Association of high pre-transplant Caveolin-1 serum concentrations with attenuated acute cellular rejection in kidney transplantation

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Abstract

Acute and chronic transplant rejections due to alloreactivity are essential contributors to graft loss. However, the strength of alloreactivity is biased by non-immunological factors such as ischemia reperfusion injury (IRI). Accordingly, protection from IRI could be favorable in terms of limiting graft rejection.

Caveolin-1 (Cav-1) is part of the cell membrane and an important regulator of intracellular signaling. Cav-1 has been demonstrated to limit IRI and to promote survival of a variety of cell types including renal cells under stress conditions. Accordingly, Cav-1 could also play a role in limiting anti-graft immune responses.

Here, we evaluated a possible association between pretransplant serum concentrations of Cav-1 and the occurrence of rejection during follow up in a pilot study. Therefore, Cav-1-serum concentrations were analyzed in 91 patients at the time of kidney transplantation and compared to the incidence of acute and chronic rejection. Higher Cav-1 levels were associated with lower occurrence of acute rejection episodes. Moreover, Cav-1 could be therapeutically useful for attenuating graft rejection.

Keywords: Caveolin-1, kidney transplantation, graft rejection, ischemia, and reperfusion injury (IRI)

1. Introduction

The success of organ transplantation depends essentially on the control of acute and chronic rejection of the graft. Suchlike alloresponses are initiated by the presentation of disparate alloantigens by either the donor's or the recipient's antigen presenting cells to the host's immune system. Both pathways trigger the proliferation of allospecific T-cells and B-cells producing donor-specific antibodies directed against the graft endothelium (Valenzuela, McNamara et al. 2014, Lin and Gill 2016). However, alloreaction is not a stereotypical process and its strength is confounded by several non-immunological factors.

Particularly ischemia and reperfusion injury (IRI) play a detrimental role in modulating adaptive and innate alloresponses (Mikhalski, Wissing et al. 2008, Eltzschig and Eckle 2011, Fuquay, Renner et al. 2013, Rao, Lu et al. 2014, Postalcioglu, Kaze et al. 2018). This process is closely related to the up-regulation of intra-graft Toll-like-receptor (TLR) expression during IRI (Wu and Chadban 2014). In particular, TLR4 signaling furthermore amplifies graft damage by apoptotic induction of tubular endothelial cells as reflected by the presence of activated effector caspase 3 and cleaved Bax (Wu, Chen et al. 2007, Goncalves-Primo, B. et al. 2014, Nieuwenhuijs-Moeke, Pischke et al. 2020).

Caveolins are components of the plasma membrane invaginations called Caveolae, which are engaged in endothelial cell function and signaling (Sowa 2012). Caveolin signaling has been demonstrated to protect several cell types from IRI (Kang and Lee 2014, Liu, Wu et al. 2018). Since IRI is closely related to cell death, this effect might be due to the antiapoptotic function of Caveolins (Das, Cui et al. 2007, Wang, Jia et al. 2008) . Particularly, for renal cells the survival-promoting function of Caveolin-1 (Cav-1) has been demonstrated in response to apoptotic stimuli (Percy, Pat et al. 2008, Chen, Lin et al. 2016).

In addition, Cav-1-containing extracellular vesicles modulate cellular signaling pathways and crosstalk between endothelial cells and distal cellular system (Crewe, Joffin et al. 2018). Therefore, we speculated that the presence of Cav-1 in serum might be relevant in renal transplantation.

In order to address this question, we conducted a pilot study having analyzed Cav-1 serum concentrations in a cohort of 91 patients prior to kidney transplantation and examined its association with HLA-mismatches (A, B, DR), patient sex, age at serum drawn and histology, including histological features of graft rejection according to the Banff-classification. Elevated Cav-1-serum concentrations correlated significantly with reduced incidence of cellular rejection episodes. Our data suggests pre-transplantation Cav-1 serum concentrations could be a useful tool for the identification of transplantation patients at risk for acute cellular rejection.

2. Results

Patients

Serum samples were collected between 2003 and 2017. Kidney biopsies were collected between 2004 and 2018. The cohort consisted of 91 HLA-matched patients, males (n=60) being the majority, females (n = 31) the minority. 77 patients received a first renal transplant, whereas 13 underwent second and one third transplantation. Data of age at Cav-1 serum draw and kidney biopsies, Cav-1 serum level, active

interstitial and tubular inflammation, active glomerular inflammation and chronic interstitial scarring was available from all 91 patients. Data of active vascular inflammation was available from 90 patients, chronic tubular atrophy from 89 patients and HLA mismatches from 81 patients (HLA-A, -B and -DR). The overview of the descriptive statistics is summarized in Table 1 for the patients' characteristics and in Table 2 for the histological parameters.

Table 1: Descriptive statistics of patients' values

	HLA-A	HLA-B	HLA-DR	Sum HLA	Cav-1	Cav-1	Sex	Age S	Age B	Days SB
count	86	86	86	86	91	91	91	91	91	91
mean	0.79	0.95	0.97	2.71	1029.23	0.35	1.34	47.78	50.96	1108.88
std	0.74	0.65	0.69	1.63	1180.46	0.67	0.48	14.38	14.14	1354.83
min	0	0	0	0	3.57	0	1	7	15	8
25%	0	1	0.25	2	117.39	0	1	39	43	51.5
50%	1	1	1	3	586.65	0	1	50	53	435
75%	1	1	1	4	1543.82	1	2	59.5	62	1644.5
max	2	2	2	6	5882.83	3	2	72	75	4657

HLA-A	HLA-Mismatch A
HLA-B	HLA-Mismatch B
HLA-DR	HLA-Mismatch DR
Sum HLA	Sum HLA-Mismatch
Cav-1	Serum level Cav-1(pg/ml)
Cav-1 class	Caveolin classifier (pg/ml; < 1500 = 0, 1500-2999 = 1, 3000-4499 = 2, < 4499 = 3)
Sex	Sex (male = 1, female = 2)
Age S	Patients' age at serum draw
Age B	Patients' age at sole or latest biopsy
Days SB	Number of days between serum draw and latest biopsy

Table 2: Descriptive statistics of histological parameters

	i	t	v	g	ptc	ti	i-	C4d	cg	mm	ah	aah	cv	ci	ct	acr	amr	hrs
count	91	91	90	91	70	46	44	77	91	91	88	67	89	91	89	91	65	82
mean	0.38	0.51	0.16	0.31	0.23	0.57	1.14	0.38	0.56	0.67	0.86	0.63	0.39	0.69	0.90	0.93	0.60	0.87
std	0.68	0.83	0.45	0.71	0.62	0.78	1.09	0.76	1.06	1.00	1.12	1.11	0.78	0.80	0.71	1.08	0.86	1.48
min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50%	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0
75%	1	1	0	0	0	1	2	0	0.5	1	2	1	1	1	1	2	1	1
max	3	3	2	3	2	3	3	3	3	3	3	3	3	3	3	3	2	8

i	Acute interstitial inflammation according to Banff score
t	Acute tubulitis according to Banff score
v	Acute vascular rejection according to Banff score
g	Acute glomerulitis according to Banff score
ptc	Acute peritubular capillaritis according to Banff score
ti	Total inflammation according to Banff score
i-IFTA	Inflammation in interstitial fibrosis and tubular atrophy, according to Banff score
C4d	C4d according to Banff score
cg	Tx glomerulopathy according to Banff score
mm	Mesangial matrix increase according to Banff score
ah	Arteriolar hyalinosis according to Banff score
aah	CNI type arteriolar hyalinosis according to Banff score
cv	Tx vasculopathy according to Banff score
ci	Interstitial fibrosis according to Banff score
ct	Tubular atrophy according to Banff score
acr	Acute cellular rejection score: none (=0), borderline (=2), acute cellular rejection (=3)
amr	Acute antibody mediated rejection score: none, borderline, acute antibody mediated rejection
hrs	Humoral rejection score: at least two of g, ptc, C4d values present and summarized

Cav-1 serum concentration in patients prior to kidney transplantation

Serum concentration varied in the range of 3.6 to 5882.8 pg/ml and herewith in the range of a previous report on healthy individuals (Tahir, Ren et al. 2003). The mean concentration was 1029.2 pg/ml; median was 586.7 pg/ml. Cav-1 serum levels were categorized in a four-tiered classifier to harmonize the scale levels to the Banff classification scheme (see also Materials and Methods). According to the Cav-1 classifier (CC), the distribution was as follows: $n0 = 67$, $n1 = 18$, $n2 = 4$, $n3 = 2$.

Higher caveolin serum levels are associated with lower intensities of acute cellular tubulointerstitial rejections but not with tubular atrophy

Histological items classified according to the Banff classification scheme were correlated with Cav-1 serum levels, using the Spearman rho correlation coefficient. One biopsy per patient has been considered, which was either the only one or the last one. The acute T cell-mediated rejection (ACR) score was classified as either no cellular rejection, borderline (suspicious for cellular rejection) or manifest cellular rejection, which were defined through the sum of square roots of t and i values from the Banff classifications, respectively. 51 patients did not show a cellular rejection, 35 patients displayed a borderline status, and 5 patients showed a manifest cellular rejection, which were defined through the sum of square roots of t and i values from the Banff classifications, respectively. Due to the low number of acute cellular rejections, a further subcategorization of T cell mediated rejection into one of the types IA-III (Jeong 2020) was not performed. Higher Cav-1 serum levels are associated with lower ACR scores (acute cellular rejection score (ACR) vs. CC: r=-0.257, p=0.014; n=91) and lower acute cellular rejections in the interstitial compartment (i vs. CC r=-0.241, p=0.021, n=91).

The CC positively correlates with the Tx age, measured in days from the serum draw until the sole or latest biopsy (CC vs. days r=0.25, p=0.017, n=91), indicating higher CC is associated with higher Tx age. Acute cellular rejection in the interstitial compartment negatively correlates with the number of days between serum draw and sole or latest biopsy (i vs. days r=-0.24, p=0.022, n=91), which is also the case for the ACR score (ACR vs. days r=-0.23, p=0.028, n=91), indicating cellular rejections being an earlier event in our cohort. The opposite is the case for acute glomerulitis (g vs. days r=0.217, p=0.039, n=91).

In contrast, neither parameter of chronic damage according to the Banff classification scheme, such as interstitial fibrosis or tubular atrophy correlated significantly with absolute Cav-1 serum levels or the CC, nor acute humoral rejections. Figure 1 provides an overview of scatter plots with Cav-1 vs. histological parameters according to the Banff classification scheme. Tubular atrophy and interstitial scarring correlate with transplantation glomerulopathy, chronic arteriolar damage (arteriolohyalinosis) and transplantation vasculopathy and the number of days between the serum draw and the latest biopsy. Peritubular capillaritis correlates with ACR and acute antibody mediated rejection. A detailed list of the respective correlation coefficients is given in the supplemental table.
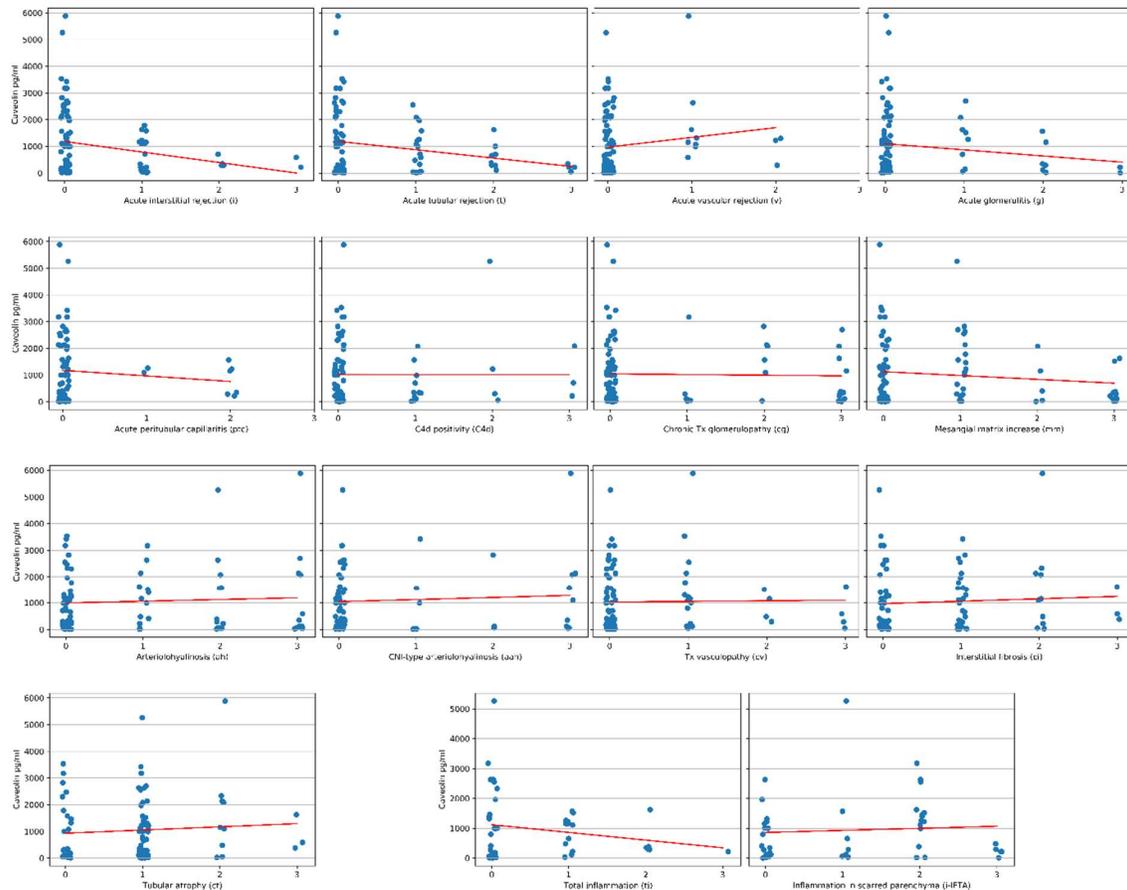
Figure 1: Scatterplots of Cav-1 serum levels in pg/ml vs. items according to the histological categories of the Banff classification

Pretransplant Cav-1 serum levels are better than pretransplant HLA mismatches in predicting acute cellular tubulointerstitial rejections in the posttransplant setting

Data of HLA mismatches was available from 81 patients. None of the HLA mismatches (HLA-A, -B, -DR) significantly correlated with acute or chronic cellular rejections, according to the Banff classification, which was also true for the sum of HLA mismatches (supplemental table). To exclude biases due to differing scale levels or non-monotonic relations, an entropy reduction based decision tree analysis was conducted as multivariable analysis to predict the classifiers "no cellular rejection", "borderline cellular rejection" or "active cellular rejection". As in other multivariable analyses, the dataset must be complete (i.e. without missing or "empty" values), which was the reason to perform three decision tree analyses to deal with empty or missing values. The first decision tree analysis (max. depth=4, min. samples per leaf=5) included the HLA mismatches (n=81) as part of the following items: 'HLA-mismatch A', 'HLA-mismatch B', 'HLA-mismatch DR', 'Sum mismatches', 'Cav-1 (pg/ml)', 'Sex (m=1, f=2)', 'Patient age at serum draw', 'Patient age at latest biopsy', 'Days between serum draw and latest biopsy', 'v', 'g', 'cg', 'mm', 'cv' and 'Sum chronic damage: ci+ct'. The only HLA mismatch playing a role in our cohort was HLA-DR as a weak level 3 predictor of lower (1/8), if no mismatch, or higher (13/27), if mismatch, proportion of borderline cellular rejection. Level 2 was patient's age at

serum draw ≤ 45; level 1 was acute glomerulitis of Banff grades g0, g1. Cav-1 serum level > 2107.46 pg/ml was the level 0 predictor of no rejection (15/16; Figure 2).

To check whether there might be other factors than Cav-1 serum levels, a second decision tree analysis (max. depth=4, min. samples per leaf=5) was conducted without HLA mismatch parameters, allowing the inclusion of more patients of the cohort being part of this analysis (n=86). The following items were included: 'Cav-1(pg/ml)', 'Sex (m=1, f=2)', 'Patient age at serum draw', 'Patient age at latest biopsy', 'Days between serum draw and latest biopsy', and according to the Banff classification: 'v', 'g', 'cg', 'mm', 'cv', 'sum chronic damage: ci+ct'. Here, Cav-1 serum level ≤ 2107.46 pg/ml was a level 1 predictor of higher borderline and ACR (31/61 vs 1/16 if the Cav-1 serum level is higher; Figure 3). Finally, a third decision tree analysis was performed, including only patients with all histological parameters of acute humoral rejection (n=64). The following items were included: 'Cav-1(pg/ml)', 'patient age at serum draw', 'patient age at latest biopsy', 'days between serum draw and latest biopsy', 'v', 'g', 'ptc', 'C4d', 'cg', 'mm', 'ah', 'cv', 'sum chronic damage: ci+ct'. Again, Cav-1 (serum level ≤ 2593.73 pg/ml) was a level 1 predictor of borderline and ACR (Figure 4). Decision trees 1 and 2 were stable over 1'000 runs. The third decision tree (Figure 4) displays the parameters "Patient age at latest biopsy" and "Patient age at serum draw" being almost equivalent predictors at level 3 (49.2% for the first vs 50.8% for the latter in 10'000 runs). All other parameters tested remained stable, including the Cav-1 serum level.
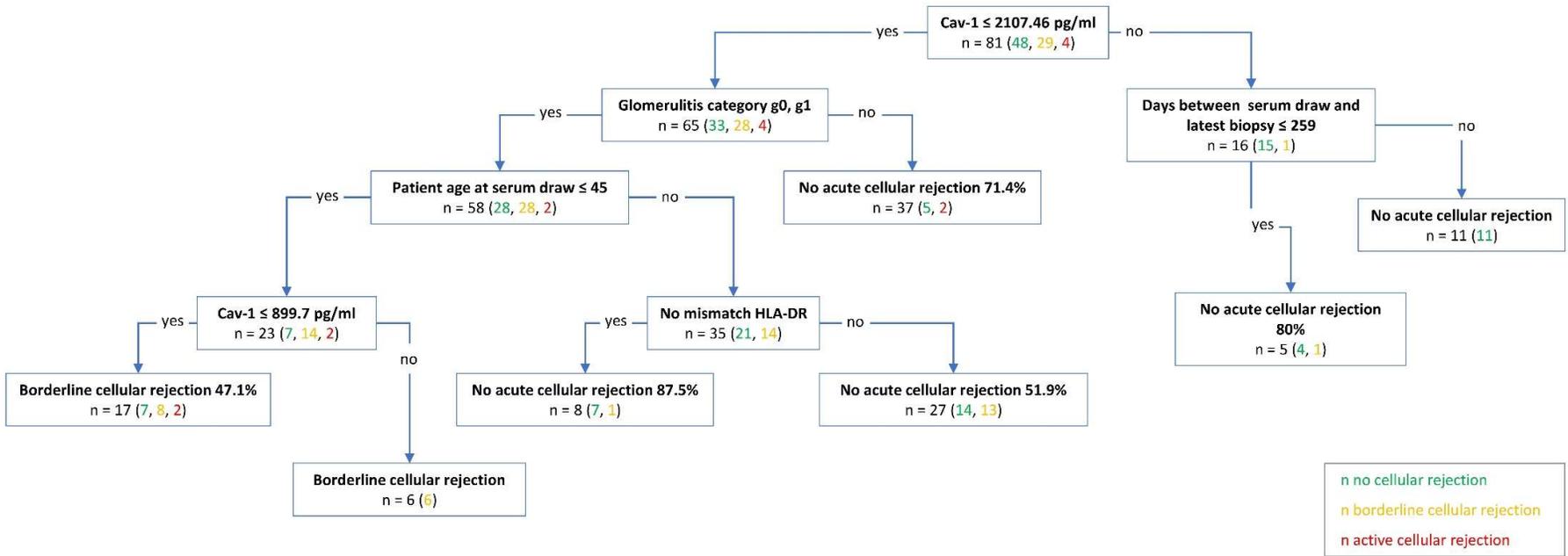
Figure 2: Decision tree analysis for acute cellular rejection, measured by combination of sum of square root of interstitial inflammation and tubulitis, being classified as no rejection, borderline cellular rejection and acute cellular rejection. HLA values were included (n=81), max. depth 4, min. samples per leaf 5. Tested items: HLA mismatch A, HLA mismatch B, HLA mismatch DR, sum HLA mismatch, Cav-1(pg/ml), sex (m=1_ f=2), 'Patient age at serum draw', 'Patient age at latest histology', ,Days between serum draw and latest biopsy', and according to the Banff classification: 'v', 'g', 'cg', 'cv', 'mm', 'sum chronic damage: ci+ct'. Decision tree was stable over 1'000 runs.
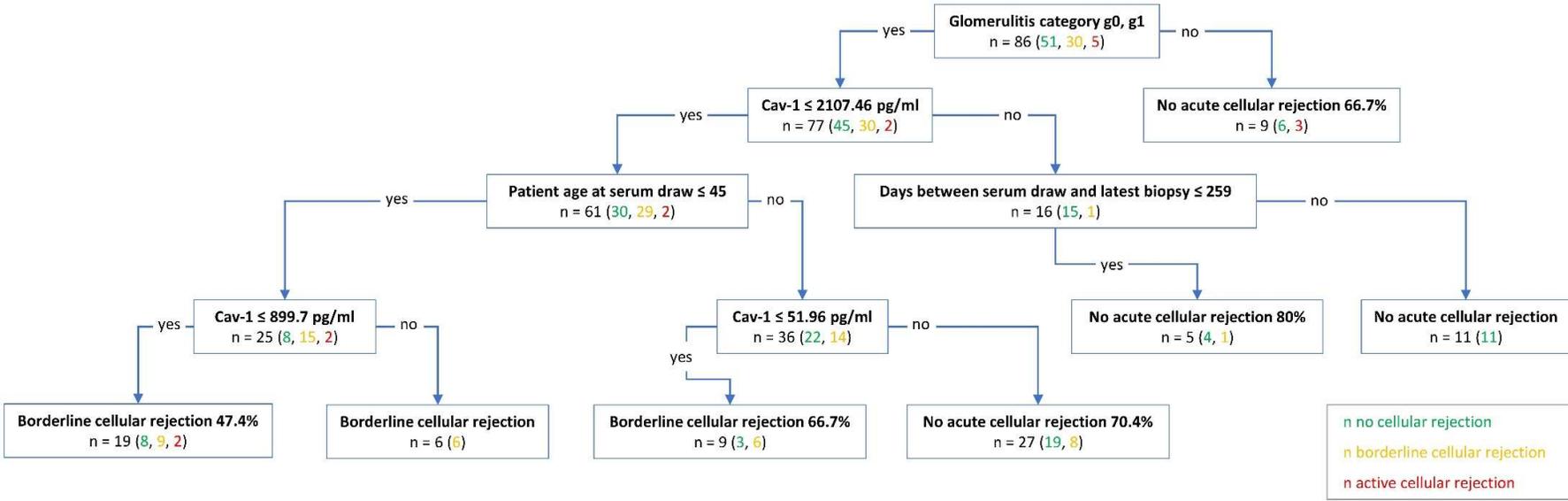
Figure 3: Decision tree analysis for acute cellular rejection, measured by combination of sum of square root of interstitial inflammation and tubulitis, being classified as no rejection, borderline cellular rejection and acute cellular rejection. No HLA values were included (n=86), max. depth 4, min. samples per leaf 5. Tested items: 'Cav-1(pg/ml)', 'Sex (m=1, w=2)', 'Patient age at serum draw', 'Patient age at latest histology', 'Days between serum draw and latest biopsy', and according to the Banff classification: 'v', 'g', 'cg', 'mm', 'cv516', 'sum chronic damage: ci+ct'. Decision tree was stable over 1'000 runs.
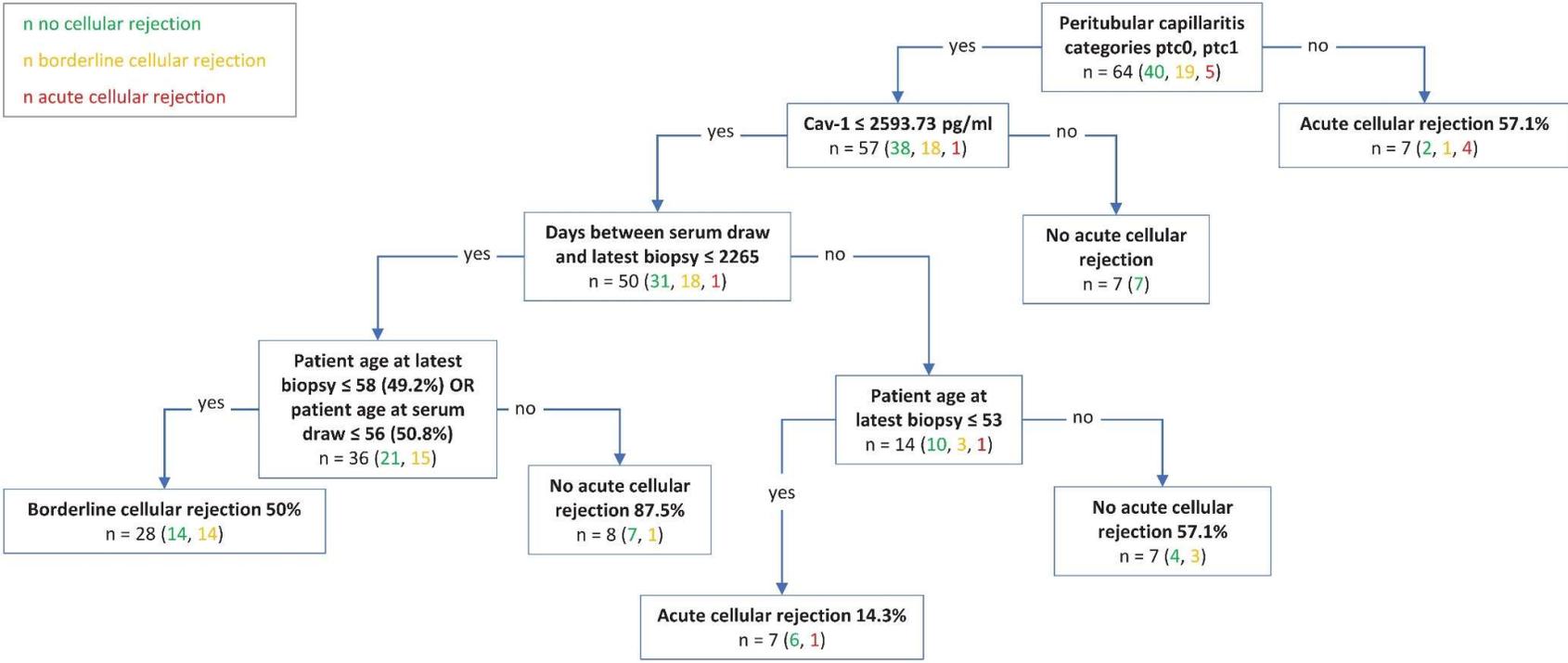
Figure 4: Decision tree analysis for acute cellular rejection, measured by combination of sum of square root of interstitial inflammation and tubulitis, being classified as no rejection, borderline cellular rejection and acute cellular rejection. Only patients without HLA values were included (n=64). Max depth = 4; min samples leaf = 5. Decision tree was instable over 10'000 runs for a level 3 node on the left side of the figure (frequency distribution as displayed). Tested items: 'Cav-1(pg/ml)', 'patient age at serum draw', 'patient age at latest biopsy', 'days between serum draw and latest biopsy', 'v', 'g', 'ptc', 'C4d', 'cg', 'mm', 'ah', 'cv', 'sum chronic damage: ci+ct'

3. Discussion

In this work we show that high serum concentrations of Cav-1 at the time of transplantation are associated with a reduced occurrence of acute cellular rejection of renal grafts, which is still true when normalizing the correlation for the time between serum draw and the biopsy rated (suppl. table). Furthermore, we found a negative correlation between ACR and the time between the serum draw and biopsy, indicative of ACR being more frequent in earlier points in time after transplantation (suppl. table). This finding is supported by the known protecting properties of Cav-1 in ischemia reperfusion injury (IRI), an inevitable complication of organ transplantation.

IRI has been shown to modulate both innate and adoptive immune responses and to promote long term graft damage (Mikhalski, Wissing et al. 2008, Eltzschig and Eckle 2011, Fuquay, Renner et al. 2013, Rao, Lu et al. 2014, Postalcioglu, Kaze et al. 2018). One mechanism of action is the up-regulation of allogenic cell surface markers including HLA I and II thereby enhancing graft immunogenicity (Daemen, Cornelis van¢t Veer et al. 1999). IRI promotes also the expression of toll-like receptors (TLRs) on epithelial and endothelial graft cells, which are in turn activated by endogenous ligands (Chen, John et al. 2011, Wu and Chadban 2014). TLR recruitment has been demonstrated to be a major determinant of allograft damage and abrogation of TLR pathways induces rejection tolerance (Zhang, Beduhn et al. 2012).

The resulting graft damage can be amplified by unmasking cryptic autoantigens released as vesicle-packed apoptotic particles, which are subsequently presented and trigger autoantibody production (Dieudé 2015, Cardinal, Dieude et al. 2017). Indeed, a number of antibodies beside anti-HLA have been described to contribute to the complexity of allorecognition. The deleterious effects of non-HLA antibodies have been confirmed in patients with rejection and might explain poor outcome even in the absence of HLA-donor specific antibodies(DSA) (Zhang and Reed 2016). Accordingly, apoptosis protection of the graft could be factorable in terms of limiting allo- and autoimmune responses. Indeed, therapies enabling protection from IRI and apoptosis have been promising in attenuating anti-graft immune responses thereby reducing the incidence of transplant complications (Powell, Tsapepas et al. 2013).

Cav-1 has been demonstrated to protect renal cells from undergoing apoptosis under stress conditions (Percy, Pat et al. 2008, Chen, Lin et al. 2016). One mechanism by which Cav-1 might exert its graft protective function is its negative effect on TGF-β-mediated signaling (Razani, Zhang et al. 2001). TGF-β transcription is found in rejected tissues and elevated TGF-β excretion in the urine with poor long-term outcome (Teppo, Honkanen et al. 2004, Willet, Pichitsiri et al. 2013). Particularly in renal cells, TGF-β amplifies apoptosis under stress conditions (Dai, Yang et al. 2003, Xu, Yang et al. 2012). Of note, TGF-β has also been shown to promote fibrosis, a common complication after kidney transplantation (Granata, Benedetti et al. 2020) .

Our findings raise the question how a host derived protein can impact the survival of the transplanted tissue. One hint to answer this question might be the recent finding of intercellular trafficking of Cav-1 between distant cellular types such as adipocytes and endothelial cells (Crewe, Joffin et al. 2018). This idea provides an attractive model how Cav-1 originated from the host could be incorporated into the graft endothelium thereby executing beneficial functional effects.

Cav-1 has already been evaluated as a therapeutic target in several disease models (Young, Ikeda et al. 2001, Feng, Guo et al. 2010, Sellers, Trane et al. 2012, Yang, Ma et al. 2016, Wang and Head 2019). In the context of the present work, it should be noted that a recombinant protein containing the caveolin scaffolding domain (CSD) protected renal endothelial cells from damage induced by angiotensin II (AT-II) (Chinnakkannu, Reese et al. 2018). This is of particular interest for patients with HLA-DSA negative humoral rejections, since AT-II type 1–receptor activating antibodies are strong inducers of allograft rejection and vasculopathy in patients without detectable HLA-DSA (Dragun, Müller et al. 2005).

Our data propose Cav-1 serum concentrations as a potential tool for the identification of patients at increased risk of acute graft rejection. However, this study does not include a detailed analysis of different immunosuppressive regimes and the underlying kidney disease impacting transplant outcome. Furthermore, a higher temporal resolution and standardized manor of serum draws would lead to a better understanding of the kinetics of factors mediating the transplant damage, as kidney biopsies might not be taken under these conditions due to ethical restrictions. It should be stressed as well, that a higher temporal resolution could better unveil non-linear or non-monotonic relations, which could explain the discrepancy between factors of humoral rejection in the Spearman correlation (not significant) vs. the decision tree analysis (relevant to predict acute cellular rejection and stable over 1'000 runs).

Even though these findings must be corroborated by a larger patient cohort, one could speculate that Cav-1 measures could be used to avoid transplantation of patients with low level Cav-1 with only poorly matched organs based on our pilot study. Moreover, encouraging data on patients with other kidney diseases suggest Cav-1 as an attractive therapeutic tool to attenuate the apoptotic load of the graft, thereby limiting alloresponses.

4. Materials and Methods

Patients' characteristics

The patients included in this study take part in the Collaborative Transplant Study (CTS) conducted by the Institute of Immunology, Heidelberg University, Germany, which includes written informed consent, ethical approval and allows subsequent analyses. Kidney transplantations performed and serum samples were taken between 2003 and 2017 at the Medical Center Freiburg, Germany.

HLA typing data were taken from the ENIS database (Eurotransplant). According to Eurotransplant rules, HLA-antigen mismatches for HLA-A and -B were calculated based upon broad antigens and HLA-DR on split HLA antigens with the exception of DR17/DR18 which were regarded as broad DR3 antigen.

Caveolin serum analyses

Sera from patients of the study cohort with matching FFPE tissue samples in the pathological department (n=91) (Institute for Surgical Pathology, University Medical Center Freiburg) were analyzed for Cav-1 levels by ELISA. ELISA was performed using the Human Cav-1 ELISA Kit (DEIA-XYA351V2) (Creative Diagnostics, New York, USA). To increase the sensitivity, the assay was combined with the ELAST ELISA Amplification System (Perkin-Elmer; Waltham, MA, USA) following the manufacturer's recommendations.

Histological analyses

Periodic acid Schiff (PAS) stained sections of 1.5 µm thickness were analyzed microscopically (Leica DM 2500; Leica, Wetzlar, Germany) and scored according to the Banff meeting report from 2015 where no previous classification was available from the reports (Loupy, Haas et al. 2017). All histological sections were derived from indication biopsies.

Statistical analysis

Scientific data was collected in Microsoft Excel (Office 365 package). As the data was not normally distributed, statistical testing was performed by Spearman test for correlations coefficients, using a Python based solution (Python 3.7, Pandas, Numpy and SciPy package; script can be provided upon request by the authors). With respect to possible analytic conflicts due to different scales of the correlated categories (four possible stages in the Banff classification vs. theoretically unlimited positive stages in the values of Cav-1 serum-levels), a Cav-1 classifier (CC) was defined, reducing the number of different stages to four, as defined by the range of the Cav-1 serum-levels (min 3.6 pg/ml, max 5882.8 pg/ml): < 1500 pg/ml = 0, 1500-2999 pg/ml = 1, 3000-4499 pg/ml = 2, > 4499 pg/ml = 3. For multivariable analysis, an entropy based decision tree classification was performed to look for non-monotonic relationships and/or relationships between variables of different scales (Python 3.7, Pandas, Numpy and SKlearn package; script in jupyter notebook can be provided upon request by the authors). Parameters were included to predict the acute cellular rejection score (ACR) as either "no cellular rejection", "borderline cellular rejection" or "active cellular rejection". Therefore, the values of active interstitial (i) and tubular (t) inflammation according to the Banff classification (Loupy, Haas et al. 2017) were summarized as square roots, which allows a clear numerical separation of the borderline categories. Of note, the decision tree classifier needs a complete dataset without "empty" values, which was the reason to perform three decision tree classifications with different compositions of items.

Ethics approval

The work of the CTS is approved by the Ethics Committee of the Medical Faculty of Heidelberg University (No. 083/2005Ä) and performed in accordance with the World Medical Association Declaration of Helsinki Ethical Principles in the currently valid version.

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