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The Significance of Intragraft CD138+ Lymphocytes and p-S6RP in Pediatric Kidney Transplant Biopsies

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Abstract

Background—We have previously shown that intragraft CD20+ B cells are associated with acute cellular rejection (ACR) and allograft loss. Phosphorylation of S6 ribosomal protein, a downstream target of the PI3K/Akt/mTor pathway, promotes growth and proliferation of cells and could identify metabolically active cells such as alloantibody secreting plasma cells. Since CD20+ lymphocytes can differentiate into CD138+ plasma cells, we aimed to identify functionally active

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plasma cells by using intragraft CD138 quantification and p-S6RP staining and correlate these results with allograft rejection, function, and survival.

Methods—We examined 46 renal transplant biopsies from 32 pediatric patients who were biopsied for clinical suspicion of rejection. Immunohistochemical staining for C4d, CD20, CD138, and p-S6RP was performed. Patient creatinine clearance and graft status was followed post-biopsy.

Results—Patients with ≥ 6 CD138+ cells/hpf had worse graft survival with a HR of 3.4 (95% CI 1.3, 9.2) 2 years post-biopsy compared to those with 0–5 cells/hpf ($p=0.016$). CD138+ cells stained for p-S6RP, indicating functionally active plasma cells. They were associated with ACR ($p=0.004$) and deteriorating graft function ($R^2=0.22$, $p=0.001$). Intragraft CD20+ and CD138+ cells found together in ACR were associated with poorer graft survival than either marker alone, HR 1.5 (95% CI 1.1, 2.2, $p=0.01$).

Conclusions—A threshold of ≥ 6 CD138+ metabolically active plasma cells/hpf, coexisting with CD20+ B cells, was associated with poor allograft function and survival. This may represent an additional antibody-mediated process present in the setting of ACR and could play an important role in characterization and treatment of transplant rejection.

Keywords

plasma cells; rejection; transplant biopsies; children

Introduction

Renal transplantation remains the treatment of choice for children with end-stage renal disease. With improving immunosuppressive management, short and medium term graft survival have been enhanced in pediatric and adolescent recipients. However, despite the continuing optimization of immunosuppressive regimens, long-term graft survival remains virtually unimproved. This seems to be due to a number of both immunological and non-immunological factors. Two important factors that heavily affect graft loss include calcineurin inhibitor nephrotoxicity and clinical or sub-clinical rejection.

Current immunosuppressive regimens are tailored more towards targeting T-cell mediated mechanisms of rejection. Recently, intrarenal B-cells have been implicated in renal allograft rejection and poor graft survival. A growing body of evidence seems to suggest a role for intragraft CD20+ B lymphocytes in acute renal allograft rejection (1–4). When we examined the impact of intragraft B lymphocytes, we did not find an association between intragraft CD20+ B lymphocytes and antibody-mediated rejection (AMR) (3). Rather we, and others, have identified an association between intragraft CD20+ B cells and acute cellular rejection (ACR) (1–4). CD20+ lymphocytes have the potential to differentiate into CD138+ plasma cells. Several studies have shown that CD138+ plasma cell-rich rejections are associated with AMR, are less responsive to standard anti-rejection therapy, and may portend poor graft outcome (5–9).

Despite these reports, it still is unknown whether intragraft B cells are active participants in allograft rejection. If they are involved, the mechanism of their action remains unclear. B cells may act in antigen presentation, alloantibody production by differentiating into plasma cells, or by another mechanism that has not yet been identified. Conversely, the presence of CD20+ and/or CD138+ plasma cells may simply represent a nonspecific epiphenomenon and their presence in the renal interstitium a marker of severe immunological attack and inflammation.

B cells are known to be efficient antigen-presenting cells (10,11). Sarwal et al found a subset of CD20+ lymphocytes that present MHC Class II antigen to CD4+ T cells (1,12). These B cells were not associated with immunoglobulins or C4d deposition in the biopsies (1). Our group, in addition to others, further supported the potential role of CD20+ infiltrates as antigen presenting cells by finding an association with only ACR (1–4).

B cells also play a pivotal role in the production and maintenance of alloantibody responses. Clonal expansion of B cells occurs upon exposure and interaction with alloantigen which involves differentiation into alloantibody-producing CD138+ plasma cells (13). It would seem logical that CD20+ infiltrating cells differentiate into CD138+ plasma cells which might be expected to mediate AMR. Recently, Xu et al found that CD138+ plasma cells were associated with AMR and hypothesized that the allograft was damaged through local antibody secretion. However, this group did not look at any biomarkers to support this hypothesis (9). Other groups have encountered difficulties in successfully enumerating intragraft plasma cells using immunohistochemistry (IHC) for CD138, so the influence of intragraft CD138+ cells is still unclear (12). The difficulties include high background staining of tubular epithelial cell cytoplasm and distinguishing viable areas of cortex from areas of tubulointerstitial scarring on the immunoperoxidase stains.

The phosphorylation of S6 ribosomal protein (p-S6RP), a downstream target of the PI3K/Akt/mTor pathway, promotes growth and proliferation of cells. Akt is known to activate mTOR, which in turn regulates protein synthesis and cell proliferation through the phosphorylation and activation of S6 kinase and S6RP (14). Thus, p-S6RP identifies cells that are synthesizing proteins and are metabolically active. Furthermore, p-S6RP has been associated with antibody-mediated rejection in heart allografts (14). Therefore, p-S6RP may be a useful biomarker to establish that intragraft CD138+ plasma cells are functionally active and important in rejection pathogenesis. To date, the role of both CD138+ plasma cells and p-S6RP as biomarkers for acute renal allograft rejection is unclear.

Since CD20+ B cells may differentiate into CD138+ plasma cells, we investigated the significance of CD138+ plasma cells within the same allograft biopsies in which we found the association between CD20+ cells and poor graft outcome. The aim of our study was to quantify CD138+ B lymphocytes in renal allograft biopsies and correlate the results with allograft rejection, function, and survival. Additionally, we wanted to determine the clinical relevance of p-S6RP as an intragraft biomarker in relation to allograft rejection, function, and survival. Lastly, we sought to evaluate which intragraft biomarker, CD20, CD138, or p-S6RP had the most deleterious impact on allograft survival.

Materials and Methods

We studied 46 renal transplant biopsies from 32 pediatric patients who received kidney transplants at the Mattel Children's Hospital at UCLA. All biopsies were performed between November 2001 to November 2004 for the clinical suspicion of rejection. During this time, a total number of 97 pediatric transplants were performed at our center; 55 were from deceased donors and 42 were living donor transplants. Biopsies were included in our study if they had been stained for C4d at the time of biopsy and if there was archived tissue available for IHC staining. For all patients, the immunosuppressive regimen consisted of daclizumab induction and maintenance prednisone, mycophenolate mofetil, and either tacrolimus or cyclosporine. Non-adherence based upon self-report was assessed for each patient at every clinic visit. Non-adherence based upon drug levels was not used because tacrolimus trough and cyclosporine peak levels could not be compared. This study was approved by the UCLA Institutional Review Board.

Biopsy results were classified by Banff 2005 updated criteria as no rejection, ACR, or AMR (15–24). Additionally, the presence or absence of interstitial fibrosis and tubular atrophy (IFTA) was documented (24). Creatinine clearance (CrCl) was calculated using the Schwartz formula for each patient at the time of biopsy and followed for 2 years post-biopsy (25). Patient graft status and treatment response was followed post-biopsy and documented for graft survival or failure using the cut-off date of December 31, 2005. Since there were no patient deaths during the observation period, we assigned the date of graft failure as the date on which patients started dialysis.

Histopathology

IHC staining for C4d, CD20, CD138, and pS6RP was performed on a biopsy specimen from each patient. At the time of biopsy, C4d was prepared by staining 1.5 µm frozen sections with mouse anti-complement C4d from Quidel (San Diego, CA), using a DAKO autostainer. CD20 and CD138 stains were prepared from archived biopsy tissue for light microscopy in paraffin blocks, sectioned into 4 µm slices, and manually stained, using mouse anti-CD20 and anti-CD138 monoclonal antibody from DakoCytomation (Carpenteria, CA). P-S6RP stains were deparaffinized in xylene and rehydrated in graded alcohols. Antigen recovery was performed by placing tissue sections in a steamer with 10 mM sodium citrate buffer (pH 6.0) for 25 min. Endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxidase in methanol for 15 min. Sections were then blocked for 30 min at room temperature with 10% normal goat serum (NGS) diluted in PBS. Phospho-S6 ribosomal protein (Serine^{235/236}) antibody (Cell Signaling Technology) was diluted 1/50 in 3% NGS and 100 µL was added to each section. The primary antibody was incubated overnight at 4°C. The secondary antibody, a biotinylated goat anti-rabbit IgG (Vector) diluted 1:200 in 3% NGS was incubated for 40 min at room temperature. After three washes in PBS, sections were incubated for 30 min with horseradish peroxidase avidin D (HRP, Vector) diluted 1:1000 with PBS. After three 5-min washes of PBS, the sections were developed with DAB kit (Vector). Slides were counterstained with dilute hematoxylin, rinsed with ammonia and then with tap water. Sections were dehydrated with graded ethanol, cleared in xylene and then cover slipped.

For CD20+ and CD138+ determinations, entire cores were scanned by 2 independent observers (EWT and WDW). The scoring was first performed by a single blinded observer (EWT) and separately validated with a second blinded observer (WDW). The readings were compared with weighted kappa showing excellent agreement 0.8 ($p < 0.001$). Areas of cortical scarring were discounted but nodular lymphoid aggregates not associated with obvious scarring were included. The number of CD20+ and CD138+ cells was counted per high power field (hpf) under 400× light microscopy for each specimen. The number of high-power fields in the renal cortex was enumerated per biopsy. Then mean CD20+ and CD138+ cell density per high-power field were calculated for each core. Positive staining for p-S6RP was scored on scale 0–4 (0= no staining, 1= rare staining, 2=focal staining, 3=multifocal staining, 4=diffuse staining). A score of 3 or greater was considered positive and renal biopsies were scored by two blinded observers (14). C4d was read as positive if ≥50% of the peritubular capillaries stained strongly for C4d.

Statistical analysis

Because the threshold for CD138 positivity was unknown, the results of the patient samples were evenly distributed into statistical tertiles by CD138+ counts 0–1, 2–5, and 6 or greater cells/hpf, with 0–1 cells/hpf being the lowest tertile (3). Patients with CD138+ 0–1 and 2–5 cells/hpf were analyzed separately and then grouped together since they had very similar graft rejection and survival values. Patient demographics were analyzed in both CD138 negative (0–5 cell/hpf) and CD138 positive (≥6 cells/hpf) groups with Fisher's exact and

Wilcoxon rank-sum tests to detect differences. Kaplan-Meier curves were analyzed with a Cox model to compare survival rates based upon the number of CD20+ cells/hpf, CD138+ cells/hpf, and +p-S6RP cells. Wald's test was used to determine the p-value for the scatter plot, showing the relationship between log CD138 count and CrCl. A multivariate model, followed by backward stepwise selection, was used to determine whether IFTA and length of time from transplant to biopsy influenced graft survival and CrCl independent of the presence of CD138+ cells. Differences between CD138+ lymphocytes and p-S6RP and ACR versus AMR were analyzed with Wilcoxon rank-sum test. ROC analysis was performed to optimize the relationship between CD138+ lymphocytes and ACR. All analyses were performed with Stata (V 9.0, College Station, TX). All *P* values were two sided. A *P* value less than 0.05 was considered statistically significant.

Results

Table 1 shows patient demographics in relation to CD138+ cells in the biopsies. Non-adherence was significantly higher in the CD138 positive (≥ 6 cells/hpf) group compared to the CD138 negative (0–5 cells/hpf) group. Additionally, CD138 positive patients tended to be biopsied later post transplant, at a median of 29 months while CD138 negative patients tended to have been biopsied earlier, at a median of 13 months. While this difference did not reach statistical significance, it was noted here because it may give some circumstantial information when considering the implications of CD138+ cell infiltrates.

Of the forty-six biopsy specimens that were assessed, thirty-one had no histological evidence of rejection; ten had ACR only; one had AMR only; and four had both ACR and AMR (Table 2). When present, the ACR specimens were scored as either Banff I or II. Table 2 shows the breakdown of CD20, CD138, and p-S6RP in association with ACR, AMR, and no rejection. Fourteen biopsies showed IFTA. IFTA occurred in nine patients without rejection. Of the five patients with IFTA and rejection, three had ACR and two had both ACR and AMR. There was no significant predilection for IFTA in association with any positive IHC staining feature (CD20, CD138 and p-S6RP) or with any histological type of rejection.

Of the ten biopsies with ACR only, nine had concomitant intragraft CD20+ and CD138+ cells. Five of the nine stained positive for p-S6RP (Table 2). Fourteen biopsies were performed in eleven patients with ACR and intragraft B cells, including the four with concomitant AMR. Nine of eleven patients were CD138 positive on their initial biopsy. Two of eleven patients converted to CD138 positivity on subsequent biopsies. Table 3 shows allograft status and treatment response for CD138+ positive patients. Overall, ten of eleven patients were steroid-resistant and lost their allografts within 3 years of the biopsy. Additionally four of the ten patients were also resistant to Thymoglobulin, IVIG, or plasmapheresis. Three of ten patients had repeat biopsies and CD138 positivity did not improve with treatment. Only one of eleven patients was steroid-responsive and had full allograft recovery.

Figure 1 illustrates Kaplan-Meier kidney graft survival curves from the time of initial biopsy as a function of CD138+ lymphocyte count for all patients ($n=32$). Patients with ≥ 6 CD138+ cells/hpf had a significant 3.4-fold greater risk of graft loss with a hazard ratio (HR) of 3.4 (95% CI 1.3, 9.2) when compared with those with 0–5 cells/hpf ($p=0.016$). In the full multivariate model, before variable selection, IFTA and time from transplant to biopsy did not have a significant effect on graft survival beyond biopsy. Stepwise selection process eliminated both variables leaving the model shown in Figure 1 ($p=0.016$).

As shown in Figure 1, there was an immediate early separation of graft outcome between patients with 0–5 and ≥ 6 CD138+ cells/hpf post-biopsy, suggesting that the rejection episodes containing ≥ 6 CD138+ B cells had important acute clinical characteristics that impacted long term outcome. Figure 2 depicts the relationship between CrCl and CD138+ counts at the time of biopsy. Biopsies with higher CD138+ counts, shown as $\log(\text{CD138+})$, had worse graft function than those that had fewer counts ($R^2 = 0.22$, $p = 0.001$). Using multivariate analysis, IFTA and length of time from transplant to biopsy were insignificant in affecting kidney function at the time of rejection episode. Furthermore, CD138+ plasma cells were significantly correlated with ACR ($p = 0.004$), but not with AMR ($p = 0.68$). However, the low number of AMR in this study can not eliminate the possibility of finding an association between CD138+ plasma cells and AMR in a larger study. ROC analysis confirmed a cut-off point of ≥ 6 CD138+ cells/hpf as strongly associated with ACR with 89% sensitivity, 83% specificity, correctly classifying 84% and comprising a total ROC area 0.92 (95% CI 0.8,1)(Figure 3).

Furthermore, the CD138+ cells were often present in small clusters or in association with lymphoid aggregates (Figure 4). We found that p-S6RP stained a subset of interstitial CD138+ plasma cells in renal biopsies (Figure 4). P-S6RP did not stain peritubular capillaries, glomerular endothelial cells, arterioles, or lymphocytes. Staining for p-S6RP of plasma cells was significantly associated with C4d-negative ACR ($p < 0.03$), but not associated with C4d-positive AMR ($p = 0.22$). Again, the low number of AMR in this study can not eliminate the possibility of finding an association between +p-S6RP plasma cells and AMR in a larger study. Although some patients with no rejection or IFTA had CD20, CD138, and/ or p-S6RP within their biopsies, these associations were not statistically significant. Intra-graft staining for CMV, EBV, BK, and parvovirus was not detected in any patients with rejection.

To determine which biomarker, CD20, CD138, or p-S6RP had the most deleterious impact on allograft survival, Kaplan Meier survival analysis with cox modeling was performed. Due to small numbers, p-S6RP alone was not found to significantly impact graft survival. Patients with both intra-graft CD20+ and CD138+ cells had a significant 1.5 fold greater risk of graft loss with a HR of 1.5 (95% CI 1.1,2.2) when compared to those with no B cells or either CD20+ or CD138+ cells ($p = 0.01$)(Figure 5).

Discussion

In recent years, intra-graft B lymphocytes have been recognized for their possible role in allograft rejection. Most studies dealt solely with intra-graft CD20+ cells. Our current study identifies the novel association between intra-graft metabolically active CD138+ cells, ACR, and poor graft outcome. We found that similar to biopsies with higher CD20+ counts, biopsies with higher CD138+ plasma cells, shown as $\log(\text{CD138+})$, and p-S6RP staining had poorer graft function at the time of biopsy ($R^2 = 0.22$, $p = 0.001$)(3). These metabolically active CD138+ plasma cells coexisted with CD20+ cells and together had a more deleterious impact on graft survival.

It is known that B lymphocytes can differentiate into alloantibody producing CD138+ plasma cells. Xu et al suggested that plasma cells could infiltrate a rejecting kidney and mediate humoral rejection through local antibody secretion (9). Charney et al described plasma-cell rich rejections that were associated with local secretion of IgG and prone to graft failure (5). CD20+ cells, through CD38+ plasmablasts, can terminally differentiate into CD138+ plasma cells, which secrete alloantibody (12–13). In our study p-S6RP staining of interstitial CD138+ plasma cells was highly correlated with ACR and not AMR, but the number of AMR cases was too few to exclude an association. These CD138+ plasma cells

were metabolically active and could be secreting alloantibody. Interestingly, all five cases of AMR were associated with intra-graft B cells and three of five had positive p-S6Rp staining. This may represent an additional presence of an antibody-mediated phenomenon in the setting of ACR. Zarkhin et al also found evidence that at least a proportion of invading B lymphocytes expressed CD38 and appeared to mediate AMR that coexisted with CD20+ infiltrates (12). However, Zarkhin et al could not evaluate intra-graft CD138+ cells due to inadequate staining techniques (12). Additionally, Xu and Zarkhin studies used semi-quantitative methods to identify CD38+ plasmablasts or plasma cells (9,12). A larger study examining local antibody-secretion, p-S6RP, and intra-graft B cells should be performed to explore the utility of p-S6RP as a functional marker for antibody secretion.

The technique to enumerate CD138+ cells in our study was labor intensive. Each reading was validated by two independent observers with excellent agreement. In this way, we were able to establish uniformity and consistency of quantification. Additionally, by meticulous reproducible examination, we were able to clearly identify CD138+ cells from nonspecific tubular epithelial cell staining and areas of scarring. Plasma cells often clustered together in small groups and were not evenly distributed throughout the biopsy. Therefore, the entire biopsy was evaluated and an average concentration determined.

The lack of uniformity in quantifying plasma cells may contribute to some of the confusion in other studies about the relevance of CD20+ and CD138+ cellular infiltrates and poor graft outcome. Some groups have used semi-quantitative techniques (9,12) while we have used strictly quantitative techniques (3). Moreover, the numerical threshold described in other studies to define CD138 positivity vary widely; in all prior studies, the threshold was ten to fifty times higher than we found in our study. Such differences will result in vastly different sensitivities between studies. This variability is likely to be another reason why various groups reach divergent opinions about the importance of intra-graft CD138+ B cells, as well as CD20+ B cells (26–27).

While most studies evaluated renal transplant biopsy tissue, some included explants from rejecting kidneys for their assessments (1,9,12). Evaluation of such end-stage tissue is often obtained from transplants for which immunosuppression has been halted for an important period of time. In addition, it is impossible to learn about time to graft failure from explanted tissue. Thus, there continues to be disagreement about the role and mechanism of intra-graft B cells in rejecting allografts (12,26–29).

Using our quantitative IHC staining techniques from biopsy tissue, not explants, we found a numerical threshold of ≥ 6 CD138+ plasma cells/hpf to be associated with ACR and a 3.4 fold increased risk of graft failure (3). We found that a combination of intra-graft CD138+ plasma cells and CD20+ lymphocytes had a more deleterious impact on allograft survival than either marker alone. Additionally, patients with CD138 positivity had significantly more self-reported nonadherence and their rejection episodes were refractory to steroids, IVIG, thymoglobulin and plasmapheresis. Scattered CD20+ B cells left untreated or inadequately immunosuppressed may eventually develop into antibody-producing CD138+ plasma cells, representing an additional antibody-mediated influence in ACR.

Alternatively, plasma cells may home to the graft due to secretion of B-cell activating factor (BAFF) and/or APRIL (a proliferation-induced ligand) by inflammatory cells such as neutrophils and macrophages. BAFF and APRIL are members of the TNF family and contribute to survival of bone marrow plasma cells. Recent studies have shown up-regulation of BAFF in peripheral blood of patients with AMR (30–31). CD138+ plasma cells could still be associated with AMR that was missed in our study due to small sample size and inability to examine DSA, BAFF, or APRIL.

Sarwal's group found a subset of intrarenal CD38+ plasmablasts, coexisting but independent of CD20 infiltrates, that were associated with markers of AMR including, donor specific antibody (DSA) and C4d (12). They found no correlation with CD138+ plasma cells and acute rejection. They note, however, that there were problems with quantification of intra-graft CD138 cells which could have been due to inadequate staining technique (12). Therefore, the presence of CD138+ plasma cells with CD20+ B cells could represent a separate form of AMR coexisting with ACR.

Lastly, since patients with IFTA and no rejection trended toward having intra-graft B cells, their presence may be simply a marker of chronic damage and an aging allograft (27). Additionally, patients with intra-graft CD138+ plasma cells tended to be biopsied later than those without, possibly reflecting severe immunological attack and inflammation (27). However, it is important to realize that this "time effect" was not statistically significant, so that this association with putative inflammation is at best a hypothetical relationship. Larger, prospective biopsy studies in protocol biopsies and rejecting allografts, excluding explants will be needed to further explore the association and role of metabolically active plasma cells in allograft rejection. Our findings suggest that such studies should include T cell subsets, DSA, BAFF, APRIL, CD38+ plasmablasts, CD138+ plasma cells, p-S6RP, local antibody secretion, CD20+ B lymphocytes, and nonadherence measures.

Our study may argue for an anti-B cell agent in the treatment of transplant rejection where IHC of the graft biopsy shows intra-graft CD20+ and/or CD138+ B cells. Current histological classifications of rejection do not always correlate with responsiveness to conventional treatment and may not appropriately identify B-cell rich rejections, delaying targeted anti-B cell therapy. Rituximab may be effective in targeting CD20+ infiltrates associated with ACR that are refractory to steroids and thymoglobulin, but is not an ideal choice for CD138+ B-cell infiltrates because mature plasma cells lose expression of the CD20 antigen (32–34). Meehan et al found that rejections marked by plasma cell rich infiltrates were refractory to steroids, tacrolimus and anti-T cell antibodies (6). Only one case series involving two patients has examined the efficacy of treating plasma-cell rich rejection (35). In this report, Adrogué et al found that plasma-cell rich rejections responded to IVIG with improvement of graft function and prevention of graft loss (35). The role of IVIG in CD138+ rich rejections may be to modulate antibody production and interfere with the maturation of peripheral B cells into plasma cells (35–36). Additionally, plasmapheresis may enhance antibody removal (37). Recently, Bortezomib, a proteasomal inhibitor which targets plasma cells, has been an effective treatment for adult renal transplant patients with both ACR and AMR (38).

Intra-graft, metabolically active CD 138+ cells may represent an additional antibody-mediated phenomenon in the setting of ACR. More importantly, metabolically active CD138+ cells together with CD20+ cells are associated with poorer graft survival than either marker alone. It is reasonable to suggest that immunohistochemical staining for intra-graft B cells can potentially be utilized in conjunction with other known markers to prospectively identify severe, refractory rejection episodes that might benefit from therapies which target B cells, such as Rituximab, IVIG, plasmapheresis or Bortezomib.

Abbreviations

ACR	acute cellular rejection
AMR	antibody-mediated rejection
CrCl	creatinine clearance

Hpf	high power field
HR	hazard ratio
IFTA	interstitial fibrosis and tubular atrophy
IHC	immunohistochemistry
p-S6RP	phosphorylation of S6 ribosomal protein

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Figure 1. Kaplan-Meier graft survival grouped by CD138+ counts post-biopsy. Patients included (n=32).

Figure 2.
Relationship between intragraft CD138+ counts and CrCl (ml/min/1.73m²).

Figure 3.
ROC curve for intragraft CD138+ plasma cells associated with ACR

Figure 4.

A) Biopsy with CD138+ plasma cells associated with ACR. Arrows highlight clusters of CD138 positive plasma cells (CD138 immunoperoxidase stain; original magnification 400 $\times$).

B) Biopsy with ACR and positive S-6RP staining in interstitial plasma cells. Arrow highlights plasma cell with eccentric nucleus and perinuclear halo (s6RP immunoperoxidase stain; original magnification 400 $\times$).

Figure 5. Kaplan-Meier graft survival grouped by no intra-graft biomarkers, either CD20 or CD138, and both CD20 & CD138. Patients included (n=32).

Table 1

Demographics (Patients, n= 32)

	CD138+ cells/hpf		p
	0–5 (n=23)	≥6 (n=9)	
Median Age (range)	15 (5–22)	14 (4–21)	0.70
Male	19 (82.6%)	4 (44.4%)	0.08
White	21 (91.3%)	8 (88.9%)	0.99
Primary	19 (82.6%)	8 (88.9%)	0.99
Median Months to 1 st biopsy (range)	13(0–78)	29 (5–43)	0.16
Living Related	13 (56.5%)	4 (44.4%)	0.70
Non-adherence	2 (8.7%)	7 (77.8%)	0.003
Mean PRA%			
Class I	8%	3.6%	0.85
Class II	8.8%	12.3%	0.86
HLA Mismatch			
0	3 (13%)	0 (0%)	0.28
1–2	8 (34.8%)	1 (11.1%)	
3–4	7 (30.4%)	6 (66.7%)	
5–6	5 (21.7%)	2 (22.2%)	

Table 2
CD20, CD138 and pS6RP staining in renal biopsies with ACR, AMR, and no rejection (N=46)

	Biomarkers						Rejection Status Total
	CD20 Only	CD138 Only	CD20 CD138	CD20 CD138 pS6RP	CD20 pS6RP	Negative	
No Rejection	12	1	1	1	1	15	31
ACR	1		4	5			10
AMR	1						1
ACR + AMR	1			2	1		4
Biomarker Total	15	1	5	8	2	15	46

Table 3

Treatment and Graft Status post-biopsy for CD138 positive patients (n=11)

	Treatment			
	Steroids Alone	Steroids & Thymoglobulin	Steroids & IVIG	Steroids, IVIG & Plasmapheresis
Full Recovery	1			
Graft Loss within 3 Months	2		1	
Graft Loss within 1 Year		1		1
Graft Loss within 2 Years	2		1	
Graft Loss within 3 Years	2			