Acute renal allograft rejection: immunohistochemical staining of mononuclear inflammatory cells and histopathologic pattern

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Introduction

Regarding the critical role of B cells in not only antibody mediated immune reaction, but also in the antigen presenting actions for stimulation of cellular immunity, several studies were designated to investigate whether these CD20+ B cells infiltration are associated with poorer clinical outcome or even whether anti B cell therapy with Rituximab would prevent allograft cellular rejection or not.

Aims and objectives

In this study we investigated the histologic pattern (including severity and site of infiltration and cluster formation) of T cells and B cells infiltration in biopsy proven TCMR and ABMR. The main goal of current study was to find a logical correlation between mentioned pattern of inflammatory cells and histopathologic findings in early and late renal allograft rejection.

Materials and Methods

- This cross sectional study was performed on all renal allograft biopsies evaluated in our referral nephro-pathology service of Nobel Institute (Isfahan-Center of Iran) during years 2017-2019.
- All slides and paraffin-embedded blocks of mentioned specimens were retrieved from the archive of the laboratory
- IHC staining for the inflammatory cells markers including CD3, CD4, CD8, CD20 and CD138 performed in all specimens

Materials and Methods

- All of the biopsies were categorized according to Banff classification into ten groups of

  1) TCMR-IA, 2) TCMR-IB, 3) TCMR-IIA,

  4) TCMR-IIB, 5) TCMR-III,

  6) ABMR-ATN, 7) ABMRTMA, 8) ABMR and TCMR,

  9) BK virus nephropathy, 10) BK virus nephropathy and TCMR.

Statistical analysis

- IBM SPSS Advanced Statistics 21.0 (IBM corporation, Armonk, New York- USA) was used for statistical analysis.
- Chi-square, ANOVA and Mann-Whitney tests were performed for descriptive and comparative evaluations
- Spearman correlation test was performed to find correlations between variables.
- P-values<0.05 was considered significant.

Histological scoring of IHC positive cells

Histological scoring	No. positive cells per high power field
0	0-2
1	3-10
2	11-20
3	21-40
4	41-80
5	>80

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Cluster formation:

- Dense aggregation of more than 30 positive cells without inter-positioning of tubules.

-For more accurate evaluation of clusters of inflammatory cells and better correlation with histopathologic changes obtained by light microscopy, we considered "cluster" as "score 6" in mentioned above scoring system

Results

- A total number of fifty one renal allograft biopsies were included in this study of which thirty and eleven cases met the criteria of TCMR and ABMR respectively and the other biopsies diagnosed as other entities which can be seen in (table. 1).

Renal allograft biopsies and pattern of mononuclear inflammatory cells infiltration

-There were greater IHC's markers expression scoring number for CD20, CD3 and CD8 in the TCMR group, while there is greater IHC's marker expression of CD138 in ABMR group. but no differences was seen between mentioned groups regarding CD4.

Results

Correlation between mononuclear inflammatory cells infiltration of renal allograft biopsies and other findings

- Spearmen correlation test showed that in the group of TCMR diagnosed biopsies there are no correlation between cellular infiltration of renal allograft biopsies by CD20, CD138, CD3, CD4 and CD8 positive cells and post-transplantation time, whereas in the group of ABMR diagnosed biopsies CD138+ cells (r=0.761, p-value=0.007) and CD4+ cells (r=0.793, p-value=0.004) are correlated with post-transplantation time of biopsy.

Results

- **Inflammatory cells cluster formation**

  - Clusters formation by inflammatory cells were seen in 24 out of 30 (80%) of TCMR diagnosed biopsies versus 4 out of 11 (36.36%) of ABMR diagnosed biopsies (P-value=0.013).

  - All of clusters of ABMR group were in the interstitium, while clusters of TCMR group were in periglomerular area (20.83%), Perivascular area (4.16%), Interstitium (4.16%), Periglomerular and perivascular area (45.83%), Periglomerular area and interstitium (20.83%) and subcapsular area (4.16%).

  - Comparison of cluster's formation site between TCMR and ABMR diagnosed groups revealed a statistical difference (p-value=0.001)

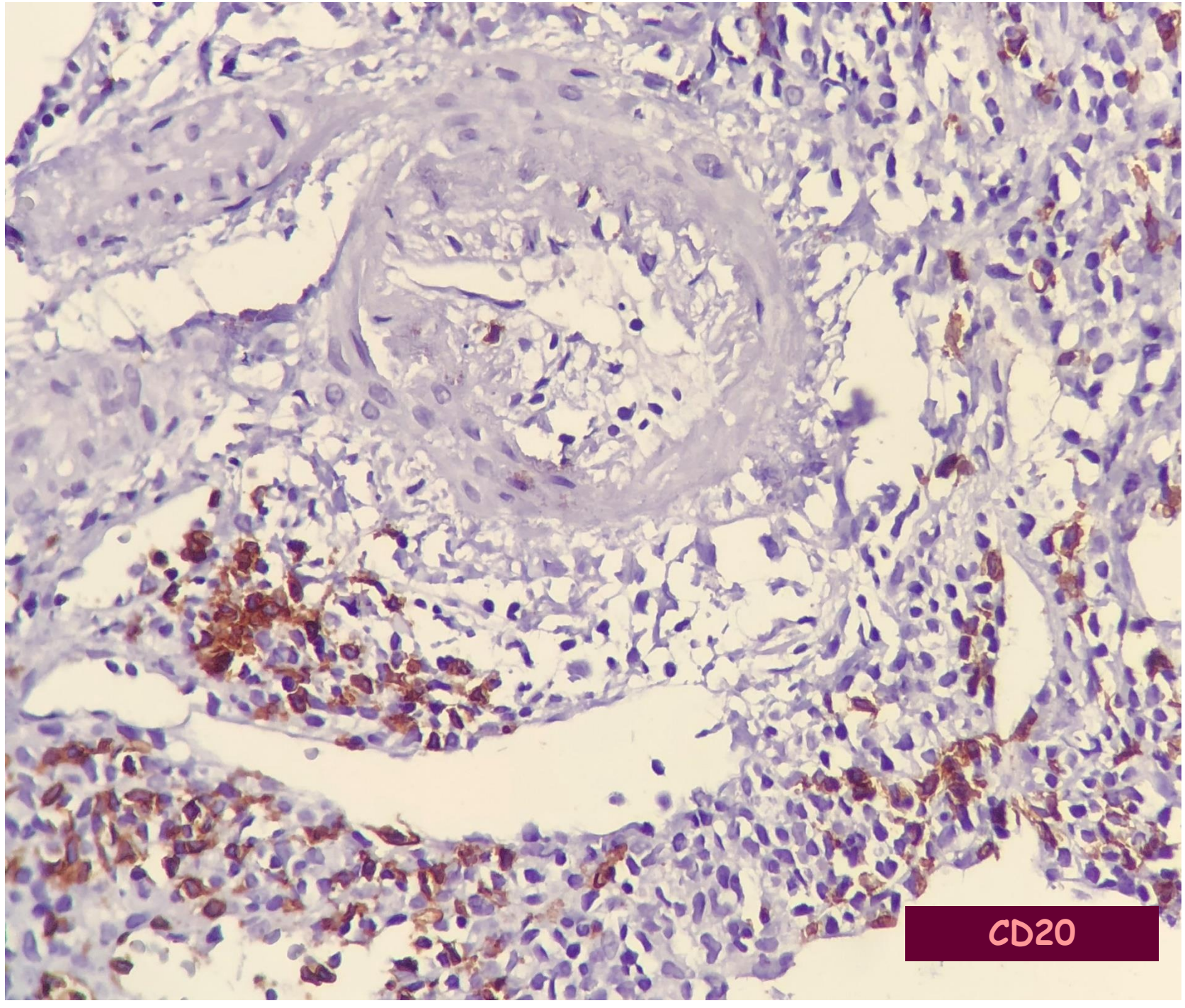
  - There were no correlation between presence of inflammatory cells clusters and post-transplantation time of biopsy not only in the TCMR group (r=0, p-value=1), neither in the ABMR group (r=0.076, p-value=0.824).

Type of rejection		TCMR IA	TCMR IB	TCMR IIA	TCMR IIB	TCMR III	ABMR (ATN)	ABMR (TMA)	ABMR and TCMR	BK	BK and TCMR	Total	P-value
number		12	8	7	2	1	7	4	8	1	1	51	-
Age		36.41±12.50	48±11	40.28±8.93	46±8.48	41	49±17.27	40.50±13.12	30.75±5.33	73	63	41.62±13.49	0.01
sex	Male	6 (50%)	8 (100%)	3 (42.85%)	1 (50%)	1 (100%)	4 (57.14%)	1 (25%)	5 (62.5%)	1 (100%)	0 (0%)	30 (58.82%)	0.23
	Female	6 (50%)	0 (0%)	4 (57.14%)	1 (50%)	0 (0%)	3 (42.85%)	3 (75%)	3 (37.5%)	0 (0%)	1 (100%)	21 (40.38%)	
Donor	Living related	0 (0%)	2 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (3.92%)	0.58
	Living Unrelated	4 (33.33%)	1 (12.5%)	3 (42.85%)	1 (50%)	0 (0%)	2 (28.57%)	2 (50%)	1 (12.5%)	0 (0%)	0 (0%)	14 (27.45%)	
	Cadaveric	8 (66.66%)	5 (62.5%)	4 (57.14%)	1 (50%)	1 (100%)	5 (71.42%)	2 (50%)	7 (87.5%)	1 (100%)	1 (100%)	35 (68.62%)	
Post Transplant time	Less than 2 weeks	2 (16.66%)	0 (0%)	2 (28.57%)	0 (0%)	1 (100%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	6 (11.76%)	0.54
	Between 2 and 6 months	2 (16.66%)	1 (12.5%)	1 (14.28%)	1 (50%)	0 (0%)	1 (14.28%)	1 (25%)	2 (25%)	0 (0%)	0 (0%)	9 (17.64%)	
	More than 6 months	8 (66.66%)	7 (87.5%)	4 (57.14%)	1 (50%)	0 (0%)	6 (85.71%)	2 (50%)	6 (75%)	1 (100%)	1 (100%)	36 (70.58%)	
GFR		37.08±12.42	43.25±18.39	45.14±11.12	22±5.65	49	32.85±19.55	32.25±21.57	29.12±17.72	56	35	36.92±16.23	0.42

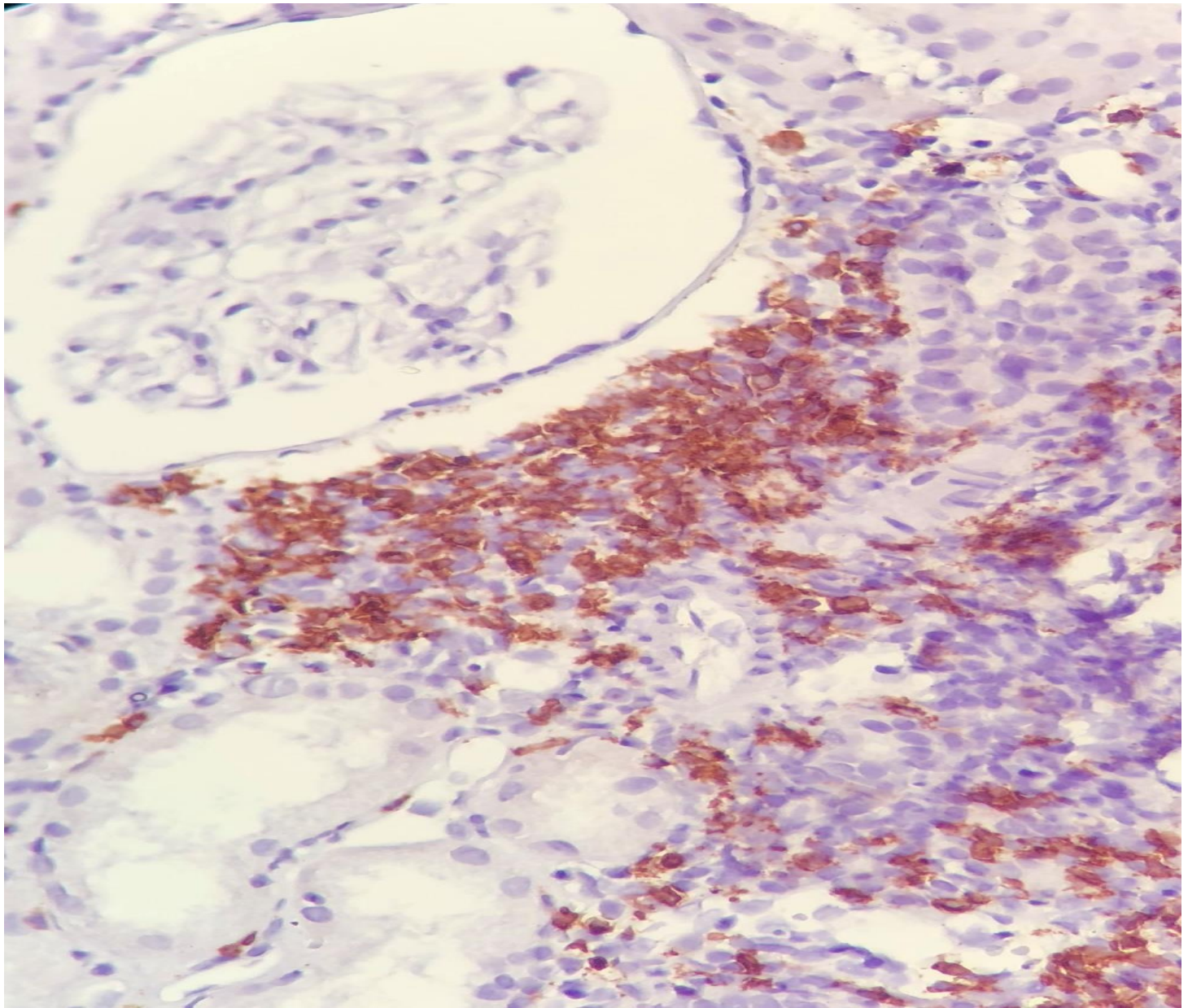
(Table-1); Demographic data, Donor type, Post-transplantation time of biopsy and GFR of all participants

Infiltration Rejection	CD20	CD138	CD3	CD4	CD8
TCMR IA	4.75±1.65	0.58±0.51	3.50±1.31	1.91±1.50	2.75±1.05
TCMR IB	5.25±1.48	0.75±1.03	4.87±1.64	1.50±1.51	3.50±1.41
TCMR IIA	5.71±0.75	0.85±0.89	4.85±1.46	1.14±1.21	3.71±1.97
TCMR IIB	6	0.50±0.70	3.50±0.70	2.50±0.70	2.50±0.70
TCMR III	6	0	6	3	5
ABMR (ATN)	2.14±1.77	3.57±1.71	3±1.82	1.85±1.21	0.71±1.11
ABMR (TMA)	1±0.81	3.25±3.20	2.75±2.50	1.75±2.06	1±0.81
ABMR&TCMR	4.37±1.68	3.12±1.80	5.75±0.70	1.50±0.75	4.12±1.12
BK	6	6	6	1	4
BK&TCMR	6	1	5	1	2
Total	4.37±2.01	1.76±2.01	4.25±1.74	1.66±1.29	2.84±1.68
P-value	<0.001	<0.001	0.01	0.901	<0.001

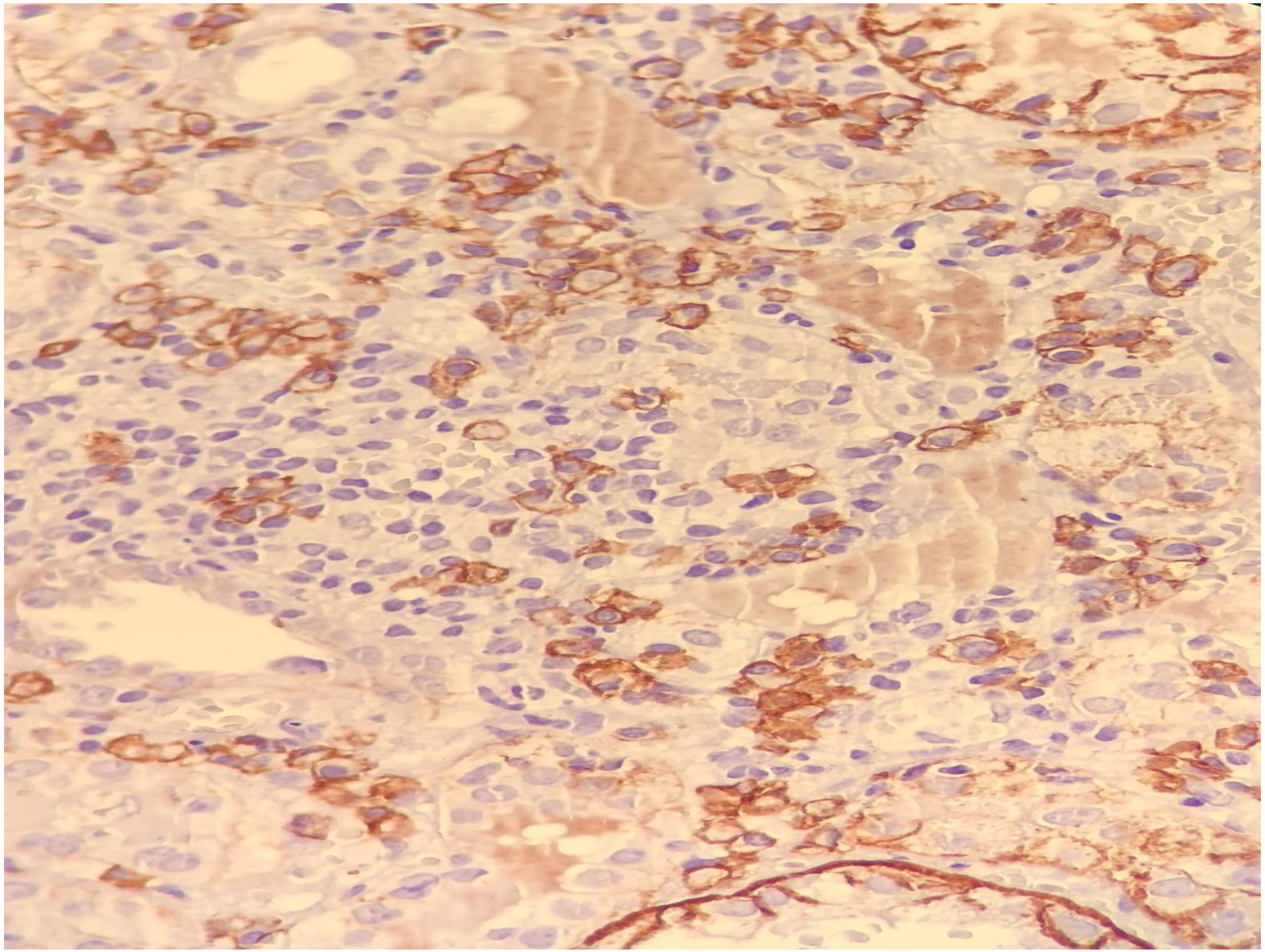
Table-2; Inflammatory cells infiltration in renal allograft biopsies


CD20

ATCMR IB- Cluster CD20



ABMR+ TCMR- CD138



Discussion

- Sarwal et al revealed an association between dense CD20+ B cells infiltration and both graft loss and glucocorticoid resistance.
- Hippen et al also suggested that CD20 positive clusters of B cells could be indicators of a more severe subcategory of TCMR leading to reduced graft survival.

They hypothesized that considering the fact that antibody producing B cells don't express CD20 marker and also absence of C4d positivity in the group of patients with diffuse and strong positivity for CD20, it's obvious that these clusters of B cells are not associated with humoral rejection and they may play an intermediating role into the process of cellular rejection.

Discussion

Dr. Bagnasco from Johns Hopkins in 2007 defined no association of CD20-positive lymphocyte infiltrates in allograft biopsies, with worse short-term or long-term outcome in those patients who develop acute rejection type I and/or type II within the first year of transplantation.

Dr Kayler from Pittsburgh Medical Center suggested that renal allograft biopsies with lymphoid clusters containing variable proportions of Bcells represent a heterogeneous collection rather than a distinct clinicopathologic entity.
In the mean while,changes in immunosuppression protocols based solely on the demonstration
Of B cells does not seem justified.

Conclusion

In summary, it's believed that each type of mononuclear inflammatory cell plays an important role in the process of allograft rejection. The importance of CD20+ B cells in progression of TCMR and CD138+ plasma cells in ABMR are discussed, but correlation between severity of the inflammation and site of cluster formation with the prognosis of renal transplantation needs further studies.

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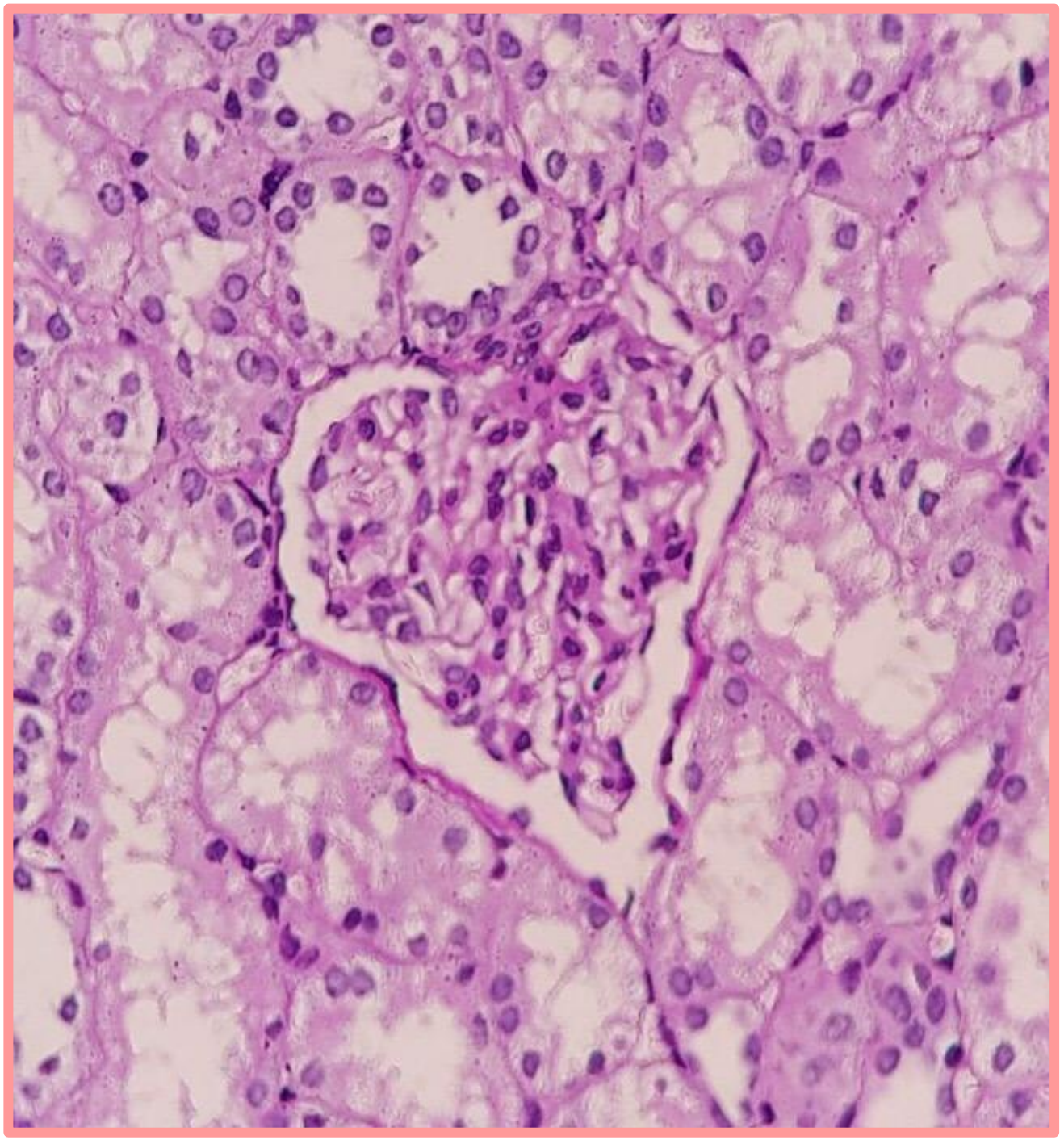

Thank You For Your Attention