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ORIGINAL ARTICLE

Isolated v-lesion represents a benign phenotype of vascular rejection of the kidney allograft – a retrospective study

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SUMMARY

While the detrimental impact of the humoral acute vascular rejection (AVR) phenotype is recognized, the prognostic significance of isolated v-lesion (IV) remains unclear. In this retrospective single-centre study, AVR was found in 98 of 1015 patients (9.7%) who had undergone kidney transplantation in 2010–2014, with donor-specific antibodies (DSA) evaluated in all of them. The outcome of four AVR phenotypes was evaluated during median follow-up of 59 months; in 25 patients with IV, 18 with T-cell-mediated vascular rejection (TCMVR), 19 with antibody-mediated vascular rejection (AMVR) and 36 with suspected antibody-mediated rejection (sAMVR). AVR was diagnosed mainly by for-cause biopsy (81%) early after transplantation (median 19 POD) and appeared as mild-grade intimal arteritis. IV occurred in low-sensitized patients after the first transplantation (96%) in the absence of DSA. IV responded satisfactorily to treatment (88%), showed no persistence of rejection in surveillance biopsy, and had stable graft function, minimal proteinuria and excellent DCGS (96%). Contrary to that, Kaplan–Meier estimate of 3-year DCGS of AMVR was 66% (log-rank = 0.0004). Early IV represents a benign phenotype of AVR with a favourable outcome. This study prompts further research to evaluate the nature of IV before considering any change in the classification and management.

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Key words
antibody-mediated rejection, intimal arteritis, isolated v-lesion, kidney transplantation, vascular rejection

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Introduction

Acute vascular rejection (AVR) has been traditionally suggested to be a predictor of poor kidney allograft outcome [1]. It is defined as the presence of intimal arteritis in muscular arteries, characterized by subendothelial inflammatory infiltration. Based on the severity of inflammation, three grades of intimal arteritis (v) are distinguished, from mild endothelialitis (v1) through severe endothelialitis (v2) to transmural fibrinoid necrosis (v3) [2]. Conventionally, vascular rejection was considered to be a T-cell-mediated process except for the severest grade (v3) judged as antibody-mediated rejection (AMR) in cases where other diagnostic criteria of AMR were met [3]. This paradigm has recently changed after a significant association of v-lesion with donor-specific

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antibodies (DSA) was documented. As a consequence, intimal arteritis of any grade became a diagnostic criterion of histological evidence of acute tissue injury in AMR in the Banff 2013 classification [4].

It is already known that such a humoral phenotype of vascular rejection seems to have the worst prognostic outcome exceeding that of other rejection phenotypes. Explanations might lie in inaccurately targeted treatment of an unrecognized humoral phenotype of AVR. Grafts treated inappropriately with T-cell-targeted agents were at higher risk of graft loss compared to those treated correctly with antibody-targeted therapy [5].

Isolated v-lesion defined as intimal arteritis with minimal tubulointerstitial inflammation represents a histological finding of unclear clinical and prognostic significance [6–10]. According to the Banff classification, presence of intimal arteritis is sufficient to classify IV as type II or III acute TCMR [2]. Moreover, the 2013 update of the Banff classification introduced intimal arteritis as a morphological feature of histological evidence of acute tissue injury, one of three features needed for AMR diagnosis [11]. However, the role of the immune response in the pathophysiology of IV remains unclear. Furthermore, recent studies report diverse findings on the response to antirejection treatment, clinical and prognostic significance and transcriptome analysis of IV [6–10,12].

Therefore, this single-centre retrospective study was conducted to evaluate the incidence and significance of acute vascular rejection phenotypes with particular emphasis on the isolated v-lesion. Thorough analysis of diagnostic and therapeutic approach to AVR phenotypes in all patients transplanted at a high-volume transplant centre in 5 years was performed. Histological findings of surveillance kidney allograft biopsies were studied, and kidney graft survival was compared between the study groups. The added value of this study includes heterogeneity in the diagnostic and therapeutic approach, inclusion of suspected and sublinical AVR, and assessment of the functional and morphological therapeutic response, that is in a real-life setting.

Materials and methods

Study design and population

A retrospective analysis of patients undergoing single kidney transplantation at our centre between January 2010 and December 2014 was performed. Those experiencing at least one episode of AVR diagnosed either by protocol or for-cause biopsy were identified and enrolled for further investigation. Clinical data were collected from the patients′ medical records.

Histopathology and definition of vascular rejection

Kidney allograft biopsy samples were obtained using a percutaneous ultrasound-guided 16G biopsy needle for for-cause or protocol biopsy, performed routinely at 3 months post-transplant in our centre. Until 2013, surveillance biopsy was performed only in specific clinical settings or as protocol biopsy at 3 months after transplantation. Since 2013, surveillance biopsies have been performed routinely at 3 months after diagnosis of intimal arteritis. Histological slides were retrospectively reviewed by two pathologists (JM, EH) experienced in the field of organ transplantation according to the updated Banff working classification criteria [4]. Inconsistent histological findings (n = 9, 9.2%) were re-evaluated by a senior pathologist to reach an agreement.

Biopsy samples were assessed for Banff scored lesions as glomerulitis (g), peritubular capillaritis (ptc), transplant glomerulopathy (cg), intimal arteritis (v), interstitial inflammation (i), tubulitis (t), mesangial matrix increase (mm), vascular intimal fibrosis (cv), arteriolar hyaline thickening (ah), interstitial fibrosis (ci) or tubular atrophy (ct). The microvascular inflammation (MI) score was defined as the sum of glomerular (g) and peritubular capillary inflammation (ptc). Immunofluorescence detection of C4d was performed in all cases.

Acute vascular rejection was defined as the presence of intimal arteritis in muscular arteries of the kidney allograft characterized by subendothelial inflammatory infiltration. According to the Banff classification, the quantitative criteria for intimal arteritis are defined as follows: v0 – no arteritis, v1 – mild-to-moderate intimal arteritis in at least one arterial cross section, v2 – severe intimal arteritis with at least 25% of the luminal area lost in at least one arterial cross section, v3 – transmural arteritis and fibrinoid changes and medial smooth muscle necrosis with lymphocytic infiltration in the vessel [2].

Anti-HLA antibody testing and cross-matches

Identification of circulating donor-specific anti-HLA antibodies (DSA) was performed by Luminex bead-based assay (One Lambda Inc., Canoga Park, CA, USA). Negative complement-dependent cytotoxicity cross-match at the time of transplantation was obligatory to undergo kidney transplantation. All patients signed informed consent with serum storage and evaluation.

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Definition of AVR phenotype categories

Patients with AVR were divided into four groups according to the histopathological finding and presence or absence of DSA. T-cell-mediated vascular rejection (TCMVR) was characterized by tubulointerstitial inflammation (TI) in the absence of C4d and DSA.

Antibody-mediated vascular rejection (AMVR) included features of at least moderate MI, that is glomerulitis and peritubular capillaritis (g + ptc ≥ 2) and/or C4d positivity and DSA positivity in addition to v-lesion.

The category of suspected antibody-mediated vascular rejection (sAMVR) was defined as the presence of intimal arteritis plus evidence of antibody interaction with the vascular endothelium [positive C4d staining or at least moderate MI (g + ptc > 2)] or serologic evidence of DSA implying that only two of three criteria of AMR were met [4]. In other words, suspected AMVR included intimal arteritis but lacked either C4d positivity/MI or DSA positivity.

Isolated v-lesion (IV) was defined as a unique group of AVR with minimal TI, no MI, C4d negativity and DSA negativity. Acute vascular rejection phenotype characteristics are summarized in Table 1.

Immunosuppressive therapy

Maintenance immunosuppressive treatment consisted of calcineurin inhibitors (CNI) (mainly tacrolimus, rarely cyclosporine A), mycophenolate mofetil or mycophenolic acid, and prednisone (Table 2). Primary kidney transplant recipients with peak panel reactive antibodies (PRA) < 20% and negative DSA received no induction or basiliximab while other patients were on rabbit antithymocyte globulin (rATG). Patients with preformed DSA had plasmapheresis (PP) before transplantation and received intravenous immunoglobulin (IVIG) on post-transplant Days 1, 3 and 5 in addition to rATG. Patients at the highest immunologic risk with preformed DSA and a history of AMR in their previous transplant received rituximab on Day 2 and had intensive PP or immunoadsorption procedures after transplantation.

Table 1. Characteristics of acute vascular rejection phenotypes.

Phenotype	MI (g + ptc)	i	T	v	C4d	DSA
IV	0	<2	<2	1–3	0	Neg
TCMVR	0	0–3	0–3	1–3	0	Neg
AMVR	0–6	0–3	0–3	1–3	0–3	Pos
sAMVR*	0–6	0–3	0–3	1–3	0–3	Neg/pos

AMVR, antibody-mediated vascular rejection; DSA, donor-specific antibodies; g, glomerulitis; i, interstitial inflammation; IV, isolated v-lesion; MI, microvascular inflammation; ptc, peritubular capillary inflammation; sAMVR, suspected antibody-mediated vascular rejection; t, tubulitis; TCMVR, T-cell-mediated vascular rejection; v, intimal arteritis.

*Patients with intimal arteritis meeting two of three criteria of antibody-mediated rejection (AMR) according to the recent Banff classification [4].

Response to treatment definition

Functional response to antirejection treatment was defined as an improvement of renal function (at least 10% decrease in serum creatinine) between diagnostic biopsy and 2 weeks after the time point. Patients with subclinical rejection were judged as responsive if renal function remained stable and a normal histological finding was made in surveillance biopsy.

Statistical analyses

Continuous variables are expressed as median and interquartile range (IQR). Categorical variables are expressed as n and percentage of total. The chi-square, ANOVA and Kruskal–Wallis tests were used for hypothesis testing when appropriate. P values <0.05 were considered statistically significant. Survival analyses were performed with the Kaplan–Meier method using the log-rank test. Follow-up is described as Kaplan–Meier estimate of potential follow-up ("reverse Kaplan–Meier") with the use of the median and further quartiles [13]. Due to the low number of graft failures (n = 18), which is much lower than the recommended 50 events per variable for variable selection, multivariate Cox regression was not performed [14]. Data analyses were performed using IBM SPSS 22 statistical software (IBM Corp., Armonk, NY, USA).

Results

Demographics and clinical characteristics

Among the 1015 patients receiving kidney transplants during the study period, 98 (9.7%) patients experienced at least one episode of AVR. The patients were divided into the IV (n = 25), TCMVR (n = 18), sAMVR (n = 36) and AMVR (n = 19) groups based on the above definition. Their baseline characteristics are

Table 2. Demographic and clinical characteristics of the study population.

	Total (n = 98)	IV (n = 25)	TCMVR (n = 18)	sAMVR (n = 36)	AMVR (n = 19)	P value
Follow-up (months)	59 (49–79)	51 (46–68)	66 (49–86)	61 (52–80)	68 (43–71)	0.506
Age (years)	53 (43–59)	58 (50–62)	55 (38–58)	51 (41–57)	51 (39–57)	0.393
Gender (male), n (%)	69 (70.4)	19 (76.0)	14 (77.8)	27 (75.0)	9 (47.4)	0.109
BMI (kg/m^2)	27.2 (23.7–30.5)	29.2 (25.7–31.6)	28.2 (24.2–33.5)	26.3 (23.3–28.4)	25.5 (23.9–29.5)	0.297
Original disease, n (%)						
Diabetes	11 (11.2)	6 (24.0)	3 (16.7)	1 (2.8)	1 (5.3)	0.048
Hypertension	13 (13.3)	5 (20.0)	2 (11.1)	5 (13.9)	1 (5.3)	0.546
Glomerulonephritis	24 (24.5)	3 (12.0)	1 (5.6)	13 (36.1)	7 (36.8)	0.020
Polycystic kidney disease	10 (10.2)	3 (12.0)	4 (22.2)	2 (5.6)	1 (5.3)	0.233
Other	40 (40.8)	8 (32.0)	8 (44.4)	15 (41.6)	9 (47.3)	0.741
Dialysis vintage, months	24 (9–42)	27 (10–39)	16 (6–41)	20 (9–30)	36 (12–128)	0.165
Retransplantation, n (%)	19 (19.4)	1 (4.0)	1 (5.6)	8 (22.2)	9 (47.4)	0.001
Deceased donor, n (%)	71 (72.4)	19 (76.0)	13 (72.2)	26 (72.2)	13 (68.4)	0.957
Cold ischaemia time (hours)	15 (1–18)	16 (2–19)	16 (1–20)	15 (1–18)	15 (1–18)	0.964
Delayed graft function, n (%)	36 (36.7)	9 (36.0)	6 (33.3)	14 (38.9)	7 (36.8)	0.983
ABO-incompatible Tx, n (%)	2 (2.0)	0 (0)	1 (5.6)	1 (2.8)	0 (0)	0.547
Peak panel reactive antibodies	6 (0–26)	4 (1–11)	8 (2–29)	4 (0–22)	22 (2–44)	0.147
HLA mismatches, total	4 (3–5)	3 (3–4)	4 (3–5)	4 (3–5)	4 (3–5)	0.132
DSA positivity, n (%)						
Prior to transplantation	24 (24.5)	1 (4.0)	0 (0)	10 (27.8)	13 (68.4)	<0.001
At the time of diagnosis	24 (24.5)	0 (0)	0 (0)	6 (16.6)	19 (100.0)	<0.001
de novo DSA	9 (9.2)	0 (0)	0 (0)	3 (8.3)	6 (31.6)	0.001
Induction therapy, n (%)						
None	21 (21.4)	2 (8.0)	5 (27.8)	11 (30.6)	3 (15.8)	0.154
Basiliximab	44 (44.9)	16 (64.0)	9 (50.0)	13 (36.1)	6 (31.6)	0.095
rATG	17 (17.3)	5 (20.0)	2 (11.1)	7 (19.4)	3 (15.8)	0.861
rATG +PP+IVIG+(RTX)	16 (16.3)	2 (8.0)	2 (11.1)	5 (13.9)	7 (36.8)	0.054
Maintenance IS, n (%)						
Tac, MMf/MPA, steroids	93 (94.9)	25 (100.0)	17 (94.4)	32 (88.9)	19 (100.0)	0.168
CyA, MMf/MPA, steroids	5 (5.1)	0 (0)	1 (5.6)	4 (11.1)	0 (0)	
Tac, trough level (μg/l)	10 (7–14)	10 (8–14)	9 (6–11)	10 (6–13)	11 (9–16)	0.315
Previous rejection, n (%)	18 (18.4)	2 (8.0)	1 (5.6)	5 (13.9)	10 (52.6)	<0.001
Biopsy n (%)						
For-cause	79 (80.6)	15 (60.0)	13 (72.2)	32 (88.9)	19 (100.0)	0.003
Protocol	19 (19.4)	10 (40.0)	5 (27.8)	4 (11.1)	0 (0)	

Table 2. Continued.

	Total (n = 98)	IV (n = 25)	TCMVR (n = 18)	sAMVR (n = 36)	AMVR (n = 19)	P value
Time of biopsy (days after Tx)	19 (7–98)	15 (8–96)	26 (6–107)	18 (6–101)	22 (11–77)	0.913
Intimal arteritis grade, n (%)						
v1	67 (68.4)	21 (84.0)	12 (66.6)	23 (63.8)	11 (57.9)	0.246
v2	27 (27.5)	3 (12.0)	5 (27.8)	11 (30.6)	8 (42.1)	0.157
v3	4 (4.1)	1 (4.0)	1 (5.6)	2 (5.6)	0 (0.0)	0.775

Data are expressed as median (interquartile range), or frequency (percentage).

AMVR, antibody-mediated vascular rejection; CyA, cyclosporine A; DSA, donor-specific antibodies; IS, immunosuppression; IV, isolated v-lesion; IVIG, intravenous immunoglobulin; MMf, mycophenolate mofetil; MPA, mycophenolic acid; PP, plasmapheresis; rATG, rabbit antithymocyte globulin; RTX, rituximab; sAMVR, suspected antibody-mediated vascular rejection; Tac, tacrolimus; TCMVR, T-cell-mediated vascular rejection; Tx, transplantation; v, intimal arteritis.

shown in Table 2. The patients showed no significant differences in age, gender and body constitution. Time on dialysis, cold ischaemia time (CIT) and incidence of delayed graft function (DGF) were comparable among the groups. A significantly higher prevalence of glomerulonephritis as the original kidney disease was observed in patients with humoral phenotypes of AVR in comparison with the other AVR subgroups (P = 0.02).

The majority of AVR cases were diagnosed by for-cause biopsy (n = 79, 81%). Subclinical AVR (n = 19) diagnosed by protocol biopsy was more frequent in IV (n = 10, 40%) and TCMVR (n = 5, 28%) than in sAMVR (n = 4, 11%) and AMVR (n = 0) (P = 0.003). The median of AVR diagnosis was 19 days post-transplant with no difference between the study groups. Most v-lesions were assessed as mild; 67 (68%) patients suffered from mild-to-moderate intimal arteritis (grade v1); severe intimal arteritis (grade v2) was found in 27 (28%); and transmural intimal arteritis (grade v3) was found in only four (4%) patients.

Regarding factors affecting immunologic risk as peak PRA and number of total HLA mismatches, the groups did not differ significantly. Isolated v-lesion occurred mainly in low-risk first-transplant recipients (96%). On the contrary, sAMVR and AMVR patients underwent more often retransplantation (P = 0.001) and had positive DSA prior to transplantation (P < 0.001). Furthermore, 10 (53%) patients in the AMVR group experienced a previous episode of rejection of aetiology other than vascular (P < 0.001) (Table 2).

Therapeutic approach

The most frequent first-line treatment were steroid pulses: 20 (80%) patients in the IV, 14 (78%) TCMVR, 18 (50%) sAMVR and two (11%) AMVR (P = 0.001) received pulses of methylprednisolone. T-cell-targeted depletive treatment with rATG was initially used in three (12%) patients in the IV, three (17%) TCMVR, 10 (28%) sAMVR and two (11%) patients in the AMVR group (P = 0.306). Plasmapheresis/IVIG therapy was instituted in one (4%) patient in the IV, one (6%) in TCMVR, and more often in the sAMVR and AMVR groups (n = 8, 22% and n = 15, 79%, respectively) (P = 0.001). In six (32%) cases of AMVR, PP and IVIG were followed by rituximab and bortezomib treatment (Table 3). The functional response to initial treatment was comparable among the groups (n = 22, 88% IV; n = 18, 100% TCMVR; n = 35, 97% sAMVR and n = 16, 84% AMVR, P = 0.14).

Table 3. Overview of first-line treatment modalities according to acute vascular rejection phenotype.

Treatment, *n* (%)	Total (*n* = 98)	IV (*n* = 25)	TCMVR (*n* = 18)	sAMVR (*n* = 36)	AMVR (*n* = 19)	*P* value
None	1 (1.0)	1 (4.0)	0 (0)	0 (0)	0 (0)	0.399
Methylprednisolone	54 (55.1)	20 (80.0)	14 (77.8)	18 (50.0)	2 (10.5)	0.001
rATG	18 (18.4)	3 (12.0)	3 (16.6)	10 (27.8)	2 (10.5)	0.306
PP + IVIG + (RTX) + (BTZ)*	25 (25.5)	1 (4.0)	1 (5.6)	8 (22.2)	15 (79.0)	0.001

AMVR, antibody-mediated vascular rejection; IV, isolated v-lesion; IVIG, intravenous immunoglobulin; PP, plasmapheresis; rATG, rabbit antithymocyte globulin; RTX, rituximab; sAMVR, suspected antibody-mediated vascular rejection; TCMVR, T-cell-mediated vascular rejection.

*Six patients in the AMVR group received RTX + BTZ in addition to PP + IVIG.

Table 4. Histological findings in surveillance biopsy according to acute vascular rejection phenotype.

	Total (*n* = 71)	IV (*n* = 17)	TCMVR (*n* = 13)	sAMVR (*n* = 24)	AMVR (*n* = 17)	*P* value
Median time of surveillance biopsy after diagnosis, days (IQR)	74 (22–91)	76 (24–91)	79 (34–93)	80 (16–115)	67 (18–77)	0.313
Finding in surveillance biopsy						
Normal, *n* (%)	42 (59.2)	15 (88.2)	9 (69.2)	12 (50.0)	6 (35.3)	0.015
AMR, *n* (%)	21 (29.6)	0 (0)	1 (7.7)	10 (41.7)	10 (58.8)	<0.001
TCMR, *n* (%)	3 (4.2)	0 (0)	3 (23.1)	0 (0)	0 (0)	0.003
Intimal arteritis*, *n* (%)	6 (8.5)	0 (0)	1 (7.7)	2 (8.3)	3 (17.6)	0.329
Infectious complications†, *n* (%)	5 (7.0)	2 (11.8)	0 (0)	2 (8.3)	1 (5.9)	0.646

AMR, antibody-mediated rejection; AMVR, antibody-mediated vascular rejection; IV, isolated v-lesion; sAMVR, suspected antibody-mediated vascular rejection; TCMR, T-cell-mediated rejection; TCMVR, T-cell-mediated vascular rejection.

*Cases with intimal arteritis are concurrently classified either as AMR or TCMR.

†The infectious complications category includes BK virus-associated nephropathy and pyelonephritis.

Surveillance biopsy

Surveillance biopsy, performed in 72% patients over a period of 16–115 days after diagnosis (Table 4), revealed a normal histological finding in the majority of IV and TCMVR patients (88%, 69%, respectively) while normal histology was less frequent in sAMVR and AMVR (50%, 35%, respectively) ($P = 0.015$). A total of 10 (42%) sAMVR and 10 (59%) AMVR surveillance biopsies documented the presence of AMR, in comparison with a lower frequency in the TCMVR ($n = 1$, 8%) and none in IV group ($P < 0.001$) (Table 4). Intimal arteritis resolved completely in all IV patients while rarely persisted in other groups ($n = 1$, 8% in the TCMVR; $n = 2$, 8% in sAMVR and $n = 3$, 18% in AMVR groups $P = 0.329$). No progression of transplant arteriopathy was observed in surveillance biopsies of IV (cv, $P = 0.76$; ah, $P = 0.317$). A detailed overview of histological findings according to the Banff classification in all diagnostic and surveillance biopsies is available in Table 5.

Kidney graft function and survival

Overall median follow-up time was 59 months [IQ range (49–79)] and did not differ between the study groups [51 (46–68), 66 (49–86), 61 (52–80), 68 (43–71) for the IV, TCMVR, sAMVR and AMVR groups, respectively, $P = 0.506$]. Renal function at biopsy was comparable among all AVR phenotypes ($P = 0.192$) (Table 6). One-year results showed comparable improvement in renal function among all study groups. Renal function did not significantly differ between the study groups during 3 years. Median proteinuria at biopsy was 0.4 (0.0–1.0) g/day and did not differ between the study groups during 3 years.

The Kaplan–Meier estimate of graft survival (Figure 1) was comparable between IV (96% at year 1; 96% at year 3), TCMVR (100% at year 1; 89% at year 3) and sAMVR (97% at year 1; 88% at year 3) but significantly worse in patients with AMVR (90% at year 1; 66% at year 3) (pairwise comparison using the

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Table 5. A detailed overview of histological findings according to the Banff classification in diagnostic and surveillance biopsies.

	Diagnostic biopsy (n = 98)					Surveillance biopsy (n = 71)				
Banff score 0/1/2/3	IV (n = 25)	TCMVR (n = 18)	sAMVR (n = 36)	AMVR (n = 19)	P value	IV (n = 17)	TCMVR (n = 13)	sAMVR (n = 24)	AMVR (n = 17)	P value
Glomerulitis (g)	25/0/0/0	18/0/0/0	17/9/6/4	4/6/4/5	<0.001	17/0/0/0	12/1/0/0	14/3/4/3	9/4/2/2	0.003
Interstitial inflammation (i)	18/7/0/0	1/2/14/1	21/6/8/1	11/3/3/2	<0.001	17/0/0/0	9/1/2/1	19/3/1/1	16/1/0/0	0.040
Tubulitis (t)	15/10/0/0	0/4/9/5	13/10/6/7	7/7/3/2	<0.001	10/7/0/0	6/2/2/3	13/4/4/3	11/5/1/0	0.077
Intimal arteritis (v)	0/21/3/1	0/12/5/1	0/23/11/2	0/11/8/0	0.450	17/0/0/0	12/1/0/0	22/2/0/0	14/2/1/0	0.263
Peritubular capillaritis (ptc)	25/0/0/0	18/0/0/0	25/7/3/1	12/5/2/0	0.005	17/0/0/0	13/0/0/0	20/3/1/0	11/3/1/2	0.009
Transplant glomerulopathy (cg)	25/0/0/0	18/0/0/0	34/1/0/1	17/1/1/0	0.429	17/0/0/0	13/0/0/0	18/3/3/0	13/4/0/0	0.039
Interstitial fibrosis (ci)	13/12/0/0	10/7/1/0	16/20/0/0	9/9/1/0	0.921	5/10/1/1	5/8/0/0	12/11/1/0	6/10/1/0	0.447
Tubular atrophy (ct)	9/16/0/0	4/13/1/0	13/22/1/0	3/15/1/0	0.256	5/10/2/0	1/12/0/0	6/17/1/0	4/12/1/0	0.884
Arteriolar hyaline thickening (ah)	3/14/8/0	1/10/7/0	4/23/8/1	3/9/6/1	0.842	1/12/4/0	2/9/2/0	5/11/7/1	3/10/3/1	0.901
Vascular intimal fibrosis (cv)	2/9/6/8	1/11/3/3	2/19/12/3	1/8/6/4	0.392	1/9/5/2	2/6/3/2	6/9/7/2	2/12/2/1	0.572
C4d	25/0/0/0	17/0/0/1	30/5/0/1	6/2/2/9	<0.001	17/0/0/0	11/0/1/1	20/3/1/0	9/1/2/5	0.001

AMVR, antibody-mediated vascular rejection; IV, isolated v-lesion; sAMVR, suspected antibody-mediated vascular rejection; TCMVR, T-cell-mediated vascular rejection.

chi-square test, IV $P = 0.001$, TCMVR $P = 0.020$, sAMVR $P = 0.003$; log-rank test, 0.0004).

Subclinical acute vascular rejection

Nineteen of 98 (19.4%) cases of AVR were subclinical findings in 3-month protocol biopsy (Table S1). Ten findings met the criteria for isolated v-lesion, while, in five cases, intimal arteritis was accompanied by TI and judged as TCMVR. Four subclinical AVR findings were suspicious of a humoral phenotype (sAMVR) due to the presence of MI in one case and presence of DSA at transplantation in three cases. None of the patients had had a previous rejection episode. All patients but one (n = 18, 95%) received treatment of methylprednisolone pulses. In 10 (56%) patients, surveillance biopsy provided either normal findings (n = 8) or showed borderline changes (n = 2). In nine patients, surveillance biopsy was not performed. Renal function remained stable during the 3-year follow-up [Cr at baseline, 118 (103–142) μmol/l; at 3 years, 124 (92–177) μmol/l; $P = 0.601$], and no significant increase in proteinuria was observed [proteinuria at baseline, 0.2 (0.0–0.3) g/day; at 3 years, 0.0 (0.0–0.3) g/day; $P = 0.876$]. Three-year death-censored graft survival was 100%.

T-cell-targeted treatment in humoral phenotypes of AVR

Therapeutic approach to AVR was based on the current Banff classification. Therefore, some samples with the humoral AVR phenotype might have been judged as T-cell-mediated rejection according to biopsy interpretation based on the Banff 2013 classification [4] or received T-cell-targeted treatment for clinical reasons. We aimed to analyse the impact of T-cell-targeted treatment on outcome of kidney allografts with the humoral AVR phenotype.

Four (21%) AMVR and 28 (78%) sAMVR patients received T-cell-targeted first-line treatment. Two AMVR patients were treated with steroids because of high risk of more intensive treatment due to serious infection (n = 1) or leucopenia (n = 1). Steroid treatment brought unsatisfactory results, and both grafts failed. Two AMVR patients were treated with rATG. One patient showed persistent antibody-mediated rejection and was treated with bortezomib; the other one died with a functioning graft due to a cardiovascular event.

Different outcome of T-cell-targeted treatment was observed in sAMVR patients. These patients expressed an incomplete antibody-mediated phenotype, due mostly to DSA negativity and their historical misclassification as T-cell-mediated rejection. However, treatment effect of

Table 6. Overview of renal function, proteinuria and graft failure in acute vascular rejection phenotype groups.

	Total (n = 98)	IV (n = 25)	TCMVR (n = 18)	sAMVR (n = 36)	AMVR (n = 19)	P value
Renal function (Cr-μmol/l)						
At biopsy	296 (163–522)	172 (120–452)	327 (167–481)	364 (182–587)	268 (168–566)	0.192
At 1 year	142 (117–178)	131 (109–165)	150 (117–182)	144 (118–166)	148 (137–280)	0.123
At 2 years	138 (110–170)	128 (105–165)	143 (117–163)	139 (109–156)	154 (110–291)	0.385
At 3 years	132 (114–179)	123 (103–153)	147 (115–197)	132 (111–176)	140 (123–301)	0.165
Proteinuria (g/day)						
At biopsy	0.4 (0.0–1.0)	0.3 (0.0–1.0)	0.2 (0.0–0.4)	0.5 (0.0–1.1)	0.5 (0.0–1.0)	0.428
At 1 year	0.2 (0.0–0.6)	0.0 (0.0–0.3)	0.0 (0.0–0.4)	0.2 (0.0–0.8)	0.6 (0.0–1.6)	0.058
At 2 years	0.1 (0.0–0.6)	0.0 (0.0–0.4)	0.1 (0.0–0.4)	0.1 (0.0–0.6)	0.6 (0.0–1.1)	0.332
At 3 years	0.0 (0.0–0.5)	0.0 (0.0–0.2)	0.0 (0.0–0.1)	0.0 (0.0–1.1)	0.3 (0.0–0.9)	0.154
Graft failure, n (%)	18 (18)	1 (4)	3 (17)	5 (14)	9 (47)	0.002

Data are expressed as median (interquartile range).

AMVR, antibody-mediated vascular rejection; IV, isolated v-lesion; sAMVR, suspected antibody-mediated vascular rejection; TCMVR, T-cell-mediated vascular rejection.

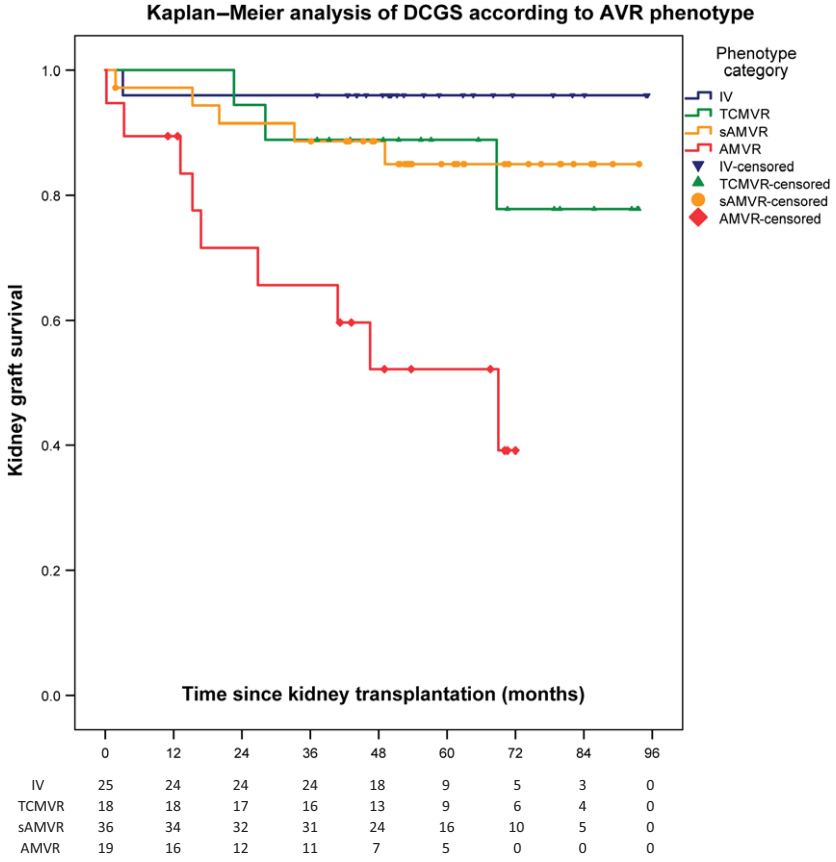

Figure 1 Kaplan–Meier analysis of death-censored graft survival according to acute vascular rejection (AVR) phenotype (log-rank 0.0004).

steroids (n = 18), rATG (n = 10) and PP/IVIG (n = 8), was not different, in terms of rejection persistence in surveillance biopsies (P = 0.610) and graft failure (6%, 30%, 13%, respectively, P = 0.199). Renal function was comparable in all groups and remained stable in 3-years follow-up (Table S2).

Discussion

The present study was designed to determine the incidence and significance of AVR after kidney transplantation in a high-volume transplant centre. Based on the updated classification, we retrospectively assessed the

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prognostic effect of four AVR phenotypes [4]. Although intimal arteritis represents a histological finding with a deleterious impact on graft survival, individual phenotypes of AVR show distinct clinical significance after kidney transplantation. Our main results are that early isolated v-lesion represents a benign clinical phenotype of AVR occurring mostly in low-sensitized patients after their first kidney transplantation in the absence of detectable DSA. Furthermore, kidneys with IV responded satisfactorily to steroid antirejection treatment showed no persistence of intimal arteritis in surveillance biopsy and had favourable graft function and survival. Our study confirmed AMVR to be a risk phenotype with a detrimental effect on kidney graft survival [5].

Our findings also demonstrate a significant incidence of AVR after kidney transplantation showing that almost 10% of patients in our centre experienced an AVR episode within the first post-transplant year. Acute vascular rejection was diagnosed mostly in the early post-transplant period (median, 19 days) and assessed as mild-grade AVR. Acute vascular rejection showed four rejection phenotypes: AMVR, suspected AMVR (sAMVR), TCMVR and isolated v-lesion (IV), with the latter including cases of intimal arteritis with none or minimal interstitial inflammation.

The humoral phenotype of AVR (AMVR, sAMVR) was most often diagnosed by for-cause biopsy. While the overall functional response to the treatment strategy was comparable across the study groups, surveillance biopsies revealed higher rates of rejection persistence in humoral phenotypes.

Contrary to the humoral phenotype, the cellular phenotype of AVR was characterized by a good response to treatment and absence of rejection findings in surveillance biopsies in more than two-thirds of cases. Moreover, its more favourable clinical pattern was confirmed by the low risk of graft failure. Isolated v-lesion was associated with a benign clinical course and a satisfactory response to steroids and no rejection persistence in surveillance biopsy.

Isolated v-lesion, defined as intimal arteritis with minimal TI, has been subject to a lively debate since 2007 when its uncertain origin was revealed by transcriptomic analysis, and its association with ischaemic reperfusion injury was suggested as the molecular profile did not correlate with T-cell-mediated rejection [12]. This view was challenged by some authors furnishing evidence that isolated v-lesion is of rejection origin as more than 80% of patients with IV responded to antirejection treatment by functional improvement comparable to that seen with TCMVR [8].

Our data show a very mild clinical course of IV in the absence of detectable DSA. A majority of patients responded to steroid treatment by functional improvement and/or histological vanishing in surveillance biopsy as no persistent rejection was observed. More than a third of IVs showed a subclinical course and represented more than half of all subclinical findings in this study. Our findings are consistent with another observation of a favourable outcome of patients with isolated intimal arteritis [7]. Also, according to a recent French study, isolated v-lesion has a very rare association with DSA, low risk of subsequent AMR development and overall good clinical prognosis [6]. The discrepancy with studies indicating that isolated v-lesion is an independent risk factor for kidney allograft failure [10] could be explained by the results of earlier analyses of v-lesions, when the incidence of steroid-resistant rejection was much higher and the humoral phenotype of AVR was poorly recognized as HLA antibody detection was less advanced and not routinely performed [9].

Some authors suggest that the severity of intimal arteritis and accompanying TI may determine the rejection phenotype [9], while others refuse this theory [10]. Halloran's group has acknowledged frequent misinterpretation and proposes a more comprehensive approach by reassessing the significance of intimal arteritis using microarray-based molecular tests [9]. They conclude that, while intimal arteritis associated with severe TI (TCMVR) certainly reflects TCMR activity, isolated v-lesions should be interpreted on the basis of time after transplantation, DSA and presence or absence of mild TI. Early isolated v-lesions developing within 1 year after transplantation with negative DSA usually do not reflect rejection, but the possibility of TCMR must be excluded.

Our results and those reported from recent studies suggest that isolated v-lesions do not always imply rejection, especially early after transplantation when IV with no DSA may reflect endothelial injury from the transplant process [15]. All our IV samples were collected within 3 months post-transplant, with no DSA detected at the time of biopsy. These early IV findings suggested an excellent response to steroid treatment and a high proportion of normal findings in surveillance biopsies. Such an uncommon success could be explained by endothelial injury from the transplant process rather than a rejection origin or very benign nature of non-DSA v-lesions. Unfortunately, the retrospective design of our study does not allow us to make any authoritative conclusions whether this resolution should be ascribed to treatment effect or a spontaneous repair

process as all our IV patients but one received antirejection therapy. The body of evidence in the relevant literature related to prognosis of subclinical and untreated IV is limited [16–18]. Further studies are warranted to investigate the nature of IV and draw conclusions regarding the treatment effect on kidney graft survival. Once such evidence is available, the therapeutic approach might be better individualized based on the AVR phenotype. This would be of high importance, as there are currently no specific guidelines for diagnostic interpretation and treatment of isolated v-lesion and the current Banff classification interprets intimal arteritis with no signs of humoral rejection as TCMR.

Additionally, the study results largely emphasize the necessity of correct assessment of the AVR phenotype and are confirmatory of previous studies [5,7,19]. While the cellular phenotype of AVR is associated with a more favourable clinical course and good response to treatment, the humoral phenotype has a detrimental effect on kidney allograft survival, especially in patients not receiving appropriate therapy [5]. After all, the results of Lefaucheur's [5] and other studies [20–22] recently led to the inclusion of intimal arteritis of any grade in the histological criteria of AMR which should prevent such a misclassification in the future [4]. In our study, T-cell-targeted treatment was applied in more than two-thirds of sAMVR and a quarter of AMVR patients. While this treatment showed a detrimental effect in the AMVR group, sAMVR patients experienced no difference in graft survival and in rejection persistence in surveillance biopsy.

The most recent Banff update also supports investigation in cases of suspected AMR with overt histological features but no DSA detected [23]. We sought to determine whether sAMVR has a detrimental effect on kidney graft survival similar to that of AMVR. While the majority of our sAMVR patients met the histological criteria of humoral rejection, DSAs were undetectable. Our data show a significantly lower risk of allograft failure from sAMVR compared to AMVR irrespective of treatment approach. Recently, Halloran *et al.* [24] showed no difference in graft survival between no-DSA and DSA subphenotypes of AMR. The inconsistency in reported data could be explained by the diagnostic challenges associated with DSA testing, for example low DSA levels, uncertainty regarding donor antigens, other alloantibodies and autoantibodies [25]. We are also aware of fact that the significantly lower risk of sAMVR in our study might be partially attributable to the relatively short-term follow-up. Further prospective studies are required to rigorously assess the risk of these subphenotypes and unify the clinicians' approach to DSA.

Admittedly, the main limitation of this study is the small number of patients with intimal arteritis reflecting the relative paucity of v-lesions. Additionally, few graft losses were reported in subgroups, potentially indicating short-term follow-up and lack of late rejections with worse graft survival [26]. Also, the retrospective design of our study has introduced historical inconsistency in diagnostic and therapeutic approaches to intimal arteritis. Donor-specific antibodies have been routinely evaluated since 2012, and in some cases, the humoral phenotype of AVR remained unrecognized and mistreated. However, the retrospective design with noncontrolled nature of the treatment and heterogeneity of our cohort depicts AVR in the real-life setting where AVR is found in for-cause and protocol biopsies with heterogeneous interpretation and treatment.

On the other hand, the strengths of our study include description of the incidence and therapeutic approach to AVR in a high-volume transplantation centre, including the category of suspected AMVR, which we initially suspected of having a poor outcome due to an unrecognized humoral phenotype and inappropriate treatment but eventually found to have a favourable clinical outcome. Moreover, this study provides a detailed overview of isolated v-lesions with a rather benign character – although treated with steroids – and uniquely describes AVR with a subclinical course. Another strength of this study is assessment of the therapeutic response including both functional and histological ones. Many studies lack such a complex evaluation, possibly due to the absence of a widely applicable definition of functional response [27].

In conclusion, this study provides single-centre evidence-based clinical data on acute vascular rejection. Our data extend current knowledge on a favourable outcome of an isolated v-lesion. Its benign clinical course might dispute the rejection phenotype of isolated v-lesions. Further studies are needed to precisely evaluate the origin of isolated v-lesions before considering a change in the diagnostic and therapeutic approach.

Authorship

MN: data gathering and processing and manuscript writing. MW: data processing and analysis, and manuscript supervisor. OV: project coordinator and manuscript supervisor. PH: serum and tissue samples processing. PV: immunogenetic examination. EH and JM: assessment of histological slides.

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Conflict of interest

The authors have declared no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article

Table S1. Characteristics of patients with subclinical AVR

Table S2. Outcome of sAMVR patients according to treatment modalities

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