–1.0) | 0.8 (0.7–1.2) |
| Kidney Donor Profile Index, median (IQR) | 43 (28–49) | **51 (47–68)** | **42 (27–46)** | 47 (33–67) | 42 (25–46) |
| Delayed graft function | 3 (14) | 1 (33) | 2 (11) | **3 (38)** | **0** |
| Warm ischemia time,[g] min, median (IQR) | 27 (21–37) | 23[g] | 26 (20–35) | 27 (20–35) | 25 (20–33) |
| Cold ischemia time, h, median (IQR) | 18 (9–29) | 14 (6–31) | 21 (10–29) | 25 (14–30) | 16 (7–30) |
| Pretransplant calculated panel reactive antibody, >80% | 1 (5) | 1 (33) | 0 | 1 (13) | 0 |
| De novo donor-specific antibodies | 5 (23) | 2 (67) | 3 (16) | 2 (25) | 3 (21) |
| CMV viremia,[h] >500 copies/ml | 1 (5) | 0 | 1 (5) | 0 | 1 (7) |
| BK viremia,[h] >10,000 copies/ml | 3 (14) | **2 (66)** | **1 (5)** | 2 (25) | 1 (7) |
| Tacrolimus level at 4 wk, ng/ml, median (IQR) | 6.8 (6.0–7.7) | 6.8 (3.2–12.8) | 6.8 (6–7.8) | 6.7 (4.1–8.6) | 6.9 (6.1–7.5) |

BMI, body mass index; CMV, cytomegalovirus; HIVAN, HIV-associated nephropathy; IQR, interquartile range; i.v. Ig, intravenous immunoglobulin; MMF, mycophenolate mofetil; n/a, not applicable.
[a]Composite outcome consisting of delayed graft function, biopsy-proven acute rejection, graft loss, serious infection, or death.
[b]Mostly presumptive diagnosis, only 2 were biopsy proven.
[c]All of the patients received antithymocyte globulin, basiliximab, and methylprednisolone for induction.
[d]None of the study subjects was lost from clinical follow-up.
[e]All deceased donors were donor after brain death.
[f]Donor creatinine was not available for the 3 patients in the rejection group.
[g]Data available for only 12 patients. Insufficient numbers in one of the columns to calculate IQR.
[h]During first year post-transplant.
Data presented as absolute number (percentage), unless specified otherwise. Fisher's exact test or Mann-Whitney test were used for comparison between groups where appropriate. Differences with P values < 0.05 are shown in bold font.

days, 3 (14%) patients developed biopsy-proven acute rejection, resulting in graft failure signified by return to long-term dialysis in 2 cases. Median time to acute rejection was 123 days (range, 59–150). Two cases were antibody-mediated and 1 was due to mixed cellular and humoral acute rejection. Capillary-positive C4d staining, a marker for antibody-dependent graft injury, was a common finding. Additional histopathological description of kidney biopsy, including Banff 07 Classification, is presented in the Supplementary Material. Median donor age and Kidney Donor Profile Index score were higher among patients who experienced acute rejection (Table 1). High-level BK viremia was documented in 3 patients (median time from transplantation to BK viremia, 106 days; range, 63–145 days) and it preceded the diagnosis of rejection in

2 patients (Table 1). There were no cases of BK nephropathy in this cohort; among patients with biopsy-proven rejection, there was no evidence of BK in the allograft by immunohistochemistry or PCR. Data on proteinuria were available for 19 patients. Except for 1 patient with proteinuria of 100 mg/dl detected 4 weeks post-transplant (who also experienced rejection and graft loss), there were no patients with significant proteinuria in this cohort.

Five (23%) patients developed an episode of severe infection[10] during the first 6 months post-transplant. These included patients with intra-abdominal infection (n = 2; Escherichia coli and Staphylococcus aureus, 1 each); surgical site infection (n = 1); community-acquired pneumonia (n = 1); and concurrent esophagitis and Clostridium difficile colitis (n = 1).

## Abnormally Elevated Levels of sCD14, LPS, and sTNF-R1 in Hemodialysis Patients Are Reduced Following Kidney Transplantation

We first aimed to characterize the immune phenotype of patients receiving chronic hemodialysis by HIV status and the impact of kidney transplantation on the levels of the specific biomarkers analyzed. Compared with the healthy HIV− control group, patients with ESRD on chronic hemodialysis treatment exhibited significantly higher levels of plasma sCD14, LPS, and sTNF-R1, regardless of their HIV status. Namely, the levels of sCD14, a marker of monocyte activation, were 3-fold higher among hemodialysis patients (median [interquartile range] 2985 [2390–3456] and 3185 [2871–4056] ng/ml for HIV− and HIV+, respectively) compared with healthy HIV− subjects (1221 [565–1852] ng/ml; P < 0.0001; Figure 1a). Similarly, the levels of LPS, a marker of microbial translocation, were higher in HIV− and HIV+ hemodialysis patients compared with healthy HIV− subjects (291 [253–309] and 280 [235–304] vs. 164 [138–197] pg/ml, respectively; P < 0.0001; Figure 1b). Plasma levels of sTNF-R1 were 25-fold higher among hemodialysis patients (26.4 [15.8–28.4] and 23.7 [16.7–30.8] ng/ml for HIV− and HIV+, respectively) compared with HIV− healthy subjects (0.88 [0.67–1.3] ng/ml; P < 0.0001; Figure 1c). There were no significant differences in the levels of CRP between groups (Figure 1d). As expected, the frequencies of circulating activated (CD38+HLADR+) CD4+ (Figure 1e) and CD8+ (Figure 1f) T cells were increased (2.34% vs. 1.72%, P = 0.01; and 2.56 vs. 1.58, P=0.003, respectively), and the frequencies of Tregs (CD4+CD25highFoxP3+, measured as percentage of CD4+CD25bright cells; Figure 1g) was reduced (60.1% vs. 73.4%, P = 0.01), among HIV+ kidney transplant candidates compared with HIV− counterparts, respectively. Notably, the abnormally elevated

levels of LPS and sTNF-R1 observed in HIV+ hemodialysis patients were reduced on paired blood samples obtained 3 months following kidney transplantation (Figure 1b and c), whereas the elevated levels of activated CD4+ and CD8+ T cells observed in HIV+ hemodialysis patients were not affected by transplantation (Figure 1e and f). Among HIV+ subjects undergoing kidney transplantation, we observed a nonstatistically significant trend toward reduced numbers of Tregs post-transplant, compared with baseline levels (48.7 vs. 61.6, respectively; P = 0.06; Figure 1g).

## Elevated Pretransplant Levels of sTNF-R1 and CRP and Frequencies of Activated CD8+ T Cells Predict Risk of Acute Rejection in HIV+ Kidney Transplant Recipients

We compared the levels of inflammatory and immune activation markers between patient groups stratified by clinical outcome. Among all the biomarkers evaluated in the cohort of HIV+ kidney transplant recipients, only the pretransplant levels of sTNF-R1 and CRP and frequencies of CD8+CD38+HLADR+ T cells were found to be significantly higher among patients who experienced biopsy-proven acute rejection versus those who did not: 44.5 ng/ml versus 22.1 ng/ml (P = 0.04), 60.2 μg/ml versus 2.9 μg/ml (P = 0.003), and 5.08% versus 2.48% (P = 0.04), respectively (Figure 2a–c). Levels of sCD14, LPS, activated CD4+ T cells and Tregs were not different between groups by rejection status (Supplementary Figure S1).

We next assessed whether the abnormally elevated levels of these biomarkers have clinical value in the prediction of acute rejection following kidney transplantation. In time to event analyses, patients with high pretransplantation levels of sTNF-R1 or activated CD8+ T cells had a significantly increased 200-day cumulative incidence of biopsy-proven acute rejection (0 vs. 38% for both; P = 0.01; Figure 2e and f). Similarly, pretransplant CRP levels higher than 5 μg/ml were associated with increased risk of acute rejection within the first 6 months post-transplantation (0% vs. 43%; P = 0.01; Figure 2g).

Because only 3 patients had the primary outcome of rejection, we also conducted similar analyses using a composite outcome consisting of delayed graft function, biopsy-proven acute rejection, graft loss, serious infection, or death. Patients in the composite outcome group had significantly higher levels of CRP compared with others (29.7 vs. 1.7 μg/ml, respectively; P = 0.001; Figure 2d). Pretransplant CRP levels higher than 5 μg/ml were associated with increased risk of experiencing the composite outcome (14% vs. 86%; P = 0.01; Figure 2h). There were no differences in the levels
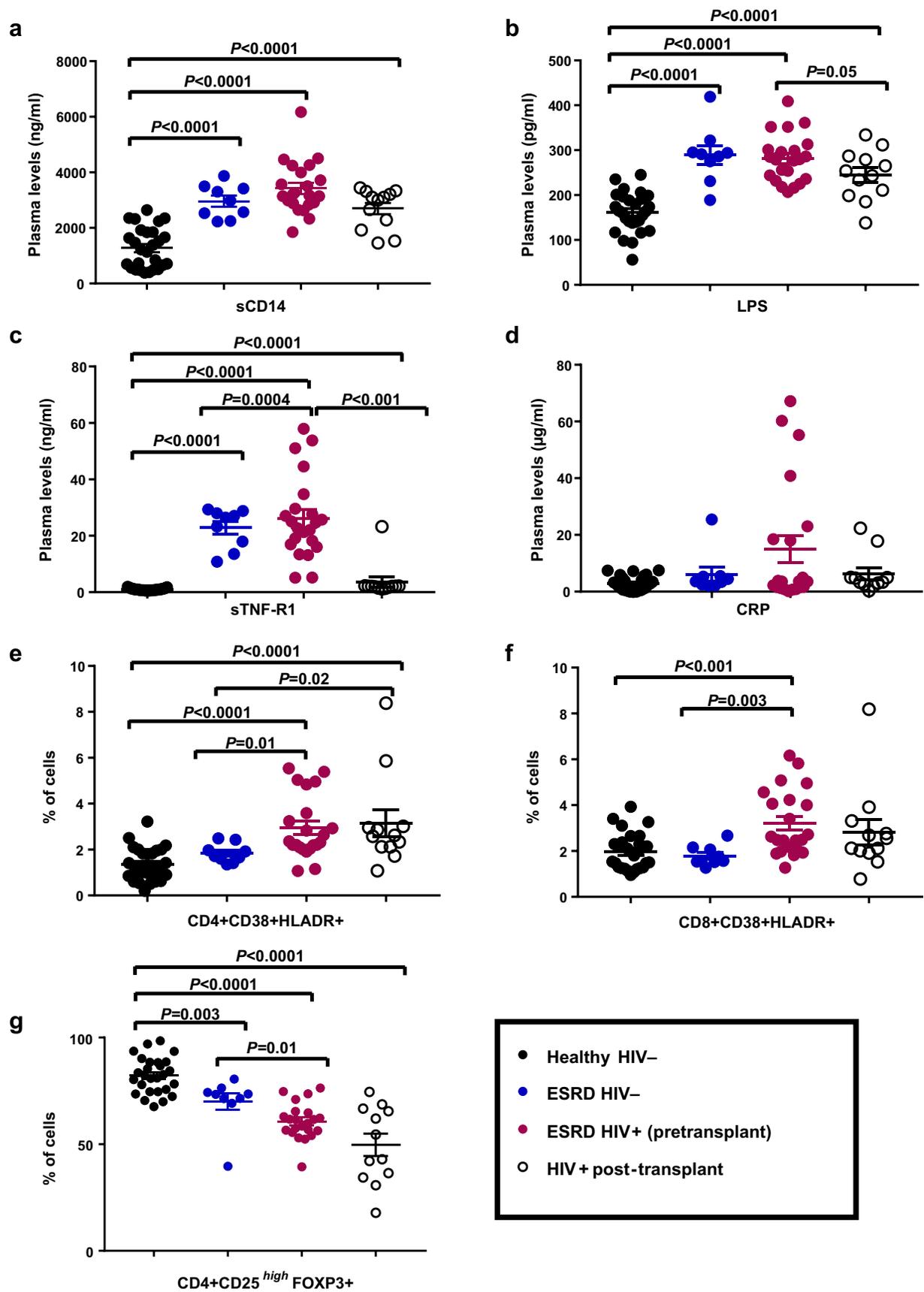
**Figure 1.** Elevated levels of soluble CD14 (sCD14), lipopolysaccharide (LPS), and soluble tumor necrosis factor receptor 1 (sTNF-R1) in HIV+ hemodialysis patients are reduced following kidney transplantation. (a–g) Scatter dot plots correspond to the circulating levels of inflammation (sTNF-R1, C-reactive protein [CRP]), microbial translocation (sCD14, LPS), and immune activation (CD38+HLADR+ T cells) (continued)

of the other biomarkers assessed between patients by the composite outcome (data not shown).

## DISCUSSION

Kidney transplant recipients with HIV infection exhibit an increased risk of allograft rejection.[4,10–12,17,19,24] Preliminary data from the HIV Organ Policy Equity in Action Study indicates that use of HIV+ donors might further increase the risk of rejection in HIV+ recipients (Durand C, *et al*. Abstract# 175, American Transplant Congress, 2018). To date, the underlying mechanisms for such predisposition to increased rejection remain poorly understood and means to identify patients at risk are lacking. In clinical practice, there is significant variation across centers in the induction immunosuppressive regimens used in transplant recipients with HIV infection ranging from no induction to administration of ATG, anti-interleukin-2R, or both.[10,12,17,19,38] Some centers advocate the use of i.v. Igs.[19] In a large study of Scientific Registry of Transplant Recipients data, patients who received ATG had the lowest rates of acute rejection.[38] ATG also seems to reduce the risk of rejection in the setting of HIV+ to HIV+ transplantation (Durand C, *et al*. Abstract# 175, American Transplant Congress, 2018). One well-documented disadvantage of T-cell depletion is the increased risk of infection associated with profound and prolonged lymphopenia, especially among those with baseline CD4 <350 cells/mm.[3,10,17,39,40] This poses a therapeutic dilemma because the salutatory effects of ATG on the increased risk of allograft rejection associated with HIV must be balanced against the higher susceptibility to opportunistic infections observed in lymphopenic ATG-treated kidney transplant recipients. Therefore, improved strategies for identification of HIV+ patients at risk of allograft rejection are needed. We hypothesized that the pathological levels of inflammation and immune activation associated with chronic HIV infection[27–34] have clinical utility in the prediction of rejection and transplant outcomes in HIV+ kidney recipients. To address this question, we studied 22 HIV− to HIV+ kidney transplant recipients who underwent biomarker assessment pretransplantation and were subsequently followed for development of biopsy-proven acute rejection. Our findings confirm

the notion that, despite well-controlled HIV infection, HIV+ kidney transplant candidates exhibit elevated levels of immune activation[32]; and consistent with our hypothesis, the pretransplant levels of sTNF-R1, CRP, and the frequencies of activated (CD38+HLADR+) CD8 T cells were found to be at significantly higher levels among HIV+ patients who experienced acute rejection, and all 3 biomarkers had robust predictive value for risk of rejection. In addition, high pretransplant levels of CRP were predictive of a composite secondary outcome of delayed graft function, rejection, graft loss, infection, or death.

CRP is secreted by hepatocytes in response to a variety of inflammatory cytokines, mainly interleukin-6, and constitutes an exquisitely sensitive systemic marker of inflammation and tissue damage.[41,42] Our findings complement previous reports in which elevated pretransplant CRP level was found to be a predictor for acute rejection in HIV− kidney transplant recipients.[43,44] Similar to our observations, pretransplant CRP levels >5 μg/ml have been associated with mortality,[45] and CRP levels >3 mg/ml associated with accelerated deterioration of graft function, in HIV− renal transplant recipients.[46] Activation of complement cascade by human CRP may opsonize and enhance phagocytosis of various ligands.[41,42] Thus, following the danger theory,[47] it is plausible that elevated levels of CRP reflect an augmented inflammatory state that predisposes T cells to alloreactivity. The mechanisms for the associations reported here require further study.

Uremia-induced impairment of the intestinal epithelial barrier structure and function contribute to systemic inflammation and uremic toxicity by accommodating the translocation of endotoxin, microbial fragments, and other noxious luminal products in the circulation.[48,49] Consistent with that notion, we observed significantly higher levels of microbial translocation (i.e., LPS), monocyte activation (i.e., sCD14), and systemic inflammation (i.e., sTNF-R1) in patients with ESRD, regardless of HIV status. Even more, in the subgroup of HIV+ patients with post-transplant samples available, the levels of LPS and sTNF-R1 were significantly reduced following kidney transplantation. Whether this is related to successful restoration of renal function by the new allograft or a result of transplant-associated immunosuppression requires further study. Plasma levels of sCD14

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**Figure 1.** (continued) biomarkers, as well as T regulatory cells (CD4+CD25highFoxP3+) in healthy HIV− subjects (black circles; *n* = 27); ESRD HIV− patients receiving hemodialysis (blue circles; *n* = 9); end-stage renal disease (ESRD) HIV+ patients receiving hemodialysis (samples obtained within 12 hours pretransplant; purple circles; *n* = 22), and HIV+ kidney transplant recipients (samples obtained 90 days post-transplant; open circles; *n* = 12). Mean with SEM are shown for each group. *P* values correspond to comparision between groups using the Mann-Whitney test, except for the comparison between ESRD HIV+ (pretransplant) and HIV+ post-transplant groups, which was performed using the Wilcoxon matched-pairs signed ranked test. Only statistically significant differences are shown.
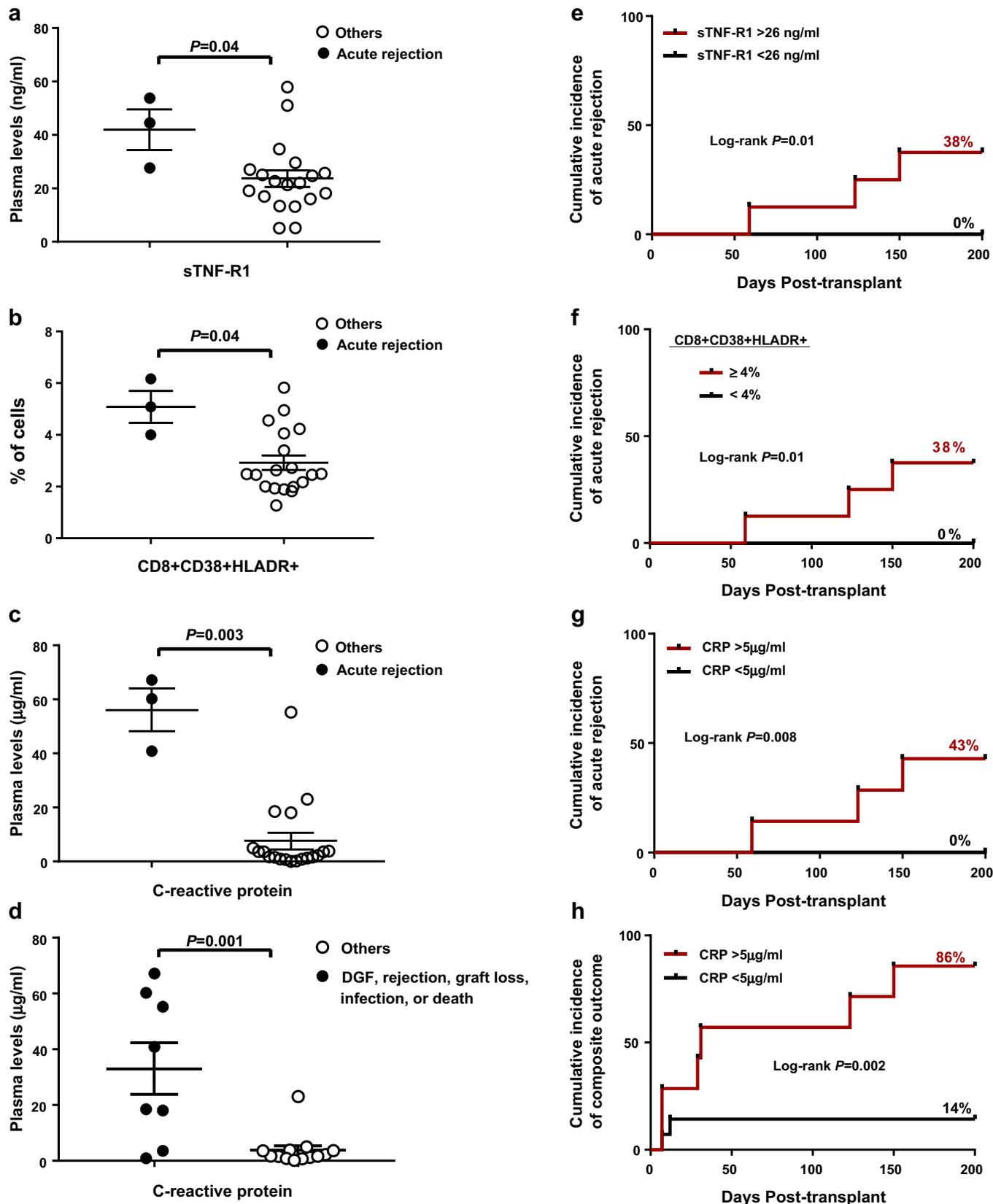
**Figure 2.** Elevated pretransplant levels of soluble tumor necrosis factor receptor 1 (sTNF-R1), C-reactive protein (CRP), and frequencies of activated CD8 T cells predict risk of acute rejection in HIV+ kidney transplant recipients. (a–d) Scatter dot plots correspond to the circulating pretransplant levels of sTNF-R1 (a), CD38+HLADR+ CD8 T cells (b), and CRP (c,d) by clinical outcome group. Note increased pretransplant levels of these biomarkers among patients who experienced rejection or the composite outcome (delayed graft function [DGF], rejection, graft loss, infection, and/or death) post-transplant. Mean with SEM are shown (continued)

independently predict mortality in HIV infection.[50] Soluble CD14 is also an independent predictor of mortality in patients with chronic kidney disease and those receiving hemodialysis[51,52]; however, data in kidney transplant recipients are lacking. Levels of sCD14 did not have predictive value for clinical outcomes in this small cohort.

Soluble TNF receptors are elevated in the setting of inflammation and chronic kidney disease.[53,54] Soluble TNF-R1 is independently associated with all-cause mortality and increased risk for cardiovascular events in advanced chronic kidney disease irrespective of the cause of kidney disease.[55] Soluble TNF-R1 predicts risk for ESRD in patients with type 2 diabetes even after adjustment for clinical covariates such as urinary albumin excretion.[56] Higher CRP and sTNF-R are independently associated with faster rates of kidney function loss in chronic kidney disease.[57] Our observation that sTNF-R1 was a robust predictor of acute rejection in HIV+ kidney transplant recipients provides additional evidence of the clinical utility of this biomarker in patients with kidney disease.

Previous studies have demonstrated increased numbers of circulating CD8+HLADR+ T cells in kidney transplant recipients with microcirculation inflammation,[58] and the infiltration of HLADR-expressing cells in renal allografts with acute rejection.[59] High levels of CD38+HLADR+ CD8+ T cells are also characteristic of patients with HIV infection and influence HIV disease progression.[29,34] Because dysregulated immune activation is a determinant of non-AIDS morbidity including renal disease,[30] it seemed logical to think that the baseline levels of T-cell activation in HIV+ candidates would correlate with their risk of acute rejection following kidney transplantation. Consistent with this concept, abnormally elevated frequencies of CD8+CD38+HLADR+ T cells measured in pretransplant blood samples were associated with increased rejection risk in HIV+ kidney transplant recipients. In a previous study, we failed to demonstrate an association between increased T-cell activation and rejection risk,[32] a discrepancy that can be due to retrospective design and lack of CD8+CD38+HLADR+ T-cell subset data.

Cytomegalovirus replication is considered a significant cause of immune activation in chronic HIV infection,[33] and a potential risk factor for acute rejection in kidney transplant recipients.[60] All the patients studied here were cytomegalovirus seropositive at the time of the transplant and received a minimum of 3 months of valganciclovir prophylaxis per protocol. This is important because administration of valganciclovir at prophylactic doses to HIV+ subjects can result in a decline in the frequency of CD8+CD38+HLADR+ T cells and reduction of CRP levels by week 8 of treatment.[33] However, despite induction immunosuppression with ATG, anti-CD25 monoclonal antibody, and pulse dose steroids followed by triple immunosuppressive therapy, and concurrent administration of valganciclovir prophylaxis, we observed no reduction in the levels of activated T cells or CRP following kidney transplant.

Protease inhibitor–containing ART regimens are associated with adverse outcomes in HIV+ kidney transplant recipients.[9,61] Pretransplant protease inhibitor–containing ART regimens were more commonly observed in the group of patients with the composite outcome (Table 1); however, only 2 patients in the entire cohort, and none of the patients who had rejection, received protease inhibitors in the posttransplant period. The serum levels of tacrolimus were within therapeutic target range for all patients. Also, none of the patients with rejection or poor transplant outcomes had HCV coinfection (Table 1). Thus, the associations between inflammatory/immune activation biomarkers and acute rejection in HIV+ recipients observed here seem to be independent of cytomegalovirus reactivation, HCV coinfection, and drug-drug interactions between ART and immunosuppressive agents.

Tregs can mediate allograft tolerance in long-term immunosuppressed kidney transplant patients.[62,63] Consistent with previous reports,[64] compared with healthy controls, the number of CD4+CD25highFoxP3+ cells was reduced in patients receiving hemodialysis, regardless of HIV status. In addition, we observed that HIV+ kidney transplant candidates had reduced numbers of circulating CD4+CD25highFoxP3+ cells at baseline compared with HIV– counterparts; this might, to some extent, explain their increased predisposition to rejection. The observed trend toward reduced number of Tregs following kidney transplantation in our cohort is not surprising because administration of anti-CD25 monoclonal antibody, alone or in combination with ATG, can reduce

**Figure 2.** (continued) for each group. *P* values correspond to comparison between groups using the Mann-Whitney test. (e–h) The 200-day cumulative incidence of acute rejection (e–g) and the composite outcome as described previously (h) by levels of sTNF-R1 (e), CD38+HLADR+ CD8 T cells (f), and CRP (g,h). Specific cutoffs for each biomarker were identified using the most significant *P*-value approach. Number of subjects in the log-rank analysis shown in (e) was 14 and 8 for low and high sTNF-R1 groups, respectively; in (f) it was 14 and 8 for low and high CD38+HLADR+ CD8 T cells groups, respectively; and in (g) and (h) it was 14 and 7 for low and high CRP groups, respectively. Note the increased cumulative incidence of poor outcomes in patients with high pretransplant levels of the corresponding biomarker.

numbers of CD4+CD25+ Tregs in the early post-transplant period.[65,66]

Limitations of our study include small cohort size, but this a very unique patient population, namely HIV+ kidney transplant recipients, and it took almost 2 years to enroll 22 patients at one of the largest US kidney transplant centers (>300 kidney transplants/year) located in Miami-Dade, one of the counties with higher incidence of HIV cases in the United States (>50 new cases/year per 100,000 population). Clinical factors, such as previous allosensitization, donor age, Kidney Donor Profile Index scores and BK viremia might have influenced transplant outcomes, but small number of events precluded the use of logistic regression models to assess whether the predictive value of novel biomarkers reported here is independent of such factors. Of note, the general characteristics of the patients and potential risk factors for poor clinical outcomes were largely similar between groups when stratifying the cohort by levels of biomarkers found to have predictive value (Supplementary Table S1). Further validation of the associations reported here will likely require a multicenter study. Only 2 time points were assessed, so we cannot establish causality between immune phenotypes and clinical outcomes. However, our main goal was to identify biomarkers with clinical utility in risk stratification of HIV+ kidney transplant recipients, and the pretransplant assessment was informative in this regard. Despite these limitations, our results shed light into immune correlates of rejection in the setting of HIV infection.

In closing, acute rejection remains a major obstacle for maintaining long-term graft survival in HIV+ kidney transplant recipients. We propose that pretransplant assessment of systemic immune activation and inflammatory status should prove useful in guiding clinical decision making and facilitate individualization of immunosuppression therapy for HIV+ patients undergoing renal transplantation. In particular, the measurement of serum CRP constitutes a rapid, inexpensive, and widely available[41] test that may be used to stratify patients at the time of transplantation according to immunological risk and help prevent rejection in this vulnerable patient population. Larger studies are needed.

## DISCLOSURE

All the authors declared no competing interests.

## ACKNOWLEDGMENTS

## AUTHOR CONTRIBUTIONS

JFC, SPal, and SPah participated in research design; JFC, MA, AR, GG, and GWB participated in the implementation of the study, patient enrollment, and the performance of the research; SPal and SPah performed laboratory experiments; JFC, SPal, and IVM participated in data collection; JFC, YN, and SPal conducted data analysis and interpretation; JFC wrote the initial version of the manuscript; and all authors reviewed and approved the final version of the article.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)
**Figure S1.** Levels of sCD14, LPS, activated CD4 cells, and Tregs do not discriminate between HIV+ kidney transplant patients who experienced acute rejection and those who did not.
**Table S1.** Baseline characteristics of study participants.
**Histopathological description, treatment, and outcome of cases of acute rejection in HIV+ kidney transplant recipients.**
**STROBE Statement.**

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