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Differential gene expression pattern in biopsies with renal allograft pyelonephritis and allograft rejection

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Abstract

Differentiating acute pyelonephritis (APN) from acute rejection (AR) in renal allograft biopsies can sometimes be difficult because of overlapping clinical and histologic features, lack of positive urine cultures, and variable response to antibiotics. We wanted to study differential gene expression between AR and APN using biopsy tissue. Thirty-three biopsies were analyzed using NanoString multiplex platform and PCR (6 transplant baseline biopsies, 8 AR, 15 APN [8 culture positive, 7 culture negative], and 4 native pyelonephritis [NP]). Additional 22 biopsies were tested by PCR to validate the results. *CXCL*9, *CXCL*10, *CXCL*11, and *IDO*1 were the top differentially expressed genes, upregulated in AR. *Lactoferrin (LTF)* and *CXCL*1 were higher in APN and NP. No statistically significant difference in transcript levels was seen between culture-positive and culture-negative APN biopsies. Comparing the overall mRNA signature using Ingenuity pathway analysis, interferon-gamma emerged as the dominant upstream regulator in AR and allograft APN, but not in NP (which clustered separately). Our study suggests that chemokine pathways in graft APN may differ from NP and in fact resemble AR, due to a component of alloreactivity, resulting in variable response to antibiotic treatment. Therefore, cautious addition of steroids might help in resistant cases of graft APN.

Keywords

acute pyelonephritis; acute rejection; CXCL1; CXCL10; CXCL11; renal allograft

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CONFLICT OF INTEREST

The authors of this manuscript have no conflicts of interest to disclose.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

1 | INTRODUCTION

Urinary tract infection complicated by acute pyelonephritis (APN) is a known complication in renal transplant recipients, especially in the early post-transplant period.[1–4] Differentiating between renal allograft APN and acute rejection (AR) based on clinical symptoms alone can be difficult. The classic tetrad of costovertebral angle tenderness, fever, elevated white blood cell count, positive urine culture used to diagnose acute pyelonephritis in the native kidney, is frequently not useful for renal allografts. Fevers and leukocytosis can be attenuated by immunosuppressive therapy. Graft tenderness may be present even in acute rejection. Urine cultures can be frequently negative in renal allograft patients with APN, because these immunosuppressed patients receive long-term prophylactic antibiotics. Lack of positive urine cultures has also been reported in a high percentage of patients with native kidney pyelonephritis in a large case series of 223 patients by Rollino et al.[5] in which they report that only 23.5% of their patients had positive urine cultures. In our previous study[6] of 49 kidney transplant recipients with APN in first two years post-transplant, we showed that only 32% (16/49) had concomitant positive urine cultures at biopsy, and in 8 of these 16 patients, colony count was less than 10^5 CFU/mL. In 14 of 49 patients, positive urine culture did not coincide with the biopsy (had positive culture beyond 10 days before or after biopsy), and in 19 of 49 patients, urine cultures were negative. Urinalysis findings in patients with APN and AR can also overlap and may not be specific.

Renal allograft biopsy therefore plays an important role in diagnosis. Even on biopsy, APN vs AR can pose a differential diagnostic dilemma.[7,8] Both conditions are associated with tubulointerstitial inflammation.[9,10] Characteristic histologic features described for APN such as tubular microabscesses are usually focal in distribution and therefore may not get sampled in the biopsy specimen. Tubular apoptotic cell debris accompanying severe acute tubular necrosis (ATN) can mimic tubular microabscesses. Neutrophil-rich inflammatory infiltrate usually associated with infection and can occasionally be seen in the inflammatory infiltrate of AR as well. Although there are well-defined Banff criteria for the diagnosis and grading of AR (based on interstitial inflammation and tubulitis),[11] tubulitis also tends to be focal. All these factors can complicate diagnosis of APN in renal allografts. APN in renal allografts can potentially affect long-term graft function by causing focal scars and reduction in functional parenchyma;[3,4] therefore, early diagnosis and treatment are important.

Ours is a preliminary exploratory study aimed at assessing differential gene expression pattern of allograft rejection (AR) and allograft pyelonephritis (APN), using archived biopsy material. We included small number of biopsies of native kidney pyelonephritis as a comparison group. Additionally, we studied patients whose biopsy shows features of APN but have negative urine culture results. These biopsies pose a diagnostic dilemma, and therefore, our second aim was to see whether these biopsies show gene expression pattern of infection or rejection. The hope was to be able to avoid unnecessary immunosuppression in such patients. Validation of gene expression by PCR, immunostaining for selected genes, and in silico functional pathway analysis using Ingenuity software were also performed.

2 | METHODS

Institutional review board (IRB) approval was obtained (Protocol number 2011H0364, 2012H0382).

2.1 | Patient cohort and biopsy samples for gene expression study by Nanostring assay

This is a retrospective study using biopsy tissue remaining in the paraffin blocks after routine pathology workup. We previously published a case series describing biopsy findings and urine culture results in 49 renal allograft recipients with APN.[6] The histologic criteria that were used for biopsy diagnosis of APN include—interstitial inflammation with predominance of neutrophils, tubular infiltration with neutrophils, and formation of tubular microabscesses.[9,10] For the present study, we used the APN biopsies from the same cohort. Biopsies with AR, native pyelonephritis (NP), and living donor baseline post-perfusion transplant biopsies were used for comparison and were selected from our pathology archival material. Selection was mainly based on adequate tissue availability for RNA isolation. All the biopsies for mRNA testing were carefully reviewed (by AS and TN). We did not include biopsies with moderate-to-extensive chronic allograft injury, because that may alter the mRNA profile.

We had a total of 33 biopsy samples, depicted in Tables 1 and 2. These were divided into four groups as shown below:

- Group I—Six post-perfusion baseline transplant biopsies designated as “B” for baseline (B2, B3, B4, B5, B7, and B8), serving as normal kidney controls.
- Group II—Eight allograft biopsies with unequivocal acute rejection designated as “R” for rejection (R1, R2, R3, R4, R5, R6, R7, and R8). These were performed within one year post-transplant (Banff Grade IB to Banff Grade IIA). These were predominantly acute cellular rejections. One of the biopsies had mixed cellular and humoral rejection.
- Group III—Three native kidney biopsies and one native kidney nephrectomy specimen with acute pyelonephritis designated as “NP” for native kidney pyelonephritis (NP1, NP2, NP3 and NP4).
- Group IV—Fifteen allograft biopsies with histologic features of acute pyelonephritis (APN) designated as “I” for infection (I1 to I15).

Among the 15 biopsies with diagnosis of APN (Table 2), eight had concomitant positive urine cultures (I1, I2, I3, I5, I6, I13, I14, and I15). The remaining 7 of 15 biopsies (I4, I7, I8, I9, I10, I11, and I12) had negative urine culture results despite the presence of histologic features of APN on the biopsy and supportive urinalysis findings. Such cases pose a diagnostic dilemma for a pathologist. We included them in this study to see whether their gene expression pattern resembled that of infection or rejection. As described in our previous study,[6] “concomitant urine cultures” was defined as within 10 days of the date of biopsy (preceding or following biopsy). This is an arbitrary cutoff, used for the purpose of this study. The urinalysis findings in all these patients are shown in Table 3.

All renal allograft recipients at our institution receive trimethoprim/sulfamethoxazole (Bactrim) prophylaxis along with nystatin and ganciclovir. Patients with biopsy diagnosis of APN received additional antimicrobial treatment to treat the episode of pyelonephritis. Ciprofloxacin is the drug most frequently used, but additional antibiotics are given depending on antibiotic sensitivity results. Induction immunosuppression consisted of high-dose solumedrol or prednisone taper over the first 5–8 days along with four to five doses of antithymocyte globulin (ATG).

2.2 | Biopsy samples for validation of Nanostring results by PCR

The 33 biopsies described in Tables 1, 2 and additional 22 samples (shown in Table 4) were subsequently tested by quantitative PCR for validation of the gene expression results obtained by Nanostring. These additional 22 samples also included biopsies with APN, AR, NP and living donor baseline biopsies as normal controls.

2.2.1 | RNA isolation—Formalin-fixed paraffin-embedded (FFPE) tissue sections from the allograft and native kidney biopsies were deparaffinized using xylene, and total RNA was prepared using the RNeasy FFPE kit (QIAGEN, Valencia, CA, USA). Total RNA quality and quantity were assessed by Nanodrop spectrophotometry (ThermoFisher Scientific, Waltham, MA, USA).

2.2.2 | mRNA NanoString assay—The digital multiplexed NanoString nCounter Gx Human Immunology Kit (NanoString Technologies Seattle, WA) with a code set of 513 genes was used. Major classes of cytokines, their receptors, chemokine ligands and receptors, interferons and their receptors, the TNF receptor superfamily, and the KIR family genes are represented. Details are provided in supplemental data Table S1. It allows 12 samples per cartridge. Therefore, 12 of 33 samples were tested on this kit (B2, B3, B4, I1, I2, I3, I6, R1, R2, R3, R4, R5). However, during the course of this study, NanoString updated this kit to nCounter Human Immunology V2 panel which retained the original genes on the panel but had an expanded code set of 581 genes. Therefore, the remaining 21 samples were tested on the newer expanded V2 kit. Comparison list of genes on each panel is provided in Supplemental data (Table S2) Three of the 12 initial samples were rerun on the V2 kit, and we confirmed reproducibility of the results (Supplemental data Table S3).

2.2.3 | Normalization and statistical analysis—Normalization and data analysis were performed separately for the 12 samples that used the GX Immunology kit and the 24 samples that used the updated V2 Immunology kit. Technical normalization was performed using positive controls to adjust counts for each gene target in the assay. Data after technical normalization were log2-transformed first. To reduce the false-positive rate, genes of over 80% samples with an expression level 2 SD below the mean expression of the negative controls were excluded from further analysis. Quartile normalization was used for normalization across samples. Overall 510 genes from GX kit and 572 genes from the V2 kit were analyzed for differential expression, respectively. A linear model was used to compare the gene expression between APN, NP, AR, and baseline. To improve the estimates of variability and statistical tests for differential expression, variance smoothing methods were

employed.[12] *P* values were adjusted by controlling the mean number of false positives at 5 (out of ~500 genes) (i.e., α=0.01).[13]

2.2.4 | Construction of heat maps and principle component analysis (PCA)— Heat maps were created to show median-centered expression of selected genes and clustering pattern among the biopsies, using Cluster 3.0 and JavaTreeView software algorithms. Using the normalized mRNAs expression values, PCA plots were constructed to show overall clustering pattern of the groups.

2.2.5 | Quantitative PCR validation—Total RNA from the FFPE samples were extracted as described previously[4] and reversed-transcribed into cDNA using SuperScript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) (Supplemental Methods).

2.2.6 | Immunohistochemistry—Three biopsies with APN (I1, I15, and I7) and three biopsies with acute rejection (from our biopsy archives) were stained for CXCL10 and CXCL2. Formalin-fixed paraffin-embedded (FFPE) tissue sections were immunostained using a three-step avidin–biotin complex peroxidase system (Vectastain Elite ABC Vector Laboratories, Burlingame, CA, USA).

2.2.7 | Ingenuity pathway analysis—Data were analyzed through the use of QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN, Redwood City, CA, USA, www.qiagen.com/ingenuity).[13] Top expressed genes which met both criteria of at least 1.5-fold change and *P*-value .05 were used for IPA. We identified upstream regulators that are predicted to be activated or inhibited based on activation *z*-score. The *z*-score algorithm is designed to produce either a prediction of activation or inhibition (or no prediction) as well as to reduce the chance that random data will generate significant predictions. The software does this using information in the Ingenuity® Knowledge Base to explain observed changes in expression in the various groups in the dataset. We then ran a comparison analysis of the upstream regulatory molecules for the disease groups (AR, APN, and NP). Significant molecules are sorted by score −log(*P*-value) from the Fisher's exact test across all observations. The predicted activation (or inhibition) state of these molecules is determined by *z*-score algorithm calculated by IPA. Orange color is used to indicate activation and blue color for inhibition.

3 | RESULTS

Clinicopathologic findings of the patients are shown in Tables 1 and 2. Urinalysis findings are shown in Table 3. The additional 22 cases used for validation are shown in Table 4.

3.1 | Differential gene expression by NanoString multiplexed assay

Top differentially expressed genes between APN, AR, and NP are shown in Table 5 ($P<.05$). We found *CXCL9, CXCL10, CXCL11, and IDO1* to be the most highly upregulated genes in AR (up to 40-, 39- and 25-fold, respectively, above normal) and showing largest fold difference compared to APN and NP. Other genes such as *SLAMF7, GBP5, IRF1, TIGIT, Granzyme B, CCL5, and CCL19* show only mild upregulation in AR. The only gene showing statistically significant upregulation in APN and NP as compared to AR was *LTF*

(lactoferrin). *CXCL1* was higher in APN and NP as well but did not show statistical significance. These comparisons are shown in the form of heat maps in Fig. 1A. Top differentially expressed genes between APN and NP are shown in Fig. 1B. The genes showing largest fold difference between allograft APN and NP were *CXCL9, CXCL10, CXCL11, IDO1, HLA-DRB1* and *HLA-DQA1, HLA-DQB1, GBP5, IRF1*. These genes are expressed higher in allograft APN (albeit not as much as in AR) compared to NP. Other genes showing higher expression in APN are those involved in T-cell costimulation including *KLRK1, KLRC2, LILRB1*. *CXCL1* shows statistically significant upregulation in NP over normal (2.8-fold, P=.008) but not in APN.

We performed the same analysis after separating out the culture negative cases from the APN group (Table 6). We did not find any genes showing statistically significant differences between culture-positive and culture-negative APN biopsies. The culture-negative APN biopsies also showed similar differences from AR.

3.2 | Validation by PCR

Eight of the top differentially expressed genes were validated using quantitative PCR. This was performed on all of the 33 biopsy samples as well as the 22 additional biopsies used for overall validation of the results (Fig. 2). APN culture-positive and culture-negative samples are shown separately. *CXCL10* and *CXCL11* are consistently higher in AR as compared to APN (both culture positive and culture negative) and NP. *CXCL1, CXCL2*, and *LTF* were statistically higher in APN (culture positive) compared to AR with similar trend in NP. Culture negative APN biopsies, however, did not show statistical difference in *CXCL1, CXCL2, and LTF* expression as compared to AR. Expression of *IDO1*, however, showed statistical difference only between culture-negative APN and AR. *CXCL9* did not show statistical differences as seen on NanoString. Similar to NanoString results, culture-positive and culture-negative APN did not show statistically significant differences for any of these genes. The P-values for these comparisons are shown in Table 7.

3.3 | Immunohistochemical staining

The three biopsies with AR showed diffuse staining for CXCL10 (Fig. 3A–C) but no staining for CXCL2 (Fig. 3D–F). In the three biopsies with APN, there was weak to no staining for CXCL10 (Fig. 3G–I) but strong staining for CXCL2 (Fig. 3J–L).

3.4 | Principle component analysis using NanoString data

Figure 4A shows the PCA plot of 12 samples run on the GX Immunology panel. These include 3 normal baselines, 4 culture-positive APN, and 5 AR. All four of the APN samples (I1, I2, I3, and I6) had positive urine culture results, and these patients recovered graft function with antibiotic treatment. These four APN biopsies did cluster separately from AR. Figure 4B shows PCA plot of the remaining 21 samples with all the groups (baselines, APN culture positive and negative, NP and AR). The normal baseline biopsies clustered together and the NP biopsies clustered together, distinct from allograft AR and APN. The AR and APN samples, however, showed overlap. The APN biopsies with positive and negative urine cultures also clearly showed overlapping gene expression as shown in Fig. 4C. No separate clustering was seen.

3.5 | Ingenuity Pathway Analysis—upstream regulators in acute rejection, allograft pyelonephritis, and native kidney pyelonephritis

Comparison of the major upstream regulators (with *z*-score greater than 1) between the disease groups is shown in Table 8. B-cell receptor (BCR) complex and the MAPK subfamilies—ERK1/2, MAPK1, appear to be active in native kidney pyelonephritis, in contrast to AR and allograft APN (culture-positive and culture-negative subgroups). IFN-γ is the major upstream regulatory molecule in AR along with IFN-α, IL-12 complex, IL-18, IL-27. APN shows activation of similar pathways as in AR and shows subtle differences from NP (Fig. 5A–F).

4 | DISCUSSION

Although biopsy examination remains the gold standard to diagnose allograft rejection, extensive efforts are in progress to develop less-invasive tools using blood[14–21] and urine[22–27] biomarkers. Interferon-gamma-controlled chemokines (CXCL9, CXCL10) and cytotoxic T lymphocyte granule contents (granzyme B, perforin) have been shown to be highly expressed in acute rejection. *CXCL10* has been shown to be a candidate urinary biomarker for acute rejection.[20–23,28–30] CXCL9, CXCL10, and CXCL11 are CXCR3 ligands and potent T-cell chemoattractants and are universally induced during cell-mediated immune responses.[31,32] However, no such markers have been identified specifically for graft pyelonephritis, which is a common differential diagnostic consideration in the early post-transplant period. Granzyme B mRNA levels in the urine were shown to be statistically different between UTI and AR.[25] However, gene expression in pyelonephritis (infection of the renal parenchyma) is likely to differ from that in lower urinary tract infections. In fact, our results did not show significant difference in expression of *Granzyme B* in the kidney between AR and APN. In some of the previous studies measuring chemokine levels in the urine of AR patients, the positive predictive values have been relatively low.[33] In the study by Hricik et al.,[33] using 2760 urinary supernatants, the PPV of CXCL9 to predict AR was 67.6%. Eliminating infectious etiologies improved the PPV of CXCL9 to 73.3% for AR. Also, the mean CXCL10 protein levels were found to be similar in patients with AR and infection. Therefore, it appears that there is likely to be significant overlap in gene expression between AR and APN which has not been addressed before. That is the focus of our study.

We confirm that CXCR3 ligands *CXCL9, CXCL10, CXCL11* show high expression in AR with large fold differences as compared to APN and NP. Our results support previous study results.[22–25] Additionally, we found significantly elevated IDO1 in AR (not been reported in previous studies). IDO1 is a metabolic enzyme which catalyzes the rate limiting step of tryptophan catabolism in plasmacytoid dendritic cells.[34,35] Other T-cell-associated genes (*TIGIT, SLAM7, IRF1, GNZB, GBP1*, and *GBP5*),[36–38] show only small fold difference between AR and APN and therefore may not be helpful as differentiating biomarkers. Only few genes on this NanoString panel show higher expression in APN as compared to AR. We identified *Lactoferrin* as one of them. *CXCL1* and *CXCL2* also showed higher expression in APN and NP by PCR. *CXCL1* and *CXCL2* are known to be neutrophil chemo-

attractants.[39,40] The fold differences, however, are small, and larger studies are needed to test their feasibility as potential biomarkers for graft pyelonephritis.

However, what we find interesting is that, although *CXCL10* and *CXCL11* expression is significantly elevated in AR compared to APN and NP, these chemokine genes also show significant difference in expression between graft APN and NP (native kidney pyelonephritis). They are found to be significantly higher in graft APN as compared to NP (also confirmed by PCR). Also, *CXCL9, IDO-1, Class II HLA* show higher expression in APN as compared to NP as seen on NanoString assay. Therefore, there are subtle differences in renal allograft pyelonephritis and native kidney pyelonephritis. This is better understood by studying the overall gene expression profiles of these groups with the help of principle component analysis (Fig. 4) and Ingenuity pathway analysis. It showed similarities between graft APN and AR and differences between graft pyelonephritis (APN) and native kidney pyelonephritis (NP).

The T-cell-dominant upstream regulatory molecules (IFN-γ, IFN-α, IL-18, IL-12)[37] are predicted to be activated in both AR and APN (culture positive and culture negative), but not NP. Conversely, B-cell receptor (BCR) complex and the MAPK subfamily—ERK1/2, are predicted to be active in native kidney pyelonephritis, but in allograft APN and AR, there is predicted inhibition of the ERK1/2 and MAPK1. With the p38 MAPK pathway, we found mild activation in graft APN but not in native kidney pyelonephritis. The four major MAPK subfamilies (JNKs, ERK1/2, p38MAPKs, and MAPK-1) are reported to have divergent roles in antimicrobial immune response.[41–44] Phagocytosis of bacteria activates ERK1/2 in human neutrophils and limits bacterial replication, but p38 activation was shown to have opposite effects.[45–48] Therefore, ineffective bacterial phagocytosis in APN resulting in lack of response to antibiotics in some cases may be speculated. Whether it is an effect of the immunosuppressive treatment is not known but is certainly a possibility. Also there is no predicted activation of TNF and NFκ-B in native kidney pyelonephritis as compared to graft APN and AR, probably suggesting a more controlled inflammatory reaction in NP as compared to AR and APN. Thus, the pathogenesis of allograft APN and native kidney pyelonephritis may not be exactly the same. The PCA plot (Fig. 4B) does show this overlap between APN and AR, but NP biopsies appear to cluster separately. Although two of the AR biopsies (R2 and R5) were associated with positive urine cultures, these were due to lower urinary tract infections, which is common in transplant patients. They are unlikely to interfere with gene expression analysis of the kidney tissue. Also R6 had mixed cellular and humoral rejection, but we have confirmed by statistical methods that the gene expression profiles of these three AR samples were close to or within the range of the other AR samples and by no way were they outliers. They are therefore unlikely to be the cause of the overlap seen between the APN and AR samples. Also, Rabant et al. recently published their findings showing similar interferon-gamma signature in antibody-mediated rejection.[49]

Based on these results, we propose that in allograft APN (in contrast to native kidney pyelonephritis), a combination of antimicrobial immune response and alloimmune response may be playing a role. Whether they occur simultaneously or one follows the other is difficult to postulate. It may have a temporal relation and may depend on the timing of the biopsy as to which component of the immune response is dominant and that may determine

the response to antibiotics. The possibility that infection can trigger a rejection in the graft has been postulated for a long time in older transplant literature.[50,51] Our previous study[6] showed that 10 of 30 patients with culture-positive APN had graft loss (six of them within one year post-biopsy) despite treatment with antibiotics. Four other patients showed improvement in graft function after adding steroids to their antibiotic treatment.

Toll-like receptors (TLRs), specifically TLR-4, is important in protection against ascending urinary tract bacterial infection.[52] TLRs have also been shown to be expressed by renal tubular epithelial cells and collecting duct cells in ascending urinary tract infection.[53] Our results did not show significantly elevated levels of TLR transcripts in APN or NP. However, the biopsy is only a snap shot and upregulation of receptor genes can be transitory. We did, however, find very high levels of gene expression for *lactoferrin (LTF)* in both APN and NP (but not in AR), and this may prove to be a good urinary biomarker to differentiate APN and AR. LTF is an iron-binding glycoprotein in secondary granules of polymorphonuclear leukocytes, found in various body secretions such as saliva, tears and milk. It has antimicrobial activity and interacts with molecules in the TLR4 pathway, such as CD14 and LPS-binding protein in macrophages.[54,55]

Other causes of allograft inflammation such as allergic interstitial nephritis and polyomavirus nephropathy were not investigated in the present study. Interstitial nephritis is rarely diagnosed in renal allograft especially in the early post-transplant period, and polyomavirus infection can be diagnosed by other modalities such as quantitative serum PCR and immunohistochemistry.

In summary, this is a preliminary exploratory study but it highlights two important issues about renal allograft pyelonephritis. We did find few genes with differential expression between graft APN and AR. These include *CXCL10, CXCL11, CXCL1, CXCL2*, and *LTF*. But we have to emphasize that the fold differences between graft APN and AR are small, leaving room for overlap. In fact, the overall gene expression pattern and pathway analysis reveal important similarities in the activated upstream molecules (especially interferon-gamma) between graft APN and AR, but not with native pyelonephritis. The other important issue highlighted in our study is that, a subset of allograft biopsies with histologic features of APN may have negative urine culture results and pose a diagnostic dilemma. These cases did not show any significant differences in gene expression from culture-positive APN biopsies. In fact, they show some overlap with both—APN and AR. From the practical point of view, we recommend that if transplant patients do not show improvement with antibiotics alone despite histologic features of APN, then a trial of steroids may be helpful. This is especially true if urine cultures are negative, and the chemokine profile shows a rejection pattern with high levels of interferon-γ-induced genes *CXCL10* and *CXCL11* and low *lactoferrin*. Although biomarkers may offer some hope, extensive validation studies are required before these can be used in clinical practice. This is an exploratory study with small sample numbers and it is possible that the gene expression markers identified may be non-specifically modulated by inflammation. Diagnosis and treatment of APN in renal allografts can be difficult. No single test (neither biopsy nor urine cultures nor biomarkers) can be used as a gold standard for diagnosis of APN in renal allografts or prediction of response to antibiotics. They have to be used in combination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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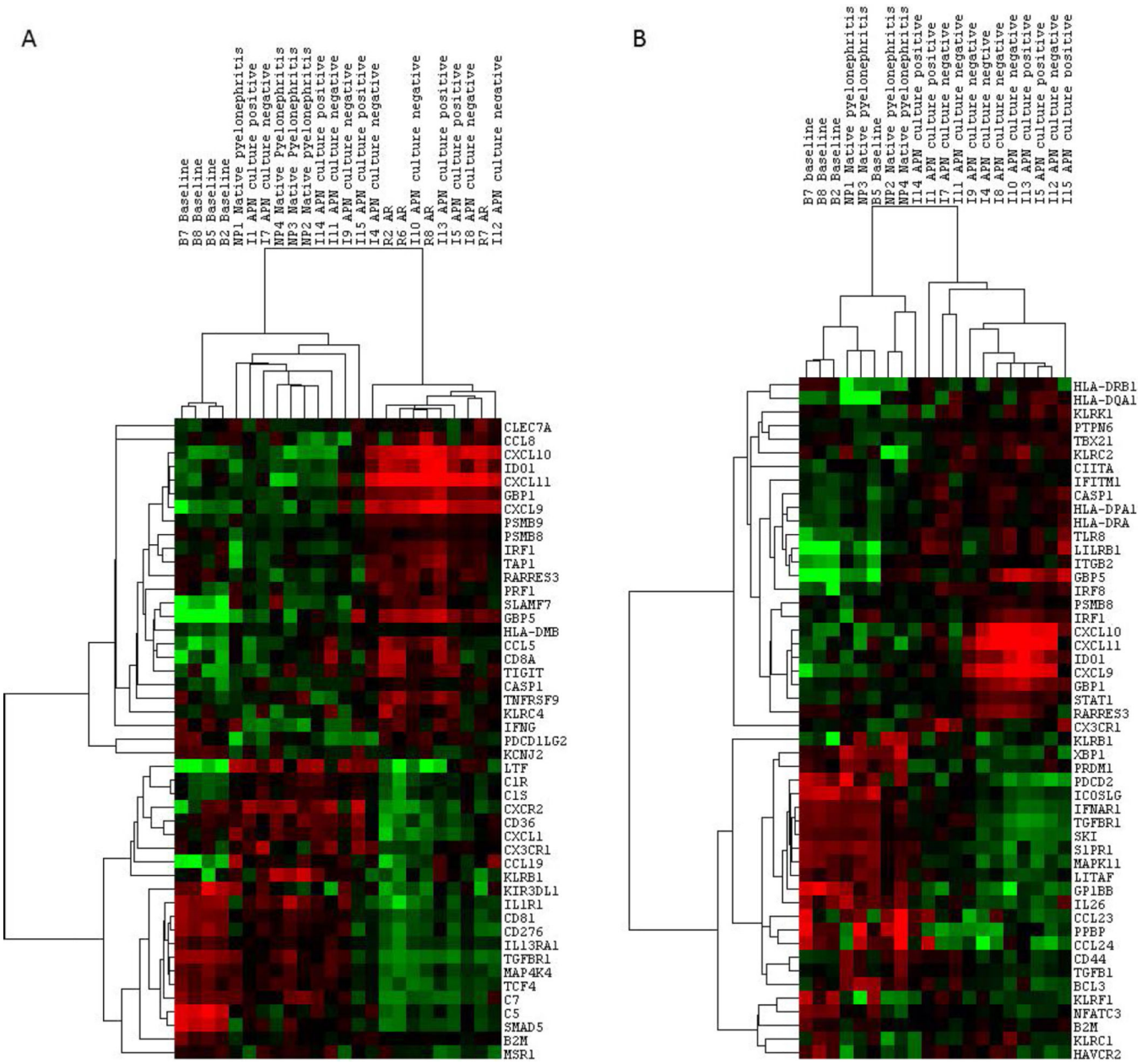


Figure 1.

(A) Unsupervised hierarchical cluster analysis showing median-centered expression of 47 differentially expressed genes between AR and APN by NanoString assay ($P<.05$). Each column represents a biopsy sample. Each row represents individual mRNA. The color in each cell reflects the relative level of expression of the corresponding mRNA. Increasing intensities of red mean higher expression, and increasing intensity of green means lower expression. All four groups (baselines, NP, APN, and AR biopsies are shown) run on the nCounter Human Immunology V2 panel are shown. The degree of relatedness is represented by the dendrogram at the top of the panel. There is good intragroup clustering among the baseline biopsies and the NP biopsies, but the APN biopsies show a spectrum. (B) Hierarchical cluster analysis showing differential gene expression between allograft APN

and NP ($P<.01$). *CXCL*9, *CXCL*10, *CXCL*11, *IDO*1 do show differential expression between APN and NP. Their expression in NP biopsies is much lower than that seen in APN biopsies. Thus, chemokine pattern in allograft APN does not exactly resemble native kidney pyelonephritis. APN, acute pyelonephritis; AR, acute rejection; CXCL, CXC chemokine ligand; NP, native kidney pyelonephritis

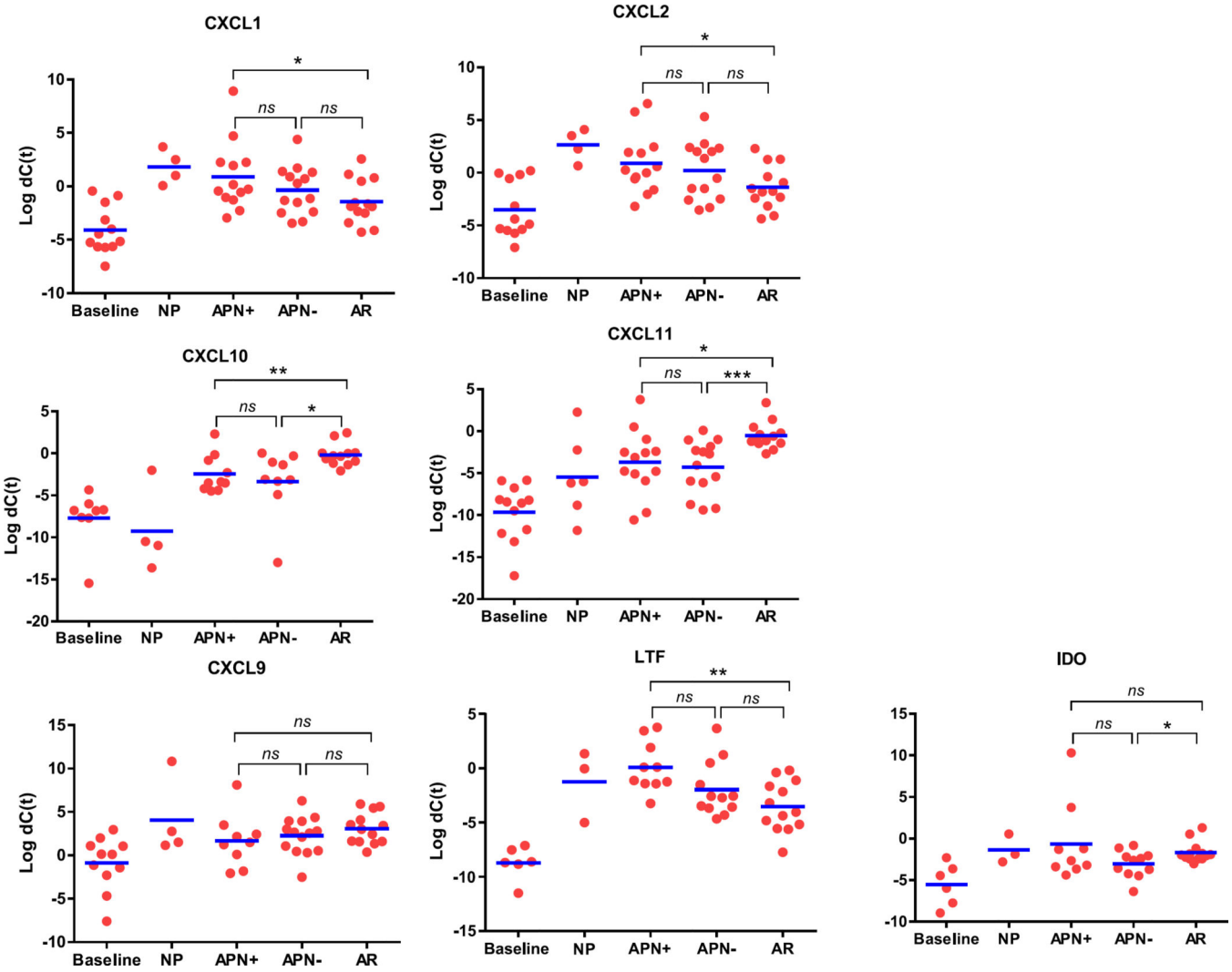


Figure 2.

Validation of eight selected genes by quantitative real-time PCR. Each dot represents one biopsy. Gene expression is represented as ΔCt after normalizing to the housekeeping gene *GAPDH*. Validation was performed on all 33 samples analyzed by NanoString as well as 22 additional biopsies. *P* values were calculated using a Student's *t* test and are shown in Table 7. ns, not significant, **P*<.05, ***P*<.01, ****P*<.001. Three samples of NP did not show amplification. Trends are similar to those seen by NanoString. *CXCL*9 and *IDO*-1, however, did not show statistical significance as seen on NanoString. APN, acute pyelonephritis; AR, acute rejection; CXCL, CXC chemokine ligand; NP, native kidney pyelonephritis

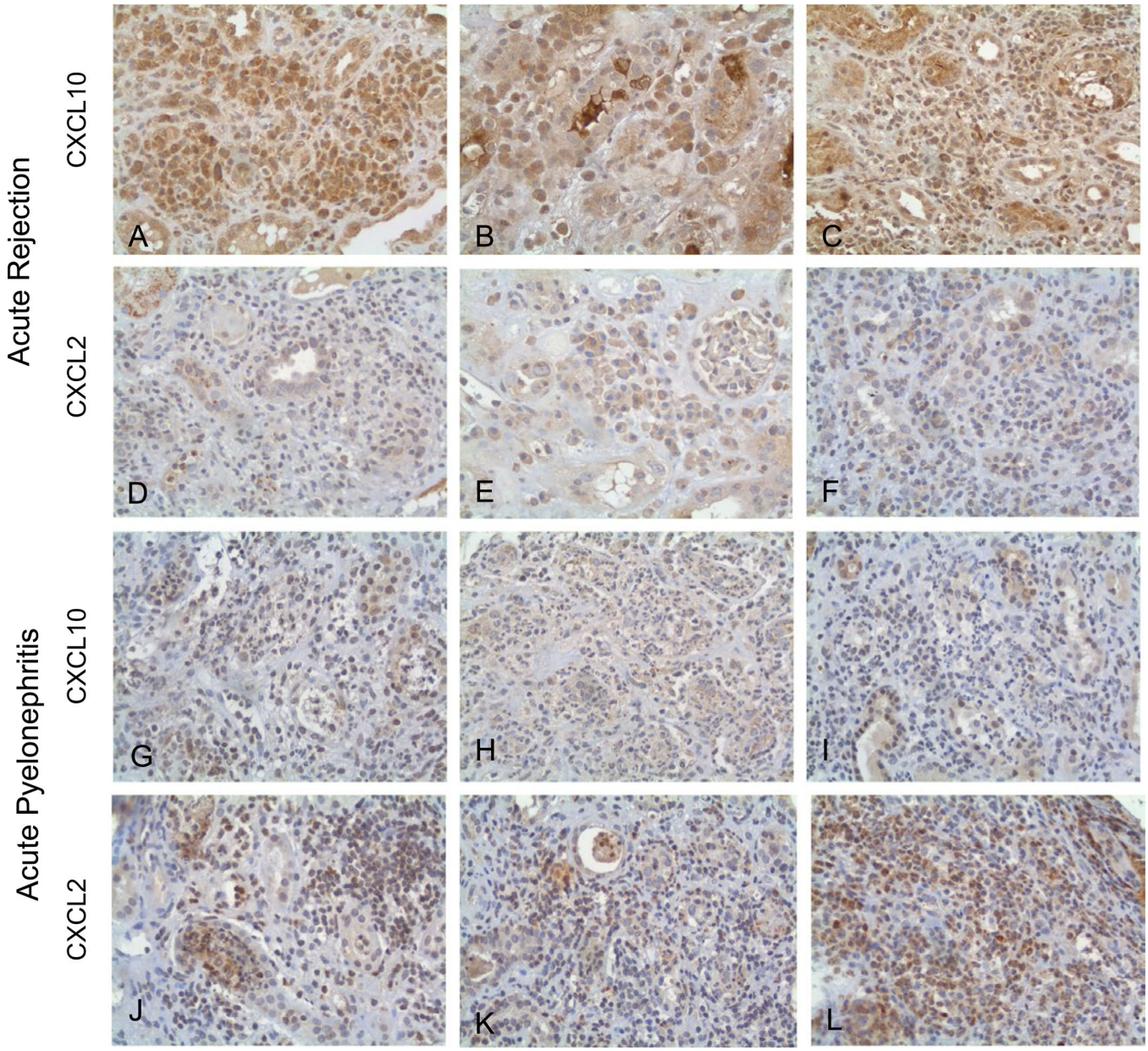


Figure 3.

Immunoperoxidase staining of FFPE tissue with rabbit anti-human polyclonal antibody to CXCL10 and CXCL2 (400×). (A–C) Three cases of acute rejection stained for CXCL10. Diffuse staining of interstitial inflammatory cells including lymphocytes and plasma cells is seen for CXCL10. Constitutive expression is seen in distal tubular epithelial cells. (D–F) The same cases of AR stained for CXCL2 show scant to negative staining. (G–I) Three cases of acute pyelonephritis (I1, I15, and I7) stained for CXCL10 show scant to negative staining. (J–L) The same three cases of APN (I1, I15, and I7) are stained for CXCL2. Prominent staining in the inflammatory cells is seen. APN, acute pyelonephritis; AR, acute rejection; CXCL, CXC chemokine ligand

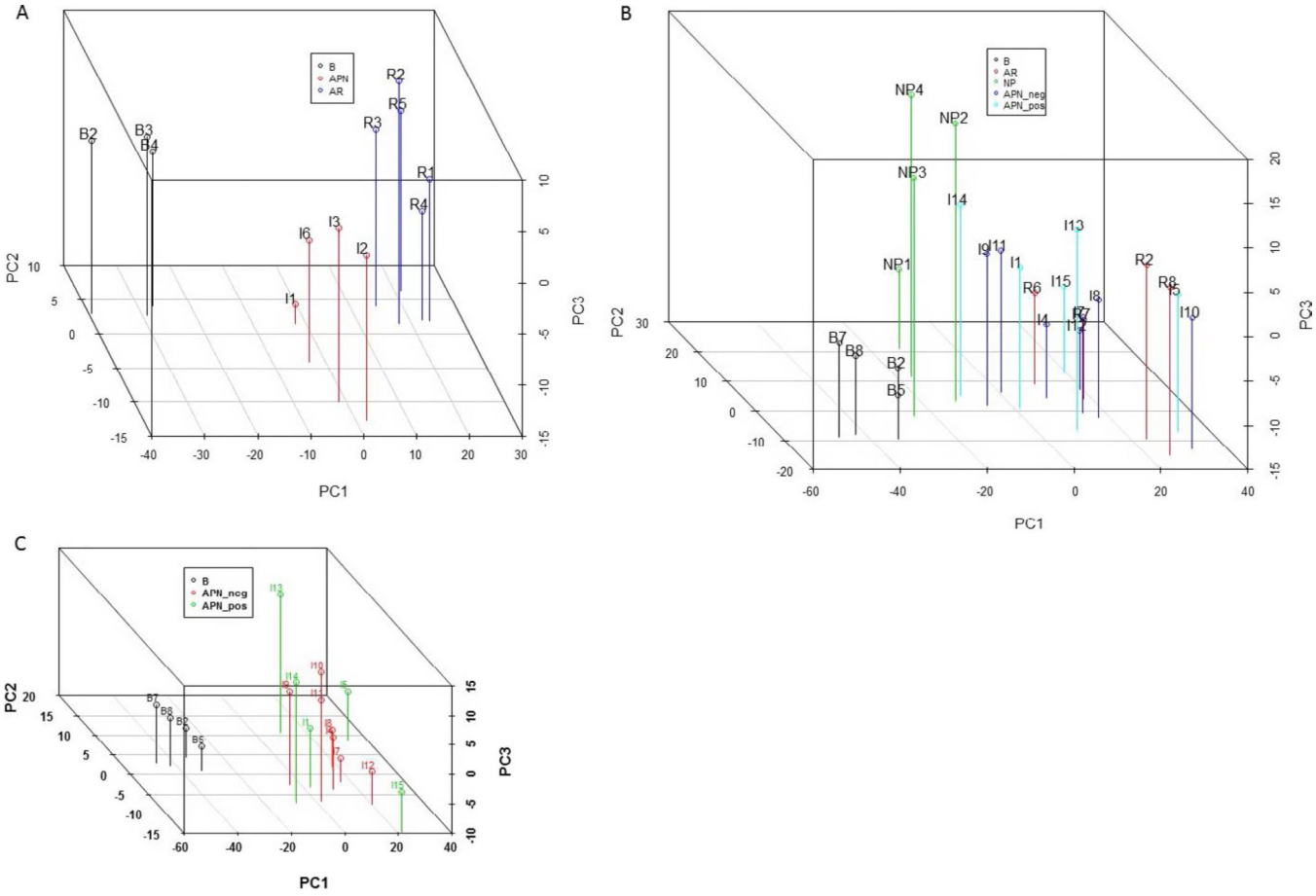
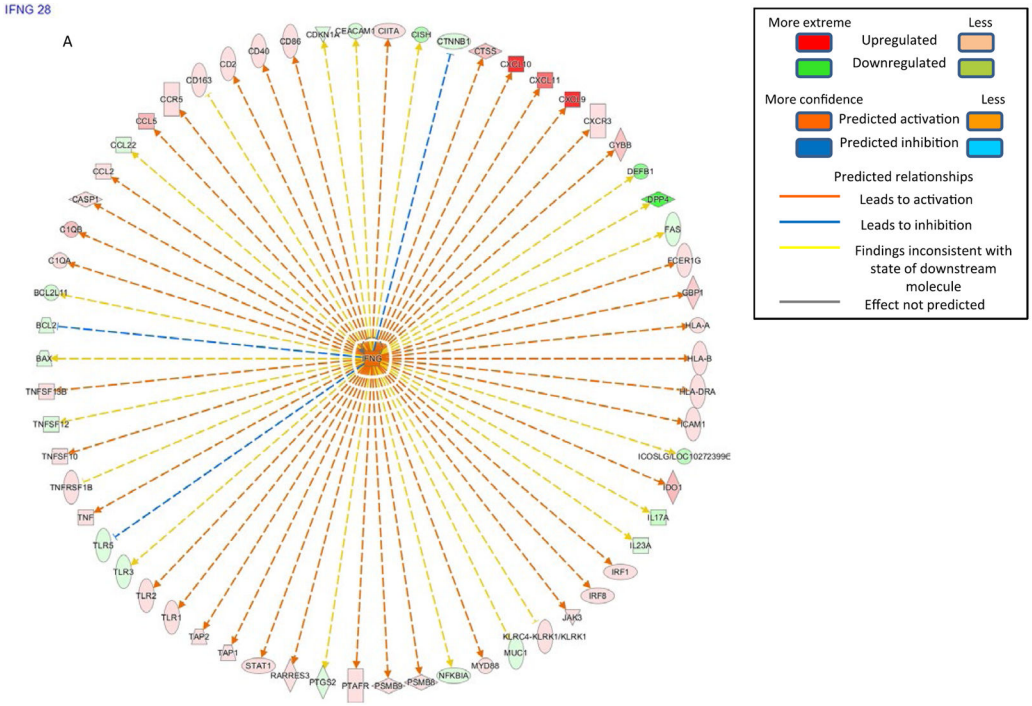
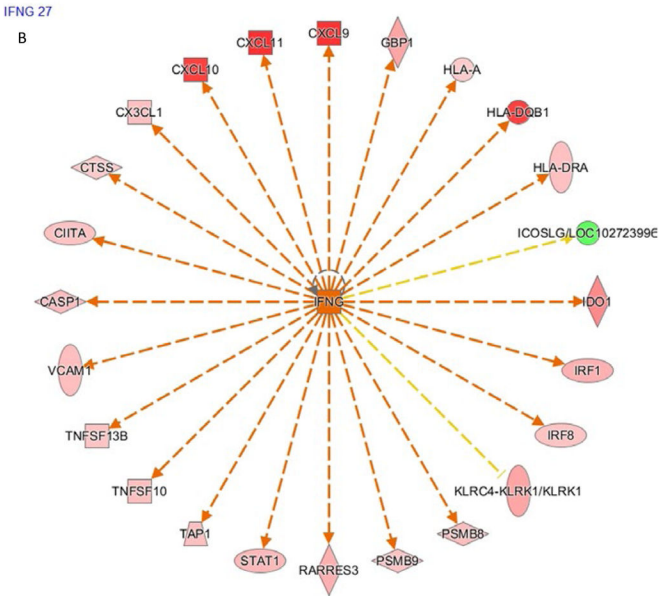
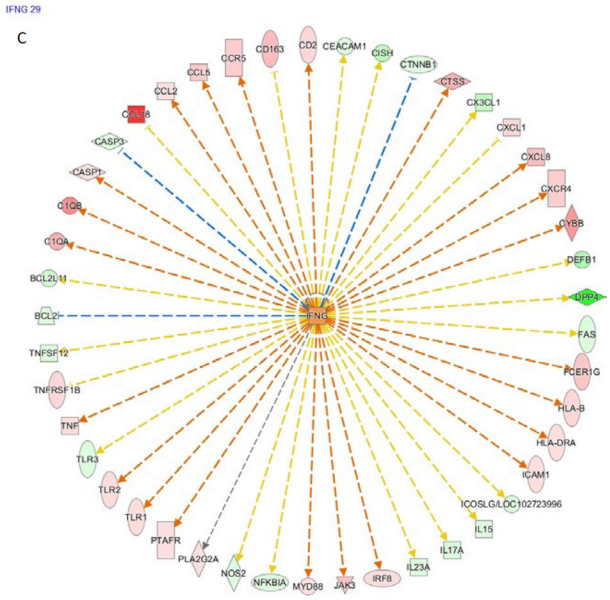


Figure 4.

Principle component analysis (PCA) of gene expression data from NanoString. This includes all genes after filtering for the low expressors. (A) Shows the 12 samples analyzed on NanoString nCounter Gx Human Immunology Kit (NanoString Technologies). The samples are labeled in the figure. The three groups—3 normal kidney baseline biopsies, 4 biopsies with allograft APN and 5 biopsies with AR clustered separately. (B) Shows remaining 21 samples (samples B2, R2, and I1 were rerun) analyzed on nCounter Human Immunology V2 panel. The normal baseline biopsies and the four samples of NP clustered separately. The biopsies with allograft APN show a spectrum and overlap with AR, irrespective of urine culture results. (C) APN culture-positive and culture-negative biopsies do not cluster separately. APN, acute pyelonephritis; AR, acute rejection; NP, native kidney pyelonephritis







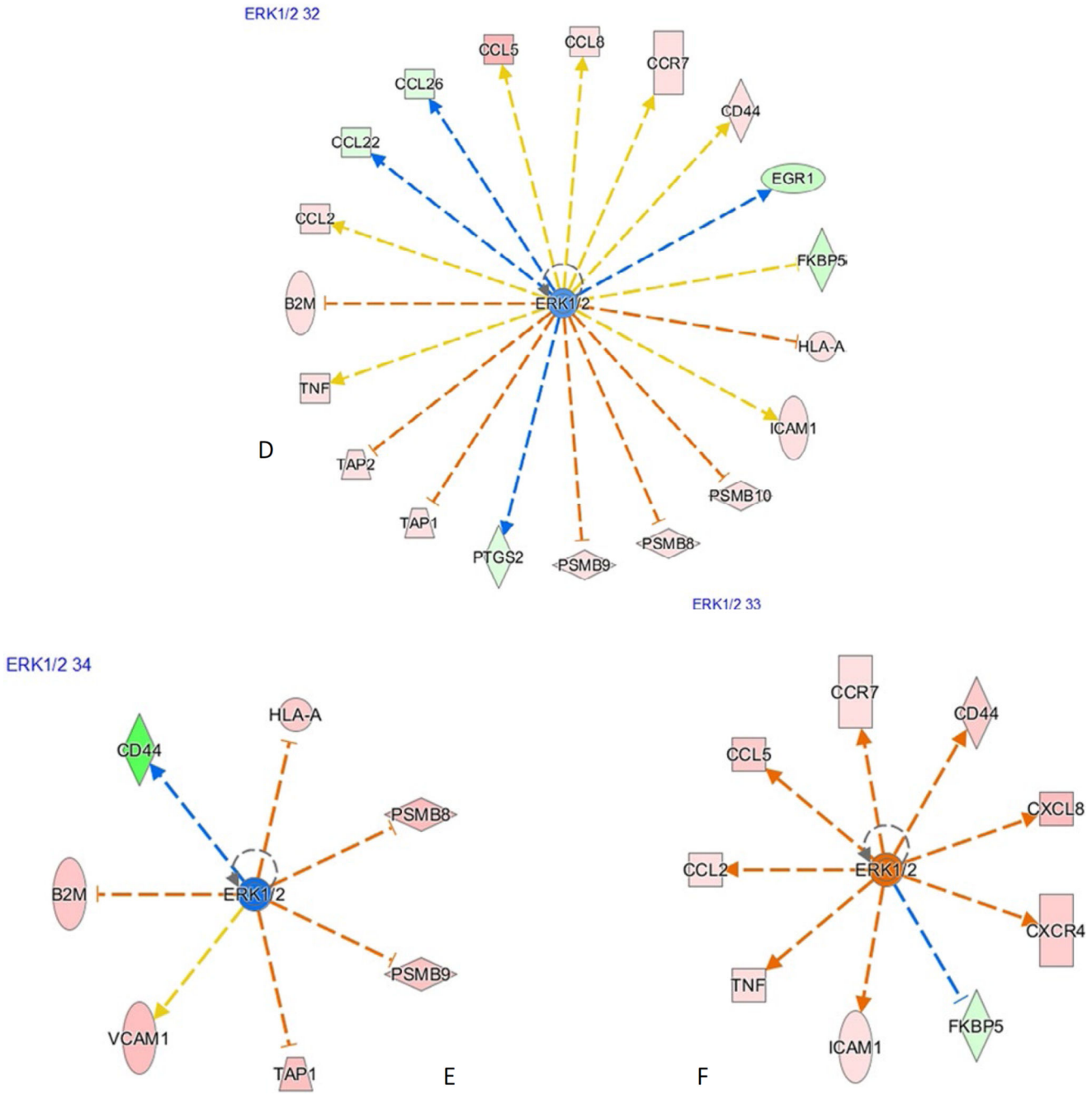


Figure 5.

Ingenuity pathway analysis results. The color code key is shown in top right corner. Downstream effectors of *IFNG* in AR (A), APN (B), and NP (C) are shown. *IFNG* and its major effectors *CXCL*9, *CXCL*10, *CXCL*11 are upregulated in AR and APN, but not in NP. Downstream effectors of *ERK*1/2 in AR (D), APN (E), and NP (F) are shown. *ERK*1/2 and most of the downstream effectors appear to be upregulated NP but not in AR and APN. In AR and APN, the effectors that are supposed to be activated by *ERK*1/2 are downregulated. The effectors that are supposed to be blocked by *ERK*1/2 are upregulated because of

inhibition of *ERK*1/2 itself. APN, acute pyelonephritis; AR, acute rejection; CXCL, CXC chemokine ligand; NP, native kidney pyelonephritis

TABLE 1

Baseline transplant biopsies (n=6), biopsies with acute rejection, AR (n=8), native kidneys with acute pyelonephritis (n=4). Biopsy diagnosis and follow-up serum creatinine levels at 1 month and 1 year after biopsy are shown

Biopsy	Age	Race	Sex	Type of transplant	Duration between transplant and biopsy	Biopsy diagnosis	PRA Class I; II	Duration between biopsy and urine culture result	Urine culture result	Colony count (CFU)/mL	Baseline S. Cr. before biopsy	S. cr. at time of biopsy	S. cr. 1 month post-biopsy	S. cr. 1 year post-biopsy
B2	48	C	F	LUD	0 days	Unremarkable renal cortex	N/A	Day of biopsy	Negative	N/A	N/A	N/A	N/A	N/A
B3	61	C	M	LRD	0 days	Unremarkable renal cortex	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B4	58	C	M	LUD	0 days	Unremarkable renal cortex	N/A	5 days after biopsy	Negative	N/A	N/A	N/A	N/A	N/A
B5	47	AA	M	LRD	0 days	Unremarkable renal cortex	N/A	Negative	N/A	N/A	N/A	N/A	N/A	N/A
B7	41	C	M	LUD	0 days	Unremarkable renal cortex	Not done	N/A	N/A	N/A	N/A	N/A	N/A	N/A
B8	47	C	M	LUD	0 days	Unremarkable renal cortex	Not done	N/A	N/A	N/A	N/A	N/A	N/A	N/A
R1	44	C	F	Cad	0 days	Acute cellular rejection Banff grade 2B; C4d negative	0; 0	On day of biopsy	Negative	N/A	2.4	8.8	1.3	1.1
R2	29	C	F	Cad	6 months	Acute cellular rejection Banff grade IIA, mild diffuse C4d	0; 0	4 days before biopsy	Positive for *Enterococcus faecalis* (UTI)	10 000–50 000	0.7	2.1	1.5	Dialysis
R3	23	C	F	LUD	4 months	Acute cellular rejection Banff grade 1B, C4d mild diffuse	0; 9	2 days before biopsy	Negative	N/A	0.9	2.4	1.2	0.9
R4	61	AA	F	Cad	19 days	Acute cellular rejection Banff grade IIB, C4d negative	95; 70	6 days before biopsy	Negative	N/A	DGF	1.3	1	1.4
R5	51	C	F	Cad	1 month, 9 days	Acute cellular rejection Banff grade 1B, C4d negative	81; 0	7 days before biopsy	Positive for MRSE; *E. coli* (lower UTI)	100 000	DGF	5.8	1.8	Dialysis
R6	45	AA	F	Cad	7 months	Combined cellular and humoral rejection (Banff grade 1B), strong diffuse C4d staining	24; 92	N/A	Negative	N/A	2.5	4.6	4.3	4.2
R7	34	AA	M	Cad	11 days	Acute cellular rejection (Banff grade 1B), C4d negative	0; 0	N/A	Negative	N/A	2.9	5.8	6.1	Dialysis
R8	44	C	F	LRD	4 days	Acute cellular rejection, Banff grade 2B; C4d negative	Not done	N/A	N/A	N/A	1.3	2	1.4	Not available
NP1	52	C	M	Native kidney biopsy	N/A	Acute pyelonephritis (neuro-genic bladder, ureteral strictures)	N/A	N/A	Positive with pyuria, esterase positive	Details not available	1.5	3.8	Not available	Not available
NP2	75	AA	F	Native kidney biopsy	N/A	Acute pyelonephritis (resected urinary bladder cancer)	N/A	N/A	Cultures performed after starting antibiotics. Negative	Details not available	1.2	4.6	Not available	Not available

Biopsy	Age	Race	Sex	Type of transplant	Duration between transplant and biopsy	Biopsy diagnosis	PRA Class I; II	Duration between biopsy and urine culture result	Urine culture result	Colony count (CFU)/mL	Baseline S. Cr. before biopsy	S. cr. at time of biopsy	S. cr. 1 month post-biopsy	S. cr. 1 year post-biopsy
NP3	48	C	F	Native nephrectomy	N/A	Acute pyelonephritis (hemipelvectomy for chondrosarcoma)	N/A	N/A	*Enterococcus faecalis*	Details not available	0.7	1.1	0.5	0.5
NP4	79	C	M	Native kidney biopsy	N/A	Acute pyelonephritis (cystictomy for bladder cancer)	N/A	7 days before biopsy	*Klebsiella pneumonia*	Not available	1.4	4 (dialysis)	Not available	Not available

B, baseline; R, rejection; NP, native kidney pyelonephritis; Ph, Phillipino; LUD, living-unrelated donor; LRD, living-related donor; Cad, cadaveric donor; S. cr., serum creatinine in mg/dL; MRSE, methicillin-resistant *Staphylococcus epidermidis;* UTI, urinary tract infection.

TABLE 2

Fifteen patients with diagnosis of acute pyelonephritis (APN) on allograft biopsy. Culture results, biopsy features, and serum creatinine levels are shown

Biopsy sample	Age	Race	Sex	Transplant type	Duration from transplant to biopsy	Biopsy diagnosis	C4d staining	PRA Class I; Class II	Duration between biopsy and urine culture	Microorganisms identified on urine culture	Colony count on culture	Treatment	Baseline S. cr. before biopsy	S. cr. at biopsy	S. cr. 10 days post-biopsy	S. cr. 1 month post-biopsy	S. cr. 1 year post-biopsy
I1	65	C	F	Cad	12 days	Acute pyelonephritis	Negative	0; 68	10 days before biopsy	*Klebsiella pneumonia*	>100 000	Antibiotics	DGF	4.3	3.5	1.1	1.49
I2	53	AA	M	Cad	1 year, 4 months	Acute pyelonephritis	Negative	18; 59	1 day after biopsy	*Enterococcus faecalis*	10 000–50 000	Antibiotics	2.5	4.3	2.6	2.6	3.73
I3	51	Ph	F	Cad	3 years, 3 months	Acute pyelonephritis	Negative	0;0	On day of biopsy	*Klebsiella oxytoca*	>100 000	Antibiotics	1.4	2.3	2.4	2.2	2.4
I4	21	AA	M	Cad	3 weeks	Acute pyelonephritis	Negative	0; 0	Negative at biopsy	*n/a*	13 days before biopsy; Candida lusitanae 1–5000	Antibiotics	DGF	DGF 12.25	9.3	3.5	2.6
I5	36	C	F	Cad	8 days	Acute pyelonephritis	Negative	0; 0	10 days before biopsy	*Enterococcus faecalis*	>100 000	Antibiotics	DGF	DGF 8.2	6.9	Lost	Lost
I6	57	C	F	LRD	6 months	Acute pyelonephritis	Negative	0; 0	4 days after biopsy	*Candida glabrata*; *Mycoplasma*	<50 000	Antibiotics	1.4	4.5	6.8	4.2	2.4
I13	32	C	F	Cad	15 days	Acute pyelonephritis	Negative	19; 0	5 days before biopsy	MRSE	<50 000	Antibiotics	1.1	3.6	1.5	1	1
I14	52	C	F	LUD	5 years, 1 months	Acute pyelonephritis	Negative	0; 0	2 days before biopsy	*E. coli*	>100 000	Antibiotics	2	3.3	2.9	2.1	1.6
I15	52	AA	F	Cad	2 years, 3 months	Acute pyelonephritis	Negative	0; 0	10 days before biopsy	*E. coli*, also sepsis	>100 000	Antibiotics	6.8	3.7	3.3	3.3	2.6
I7	30	AA	F	Cad	15 days	Acute pyelonephritis	Negative	0; 0	Negative at time of biopsy	n/a	1 month after biopsy; Klebsiella oxytoca, Citrobacter koseri <50 000	Antibiotics	DGF	DGF 8.4	8.1	Lost	Lost
I8	46	C	M	Cad	8 days	Acute pyelonephritis	Trace focal	0; 0	Negative at time of biopsy	n/a	2 months after biopsy; *Klebsiella*, Enterobacter ESBL <1000	Antibiotics, followed by corticoster-oids	DGF	DGF 10.9	5.7	8.2	2.1
I9	51	C	F	LRD	2 months	Acute pyelonephritis	Negative	0; 0	Negative at time of biopsy	Mixed flora (contamination)	n/a	Antibiotics	1.9	4.6	2.6	2.7	Expired

Biopsy sample	Age	Race	Sex	Transplant type	Duration from transplant to biopsy	Biopsy diagnosis	C4d staining	PRA Class I; Class II	Duration between biopsy and urine culture	Microorganisms identified on urine culture	Colony count on culture	Treatment	Baseline S. cr. before biopsy	S. cr. at biopsy	S. cr. 10 days post-biopsy	S. cr. 1 month post-biopsy	S. cr. 1 year post-biopsy
I10	37	C	M	Cad	15 days	Acute pyelonephritis,	Negative	77; 33	Negative at time of biopsy	n/a	n/a	Antibiotics, followed by corticoster-oids	DGF	DGF 5.3	9.3	1.1	2.4
I11	62	AA	M	Cad	20 days	Acute pyelonephritis	Weak focal	0; 0	Negative at time of biopsy	n/a	N/A	Antibiotics	8.8	6.3	4.3	1.8	2
I12	66	C	M	Cad	13 days	Acute pyelonephritis	Negative	0; 0	Negative at time of biopsy	n/a	N/A	Steroids	2.8	3.9	2.1	2.1	1.6

LRD, living-related donor; Cad, cadaveric donor; PRA, panel-reactive HLA antibodies; S. cr., serum creatinine in mg/dL; DGF, delayed graft function (dialysis within first week post-transplant); Ph, Phillipino; MRSE, methicillin-resistant *Staphylococcus epidermidis*.

TABLE 3

Urinalysis results for the transplant patients with acute pyelonephritis (n=15) and patients with acute rejection (n=8)

Biopsy	Concomitant urine culture result	Urinalysis results				
		Bacteria	WBCs/hpf	RBCs/hpf	Leukocyte esterase	Nitrites
I1	Positive	Present	20–29	Rare	Large	Negative
I2	Positive	Present	30–49	Rare	Moderate	Negative
I3	Positive	Not available	Not available	Not available	Not available	Not available
I5	Positive	Absent	5–9	30–49	Small	Negative
I6	Positive	Present	10–14	Absent	Small	Negative
I13	Positive	Present	30–49	Absent	Trace	Negative
I14	Positive	Present	>50	1–2	Moderate	Positive
I15	Positive	Absent	>50	5–9	Moderate	Negative
I4	Negative	Present	>50	3–4	Large	Negative
I7	Negative	Present	10–14	15–19	Small	Negative
I8	Negative	Present	10–14	1–2	Small	Negative
I9	Negative	Present	5–9	Absent	Negative	Negative
I10	Negative	Absent	20–29	20–29	Large	Negative
I11	Negative	Absent	Rare	20–29	Negative	Negative
I12	Negative	Absent	10–14	15–19	Small	Negative
R1	Negative	Present	3–4	1–2	Negative	Negative
R2	Positive	Absent	3–4	1–2	Moderate	Negative
R3	Negative	Absent	1–2	Absent	Negative	Negative
R4	Negative	Present	20–29	5–9	Moderate	Negative
R5	Positive	Present	20–29	Rare	Large	Negative
R6	Negative	Absent	5–9	>50	Large	Negative
R7	Negative	Absent	5–9	5–9	Trace	Negative
R8	Negative	Absent	Not available	Not available	Not available	Not available

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TABLE 4

Additional 22 biopsy samples for validation of results

Biopsy	Age (years)	Race	Sex	Type of transplant	Duration between transplant and biopsy	Biopsy diagnosis	PRA Class I; Class II	Duration between biopsy and urine culture result	Urine culture result	Colony count (CFU)/mL	Baseline S. Cr. before biopsy	S. cr. at time of biopsy	S. cr. 1 month post-biopsy	S. cr. 1 year post-biopsy
Baselines														
B8	29	AA	F	LRD	N/A	Unremarkable renal cortex	N/A	I day before	Negative	N/A	N/A	N/A	N/A	N/A
B9	64	C	M	LUD	N/A	Unremarkable renal cortex	N/A	No cultures	N/A	N/A	N/A	N/A	N/A	N/A
B10	66	C	F	LRD	N/A	Unremarkable renal cortex	N/A	No cultures	N/A	N/A	N/A	N/A	N/A	N/A
AR														
R9	53	AA	M	Cad	2 years 5 months	ACR 1B; C4d neg	0; 0	1 day before, 2 days after	Negative	N/A	2.2	3	2.3	Not available
R10	47	C	M	LRD	15 days	ACR III; C4d neg	0; 0	6 days before	Negative	N/A	1.4	3.6	1.6	Not available
R11	43	C	M	Cad	17 days	ACR 1B; C4d neg	0; 0	3 days before, same day	Negative	N/A	3.5	7.5	1.8	1.9
R12	43	C	M	Cad	7 months	ACR 1B; C4d neg	0; 0	Same day	Negative	N/A	1.6	2.6	2.6	7
R13	48	C	F	LUD	11 months	ACR 1B; C4d neg	0; 3	1 day before, 2 days after	Negative	N/A	2.7	3	2.6	3
R14	27	AA	M	LRD	1 year, 1 month	ACR 1A; C4d neg	0; 0	4 days before	Negative	N/A	2.5	3.2	3.2	2.8
APN with positive urine cultures at time of biopsy														
I16	52	C	F	LUD	2 years	Acute pyelonephritis	0; 0	1 day before	*E. coli*	>100 000	1.4	2.4	4.3	1.7
I17	32	C	F	Cad	15 days	Acute pyelonephritis	19; 0	5 days before	MRSE	50 000	1.1	3.6	3.6	1.0
I18	41	AA	M	Cad	1 month 17 days	Acute pyelonephritis	40; 0	2 days before	*Klebsiella pneumonia*	50 000	5.5 (DGF)	7.3	8.2	9.5 (failed)
I19	50	C	F	Cad	2 months 10 days	Acute pyelonephritis	0; 0	9 days before	*Serratia marcescens*	100 000	2.2	11	3.1	2.6

Biopsy	Age (years)	Race	Sex	Type of transplant	Duration between transplant and biopsy	Biopsy diagnosis	PRA Class I; Class II	Duration between biopsy and urine culture result	Urine culture result	Colony count (CFU)/mL	Baseline S. Cr. before biopsy	S. cr. at time of biopsy	S. cr. 1 month post-biopsy	S. cr. 1 year post-biopsy
APN with negative urine cultures at time of biopsy														
I20	31	Hispanic	F	LRD	7 days	Acute pyelonephritis	0; 50	1 day before, 2 days after	Negative	N/A	1.2	1.7	4.7	1.1
I21	39	C	M	Cad	7 weeks	ATN, Acute pyelonephritis	0; 0	13 days before	Negative	N/A	2.3	4.9	2.5	2.2
I22	57	C	F	LUD	10 days	Acute pyelonephritis	0; 0	2 days after	Negative	N/A	5.2	7.77	7.3	2.12
I23	67	AA	M	Cad	4 months 3 weeks	Acute pyelonephritis	0; 0	10 days before	Negative	N/A	1.8	11	Not available	Failed
I24	31	C	M	Cad	4 months	Acute pyelonephritis	0; 0	1 day after	Negative	N/A	1.5	3.1	2.1	1.7
I25	58	C	M	Cad	2 years	Acute pyelonephritis	0; 24	4 days after	Negative	N/A	2.2	2.6	2.5	Not available
Native kidney pyelonephritis (NP)														
NP5	48	C	F	Nephrectomy	N/A	Acute pyelonephritis	N/A	Day of surgery	Enterococcus	>10 000	0.6	1.1	0.9	0.6
NP6	61	AA	F	Nephrectomy	N/A	Acute pyelonephritis	N/A	1 month before surgery	Enterobacter	>100 000	1	1.2	Not available	1
NP7	46	C	F	Nephrectomy	N/A	Acute and chronic pyelonephritis	N/A	8 days before surgery	Negative	N/A	0.7	0.7	Not available	0.8

S. cr., serum creatinine; MRSE, methicillin-resistant *Staphylococcus epidermidis*; DGF, delayed graft function.

TABLE 5

Differential gene expression between AR and APN, $P<.05$

	log2 average				AR vs baseline		APN vs baseline		NP vs baseline		APN vs AR		APN vs NP	
Gene	**AR**	**APN**	**NP**	**Baseline**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**
IDO1	10.29	8.25	6.51	6.69	12.19	0	2.95	.0147	−1.13	.809	−4.17	8.00×10^{-04}	3.33	.0033
CXCL10	11.54	9.12	6.67	6.18	41.19	0	7.69	.002	1.41	.6314	−5.26	.004	5.48	.0036
CXCL11	10.36	7.92	5.31	5.68	25.47	1.00×10^{-04}	4.7	.0178	−1.29	.7227	−5.56	.0048	6.08	.0029
CXCL9	12.65	10.57	8.02	7.36	39.11	0	9.24	.0014	1.59	.5369	−4.17	.0153	−1.15	.5191
LTF	8.92	11.51	12.43	7.16	3.38	.1391	20.38	2.00×10^{-04}	33.33	1.00×10^{-04}	6.03	.0064	1.11	.5803
CD36	6.57	7.48	7.67	6.99	−1.33	.2933	1.4	.1586	1.61	.0975	1.88	.0055	−1.89	.3006
C1R	10.2	11	10.85	8.94	2.4	.0014	4.19	0	3.85	0	1.75	.0057	1.3	.1666
CLEC7A	8.29	7.51	7.13	6.65	3.11	1.00×10^{-04}	1.81	.0083	1.39	.1891	−1.72	.0076	2.55	.0076
GBP1	11.38	10.03	8.68	8.21	8.98	0	3.52	.0018	1.39	.4571	−2.56	.0077	1.1	.7216
SLAMF7	9.14	8.06	7.92	5.67	11.06	0	5.22	0	4.76	2.00×10^{-04}	−2.13	.0089	1.71	.0225
PSMB9	10.6	9.69	8.91	8.14	5.49	0	2.93	2.00×10^{-04}	1.72	.0787	−1.89	.0091	1.17	.4029
C1S	9.51	10.22	10	8.36	2.21	.0028	3.63	0	3.12	1.00×10^{-04}	1.64	.0112	1.54	.1751
CCL5	9.67	8.48	7.85	6	12.7	0	5.55	0	3.57	.0047	−2.27	.0135	3.06	.0066
GBP5	9.75	8.32	6.71	4.56	36.65	0	13.59	0	4.35	.0063	−2.7	.0144	−1.18	.2904
CD81	11.17	11.73	11.96	12.62	−2.7	0	−1.85	.001	−1.58	.0274	1.47	.0152	5.84	.0039
CX3CR1	7.13	7.91	6.94	6.59	1.45	.1845	2.48	6.00×10^{-04}	1.27	.3858	1.71	.0159	1.95	.0035
KIR3DL1	4.32	5.02	4.96	5.78	−2.78	5.00×10^{-04}	−1.69	.0198	−1.77	.0324	1.63	.0165	1.04	.8332
PSMB8	10.7	10.07	9.14	9.22	2.8	1.00×10^{-04}	1.81	.0051	−1.05	.8243	−1.54	.0174	1.9	.001

	log2 average				AR vs baseline		APN vs baseline		NP vs baseline		APN vs AR		APN vs NP	
Gene	**AR**	**APN**	**NP**	**Baseline**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**
CXCR2	5.28	6.26	6.45	5.74	−1.37	.3711	1.43	.24	1.64	.1748	1.97	.0175	−1.15	.6197
CASP1	8.7	8.14	7.22	6.52	4.51	0	3.08	0	1.61	.0261	−1.47	.0199	1.9	3.00×10^{-04}
PRF1	8.24	7.51	7.13	6.26	3.96	0	2.38	.001	1.82	.0375	−1.67	.0217	1.3	.2212
KLRC4	6.95	6.2	5.46	5.48	2.76	.0015	1.64	.051	−1.02	.9583	−1.69	.0232	1.66	.0263
TNFRSF9	6.06	5.28	5.23	4.57	2.81	.0018	1.64	.0587	1.59	.1329	−1.72	.0236	1.04	.8734
PDCD1LG2	6.75	5.92	5.73	6	1.69	.1131	−1.05	.8438	−1.21	.5608	−1.79	.0246	1.14	.5822
KCNJ2	7.5	6.93	6.53	7.07	1.35	.1846	−1.1	.6085	−1.45	.1021	−1.49	.0252	1.32	.1083
C5	5.95	6.37	6	7.87	−3.85	0	−2.86	0	−3.67	0	1.34	.0268	1.29	.0488
IRF1	9.72	8.78	7.63	7.98	3.35	.0031	1.74	.086	−1.27	.5215	−1.92	.0273	2.22	.0086
IL-1R1	6.5	7.32	7.79	8.05	−2.94	.003	−1.67	.0768	−1.2	.5861	1.76	.03	−1.39	.1938
IL-13RA1	9.12	9.54	9.53	9.63	−1.43	.044	−1.06	.648	−1.08	.6635	1.34	.0308	1.01	.9483
CCL8	7.22	6.3	6.16	5.97	2.38	.028	1.26	.4743	1.14	.7215	−1.89	.0317	1.1	.7398
TGFBR1	6.86	7.43	8.33	8.65	−3.45	0	−2.33	2.00×10^{-04}	−1.25	.3502	1.49	.0327	−1.85	.0015
HLA-DMB	10.22	9.76	9.37	8.13	4.27	0	3.1	0	2.38	1.00×10^{-04}	−1.37	.033	1.31	.0707
CD8A	8.2	7.19	6.63	5.61	6.01	2.00×10^{-04}	3	.004	2.04	.0948	−2	.0338	1.47	.2216
TAP1	8.32	7.5	6.63	6.75	2.98	.0032	1.69	.0748	−1.09	.8078	−1.75	.0342	1.83	.0241
C7	9.29	10.1	11.04	10.63	−2.56	.0095	−1.45	.1969	1.32	.4052	1.75	.0345	−1.92	.0155
KLRB1	5.99	6.72	7.86	5.58	1.33	.3436	2.2	.0044	4.76	0	1.65	.0352	−2.22	.0017
RARRES3	10.19	9.27	8.09	8.62	2.96	.0088	1.56	.1792	−1.45	.3427	−1.89	.0359	2.26	.0093
TCF4	7.89	8.34	8.84	8.51	−1.54	.0299	−1.12	.4587	1.27	.2264	1.36	.0367	−1.41	.0203

	log2 average				AR vs baseline		APN vs baseline		NP vs baseline		APN vs AR		APN vs NP	
Gene	**AR**	**APN**	**NP**	**Baseline**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**
B2M	14.81	14.27	13.59	13.96	1.79	.0153	1.24	.2733	−1.29	.2636	−1.45	.0377	1.6	.0107
MSR1	7.78	7.21	7.43	7.23	1.47	.1275	−1.02	.9411	1.15	.5658	−1.49	.0399	−1.18	.4015
SMAD5	6.85	7.32	7.03	8.74	−3.7	0	−2.7	0	−3.5	0	1.38	.0404	1.22	.2043
TIGIT	6.88	6.12	5.7	4.79	4.26	2.00×10^{-04}	2.52	.0025	1.89	.062	−1.69	.0414	1.33	.2496
MAP4K4	8.3	8.77	9.01	9.07	−1.69	.0133	−1.23	.2242	−1.04	.84	1.38	.0428	−1.19	.2718
CD276	6.89	7.36	7.42	8.14	−2.38	4.00×10^{-04}	−1.72	.0055	−1.65	.026	1.39	.0485	−1.04	.7903
CCL19	9.06	10.07	10.32	6.61	5.46	9.00×10^{-04}	11	0	12.5	0	2.01	.0488	−1.19	.618
IFNG	7.22	6.45	5.92	6.28	1.93	.0685	1.12	.6922	−1.28	.4813	−1.72	.0489	1.44	.176
CXCL1	8.87	9.7	10.4	8.86	1	.9904	1.79	.075	2.86	.0084	1.78	.0499	−1.61	.0986

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TABLE 6

Separated out culture-positive and culture-negative patients from the APN group and compared for the top differentially expressed genes. No significant differences are seen between culture positive and culture negative APN biopsies.

	APN culture positive vs AR		APN culture negative vs AR		APN culture positive vs APN culture negative	
Gene	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**
IDO1	**−3.57**	**.0074**	**−4.55**	**.001**	**−1.28**	**.5177**
CXCL10	**−5.88**	**.0086**	**−5**	**.012**	**1.23**	**.7128**
CXCL11	**−6.25**	**.0092**	**−5**	**.0145**	**1.27**	**.6723**
CXCL9	**−5**	**.0199**	**−3.7**	**.0429**	**1.39**	**.5718**
LTF	**7.09**	**.0115**	**5.37**	**.0191**	**−1.32**	**.6581**
CXCL1	**2.03**	**.0417**	**1.62**	**.128**	**−1.25**	**.443**
CLEC7A	−2	.0031	−1.54	.0396	1.31	.1607
CD36	2.08	.0061	1.75	.0214	−1.19	.4236
PSMB9	−2.13	.0068	−1.69	.0375	1.26	.307
C1R	1.85	.0092	1.67	.0182	−1.11	.5949
CXCR2	2.39	.0093	1.72	.0703	−1.39	.2339
IL-8	4.03	.0164	1.85	.2342	−2.17	.1106
MSR1	−1.72	.0165	−1.35	.1467	1.28	.188
TNFAIP6	1.94	.0169	−1.1	.685	−2.13	.0025
RARRES3	−2.27	.0216	−1.67	.1166	1.37	.2915
KLRB1	1.9	.0219	1.49	.1143	−1.27	.3008
IRAK3	1.58	.0235	1.18	.3487	−1.33	.0923

	APN culture positive vs AR		APN culture negative vs AR		APN culture positive vs APN culture negative	
Gene	**Fold change**	***P*-value**	**Fold change**	***P*-value**	**Fold change**	***P*-value**
GBP1	−2.44	.0283	−2.63	.0128	−1.08	.8382
CASP1	−1.52	.0309	−1.43	.0463	1.06	.7072
KLRC4	−1.79	.0313	−1.61	.056	1.11	.6408
C1S	1.62	.0333	1.65	.0195	1.02	.9256
CD81	1.48	.0347	1.46	.0289	−1.01	.9389
HLA-DMB	−1.43	.0412	−1.33	.0758	1.07	.6312
HLA-DPB1	−1.64	.0413	−1.12	.5848	1.45	.0724
KIR_Activating_ Subgroup_2	1.71	.0418	1.08	.7577	−1.59	.0437
CX3CR1	1.69	.0426	1.72	.0267	1.02	.94
PSMB8	−1.54	.0429	−1.56	.0305	1	.9899
TNFRSF10C	1.58	.0452	1.26	.2589	−1.25	.2475
IL-1A	−1.61	.0483	−1.19	.4248	1.35	.142
HLA-DMA	−1.41	.0486	−1.19	.2809	1.19	.2409
CR1	1.74	.0488	1.49	.1224	−1.16	.5118
CD86	−1.45	.0507	−1.18	.3468	1.23	.1953
CCL8	−2	.0508	−1.82	.0633	1.08	.7879

The genes validated by PCR are highlighted in bold.

TABLE 7

P values for the dot plot shown in Figure 2

	CXCL1	CXCL2	CXCL10	CXCL11	CXCL9	LTF	IDO
APN culture+ vs AR	0.0395	0.0288	0.0075	0.0119	0.1919	0.0012	0.4686
APN culture− vs AR	0.2088	0.1061	0.0172	0.0009	0.2967	0.1174	0.025
APN culture+ vs APN culture−	0.2563	0.5251	0.5454	0.6675	0.6051	0.0607	0.1317
APN culture+ vs NP	0.5849	0.2657	0.0184	0.4077	0.2844	0.4385	0.8027
NP vs AR	0.0127	0.0027	0.0002	0.0041	0.514	0.1694	0.7522

APN, acute pyelonephritis; AR, acute rejection; NP, native pyelonephritis. The orange shaded boxes highlight the statistically significant *P*-values. $P < 0.05$.

TABLE 8

Upstream regulatory molecules in AR, APN culture-positive, APN culture-negative, native pyelonephritis using Ingenuity pathway analysis, showing *z*-scores.

Upstream regulators	AR	APN culture positive	APN culture negative	NP
IL-27	4.658	3.8935	4.3333	3.503
EBI3	3.8312	3.4218	3.6996	2.7894
IFNG	3.2101	2.6045	2.9437	0.9913
MAPK1	−3.3264	−2.3757	−2.8673	−0.9045
IRF4	−3.05796	−2.7196	−3.3023	0.2182
Interferon-alpha	3.08814	1.6787	2.9861	0.3428
IL-12 (complex)	2.9496	1.708	2.7634	1.6843
IFNL1	2.7997	2.1783	2.6186	0.2773
ERK1/2	−1.0566	0.6602	0.4849	2.9153
IL-18	2.4275	1.8676	2.6921	1.0687
TNF	2.4727	1.7964	2.0825	0.5429
STAT1	1.66119	0.4527	1.6666	0.239
P38 MAPK	1.3962	1.5366	2.1335	0.7475
NFkB (complex)	1.35419	0.5369	0.5166	0.0143
BCR (complex)	0.63907	1.0397	1.3655	2.1801

Orange—predicted activation; blue—predicted inhibition; white—no prediction can be made and darker colors indicate higher *z*-score.