

Granzyme B positive Cytotoxic T cells are Associated with Chronic Active Antibody Mediated Rejection.

Narayan Prasad¹, Brijesh Yadav¹, Vikas Agrawal,² Vinita Agrawal³

Department of Nephrology¹, Clinical Immunology², Pathology³

Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, 226014 India

Introduction

- Chronic antibody mediated rejection respond poorly to conventional steroid therapy and remained one of major cause for graft loss in late post transplant time after transplantation.
- CABMR accounts for 20-60% of graft loss by 5 years
- CABMR is often associated with C4d deposition in peritubular capillaries and donor specific antibody against the either or both class of HLA.
- Clinically, it is associated with proteinuria, hypertension, rise in serum creatinine level decline in glomerular filtration rate
- Histologically, shows multilayering in peritubular capillary wall, glomerular basement membrane, interstitial fibrosis

Cytotoxic T cell

- Cytotoxic T cell is, Granzyme-B positive CD3+CD8+ T cell.
- On activation it secretes serine protease Granzyme B, Perforin, lymphotoxin and express surface receptor Fas L.
- Perforin form pore in target cell and disturb osmotic balance of cell.
- Granzyme B, a serine protease, cleaves procaspase in to active caspase and induce apoptosis in kidney cell.
- Granzyme-B also cleave cytoskeleton protein, metalloproteinase and IL-1 β and generate inflammatory millieue.
- FasL binds to FasR of target cell and induce apoptosis in them.
- The exact mechanism of immune injury in such conditions are not well established.

AIM

- To study the frequency of cytotoxic T cell (CD3+CD8+GzmB+), in blood, Granzyme-B level in serum, Cell intact Granzyme-B level in PBMCs culture supernatants of peripheral mononuclear cell in patients of SGF and CABMR.
- To determine the Granzyme-B mRNA and Protein expression in allograft biopsy tissue of these patient in these conditions

Material and Methods

Patient recruitment (N=42)

CABMR (N= 32)

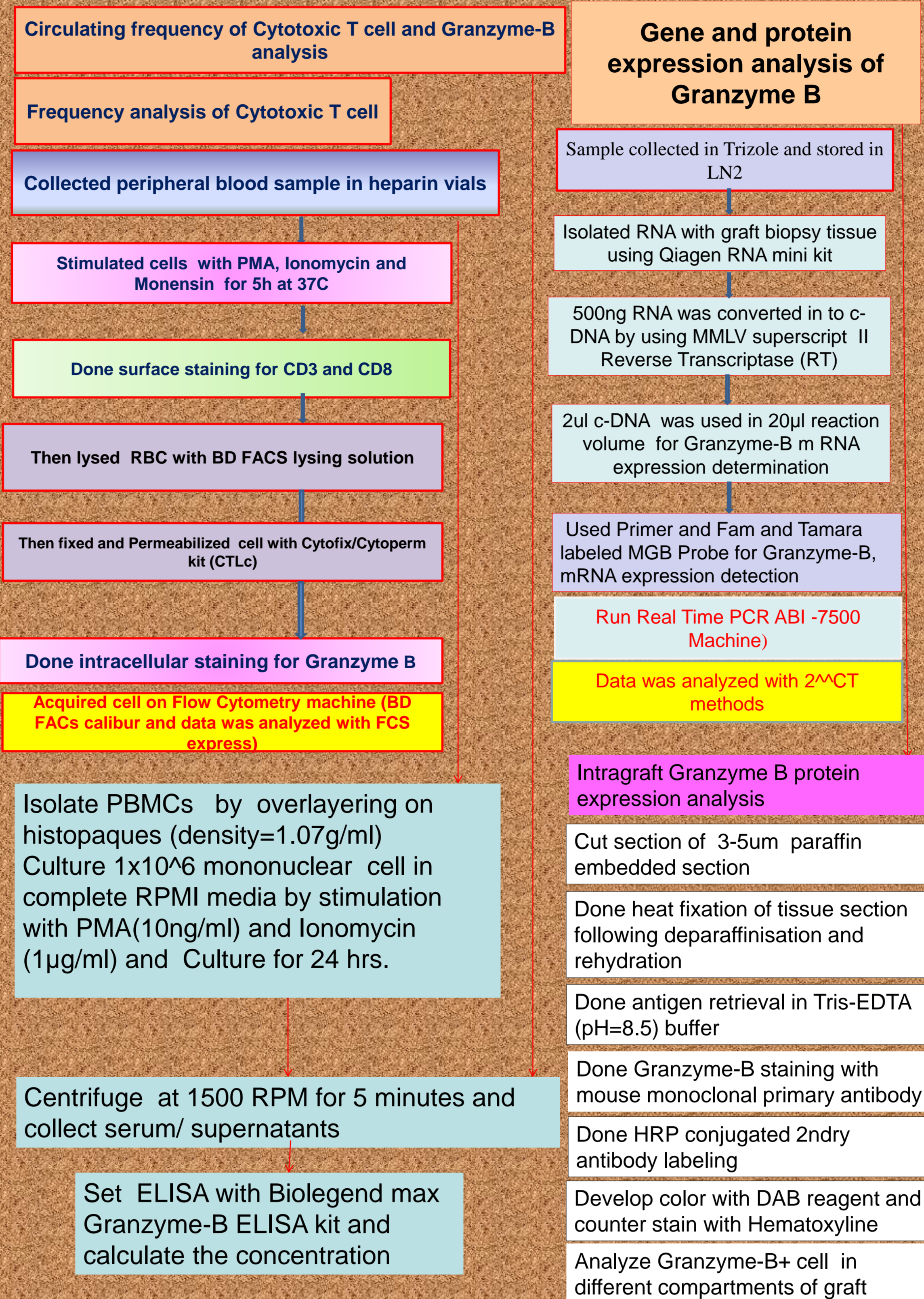
- PTCBMML (Peritubular capillary basement membrane multilayering)
- C4d+ in peritubular capillary
- DSA positive against either class of HLA
- Proteinuria, >25% rise in serum creatinine

SGF (N=10)

- <10% cortical surface with evidence of lesions
- Stable serum creatinine level in last six month
- No proteinuria
- Negative C4d staining in PTCs and absence of DSA

All patients were included in study following as per criteria of Banff-2007

Materials and Methods



Results

Table 1 | Demographic and clinical characteristics of patients

Characteristics	SGF (Mean \pm SD)	CABMR (Mean \pm SD)	P value
Pt. Gender (M: F)	10:0	21:11	0.182
Do. Gender (M: F)	2:8	7:25	0.874
Patient age (Years)	44.36 \pm 8.20	37.93 \pm 12.78	0.148
Post Tx bx interval (Months)	46.70 \pm 17.30	70.18 \pm 34.48	0.046
eGFR (mL/min/1.73 m ²)	70.62 \pm 22.14	44.60 \pm 16.62	<0.001
Tac level (ng/ml)	4.82 \pm 0.98	5.57 \pm 1.31	0.102
TLC	8.39 \pm 2.12	8.05 \pm 5.08	0.839
BUN	25.79 \pm 13.3	40.55 \pm 11.79	0.002
Baseline creatinine (mg/dl)	0.81 \pm 0.47	0.88 \pm 0.42	0.644
S. Creatinine (mg/dl)	1.21 \pm .18	2.40 \pm 0.80	<0.001
24 hour urine protein (gm)	0.16 \pm 0.085	2.94 \pm 1.54	<0.001
HLA mismatch	3.40 \pm 0.69	3.15 \pm 0.54	0.170
Induction regimen (Basiliximab)	10	32	1.00
Baseline Immunosuppression Tacrolimus+MMF+Pred	10	32	1.00
ESRD cause (MN/HTN/NOS)	6/3/1	20/9/3	0.698

Banff- 2007, Histological injury score of allograft

Characteristics	SGF(10)	CABMR(32)	P value
Peritubular capillaritis			
ptc0 (%)	9(90%)	2 (6.25%)	<0.001
ptc1 (%)	1(10%)	7 (21.8%)	
ptc2 (%)	0(0%)	8 (25%)	
ptc3 (%)	0(0%)	15(46.8%)	
Glomerulitis			
cg0 (%)	9 (90%)	3 (9.32%)	<0.001
cg1 (%)	1 (10%)	14(43.7%)	
cg2 (%)	0 (0%)	12(37.5%)	
cg3 (%)	0 (0%)	3 (9.32%)	
Interstitial Inflammation			
ti0 (%)	8 (80%)	2 (6.25%)	<0.001
ti1 (%)	2 (20%)	14(43.7%)	
ti2 (%)	0 (0%)	14(43.7%)	
ti3 (%)	0 (0%)	2 (6.25%)	
C4d staining			
cd0 (%)	10 (100%)	0 (0%)	<0.001
cd1 (%)	0 (0%)	2 (6.25%)	
cd2 (%)	0 (0%)	21 (65.62%)	
cd3 (%)	0 (0%)	9 (28.1%)	
Interstitial fibrosis			
ci0 (%)	10(100%)	3 (9.3%)	<0.001
ci1 (%)	0 (0%)	12(37.5%)	
ci2 (%)	0 (0%)	9 (28.1%)	
ci3 (%)	0 (0%)	8 (26.6%)	
Arteriolar hyalinosis			
aah0 (%)	9 (90%)	24 (75%)	<0.001
aah1 (%)	1 (10%)	7(21.8%)	
aah2 (%)	0 (0%)	1 (%)	
aah3 (%)	0 (0%)	0 (3.1%)	
Tubular atrophy			
t0 (%)	6 (60%)	2 (6.6%)	0.021
t1 (%)	4 (40%)	7(23.3%)	
t2 (%)	0 (0%)	15 (50%)	
t3 (%)	0 (0%)	6 (20%)	

Results

Table 2. Mean % of CD3+CD8+T cell and CD3+CD8+ Gzm B+ in SGF and CABMR group.

Characteristics	SGF	CABMR	P Value
CD3+CD8+Cell%	33.10 \pm 4.38	26.80 \pm 6.16	0.031
CTLc (CD3+CD8+GzmB)%	27.32 \pm 2.7	12.44 \pm 1.68	<0.001

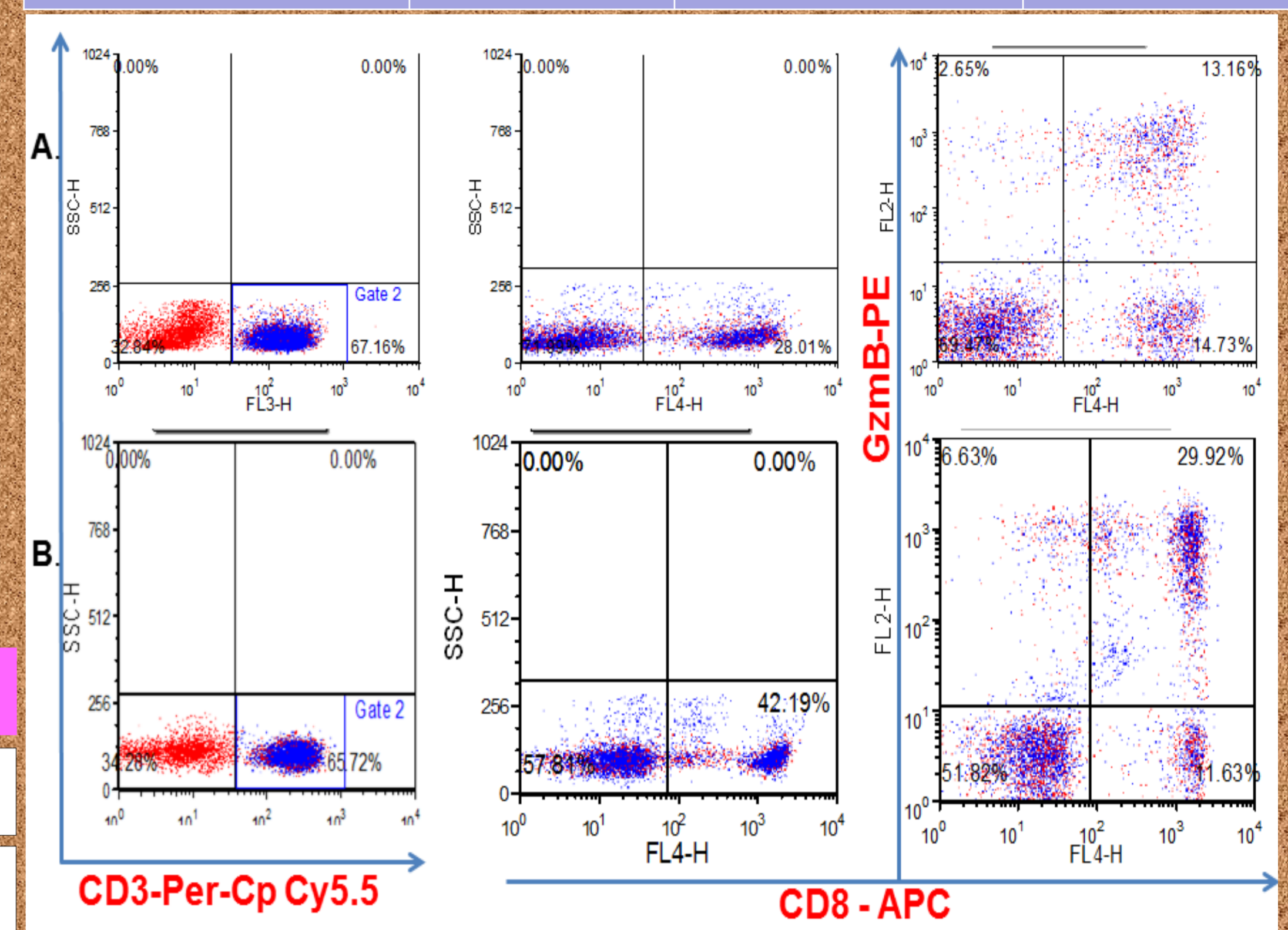


Table 3. Soluble and cell-intact Granzyme-B expression analysis in serum and PBMCs culture supernatants.

	Serum soluble Granzyme-B level (pg/ml)	PBMCs culture supernatants Granzyme-B level (pg/ml)
SGF	100.82 \pm 22.41	109.41 \pm 33.27
CABMR	177.82 \pm 48.66	82.69 \pm 19.88
P value	<0.001	0.003

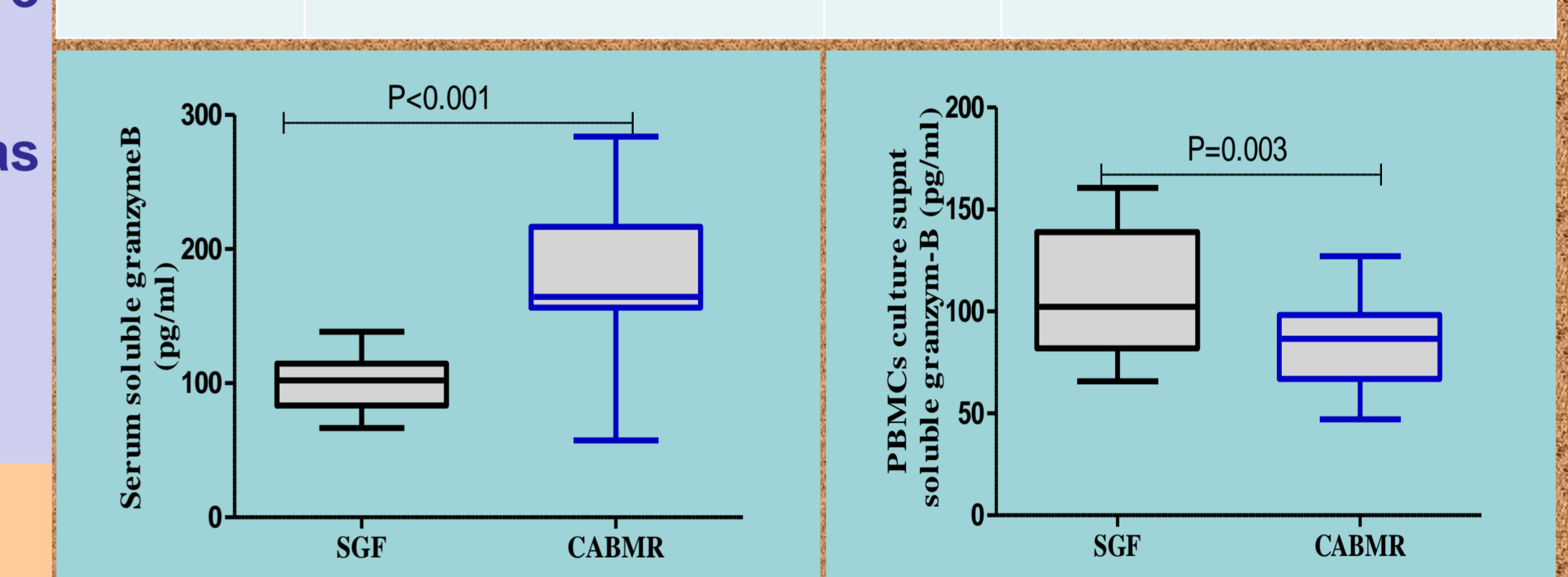


Table 4. Intragraft Granzyme-B, mRNA and protein expression analysis in serum and PBMCs culture supernatants.

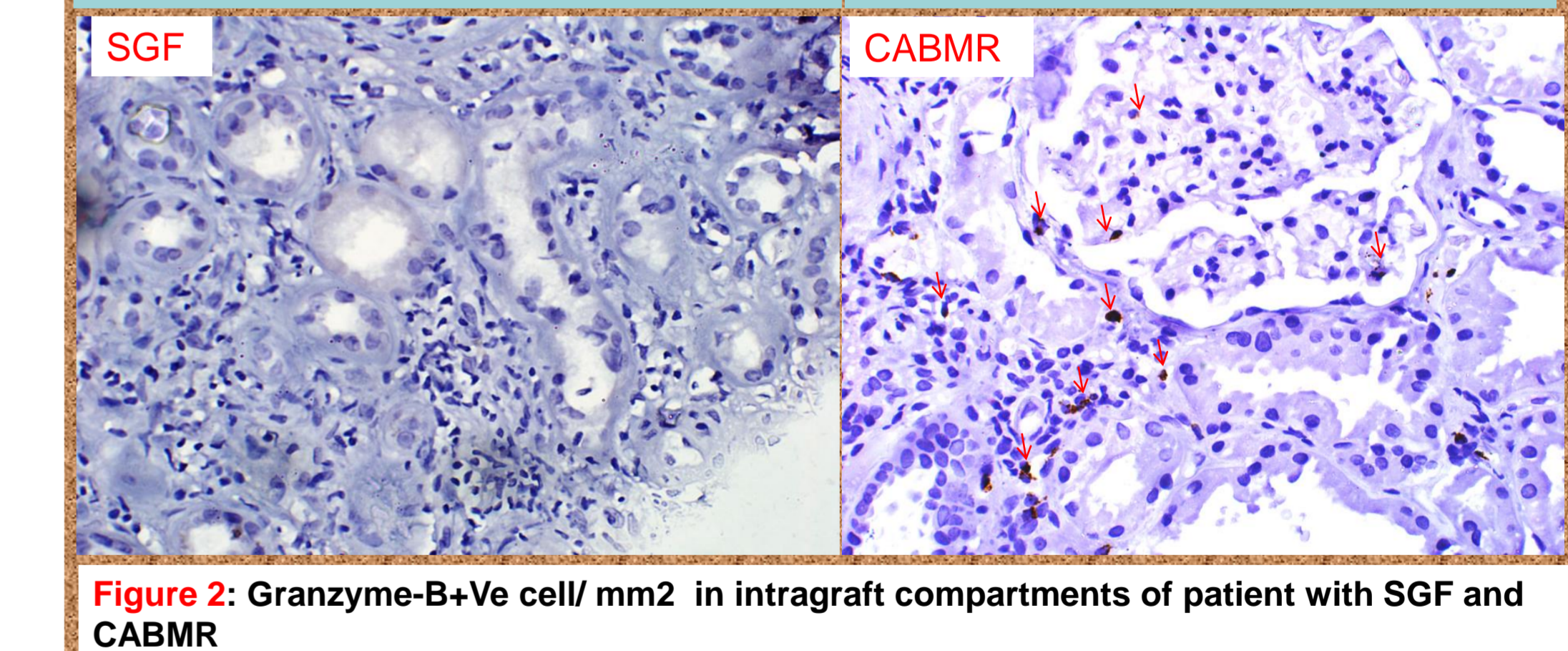
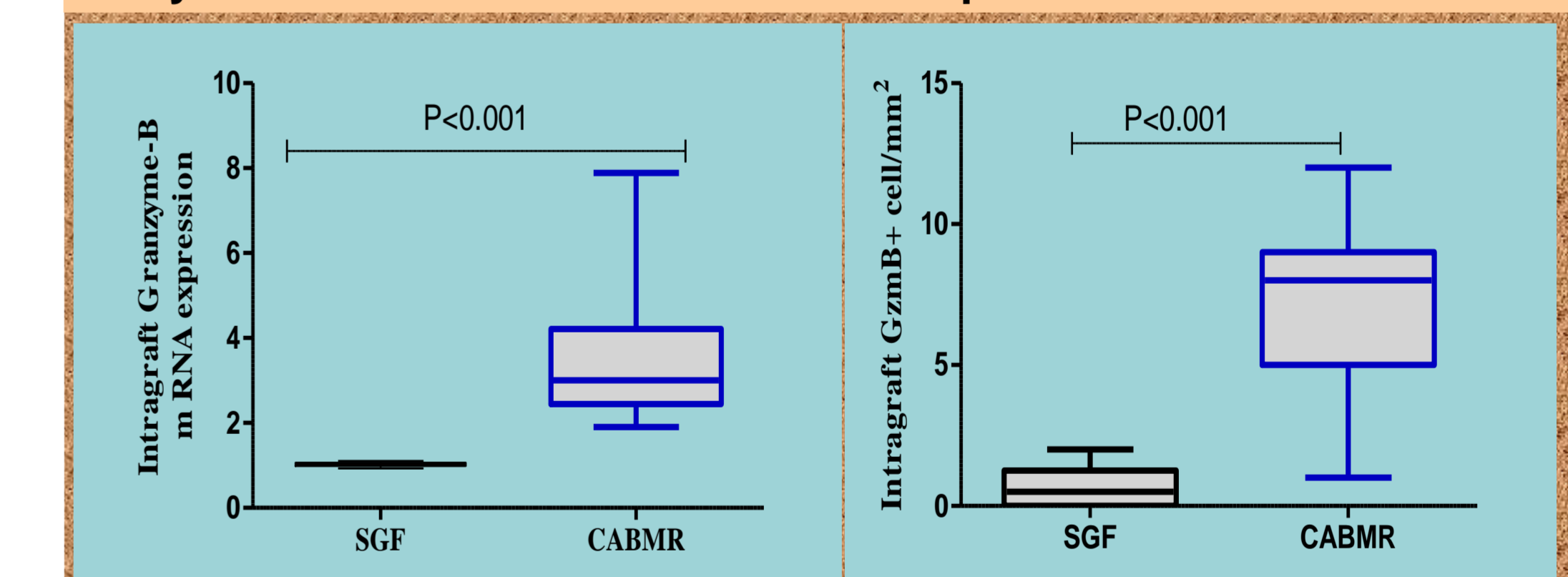


Figure 2: Granzyme-B+ve cell/ mm² in intragraft compartments of patient with SGF and CABMR

Conclusions

- The low frequency of Granzyme-B+ cytotoxic T cell and higher soluble serum Granzyme-B level in CABMR suggest activated cytotoxic T cell releases Granzyme-B in serum.
- Higher intragraft Granzyme-B, mRNA and protein expression suggest sequestration of CTLc in graft tissue from circulation.
- Granzyme-B dependent allograft injury may be the cause for higher allograft injury score in CABMR group.

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