Clinical and molecular spectrum of v-lesion

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Running title: Clinical and molecular spectrum of v-lesion

ABBREVIATIONS:

ABMR: antibody-mediated rejection

ATN: acute tubular necrosis

B-HOT: Banff Human Organ Transplant

CNI: calcineurin inhibitors

DGF: delayed graft function

DSA: donor-specific antibodies

DSAST: donor-specific antibodies associated transcripts

ENDAT: endothelial associated transcripts

ESKD: end stage kidney disease

FDR: false discovery rate

FFPE: formalin-fixed paraffin-embedded

g: glomerulitis

HLA-DSA: human leukocyte antigen donor-specific antibodies

i: interstitial inflammation

i-IFTA: inflammation within areas of interstitial fibrosis and tubular atrophy

IRI: ischemia-reperfusion injury

IQR: interquartile range

KT: kidney transplantation

mTORi: mTOR inhibitors

MVI: microvascular inflammation

PCA: principal component analysis

PNF: primary non-function

ptc: peritubular capillaritis

RRT: renal replacement therapy

SD: standard deviation

t: tubulitis

ti: inflammation in total cortical parenchyma

TCMR: T-cell mediated rejection

v: endarteritis

ABSTRACT

Isolated v-lesion presents diagnostic stratification and clinical challenges. We characterized allograft outcomes for this entity based on post-transplant time (early: ≤1 month vs. late: >1 month) and compared its molecular phenotype with other v+ rejection forms. Using the NanoString® B-HOT panel, we analyzed 92 archival FFPE kidney biopsies from three centers: isolated v-lesion (n=23), ABMR v+ (n=26), TCMR v+ (n=10), mixed rejection v+ (n=23), and normal tissue (n=10). Six gene sets (ABMR, DSAST, ENDAT, TCMR, early/acute injury, late injury) were assessed. Early isolated v-lesions had the poorest one-year death-censored graft survival compared to late isolated v-lesions or other rejections (p=0.034). Gene set analysis showed lower TCMR-related gene expression in isolated v+ groups than TCMR and mixed rejection (p<0.001). Both early and late isolated v-lesions had lower ABMR-related gene expression than ABMR, mixed rejection, and TCMR (p≤0.022). Late isolated v-lesions showed reduced DSAST and ENDAT gene expression versus ABMR (p≤0.046); and decreased early/acute injury gene expression than early isolated v+, ABMR, TCMR, and mixed rejection (p≤0.026). In conclusion, isolated v-lesions exhibit distinct gene expression patterns versus other rejection v+ forms. Early isolated v+ is associated with poorer prognosis and increased early/acute injury gene expression than late isolated v+, suggesting distinct etiologies.

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SIGNIFICANCE STATEMENTS

What is already known about this subject: Isolated endarteritis, defined as v>0 histology with minimal concurrent tubulointerstitial and/or microvascular inflammation below diagnostic Banff thresholds, is a clinically challenging entity. Limited evidence suggests the possibility of a non-rejection origin when it occurs early after transplantation.

What this study adds: The present study identifies distinct gene expression patterns in biopsies with isolated v-lesions compared to other rejection forms presenting with v-lesions. Additionally, early isolated v+ is associated with poorer prognosis and increased early/acute injury gene expression compared to late isolated v+, suggesting distinct etiologies.

What impact this may have on practice or policy: Assessing the molecular phenotype of biopsies with isolated v-lesion can improve diagnostic and thus inform therapeutic decision making.

INTRODUCTION

Intimal arteritis, also referred to as endarteritis or v-lesion, is characterized by the infiltration of mononuclear immune cells beneath the arterial endothelium.[1] The Banff Classification of Kidney Allograft Pathology first regarded this lesion as very specific and essentially diagnostic of acute T-cell mediated rejection (TCMR).[2] The 1997 modification then integrated the distinction of grades 1, 2, and 3 acute TCMR based on the presence and severity of endarteritis.[3] Rejection episodes with a vascular component (grades 2 and 3) have been identified as a severe phenotype characterized by steroid resistance and poorer outcomes.[4] Later studies indicated that these inferior outcomes might reflect, in some cases, a combination of the severity of TCMR and an antibody-mediated rejection (ABMR) component.[5,6] Therefore, based on the cohort study of Lefaucheur et al., endarteritis was included among lesions fulfilling histologic criteria for ABMR in the Banff 2013 consensus.[5,7]

Isolated v-lesion is an increasingly acknowledged entity that confers diagnostic and treatment challenges.[8–13] It is defined as a Banff lesion score of v≥1 that occurs with minimal concurrent tubulointerstitial inflammation, a low microvascular inflammation (MVI) sum score, and C4d negativity.[14–16] With the original Banff notion that even a single lymphocyte underneath the arterial endothelium is indicative of rejection, isolated v-lesions are difficult to interpret due to the limited evidence on their clinical and prognostic significance. While some authors highlight the possibility of a non-rejection origin,[11,12,17] according to the current Banff scheme, isolated v-lesion should still be considered either grade 2-3 acute TCMR or ABMR.[16]

Intragraft RNA transcript analysis enables quantitative and mechanism-based assessment of kidney transplant (KT) biopsies.[18] We hypothesized that intragraft molecular analysis could improve our understanding of isolated v-lesions, including the possibility that this entity may represent a non-rejection process. The NanoString® nCounter® gene

expression analysis system (NanoString Technologies, Seattle, WA) allows for direct molecular-histological correlation due to the reliable assessment of transcripts in formalin-fixed paraffin-embedded tissue (FFPE).[19,20] Importantly, this technology permits retrospective molecular analysis from patients with a well-known demographical and immunological background, enabling immediate correlation with long-term clinical follow-up.[18]

This study aimed to 1) characterize the short- and long-term allograft outcomes of isolated v-lesions according to post-transplant time and 2) use the Banff Human Organ Transplant (B-HOT) panel[21] to compare the gene expression profiles between early (≤1 month after KT) and late (>1 month) isolated v-lesions and other forms of rejection v+: ABMR v+, TCMR v+, mixed rejection v+, and controls.

METHODS

Study cohort and design

Observational cohort study considering all KT patients from Hospital del Mar and Hospital Universitari Vall d'Hebron with surveillance and clinically indicated allograft biopsies performed between 01/2007 and 02/2022. The only selection criteria were that 1) all cases met the histomorphological criteria of endarteritis lesion (v≥1) and 2) there was enough tissue for histology and gene expression analysis. Endarteritis was defined according to Banff criteria.[2,3,22] Isolated endarteritis was defined by Banff lesion scores[3] as an intimal arteritis score of at least 1 with a tubulitis score of 0-1 and an interstitial inflammation score of 0-1, a microvascular inflammation (g+ptc) score of 0-1, and C4d negative.[8] The ABMR v+ group included patients fulfilling the 2019 Banff diagnostic criteria and those with isolated MVI ≥2 $C4d^-$ plus endarteritis. Time post-KT was defined as early if the index biopsy was performed during the first month after KT and late if the index biopsy was performed after the first month. All biopsies performed during the first month post-KT were indication biopsies due to early graft dysfunction. Only one biopsy per patient was included, always considering the first episode of isolated v-lesion or v+ rejection with an available sample. Delayed graft function (DGF) was defined as the need for dialysis during the first week after KT followed by recovery of allograft function. Chronic graft dysfunction was defined as persistent estimated glomerular filtration rate <30 ml/min/1.73m^2 after the biopsy. Patients were followed up until 05/2022. Histologically normal implant biopsies from the University of Alberta Hospital were also included as controls. Donor-specific antibodies against human leukocyte antigen (HLA-DSA) were determined in serum samples contemporaneous to allograft biopsies.

Finally, a total of 92 archival FFPE human kidney allograft biopsies were recruited. These included 23 biopsies with isolated v-lesion, 26 ABMR v+ biopsies, 10 TCMR v+, 23 mixed rejection v+, and 10 normal implant biopsies (**Figure S1**).

Demographical, clinicopathological, and immunological data were collected until graft loss, death, or May/2022. The median follow-up time after the index biopsy was 36.5 (14.7 – 62.9) months. The Parc de Salut Mar Ethical Research Board (2021/10098) approved the study, and all patients gave written informed consent. Clinical and research activities being reported herein are consistent with the Principles of Istanbul and Helsinki Declarations. The study was conducted according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.

Sera collection and anti-HLA antibodies tests

Serum samples were collected and stored at -80ºC until analysis. Screening for anti-HLA antibodies was performed with Luminex Lifecodes LifeScreen Deluxe assay (Gen-probe®, Stamford, CT, USA). Anti-HLA alloantibody IgG identification (HLA-A, B, C, DRB1, and DQB1) was made using Luminex Lifecodes LSA Class-I and/or Class-II assays in a Luminex platform, as previously described.[23]

RNA isolation and gene expression analysis

RNA extraction and gene expression analysis were performed as previously described.[20,24,25] In brief, three consecutive 20-µm sections were obtained from each FFPE block and sent to the University of Alberta. RNA was isolated using the RNeasy FFPE Kit (Qiagen, Toronto, ON). The concentration and purity of the isolated RNA were measured using a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Gene expression was quantified with the NanoString nCounter® FLEX Analysis System (Nanostring Technologies, Seattle, WA), as per manufacturer instructions. We utilized the nCounter® B-HOT Panel, a 770-gene set designed to profile the inflammatory, innate, and adaptative immune response, including markers of three major signaling pathways: tissue damage, organ rejection,

and immune response (https://www.nanostring.com/products/ncounter-assays-panels/immunology/human-organ-transplant/).[21] Two hundred sixty-eight genes contained in this gene set were excluded from analysis due to low expression within the range of negative controls (mean expression score of <30 gene counts). This resulted in 502 genes being analyzed, including 490 experimental genes (**Table S1**) and 12 housekeeping genes. Gene annotations were determined according to the prime gene lists of previously published studies on KT and related diagnoses.[16,26–28] Hence, we defined six categories that included transcripts previously associated with ABMR, DSA (DSAST), endothelial injury (ENDAT), TCMR, early/acute injury, and late injury (**Table S2**). The functional associations of these genes are defined in **Tables S3-S6**. Quality control assessment and data normalization were performed with nSolver™ Analysis Software Version 4.0 (NanoString Technologies, Seattle, WA) using the manufacturer-recommended default settings.

Statistical analysis

According to their distribution, continuous data are presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical data are expressed as counts (percent). Comparison of continuous variables was performed by *t*-test or ANOVA for parametric data and Mann-Whitney U-test or Kruskal-Wallis test for non-parametric distributions. Nominal variables were compared using the chi-squared test or Fisher exact test, where appropriate. Log2 normalized counts were used for individual gene analysis, and mean log2 normalized counts were used for gene set analysis. Volcano plot analysis was performed using linear regression with a false discovery rate (FDR) threshold of 0.05. Principal component analysis (PCA; prcomp function in R's stats package) with 95% confidence ellipses was used on the standardized dataset (92 samples, 490 genes) to compare gene expression patterns between sample groups. Survival analyses were performed using the Kaplan-Meier

method, applying the log-rank test. Statistical significance was considered at $P<0.05$ or FDR<0.05, as appropriate. We used Stata/BE (Version 17.0, StataCorp LLC, USA) for statistical analysis and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria) for post-normalization statistical analysis and graphical presentation.

RESULTS

1. Clinical data analysis

1.1. Study population, demographics, and histology

Table 1 displays the demographic and clinical characteristics of the study cohort. We found no significant differences between groups regarding patient age, sex, ethnicity, comorbidities, cause of end-stage kidney disease (ESKD), type and time on renal replacement therapy (RRT), retransplantation, and donor characteristics. As expected, ABMR and mixed-rejection v+ groups had an increased percentage of HLA-DSA at the time of biopsy (p=0.007). Patients with ABMR v+ received more anti-thymocyte globulin as induction therapy (p=0.001). Additionally, more patients with isolated v+ early received *de novo* mTOR inhibitors (mTORi) as maintenance immunosuppression (p=0.002). Cold ischemia time was similar between groups. Median time from KT to biopsy was 13 (10 – 22) days in the isolated v+ early group, isolated v+ late: 145 (73 – 365) days, ABMR v+: 16 (13 – 195) days, TCMR v+: 73 (28 – 176) days, and mixed rejection v+: 70 (14- 172) days. Of note, 53.9% of biopsies in the isolated v+ late were protocol biopsies, compared to 0-10% in the other groups (p<0.001). Regarding antirejection treatment, TCMR, ABMR, and mixed rejection groups received more corticosteroids than isolated v+ groups (76.9-100% vs ≈40%, p<0.001). Likewise, ABMR and mixed rejection groups received more intravenous immunoglobulin and plasmapheresis than the other three groups (26.1-34.6% vs 0-7.7%, p=0.034).

Table 2 summarizes the histological findings of the index kidney biopsies by groups. Banff lesion scores, including glomerulitis (g), peritubular capillaritis (ptc), C4d deposition, interstitial inflammation (i), or tubulitis (t), differed according to the group's definition. The mean Banff v-score was similar between groups. Notably, biopsies with isolated v+ showed an increased percentage of acute tubular necrosis (ATN), and biopsies with mixed rejection v+

displayed greater inflammation within areas of interstitial fibrosis and tubular atrophy (i-IFTA) and inflammation in total cortical parenchyma (ti) scores.

1.2. Allograft and patient outcomes

Table 3 summarizes short- and long-term allograft and patient outcomes. Seventy-five percent of patients with isolated v+ early presented with DGF, followed by 58.3% of patients with ABMR v+ and 39.1% with mixed rejection v+. In contrast, only 15.4% and 10% of those with isolated v+ late or TCMR v+ had DGF (p=0.011). Similarly, 60% of patients with isolated v+ early displayed prolonged DGF that eventually became primary non-function (PNF) in contrast with the 0 – 7.7% of the other entities (p<0.001). All graft losses that occurred in the isolated v+ early group (60%) were due to the early graft dysfunction leading to this indication biopsy, which differed from the results found in the isolated v+ late (0%), ABMR v+ (19.2%), TCMR v+ (10%), and mixed rejection v+ (4.4%) groups (p=0.001). On the contrary, ABMR v+ and mixed rejection v+ showed the highest numbers of chronic graft dysfunction at follow-up (p=0.066). We found no differences in patient mortality among groups.

Next, we evaluated death-censored graft survival using Kaplan-Meier curves according to diagnosis and time after KT (**Figures 1A-B**). Patients with early isolated v+ had the worst survival one year after the biopsy (40%) in comparison with those presenting isolated v+ late (100%) or other forms of rejection v+ (82.1-93.6%; p=0.011) (**Figure 1A**). These differences persisted when we analyzed the different types of rejection v+ separately (p=0.034; **Figure 1B**).

1.3. Transcriptomic data analysis

Given the differences observed in allograft outcomes, we then asked whether transcriptomic analysis using Nanostring B-HOT Panel can distinguish between early and late isolated v-lesions and other forms of rejection v+.

We first evaluated the expression of the 490 genes in the entire cohort using PCA. **Figure 2** demonstrates significant overlap among the different v+ phenotypes, as well as significant heterogeneity within each group.

1.4. Gene expression pairwise comparison among different v+ phenotypes

Exploratory volcano plot analyses were performed in an attempt to identify novel discriminatory transcripts that can distinguish between isolated v+ early, isolated v+ late, and other forms of rejection v+. After correcting for multiple comparisons, only two injury- and repair-induced transcripts, ADAMTS1 and SERPINA3, demonstrated statistically significant higher expression in isolated v+ early versus late (FDR=0.013 and 0.022, respectively). Contrarily, only one gene involved in tissue homeostasis (SLC12A3) had significantly higher expression in isolated v+ late versus the early group (FDR=0.013) (**Figure 3A, Table 4**).

When comparing early isolated v+ with TCMR v+, we found that the former presented more upregulated transcripts related to injury-repair response (IMPDH2, SERPINA3, ABCB1, KRT19, GDF15, and ADAMTS1), endothelial injury (PDGFRB, RGS5, and TEK), oxidative stress (MET, AQP1, HYAL1, and RGN), and inflammatory response (CD24) (**Figure 3B, Table S7**). Conversely, biopsies with TCMR v+ had more upregulated TCMR-related genes (LAP3, APOL2, WARS; PLAAT4, GBP5, CD8A, and LAG3) and genes involved in interferon signaling (STAT1, GBP2, IRF1, HLA-B, GBP1, GBP4, FCGR1A, HLA-E, MX1, and HLA-DPA1). **Figure 3C** demonstrates the differences in upregulated transcripts between isolated v+ late versus TCMR. While the latter had increased expression of rejection-related genes

(ACKR1, ACVRL1, ADGRL4, ANXA1, and AOAH) and injury- and repair-induced transcripts (ABCA1, ABCB1, ALAS1, and ADAMTS1), isolated v+ late showed a less specific pattern of functional pathways including, among others, seven genes involved in interferon signaling (FCGR1A, GBP1, HLA-E, IFIT1, IFITM2, IFNGR1, and IFNGR2).

No statistically significant differential expression was identified between isolated v+ early and ABMR v+ (FDR >0.05) (**Figure 3D**). However, those genes most strongly associated with each entity, as defined by unadjusted p-values, were further examined to elucidate trends in the functional association. Seven of the top 20 genes with relatively higher expression in ABMR v+ versus isolated v+ early were ABMR-associated transcripts, including two DSA-related transcripts (PLA1A, CD74, CXCL11, GBP5, HLA-DRB3, IDO1, and IFI27). Nine of the remaining 13 genes were involved in interferon signaling (BST2, CD38, GBP1, GBP2, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, and HLA-DQB1). The top 20 genes with relatively higher expression in isolated v+ early versus ABMR demonstrated a less specific pattern of functional association, with five genes primarily involved in immune response, including antigen presentation (KLHL13), toll-like receptor signaling (HMGB1 and SIGIRR), cell proliferation (PDGFA), and T-cells (PPP3CA), five genes involved in interferon signaling (MAPK14, ABCE1, IL18, IRF6, and IFNAR1), four with injury-repair response (LTF, KRT19, SERPINA3, and ARG2), three with oxidative stress (FOXO1, HYAL1, and MET), and two with ABMR (TRIB1 and GATA3). **Figure 3E** shows molecular differences between isolated v+ late and ABMR v+. Overall, the isolated v+ late group showed an increased number of upregulated genes involved in oxidative stress (HDAC6, FABP1, ALDH3A2, and RGN) and normal cell functions, including tissue homeostasis (SLC12A3, AQP2, EHD3, and RAB40C), hematopoiesis (MME, TMEM178A, and TFRC), and metabolism (CHCHD10). Contrarily, ABMR v+ displayed more upregulated rejection- and inflammation-related (CXCL11,

FCGR3A/B, CX3CL1, CXCL10, and CXCL9) and injury-repair related genes (CTSS, SOD2, ARG2, MYD88, and NFKBIZ).

Subsequently, isolated v+ early and late were compared with mixed rejection v+ cases (Figures 3F and 3G, respectively). Isolated v+ early displayed an increased number of upregulated genes involved in injury- and repair-related transcripts (GDF15, SERPINA3, IL17RB, and ABCB1), oxidative stress (HYAL1, MET; SDC1, and AQP1), MAPK signaling pathway (ERRFI1, EGFR, and PDGFA), and endothelial cells (RGS5) when compared to the mixed rejection cases. The mixed rejection v+ group showed higher expression of ABMR and TCMR-related genes (IDO1, IFI27, WARS, LST1, CXCL11, NKG7, APOL2, CD8A, IFI30, LCP2), and transcripts involved in interferon signaling (GBP1, HLA-B, SLAMF7, HLA-DPB1, APOL1, FCER1G, GBP4). When comparing isolated v+ late with mixed rejection v+, the former displayed a heterogeneous pattern of functional pathways, including an increased number of upregulated genes involved in oxidative stress (HYAL1, ALDH3A2, FABP1, HDAC6, SDC1, ABCC2, RGN, and AQP1), tissue homeostasis (RAB40C, SLC12A3, TPMT, and UMOD), MAPK signaling (VEGFA and IGF1R) or metabolism (TPMT and CHCHD10). In contrast, mixed rejection v+ showed increased expression of ABMR and TCMR-related genes (WARS, CXCL9, CXCL11, NKG7, CXCL10, LCP2, SLAMF8, and CD8A).

Finally, an exploratory volcano plot analysis was conducted to identify discriminatory transcripts that could differentiate between patients with early isolated v+ who developed PNF and those with functioning grafts. However, no statistically significant differential expression was detected between groups, which may be attributable to the small sample size (**Figure S2**).

1.5. Gene set analysis among different v+ phenotypes

To further explore the differences and similarities between groups, transcriptional signatures previously associated with ABMR (including DSA and endothelial injury-related genes), TCMR, early/acute injury, and late injury were evaluated (**Figure 4A-F**). Gene set analysis showed lower expression of TCMR-related genes in isolated v+ groups compared to TCMR and mixed rejection ($p<0.001$). Both isolated v+ early and late had lower ABMR-related genes than ABMR, mixed rejection, and TCMR groups ($p\leq0.022$). Furthermore, late isolated v+ showed lower DSAST and ENDAT gene set expression than ABMR ($p\leq0.046$); and lower early/acute injury gene set expression than isolated v+ early, ABMR, TCMR, and mixed rejection ($p\leq0.026$). Late injury gene set expression was highest in TCMR and mixed rejection compared to the other groups ($p\geq0.034$).

DISCUSSION

Isolated v-lesion is an increasingly recognized but clinically challenging entity. Current data suggest the possibility of a non-rejection origin when it occurs early after transplantation, which could have diagnostic and treatment implications.[11,12,17] In this study, we sought to improve our understanding of this lesion by further characterizing its prognosis and molecular phenotype compared to other forms of rejection v+. We found that biopsies with isolated v-lesions display lower expression of TCMR-related genes than biopsies with TCMR and mixed rejection and lower expression of ABMR-related genes than biopsies with ABMR advocating for non-rejection mechanisms of damage involved. Moreover, early isolated v+ confers a poor prognosis and is associated with higher expression of injury- and repair-induced transcripts than late isolated v+, suggesting a different pathogenesis.

Since first being described in 2007,[29] the transplant community has attempted to elucidate the significance and prognosis of isolated endarteritis.[8–11,15,30–33] In 2015, the Banff Working Group conducted a multicenter study comparing isolated vascular rejection with TCMR v+ and controls. Their findings demonstrated that isolated vascular rejection had a 3.51-fold higher risk of allograft failure than patients without rejection.[8] Another study from Wu *et al.* similarly demonstrated unfavorable long-term outcomes in 11 cases of isolated v-lesions.[9] Conversely, the French group compared isolated endarteritis with ABMR v+, confirming better survival in the first group.[10] In the present study, however, we identified two distinct patterns of clinical behavior for the first time, depending on the post-transplant time of the isolated v+ lesion's appearance. On the one hand, early isolated v+ was associated with a poor prognosis, including a high percentage of DGF, up to 60% of PNF, and only 40% of death-censored graft survival one year after KT. The high percentage of PNF grafts in early isolated v-lesions motivated us to undertake the current study and analyze the clinical and molecular differences between these two groups and forms of rejection v+. Importantly, we observed that all graft

losses in this group were due to a prolonged DGF that eventually became PNF, leading to their pertinent indication biopsy. On the other hand, when isolated v+ occurred after a month post-KT, it was diagnosed mainly by protocol biopsies and showed favorable results, with 100% survival at one year. ABMR, TCMR, and mixed rejection v+ displayed an intermediate prognosis, although the humoral phenotypes had a more detrimental impact on graft survival. Based on these findings, we postulated that early and late isolated v+ may arise from different etiologies, with the first reflecting severe peritransplant injury. The "injury response" hypothesis suggests that stressed or injured tissues produce immunogenic substances and display increased antigenicity, leading to detrimental effects on short- and long-term KT survival.[34–36] Kidney ischemia-reperfusion injury (IRI) triggers a potent inflammatory process engaging both the innate and adaptive immune responses. This process is thought to be responsible for the initial renal injury and mediates long-term structural changes, including interstitial fibrosis or repair. Of note, while some authors have proven a significant prolonged CIT in isolated v+ cases, we did not find differences in CIT between the study groups.[8,37] Also, the discrepancy with studies indicating that isolated v+ represents a benign clinical phenotype with a satisfactory response to steroid antirejection treatment may reflect differences in group definitions (i.e., isolated v+, early isolated v+, diagnostic assignment in patients with HLA-DSA and/or g+ptc score<2),[6,11,31,38–40] the use of antirejection treatment before biopsy procurement, which could have modified the diagnosis as lesions can persist after treatment,[41] and the high percentage of expanded criteria donors in our cohort. In fact, our data showed 70% of expanded criteria donors with a mean donor age of 64 years old in patients with isolated v+ and a high percentage of cardiovascular risk factors. This demographic data was not shown in other studies with better results.[6,11] To our knowledge, no previous studies have evaluated the presence of isolated v-lesions within the course of DGF. Instead, most of our cases showing early isolated v-lesions were found in the context of prolonged DGF that eventually became

PNF. This situation poses significant therapeutic challenges when weighing the risks and benefits of immunosuppressive antirejection treatment for these patients.

Our next goal was to determine whether isolated v+ represents true vascular rejection or may illustrate a non-rejection origin such as IRI. Wohlfahrtova et al. performed transcriptomic analyses comparing early isolated v-lesions (n=6) versus TCMR (n=4) and normal findings (n=8). They demonstrated that KT biopsies featuring early isolated v-lesions reflect an injury-repair response triggered by implantation stress and may indicate endothelial injury related to ischemia-reperfusion.[12] In addition, other microarray studies have revealed weaker TCMR gene expression scores in isolated v+ cases compared to TCMR v+.[6,32] To investigate this further, we exploited the unique advantages of the NanoString® B-HOT panel to identify differences and similarities between the gene expression profiles of early and late isolated v-lesions and other forms of rejection v+. Our findings showed that KT recipients with isolated v+ (early and late) had lower expression of TCMR-related genes than TCMR v+.[6,32] Conversely, only early isolated v+ showed higher expression of injury and repair, endothelial injury, and oxidative stress-related genes than TCMR. Coupled with the fact that early isolated v+ lesions have been associated with expanded criteria donors and DGF,[8,17] these findings support the hypothesis that early isolated v+ may represent IRI.[42] What is more, exploratory differential expression analysis of individual genes between early and late isolated v+ also revealed that two acute-phase inflammatory response genes involved in the injury-repair process (SERPINA3 and ADAMTS1) displayed significantly upregulated expression in early isolated v+ compared to the latter group. On the contrary, only SLC12A3, a gene involved in tissue homeostasis, was upregulated in the late isolated v+ group.

This evidence is noteworthy as it challenges the conventional histological diagnosis through molecular techniques. A study by Salazar et al. consistently found that molecular scores questioned up to 59% of conventional diagnoses.[6] Specifically, 15 of 30 biopsies

conventionally diagnosed as pure TCMR did not exhibit pure TCMR on a molecular level. Instead, 10 cases showed no rejection, 4 had mixed rejection, and one represented pure ABMR. This approach also misclassified some cases with molecular ABMR activity as mixed rejection.[6] Although it is crucial to administer an accurate and prompt antirejection treatment to prevent the detrimental effect on kidney graft survival,[43–45] unnecessary treatment can jeopardize the patient's well-being and lead to potential complications such as infections, post-transplant lymphoproliferative disease, and cancer.[46] In our cohort, up to 40% of KT recipients with isolated v-lesions received corticosteroids, and 30% of early isolated v+ cases received antithymocyte globulin.

Given the possible concern that isolated v-lesions may have an underlying humoral component, our molecular assessment also compared the differences and similarities between early and late isolated v-lesions with ABMR and mixed rejection v+. Initial exploratory volcano plot analysis did not identify significant molecular signatures that differentiated between early isolated v-lesions and ABMR v+. Instead, late isolated v-lesions displayed significantly lower expression of ABMR-associated transcripts than ABMR v+. Nonetheless, gene set analysis among the different v+ phenotypes showed that both early and late isolated v+ had lower ABMR-related genes than ABMR, mixed rejection, and TCMR groups. Of note, TCMR v+ cases showed high expression of ABMR-related genes probably because both gene sets shared general rejection-related transcripts such as CXCL9, CXCL10, GBP5, and WARS. Finally, as expected, mixed rejection v+ cases showed higher expression of ABMR and TCMR-related genes than isolated v-lesions. However, only the early isolated v+ group displayed an increased number of upregulated genes involved in injury- and repair-related processes compared to mixed rejection. All these findings support the hypothesis that isolated v-lesions may not represent rejection, but only early isolated v-lesions may represent IRI.

The strengths of this study include the stringent case selection of isolated v-lesions, the systematic assessment of DSAs at the time of the biopsy, and the comprehensive comparison between this entity and all forms of rejection v+. Also, the combined clinical and molecular data analysis provides a more holistic understanding of this lesion. Limitations include, first, its retrospective design with a relatively small number of cases per group, which reflects the rarity of biopsy-proven examples of this lesion, particularly with residual tissue available for molecular analysis. Second, we lack data on warm ischemia time, the number of allograft arteries, and surgical complications, which could have contributed to the peritransplant injury. Finally, a maximum of only 800 genes could be analyzed with the NanoString® nCounter platform, which is significantly less than the tens of thousands of genes possible to study with microarrays and RNA sequencing. Nonetheless, previous microarray studies have demonstrated the highly stereotyped nature of inflammatory molecular signals in allograft tissue, and analysis of a carefully selected panel of representative genes, such as the NanoString® B-HOT panel, is likely adequate.

In conclusion, this study provides a novel analysis of gene expression in biopsies with isolated v-lesion according to time after transplantation and compared to other types of rejection v+. Although we do not study the expression of the individual cells causing the v lesions, our data show that KT recipients with isolated v-lesions present lower expression of TCMR-related genes than TCMR and mixed rejection and lower expression of ABMR-related genes than biopsies with ABMR v+. Early isolated v+ histology displays inferior allograft outcomes and is associated with higher expression of injury-repair genes than late isolated v+, suggesting a different etiology. These results further enhance our understanding of the potential molecular mechanisms operating in the tissue of biopsies with isolated v-lesions and suggest the need to reevaluate diagnostic categorization and therapeutic approaches of cases with isolated v-lesions.

AUTHOR CONTRIBUTIONS

Project conception: AB and MJP-S. Specific study design: AB, MM, BAA, and MJP-S. Data acquisition: AB, BC, JG, IT. Analysis and interpretation of data: AB, BAA, and MM. Draft of the manuscript: AB and BAA. Critical revision or mentoring: MJP-S, DR-P, MR, CB, JP, MM, BAA, and MC. AB was the major contributor in writing the manuscript. All the authors revised and approved the final version of the manuscript.

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DISCLOSURE

The authors declare no financial disclosures or conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1. Demographic and clinical characteristics of the study population[1].

	Isolated v+ early (n=10)	Isolated v+ late (n=13)	ABMR v+ (n=26)	TCMR v+ (n=10)	Mixed Rejection v+ (n=23)	p-value
Recipient characteristics						
Age at transplantation, years, mean ± SD	66.4 ± 8.9	57.1 ± 16.6	61.9 ± 14.7	52.7 ± 18.8	57.2 ± 14.5	0.298
Female sex, N (%)	2 (20)	7 (53.9)	12 (46.2)	2 (20)	5 (21.7)	0.230
Ethnicity, N (%)						
Caucasian	8 (80)	10 (76.9)	22 (84.6)	7 (70)	20 (90.1)	
Black	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	
Latin American	1 (10)	1 (7.7)	5 (15.4)	2 (20)	0 (0)	0.459
Asian	0 (0)	1 (7.7)	0 (0)	0 (0)	2 (9.1)	
Other	1 (10)	1 (7.7)	0 (0)	0 (0)	0 (0)	
Hypertension, N (%)	10 (100)	12 (92.3)	22 (84.6)	10 (100)	21 (91.3)	0.839
Diabetes mellitus, N (%)	4 (40)	7 (53.9)	9 (34.6)	4 (30)	8 (34.8)	0.473
Cardiovascular disease, N (%)	2 (20)	3 (23.1)	6 (23.1)	2 (20)	2 (8.7)	0.115
Cerebrovascular disease, N (%)	1 (10)	1 (7.7)	1 (3.9)	0 (0)	4 (17.4)	0.942
Peripheral vascular disease, N (%)						
None	5 (50)	8 (61.5)	21 (80.8)	9 (90)	17 (78.3)	
Mild	1 (10)	3 (23.1)	2 (7.7)	1 (10)	2 (8.7)	0.177
Moderate/severe	4 (40)	2 (15.4)	3 (11.5)	0 (0)	3 (13)	
BMI, kg/m2, mean ± SD	28.9 ± 3.9	28.5 ± 4.6	26.6 ± 5.2	28.6 ± 4.9	25.5 ± 5.4	0.058

HCV, N (%)	1 (10)	1 (7.7)	2 (7.7)	1 (10)	1 (4.4)	0.125
Cause of ESKD, N (%)						
Glomerulonephritis	0 (0)	1 (7.7)	4 (15.4)	1 (10)	3 (13)	
PKD	2 (20)	2 (15.4)	2 (7.7)	1 (10)	1 (4.4)	
Reflux/obstructive nephropathy	0 (0)	0 (0)	2 (7.7)	0 (0)	1 (4.4)	
Hypertension	0 (0)	2 (15.4)	1 (3.9)	1 (10)	0 (0)	0.433
Diabetes	3 (30)	6 (46.2)	5 (19.2)	1 (10)	7 (30.4)	
Other	1 (10)	1 (7.7)	2 (7.7)	0 (0)	3 (13)	
Unknown	4 (40)	1 (7.7)	10 (38.5)	6 (60)	8 (34.8)	
Type of RRT, N (%)						
Hemodialysis	7 (70)	9 (69.2)	22 (84.6)	8 (80)	16 (69.6)	
Peritoneal dialysis	1 (10)	2 (15.4)	1 (3.9)	2 (20)	4 (17.4)	0.957
None (pre-emptive transplant)	2 (20)	2 (15.4)	3 (11.5)	0 (0)	3 (13)	
Time on RRT, months, median (IQR)	3.4 (2.1 – 5.4)	1.4 (1.0 – 2.7)	3.2 (1.0 – 6.8)	1.5 (1.0 – 2.0)	1.6 (1.9 – 3.3)	0.183
Retransplantation, N (%)	0 (0)	1 (7.7)	6 (23.1)	1 (10)	4 (17.4)	0.269
Biopsy indication, n (%) For-cause Protocol	 10 (100) 0 (0)	 6 (46.1) 7 (53.9)	 26 (100) 0 (0)	 9 (90) 1 (10)	 23 (100) 0 (0)	**<0.001**
Immunologic profile						
HLA-A/B/DR mismatch, mean ± SD	4.5 ± 1.2	4.1 ± 1.0	4.4 ± 0.9	4.4 ± 1.1	4.0 ± 1.5	0.799
Pre-transplant HLA-DSA	0 (0)	0 (0)	5 (19.2)	0 (0)	3 (13)	0.247
Post-transplant HLA-DSA (at Bx)	0 (0)	0 (0)	8 (30.8)	0 (0)	8 (34.8)	**0.007**
Post-transplant HLA-DSA (ever after KT)	0 (0)	2 (15.4)	10 (38.5)	4 (40)	12 (52.2)	**0.019**

Donor characteristics						
Donor age, years, mean ± SD	63.7 ± 5.5	63.5 ± 14.0	64.7 ± 16.6	52 ± 20.2	59 ± 14.2	0.226
Donor sex female, N (%)	2 (20)	7 (53.9)	14 (53.9)	6 (60)	8 (34.8)	0.240
Type of donor, N (%)						
Living donor	2 (20)	0 (0)	4 (15.4)	2 (20)	5 (21.7)	
DBD	6 (60)	8 (61.5)	10 (38.5)	7 (70)	12 (52.2)	0.146
DCD (controlled)	1 (10)	5 (38.5)	12 (46.2)	1 (10)	4 (17.4)	
DCD (uncontrolled)	1 (10)	0 (0)	0 (0)	0 (0)	2 (8.7)	
Expanded criteria donor[2], N (%)	7 (70)	9 (69.2)	20 (76.9)	5 (50)	11 (47.8)	0.237
History of hypertension, N (%)	5 (50)	9 (69.2)	12 (46.2)	3 (30)	11 (47.8)	0.612
History of diabetes mellitus, N (%)	1 (10)	4 (30.8)	4 (15.4)	2 (20)	1 (4.4)	0.244
BMI, kg/m2, mean ± SD	29.3 ± 2.9	26 ± 9.6	28 ± 5.3	28.3 ± 4.7	27.8 ± 5.4	0.690
Cerebrovascular disease as death cause, N (%)	5 (62.5)	6 (46.2)	11 (50)	4 (50)	9 (47.4)	0.981
HCV positive, N (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.7)	0.374
ABOi	0 (0)	0 (0)	2 (7.7)	0 (0)	1 (4.4)	0.912
Cold ischemia time, hours, mean ± SD	14.6 ± 7.1	15.7 ± 6.8	14.5 ± 7.2	13.2 ± 5.1	11.5 ± 7.7	0.322
Baseline immunosuppression						
Induction therapy, N (%)						
None	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	
Antithymocyte globulin	0 (0)	1 (7.7)	9 (34.6)	1 (10)	0 (0)	**0.001**
Anti-CD25 monoclonal antibodies	10 (100)	12 (92.3)	17 (65.4)	8 (80)	23 (100)	
Maintenance immunosuppression						

Corticosteroids, N (%)	10 (100)	13 (100)	26 (100)	10 (100)	22 (95.7)	0.683
CNI + Mycophenolate mofetil, N (%)	4 (40)	13 (100)	23 (88.5)	10 (100)	19 (92.6)	**0.002**
CNI + *de novo* mTORi, N (%)	6 (60)	0 (0)	3 (11.5)	0 (0)	4 (17.4)	**0.002**
Rejection treatment						
Corticosteroids (IV), N (%)	4 (40)	4 (30.8)	20 (76.9)	10 (100)	20 (87.0)	**<0.001**
Antithymocyte globulin, N (%)	3 (30)	1 (7.7)	10 (38.5)	4 (40)	13 (56.52)	0.059
IVIG and plasmapheresis, N (%)	0 (0)	1 (7.7)	9 (34.6)	0 (0)	6 (26.1)	**0.034**
Rituximab, N (%)	0 (0)	1 (7.7)	6 (23.1)	2 (20.0)	5 (21.74)	0.464
Follow-up times						
Median time from KT to Bx, days, median (IQR)	13 (10 – 22)	145 (73 – 365)	16 (13 – 195)	73 (28 – 176)	70 (14- 172)	**0.002**
Follow-up time after KT, months, median (IQR)	3.0 (2.0 – 56.3)	51.6 (38.9 – 69.9)	31.7 (14.9 – 54.1)	82.7 (58.2 – 113.0)	36.3 (20.9 – 74.1)	**0.001**
Follow-up time after Bx, months, median (IQR)	2.3 (1.6 – 56.1)	46.0 (37.1 – 56.7)	24.1 (14.0 – 44.1)	68.1 (47.4 – 104.5)	35.9 (14.7 – 66.6)	**0.002**

ABMR: antibody-mediated rejection; ABOi: ABO incompatible; BMI: body mass index; Bx: biopsy; CNI: calcineurin inhibitors; DBD: donation after brain death; DCD: donation after circulatory death; DSA: donor-specific antibodies; ESKD: end-stage kidney disease; HCV: hepatitis C virus; HLA: human leukocyte antigen; IQR: interquartile range; IV: intravenous; IVIG: intravenous immunoglobulin, KT: kidney transplantation; mTORi: mTOR inhibitor; N: number; PKD: polycystic kidney disease; RRT: renal replacement therapy; SD: standard deviation; TCMR: T-cell mediated rejection; v: endarteritis.

[1]We excluded normal cases from the comparison since histologically normal implant biopsies comprised the control group, and no subsequent follow-up assessments were conducted.

[2]Expanded criteria donors were defined as any donor over the age of 60 years or a donor over the age of 50 years with 2 of the following 3 items: (1) history

of high blood pressure, (2) serum creatinine ≥1.5 mg/dl, and (3) death due to stroke.

Table 2. Comparison of histological features between groups.

	Isolated v+ early (n=10)	Isolated v+ late (n=13)	ABMR v+ (n=26)	TCMR v+ (n=10)	Mixed Rejection v+ (n=23)	Global p-value
Percentage of glomerulosclerosis, mean ± SD	13.40 ± 11.58	7.23 ±7.05	10.42 ± 12.16	7.30 ± 10.04	12.13 ± 10.45	0.430
g, mean ± SD	0 ± 0	0 ± 0	1.81 ± 1.02	0 ± 0	1.26 ± 1.25	**<0.001**
g ≥1, n (%)	0 (0)	0 (0)	23 (88.5)	0 (0)	14 (60.9)	**<0.001**
ptc, mean ± SD	0.50 ± 0.53	0.38 ± 0.51	1.46 ± 0.71	0.90 ± 0.32	1.83 ± 0.49	**<0.001**
ptc ≥1, n (%)	5 (50)	5 (38.5)	23 (88.5)	9 (90)	23 (100)	**<0.001**
MVI score (g + ptc), mean ± SD	0.50 ± 0.53	0.38 ± 0.51	3.27 ± 1.19	0.90 ± 0.32	3.09 ± 1.47	**<0.001**
MVI (g + ptc ≥2), n (%)	0 (0)	0 (0)	25 (96.2)	0 (0)	22 (95.7)	**<0.001**
C4d, mean ± SD	0 ± 0	0 ± 0	0.85 ± 1.22	0 ± 0	0.39 ± 0.94	**0.005**
C4d ≥1, n (%)	0 (0)	0 (0)	10 (38.5)	0 (0)	4 (17.4)	**0.005**
cg, mean ± SD	0 ± 0	0 ± 0	0.23 ± 0.65	0 ± 0	0.09 ± 0.29	0.273
cg, n (%)	0 (0)	0 (0)	4 (15.4)	0 (0)	2 (8.7)	0.458
v, mean ± SD	1.10 ± 0.32	1.08 ± 0.28	1.15 ± 0.46	1.20 ± 0.63	1.35 ± 0.71	0.699
v ≥1, n (%)	10 (100)	13 (100)	26 (100)	10 (100)	23 (100)	n.a.
i, mean ± SD	0.30 ± 0.48	0.23 ± 0.44	0.15 ± 0.37	2.60 ± 0.70	1.96 ± 0.88	**<0.001**
i ≥1, n (%)	3 (30)	3 (23.1)	4 (15.4)	10 (100)	23 (100)	**<0.001**
t, mean ± SD	0.30 ± 0.48	0.31 ± 0.48	0.08 ± 0.27	2 ± 0.82	1.65 ± 0.83	**<0.001**
t ≥1, n (%)	3 (30)	4 (30.8)	2 (7.7)	10 (100)	22 (95.7)	**<0.001**
Borderline (Banff 2019), n (%)	2 (20)	3 (23.1)	0 (0)	n.a.	n.a.	n.a.

ti, mean ± SD	1.25 ± 0.71	0.20 ± 0.42	0.64 ± 0.50	1 ± 0	1.94 ± 0.85	**<0.001**
ti ≥1, n (%)	8 (100, n=8)	2 (20, n=10)	7 (63.6, n=11)	2 (100, n=2)	15 (93.8, n=16)	**<0.001**
i-IFTA, mean ± SD	1.25 ± 0.71	0.50 ± 0.97	0.91 ± 0.94	0.50 ± 0.71	1.88 ± 1.20	**0.019**
i-IFTA ≥1, n (%)	8 (100, n= 8)	3 (30, n=10)	7 (63.6, n=11)	1 (50, n= 2)	13 (81.3, n=16)	**0.010**
mm, mean ± SD	0.20 ± 0.63	0.08 ± 0.28	0.12 ± 0.33	0 ± 0	0.09 ± 0.29	0.867
mm ≥1, n (%)	1 (10)	1 (7.7)	3 (11.5)	0 (0)	2 (8.7)	0.972
ci, mean ± SD	1.50 ± 0.85	1.00 ± 0.91	1.12 ± 0.71	0.70 ± 0.67	0.91 ± 0.51	0.125
ci ≥1, n (%)	10 (100)	9 (69.2)	22 (84.6)	6 (60)	19 (82.6)	0.160
ct, mean ± SD	1.00 ± 0.94	1.31 ± 0.75	0.81 ± 0.80	0.70 ± 0.67	1 ± 0.60	0.127
ct ≥1, n (%)	7 (70)	12 (92.3)	16 (61.5)	6 (60)	20 (87)	0.100
cv, mean ± SD	1.60 ± 0.84	1.31 ± 1.11	1.35 ± 0.85	0.70 ± 1.06	1.04 ± 0.71	0.131
cv ≥1, n (%)	9 (90)	9 (69.2)	22 (84.6)	4 (40)	18 (78.6)	0.068
ah, mean ± SD	1.00 ± 1.15	0.23 ± 0.60	0.65 ± 0.89	0.50 ± 0.71	0.83 ± 0.89	0.567
ah ≥1, n (%)	5 (50)	2 (15.4)	10 (38.5)	4 (40)	13 (56.5)	0.218
TMA, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	n.a.
ATN, n (%)	9 (90)	4 (30.8)	17 (65.4)	4 (40)	6 (26.1)	**0.002**
Glomerulonephritis, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	n.a.

ABMR: antibody-mediated rejection; ah: arteriolar hialinosis; ci: interstitial fibrosis; cg: chronic glomerulopathy; ct: tubular atrophy; cv: transplant arteriopathy; g: glomerulitis; i: interstitial inflammation; i-IFTA: inflammation in areas of interstitial fibrosis and tubular atrophy; mm: mesangial matrix

expansion; MVI: microvascular inflammation; ptc: peritubular capillaritis; SD: standard deviation; t: tubulitis; ti: total cortical inflammation; TCMR: T-cell mediated rejection; v: endarteritis.

Table 3. Comparison of allograft and patient outcomes between groups.

	Isolated v+ early (n=10)	Isolated v+ late (n=13)	ABMR v+ (n=26)	TCMR v+ (n=10)	Mixed Rejection v+ (n=23)	p-value
Allograft and patient outcomes						
Delayed graft function[1], N (%)	3 (75)	2 (15.4)	14 (58.3)	1 (10)	9 (39.1)	**0.011**
Primary non-function[2], N (%)	6 (60)	0 (0)	2 (7.7)	0 (0)	0 (0)	**<0.001**
Chronic graft dysfunction after the episode, N (%)	0 (0)	3 (23.1)	9 (45)	0 (0)	7 (31.8)	0.066
Graft loss due to the episode, N (%)	6 (60)	0 (0)	5 (19.2)	1 (10)	1 (4.4)	**0.001**
Global death-censored graft failure, N (%)	6 (60)	1 (7.7)	8 (30.8)	3 (30)	12 (52.2)	**0.035**
Mortality, N (%)	1 (10)	3 (23.1)	6 (23.1)	3 (30)	2 (8.7)	0.505

ABMR: antibody-mediated rejection; N: number; SD: standard deviation; TCMR: T-cell mediated rejection; v: endarteritis.

[1]Patients with PNF are excluded.

[2]Patients with prolonged DGF (>7 days) that eventually became PNF.

Table 4. Upregulated and downregulated genes in isolated v+ early (n=10) vs late (n=13).

Genes	Gene name	Functional annotation / gene sets	Fold change (isolated v+ early vs. late)	Unadjusted P-value	FDR
Upregulated genes					
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif 1	Tissue and cellular process: angiogenesis, cell-ECM interaction. IRRAT, IRITD3.	2.693	5.44E-05	0.013
SERPINA3	Serpin family A member 3 / AACT	Tissue and cellular process: cell process. Immune system: innate immune system. IRITD3, IRRAT.	4.361	3.62E-05	0.022
Downregulated genes					
SLC12A3	Solute Carrier Family 12 Member 3	Tissue and cellular process: tissue homeostasis. Organ specific: kidney. eGFR later, KT1, KT2.	0.107	1E-04	0.013

ECM: extracellular matrix; FDR: false discovery rate; N: number; v: endarteritis.

FIGURE LEGEND

Figure 1. Kaplan-Meier survival curves.

(**A**) Death-censored graft survival after grouping all cases according to isolated vs. rejection v+ profile. (**B**) Death-censored graft survival according to the study groups. Patients with early isolated v+ had the worst survival one year after the biopsy (40%) in comparison with those presenting isolated v+ late (100%) or other forms of rejection v+.

ABMR: antibody-mediated rejection; TCMR: T-cell mediated rejection; v: endarteritis.

Figure 2. Gene expression patterns among different v+ phenotypes

Principal component analysis evaluating the expression of 490 genes in the full study cohort demonstrates significant overlap among the different v+ phenotypes.

ABMR: antibody-mediated rejection; PC: principal component; TCMR: T-cell mediated rejection; v: endarteritis.

Figure 3. Gene expression pairwise comparison among different v+ phenotypes.

(**A-G**) Volcano plots show the gene expression pairwise comparison among different v+ phenotypes for 490 immune-related genes. Fold change is represented by the y-axis, and linear regression p-value is represented by the x-axis. Red, purple, green, blue, orange, yellow, and gray represent genes previously associated with ABMR, DSAST, ENDAT, TCMR, early-injury, late-injury, and other gene pathways, respectively. FDR-adjusted P value <0.05 was considered significant (dashed line).

ABMR: antibody-mediated rejection; TCMR: T-cell mediated rejection; v: endarteritis.

Figure 4. Gene set analysis among the different v+ phenotypes.

Box plots display gene set expression scores among the different v+ phenotypes. Each figure (**A-F**) represents the analysis of transcriptional signatures previously associated with ABMR, DSAST, ENDAT, TCMR, early/acute injury, and late injury, respectively. Boxes represent the interquartile range, and whiskers represent the upper and lower extremes.

ABMR: antibody-mediated rejection; TCMR: T-cell mediated rejection; v: endarteritis.