Histopathological Analysis of Infiltrating T Cell Subsets in Acute T Cell-Mediated Rejection in the Kidney Transplant
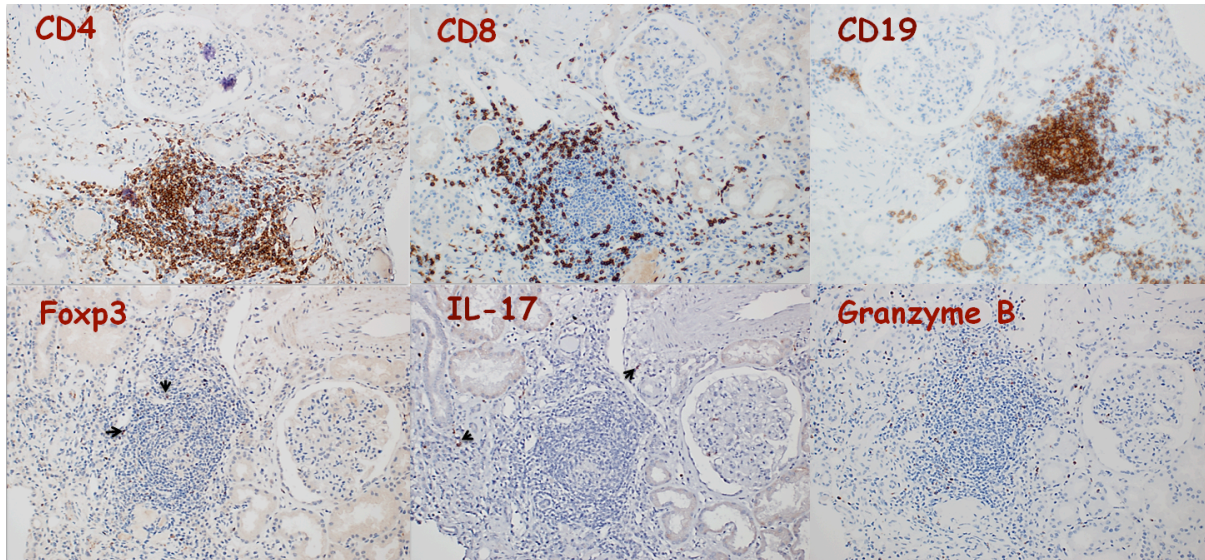
FIGURES

Figure 1: Representative T cell subsets infiltrating a kidney transplant undergoing ATCMR using antibodies to CD4, CD8, CD19, Foxp3, IL-17 and granzyme B as labeled on the pictures (the arrows indicate positive cells). All pictures derived from the same region cut at consecutive levels) [Immunohistochemistry staining, magnification 200x].
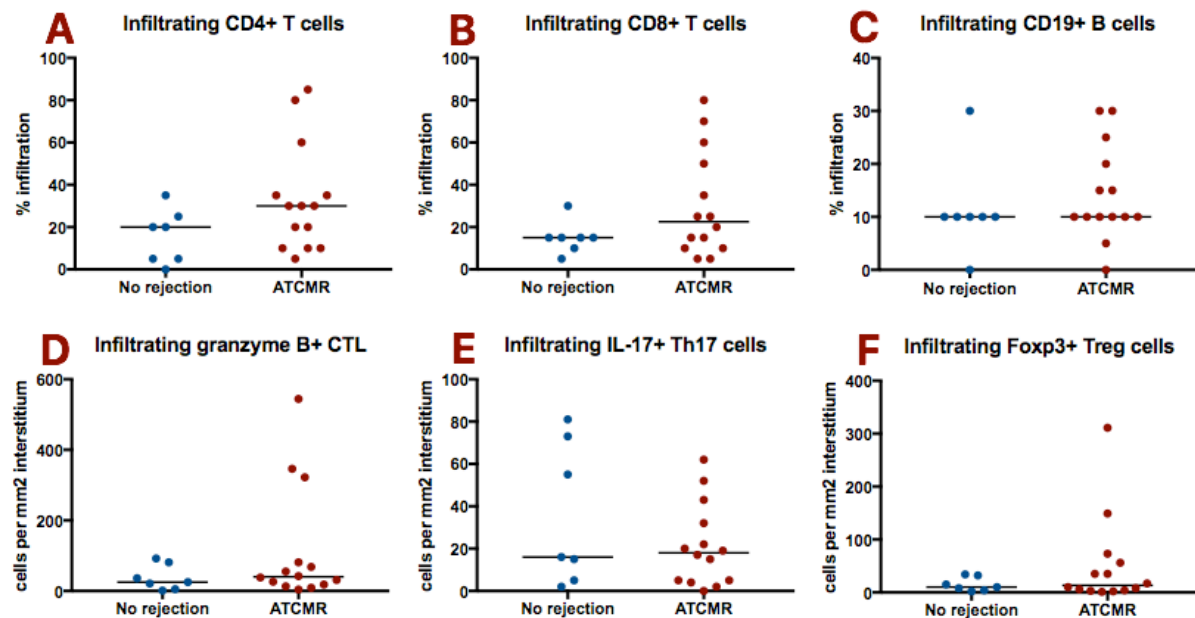

Figure 2: T cell subsets infiltrating kidney tissue, including %CD4$^+$ cells (**a**), %CD8$^+$ cells (**b**), %CD19$^+$ cells (**c**), granzyme B$^+$ cells/mm^2 (**d**), IL-17$^+$ cells/mm^2 (**e**) and Foxp3$^+$ cells/mm^2 (**f**) [all detected by immunohistochemistry] are compared between patients with ATCMR-KTx (n=14) and patients with no rejection (n=7). The horizontal lines indicate the median values. Wilcoxon rank-sum test p values for all comparisons were statistically non-significant.
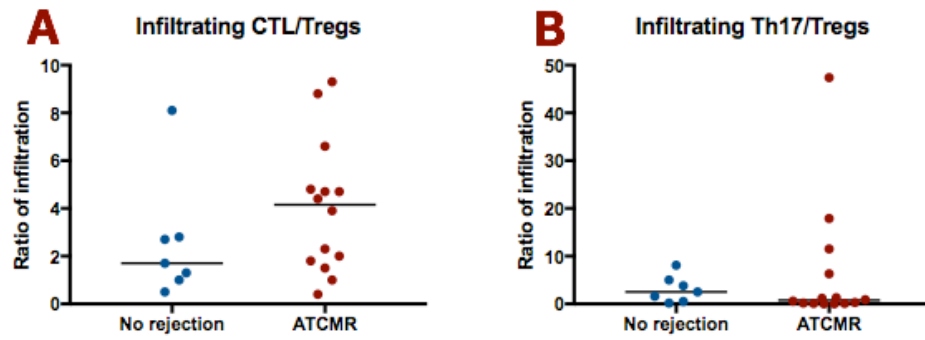
Figure 3: The ratios of (**A**) infiltrating granzyme B^{+} cells (CTL) over Foxp3^{+} cells (Tregs) and of (**B**) of infiltrating IL-17^{+} cells (Th17) over Foxp3^{+} cells (Tregs) are compared between patients with ATCMR-KTx (n=14) and patients with no rejection (n=7). All cell types were detected by immunohistochemistry. The horizontal lines indicate the median values. Wilcoxon rank-sum test p values for both comparisons were statistically non-significant.
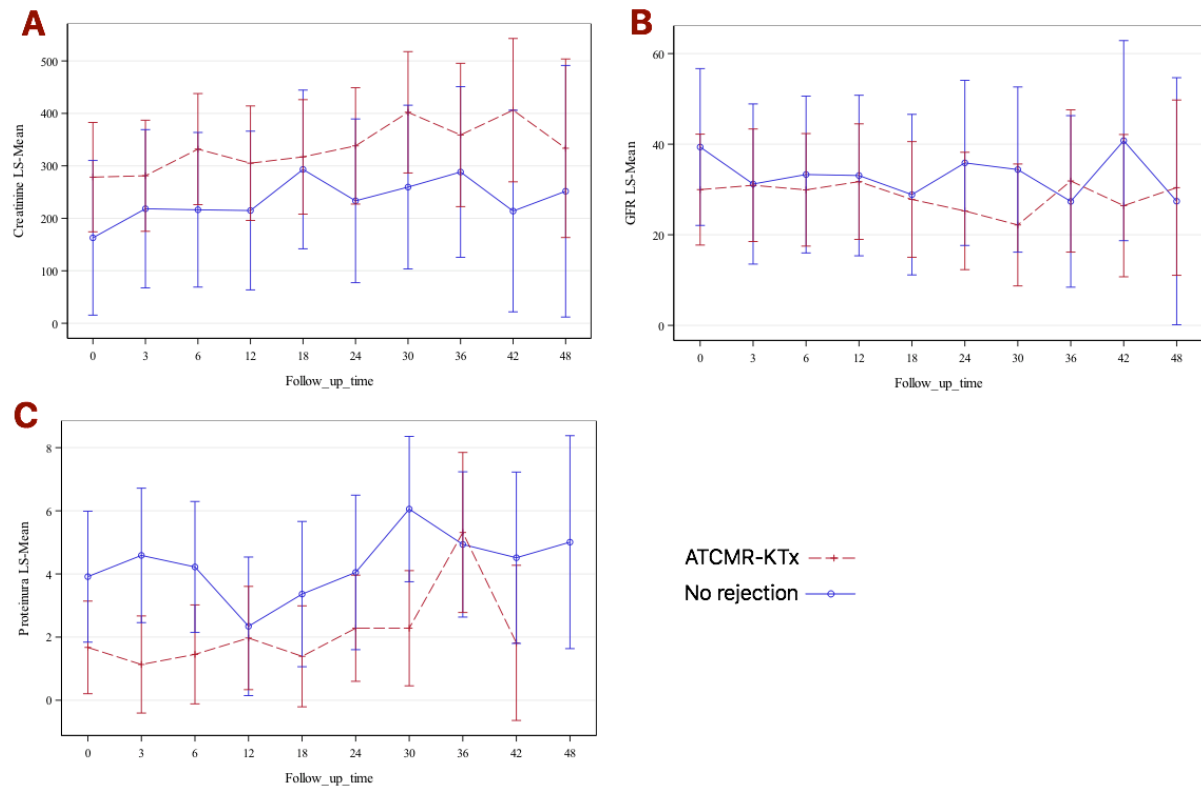

Figure 4: Longitudinal analysis comparing the dynamic changes in serum creatinine (**A**), GFR (**B**) and proteinuria (**C**) throughout the follow up period in the ATCMR-KTX (**red non-continuous line**) and non-rejection (**blue continuous line**) groups. The comparisons between overall mean values and mean values at follow-up times were statistically non-significant. Upper and lower limits for 95% confidence intervals at the different time points are indicated.
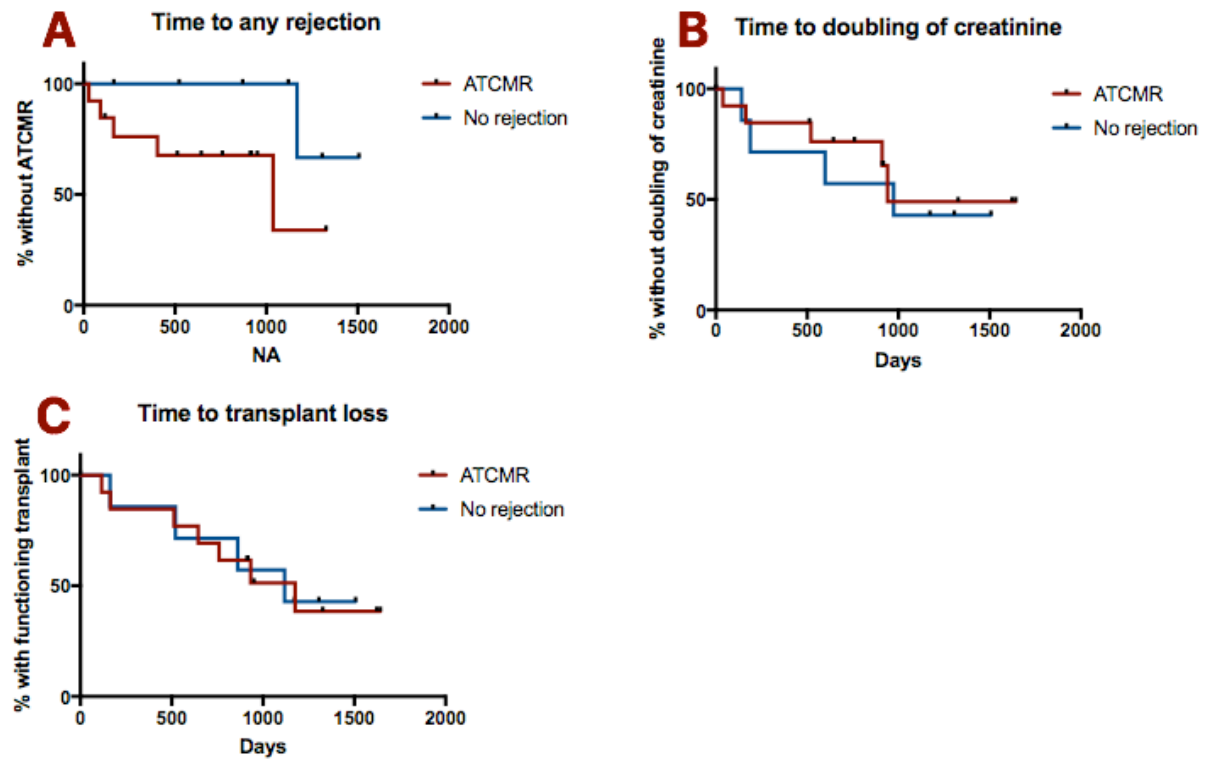
Figure 5: Time-to-event plots of (**A**) time to any rejection (borderline, ATCMR-KTx or antibody-mediated rejection) post-biopsy, of (**B**) time to doubling of creatinine post-biopsy, and of (**C**) time to confirmed or suspected immune-mediated transplant loss in patients with ATCMR-KTx (n=14) and patients with no rejection (n=7). Log-rank test p values for all the comparisons were statistically not significant.

TABLES

Table 1. Baseline clinical and demographic characteristics of the kidney transplant patients

Characteristic	n^	No rejection	n$	ATCMR	p value
Age (years)*	7	60.8	14	44.9	0.0101
Male sex (%)	7	57.14	14	71.43	0.6384
Race Chinese (%)	7	86.71	14	57.14	0.3371
Dialysis vintage (years)*	7	2.08	14	5.015	0.6888
Transplant vintage (years)*	7	13.75	14	3.935	0.0031
Deceased donor (%)	6	66.67	13	53.85	>0.9999
Delayed graft function (%)	6	33.33	12	41.67	>0.9999
Cold ischaemia time (h)	5	3	9	10	0.6973
Total HLA mismatch (#)*	6	3	11	3	0.9973
Very high immune risk (%)&	6	16.67	11	43.45	0.3334
% Panel of reactive antibodies*	3	8	9	0	0.2318
History of ATCMR (%)	7	14.29	14	50	0.1736
Re-transplant (%)	7	0	14	7.14	>0.9999
GFR at biopsy (ml/min/1.73m2)*	7	41.2	14	17.95	0.0767
Proteinuria at biopsy (g/day)*	7	3.5	14	1.23	0.2028
t score*	7	0	14	2	0.0116
I score*	7	1	14	2	0.0007
v score*	7	0	14	0	0.3371
Tacrolimus use at biopsy (%)	7	0	14	50	0.0468
Ciclosporin use at biopsy (%)	7	100	14	35.71	0.0071

MTORI use at biopsy (%)	7	0	14	14.29	0.5333
Steroids use at biopsy (%)	7	100	14	100	>0.9999
Mycophenolate use at biopsy (%)	7	57.14	14	85.71	0.2800
Azathioprine use at biopsy (%)	7	28,57	14	0	0.1000
Anti-CD25 induction (%)	5	0	12	41.67	0.2445
Prior thymoglobulin use (%)	7	14.29	14	14.29	>0.9999

ATCMR-Acute T cell-mediated rejection; GFR-Glomerular filtration rate; HLA-Human leukocyte antigen;

MTORI-Mammalian target of rapamycin inhibitor
*Results reported as median values.

[&]*According to UK Fuggle's classification based on HLA-DRB1 and HLA-B mismatches [30]*
[^]*Indicates the number of patients with available data in the non-rejection group*
[$]*Indicates the number of patients with available data in the ATCMR-KTx group*

Table 2. Correlation (R) between numbers and ratios of infiltrating immune cells and kidney transplant outcomes

Group	Immune parameter	vs	Outcome	R	P value
No rejection	Infiltrating Th17 cells		Creatinine t3	0.9429	0.0167
No rejection	Infiltrating Th17 cells		GFR t0	-0.8571	0.0238
No rejection	Infiltrating Th17/Tregs		GFR t0	-0.7857	0.0480
No rejection	Infiltrating Th17 cells		GFR t3	-0.9429	0.0167
No rejection	Infiltrating Th17/Tregs		GFR t3	-0.9429	0.0167
No rejection	Infiltrating Th17 cells		GFR t6	-0.8929	0.0123
ATCMR-KTx	Infiltrating CTL/Tregs		Creatinine t3	-0.6694	0.0145
ATCMR-KTx	Infiltrating Th17 cells		Creatinine t24	0.6485	0.0490
ATCMR-KTx	Infiltrating Th17 cells		Creatinine t30	0.7619	0.0368
ATCMR-KTx	Infiltrating Th17 cells		GFR t30	-0.8333	0.0154
ATCMR-KTx	Infiltrating Th17 cells		Proteinuria t12	0.8095	0.0218

Table 3. Comparison of time to transplant outcomes in the kidney transplant patients

Outcomes	Group	Median time-to-event	P values
	ATCMR	1037	
Any rejection			0.0941
	No rejection	Undefined*	
	ATCMR	941	
Doubling of creatinine			0.7452
	No rejection	974	
	ATCMR	1176	
Transplant loss			0.9560
	No rejection	1118	

Median time-to-event was not obtainable (see Figure 4A)

Table 4. Effect of immune and clinical variables on kidney transplant outcomes

Outcomes	Risk factor	Hazard ratio	95% CI	P value
Time to any rejection	Age	0.898	0.821 , 0.983	0.0193
Time to doubling of creatinine	Infiltrating Th17 cells	1.031	1.002 , 1.061	0.0359
Time to doubling of creatinine	Proteinuria	1.382	1.087 , 1.757	0.0083
Time to transplant loss	Infiltrating Th17 cells	1.026	1.000 , 1.052	0.0472
Time to transplant loss	Serum creatinine	1.009	1.003 , 1.016	0.0036
Time to transplant loss	Delayed graft function	5.456	1.238 , 24.036	0.0160

CI-Confidence intervals