T-Cell Efflux Dynamics: The Role of P-Glycoprotein and MRP-1 in Pediatric Steroid-Resistant Nephrotic Syndrome

Presented By

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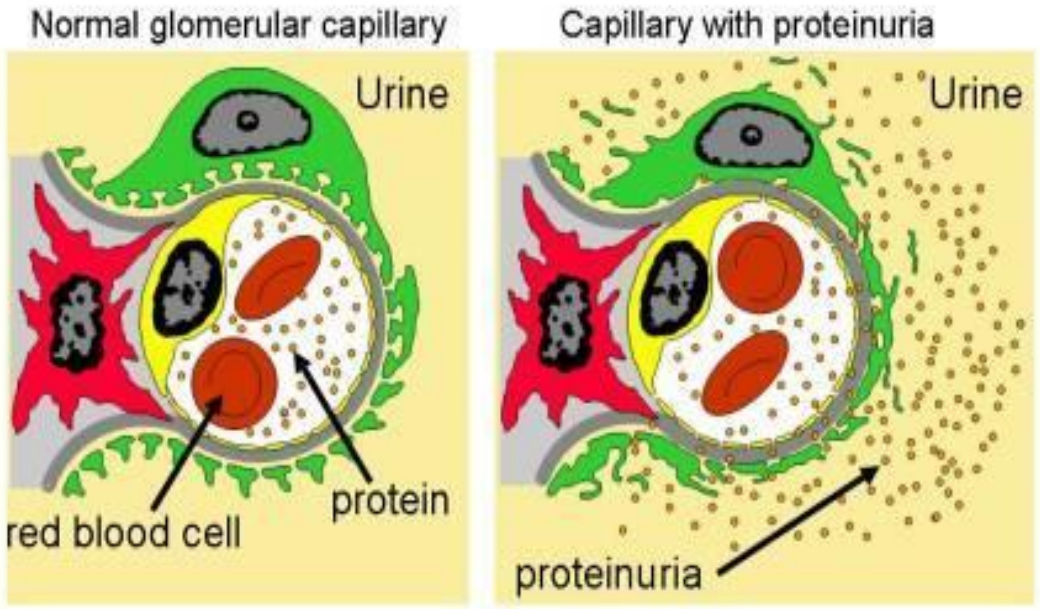
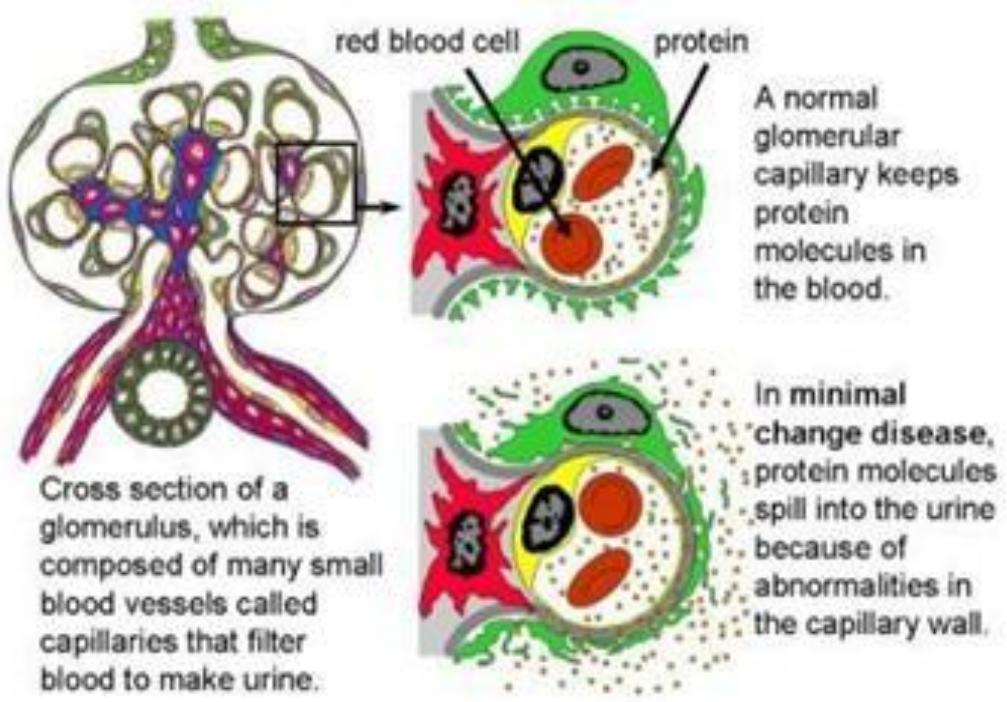
Introduction

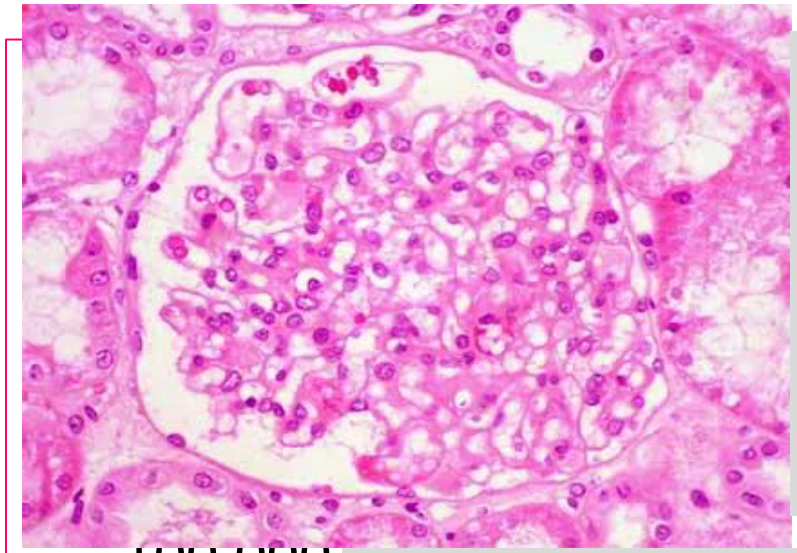
How we characterize Nephrotic syndrome?

NS is characterized by proteinuria greater than 40 mg /m^2/h, hypoalbuminemia less than 2.5g/dL and edema.





Introduction



Minimal change disease.
Glomeruli appear to be normal with normal capillary walls and normal cellularity on Light microscope.

Immunofluorescence was negative.

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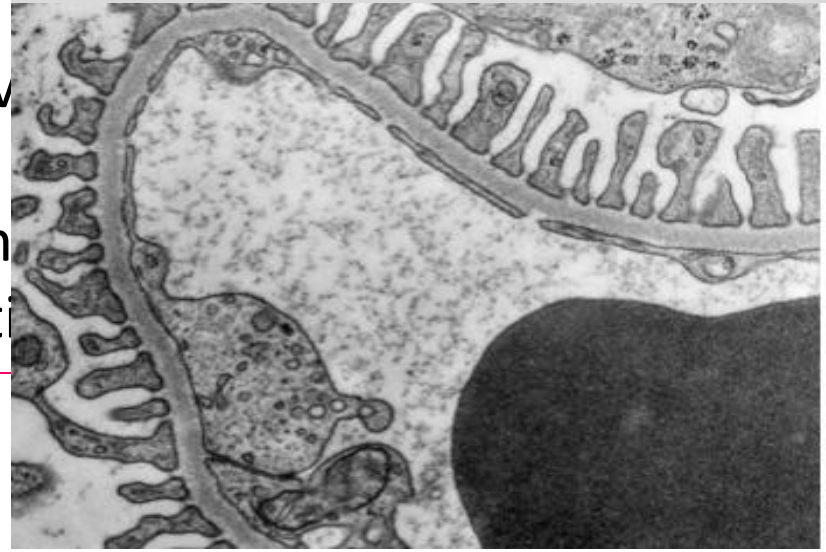
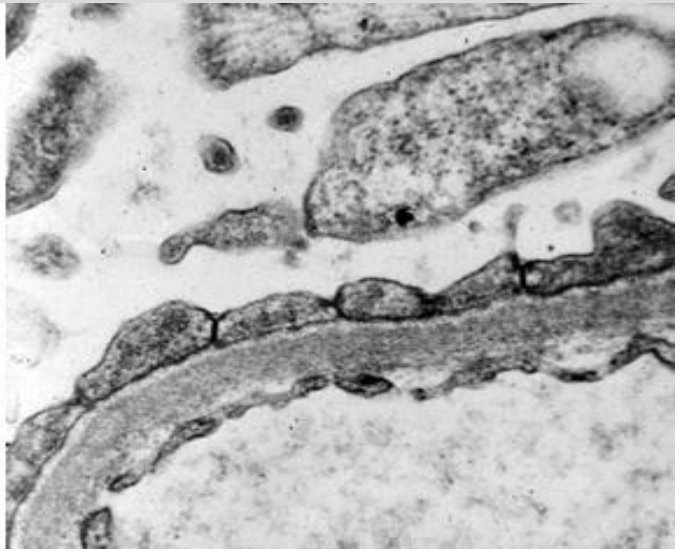
Normal podocyte foot process



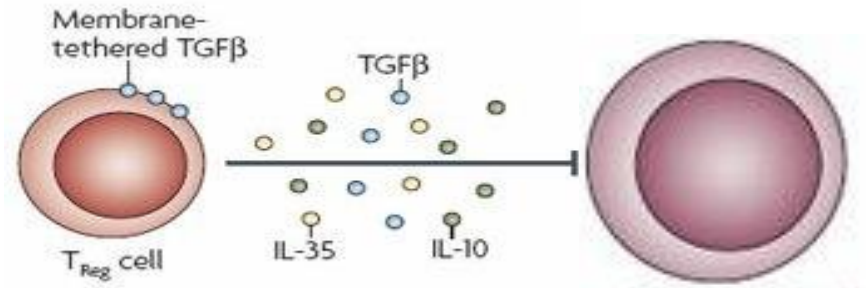
Diffuse effacement of podocyte



Pathogenesis of Nephrotic Syndrome

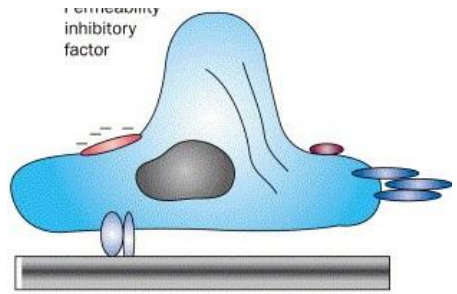
- Indirect evidences suggests that nephrotic syndrome (NS) is consequence of dysfunction of CD4+ T cells.

Shalhoub RJ . Lancet .7:556-560.



Regulatory Cytokines
(suppress T effector cells)

Proinflammatory Cytokines (IFN-ϒ, IL-4,IL-13, IL-17, IL-23)
(increases permeability to plasma protein)

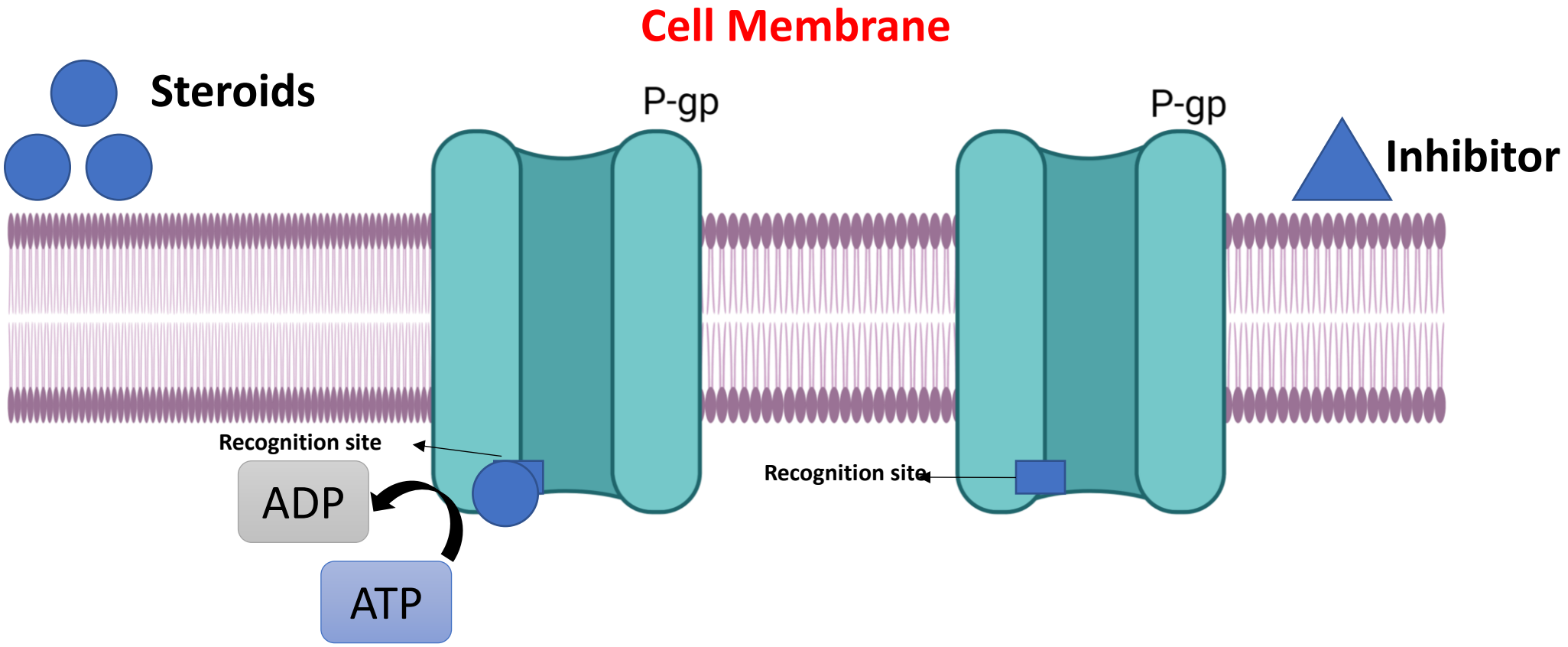


Introduction

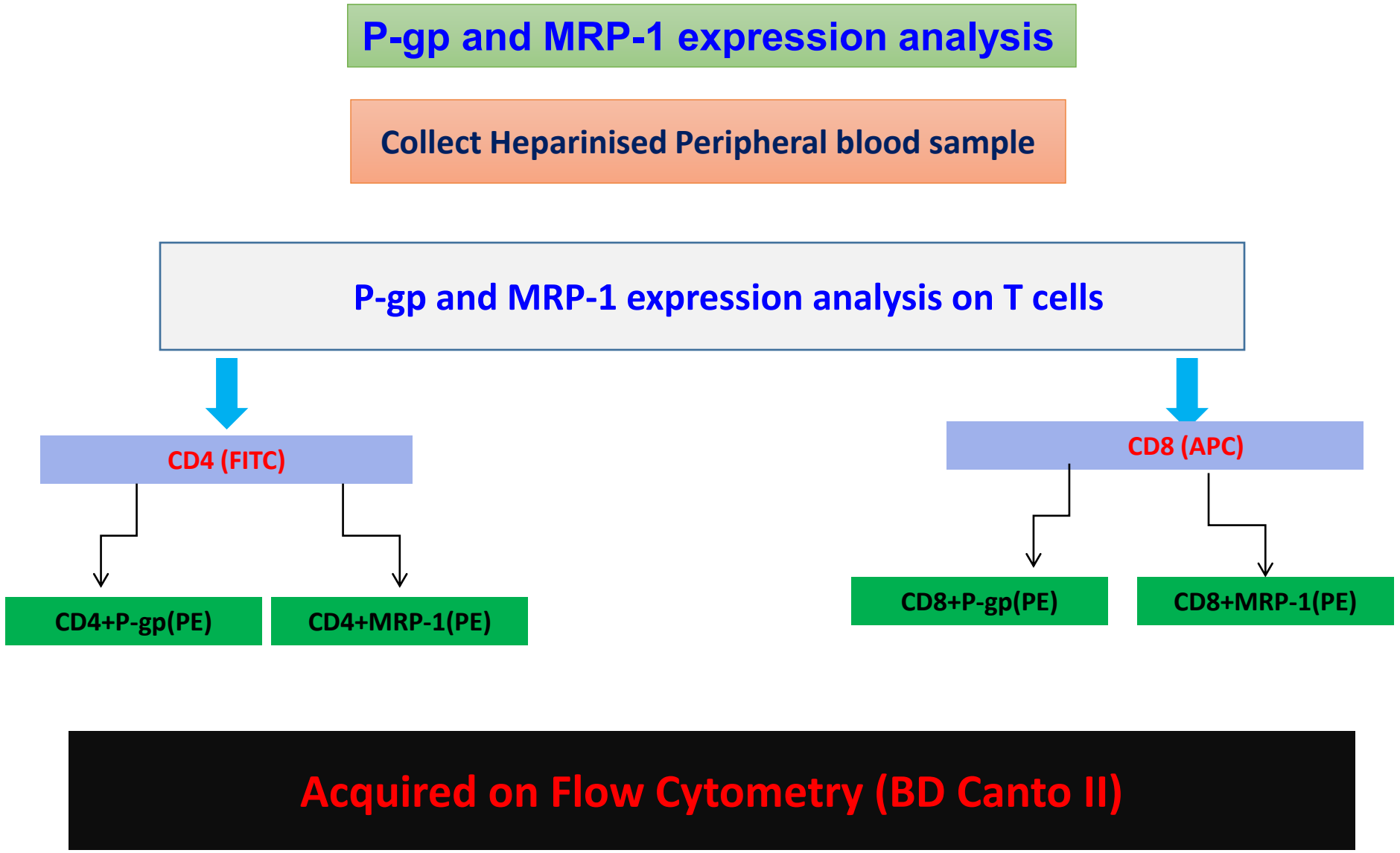
- P-glycoprotein is a member of the ATP-binding cassette (ABC) transporter family; encoded by **MDR1 (ABCB1) gene**.
- Functions as an **ATP-dependent efflux pump**, exporting a wide range of xenobiotics and drugs across cell membranes.
- Highly expressed in **intestinal epithelium, liver, kidney, blood–brain barrier, placenta, and immune cells**.
- Plays a key role in **drug absorption, distribution, metabolism, and excretion (ADME)**
- Another important member of the **ABC transporter family**, encoded by the **ABCC1 gene** is Multi Drug Resistant protein-1 (MRP-1)
- Acts as an **ATP-dependent efflux transporter** with strong affinity for **organic anions and glutathione-conjugated drugs**.
- Widely expressed in lung, testis, placenta, immune cells, and tumor cells

Ricky W Johnstone et al.. Cell (2000).25:1;1-6

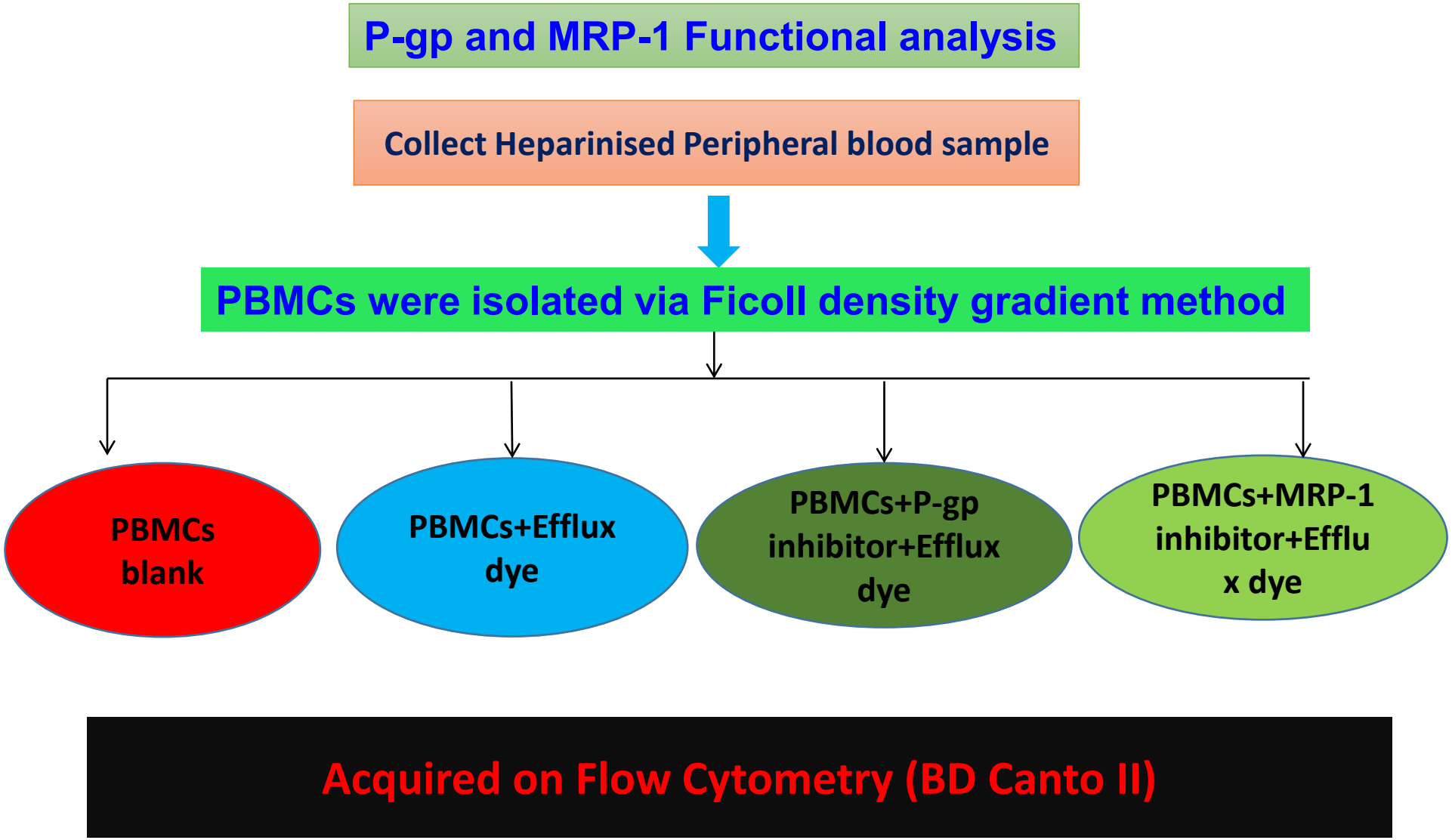
P-Glycoprotein



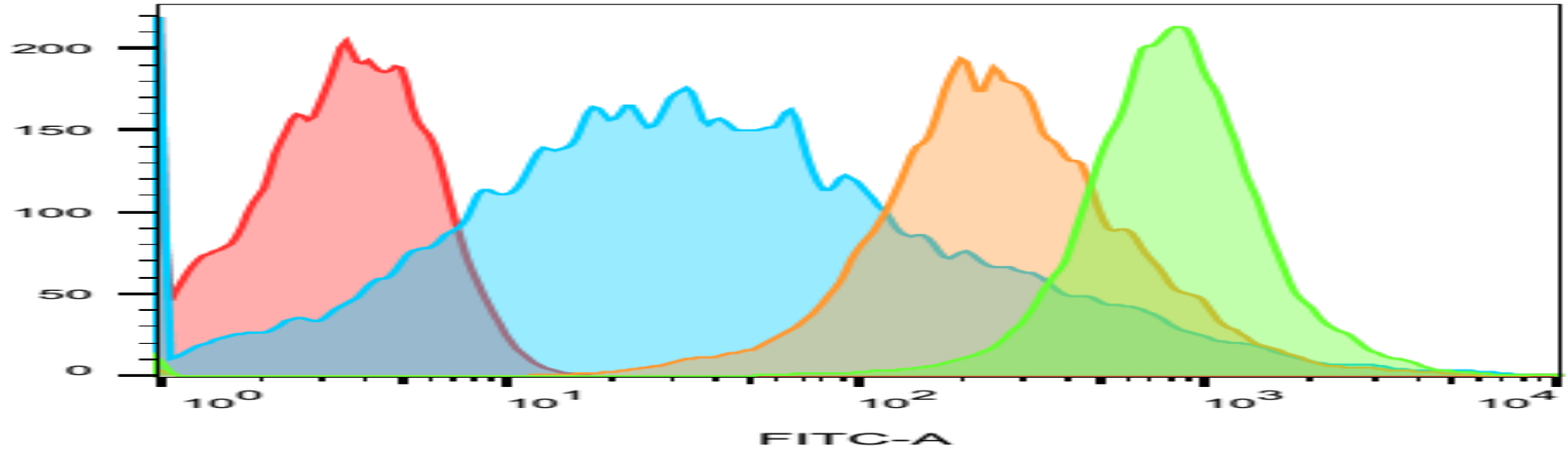
Methods


P-gp and MRP-1 expression analysis
Collect Heparinised Peripheral blood sample
P-gp and MRP-1 expression analysis on T cells
CD4 (FITC)
CD8 (APC)
CD4+P-gp(PE)
CD4+MRP-1(PE)
CD8+P-gp(PE)
CD8+MRP-1(PE)
Acquired on Flow Cytometry (BD Canto II)

Material and methods



Flowcytometric overlay histogram for functional analysis.



Sample Name	Subset Name	Count	Mean : FITC-A
functional_MRP + I.fcs	Lymphocytes	11350	951
functional_PGP + I.fcs	Lymphocytes	10681	391
functional_DYE 37.fcs	Lymphocytes	11259	129
functional_UNSTAINED.fcs	Lymphocytes	10320	3.38

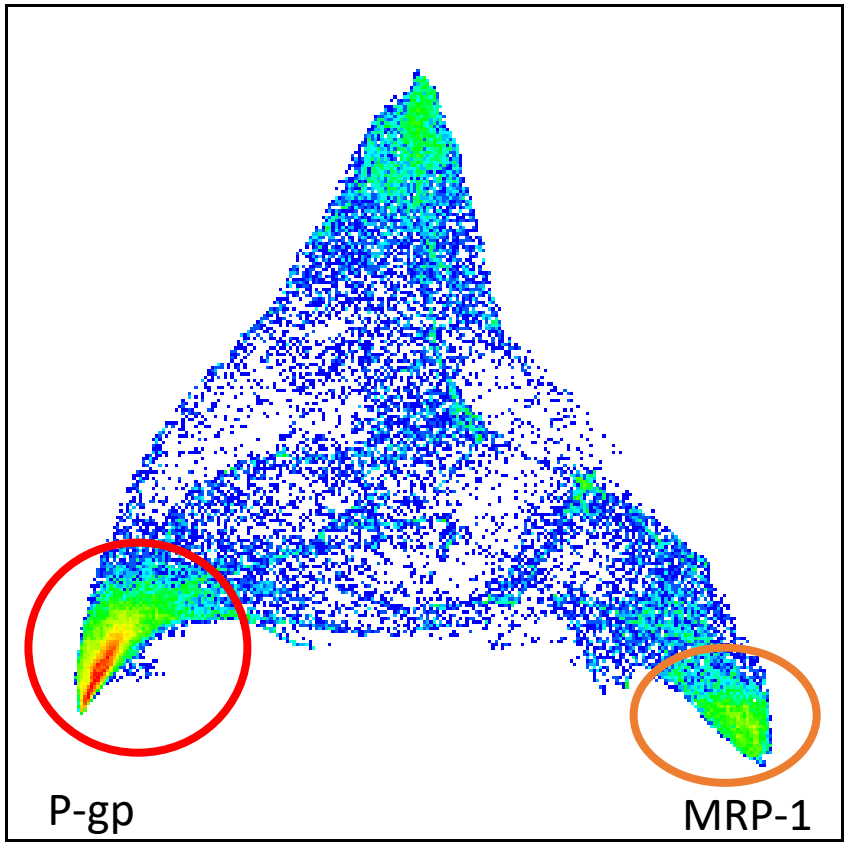
Multi resistance activity factor (MAF) for each transporter, was calculated using formula (MAFMDR1=100× (FMDR1-F0)/FMDR1)

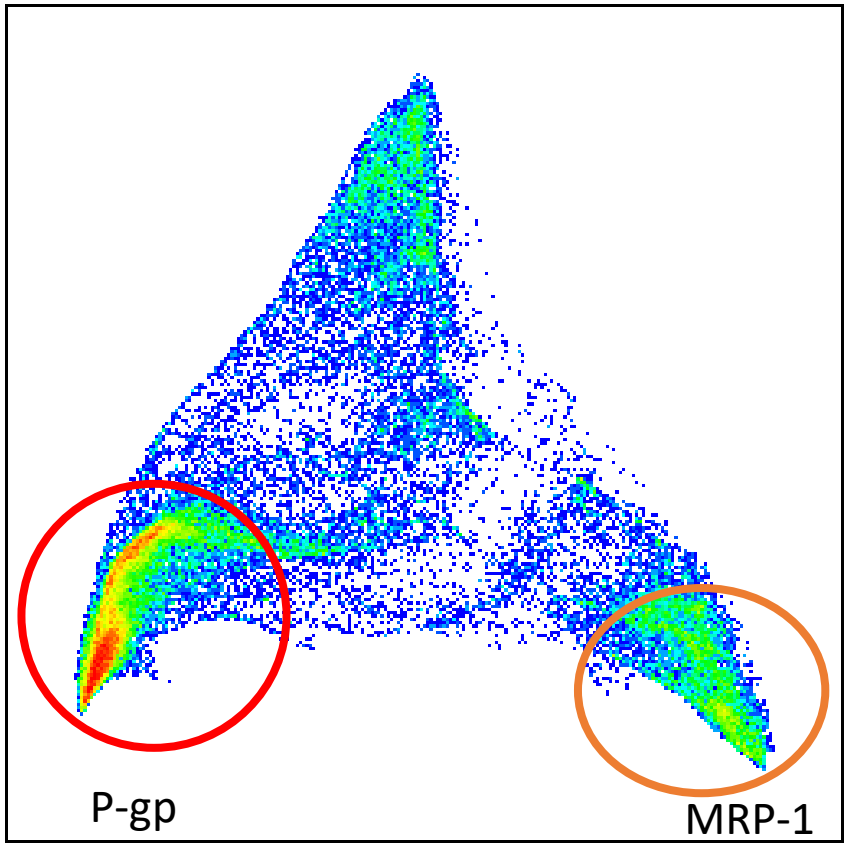
Results

Demographic and Biochemical Parameters

Demographic Profile	Remission(N=171)	Resistant(N=83)	p value
Age (Years)	5.82±3.5	9.43±4.8	0.267
Height (cm)	109.21±17.12	126.13±20.10	0.329
Weight (kg)	26.61±12.41	28.13±6.27	0.512
Biochemical Parameters			
Haemoglobin	10.15±1.43	11.45±2.40	0.296
Systolic	96.12±7.86	100±14.16	0.622
Diastolic	67.96±7.99	67.57±9.12	0.655
TLC	7894.84±2987.80	8124.59±2978.60	0.824
Urine Protein (mg/dl)	**17.39±2.91**	**294.36±99.4**	**<0.001**
Serum BUN	13.40±5.01	12.39±4.67	0.784
Serum Creatinine	0.71±.29	0.72±0.17	0.864
Total Chloestrol(TC) (mg/dl)	**287.54±168.16**	**583.63±167.21**	**<0.001**
TriGlyceride (TG) (mg/dl)	**247.77±147.96**	**597.77±324.91**	**<0.001**
Albumin	**2.67±.88**	**1.97±.89**	**0.013**

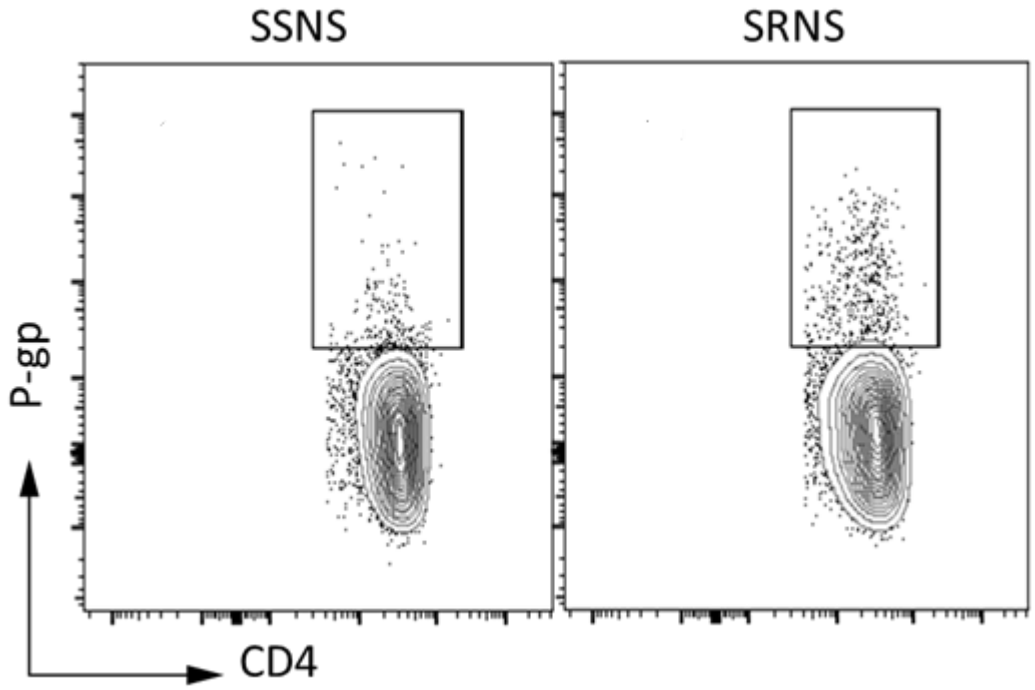
Results

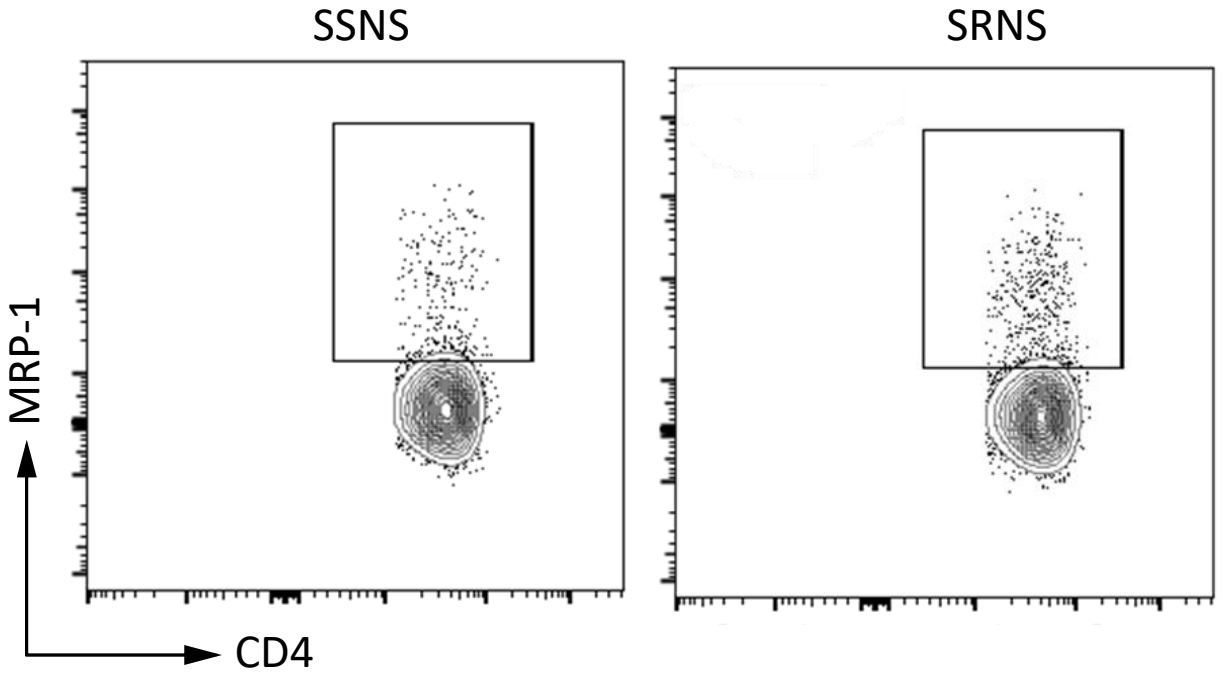




A tSNE representation of the expression of P-gp and MRP-1 on CD3 positive T cells, the plots were generated using flo-jo software, BD Biosciences

CD 4 Pg-p and MRP-1 gating Strategy





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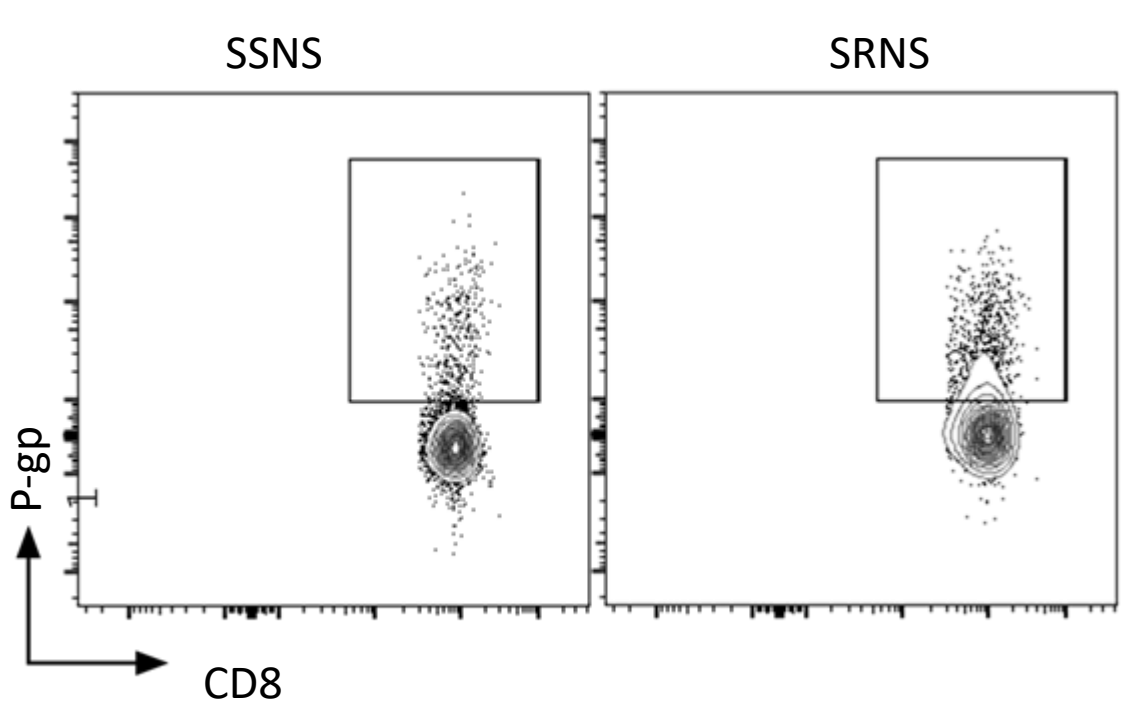
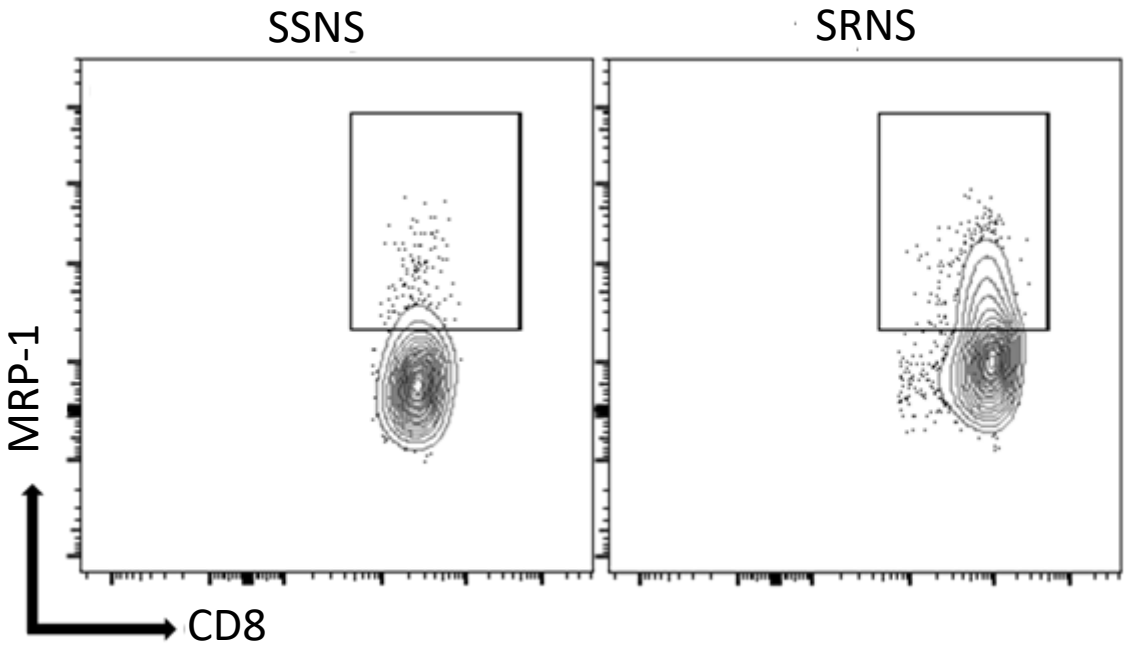
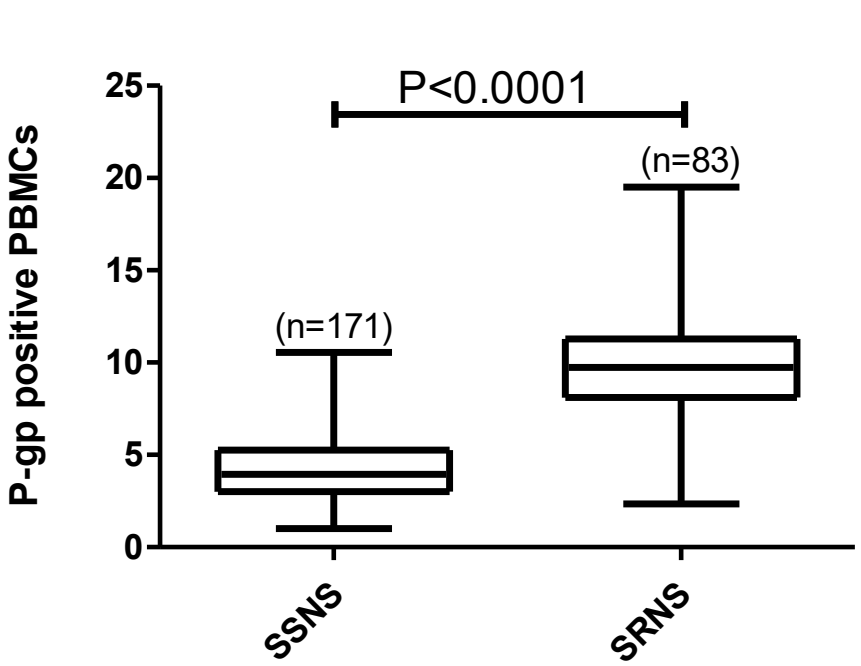
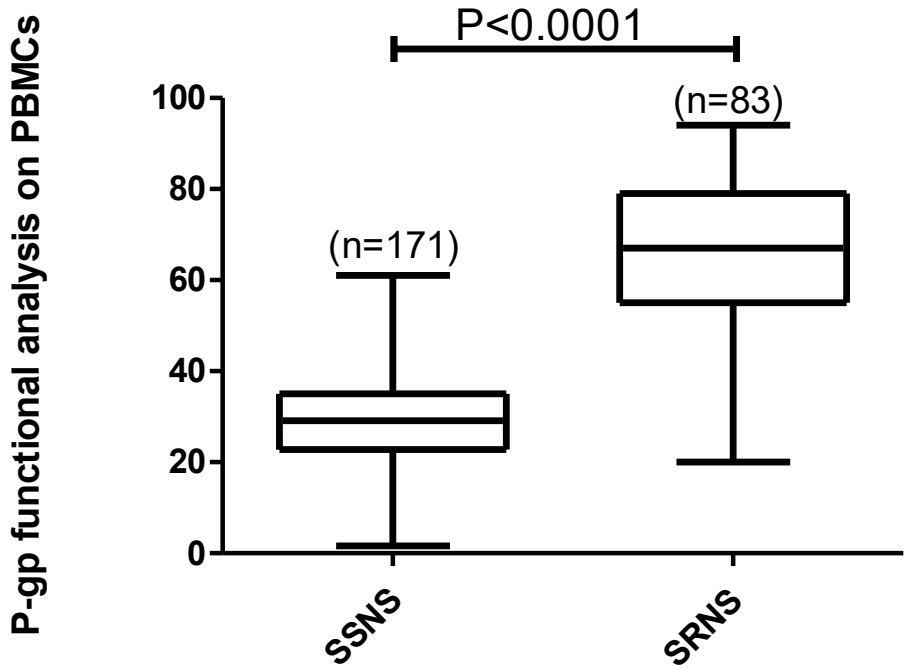




Results

P-gp expression and functionality

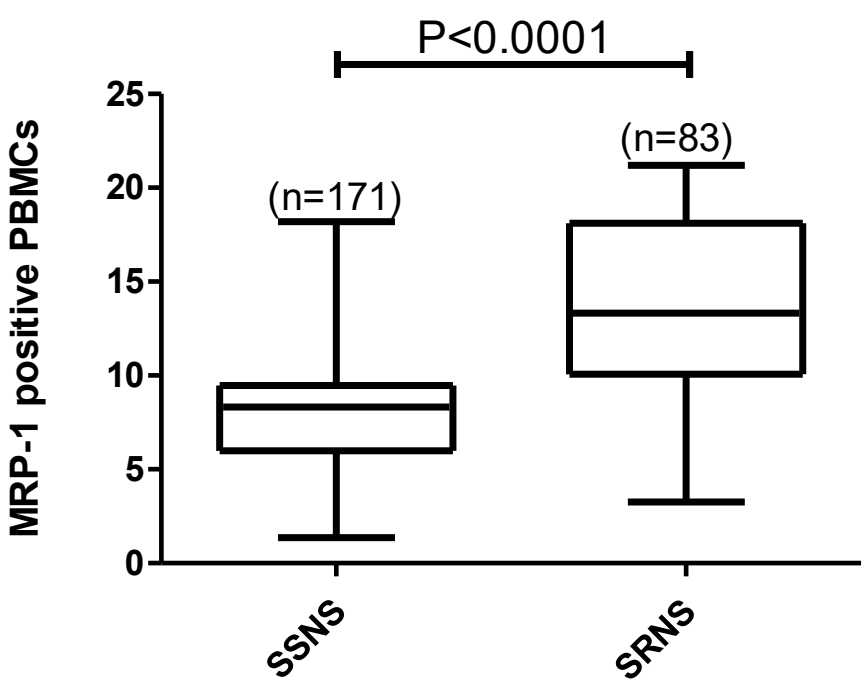


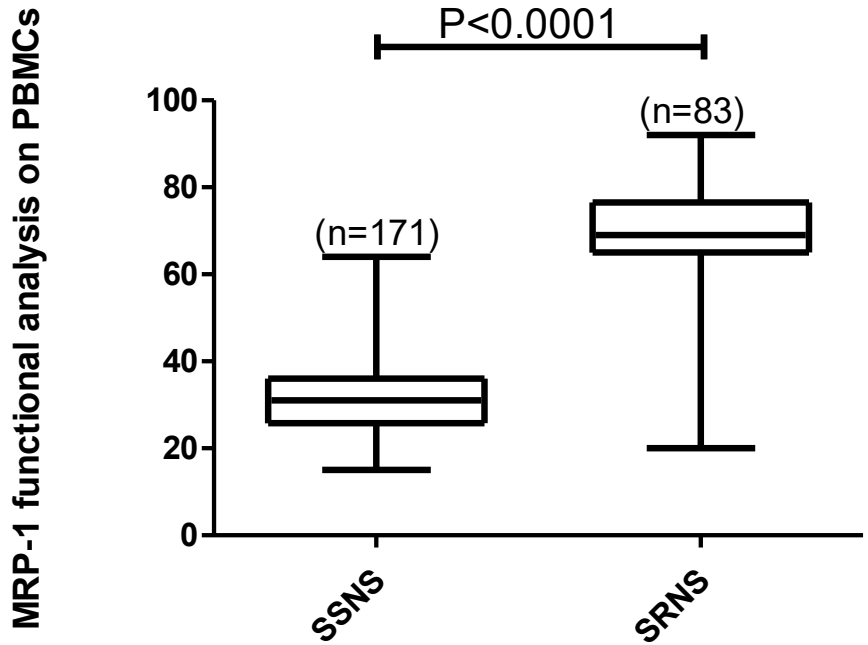


Harshit et al, The Pharmacogenomics journal.

Results

MRP-1 expression and functionality





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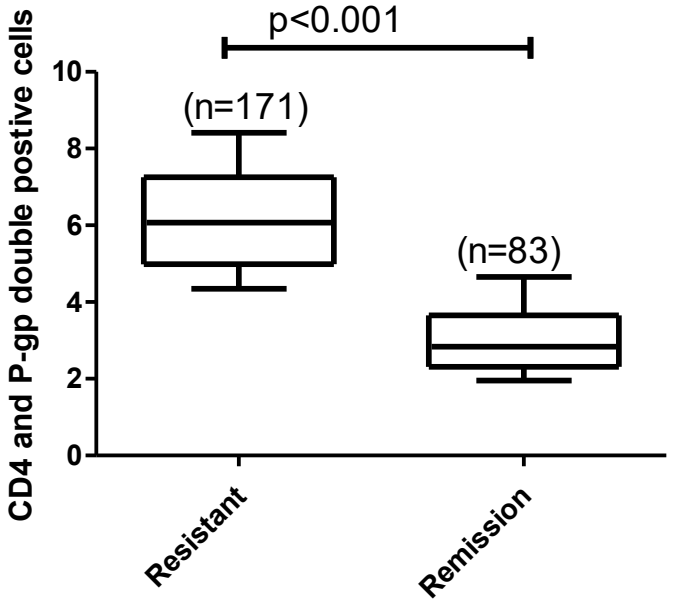
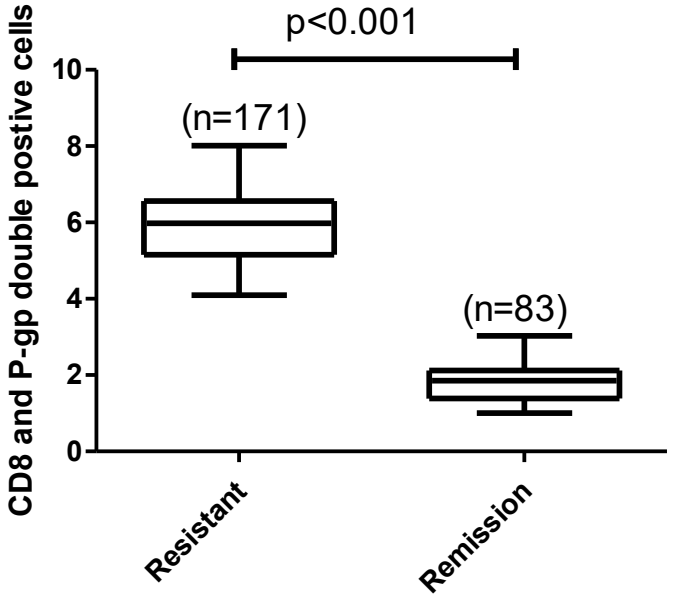
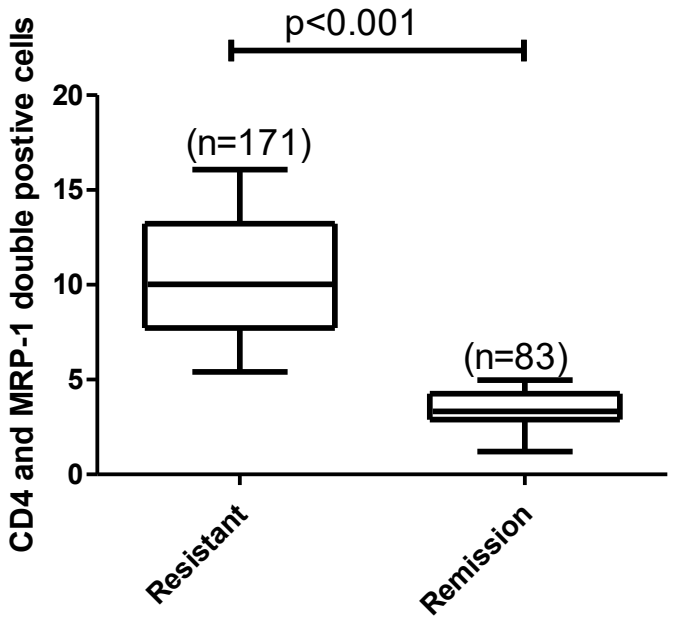
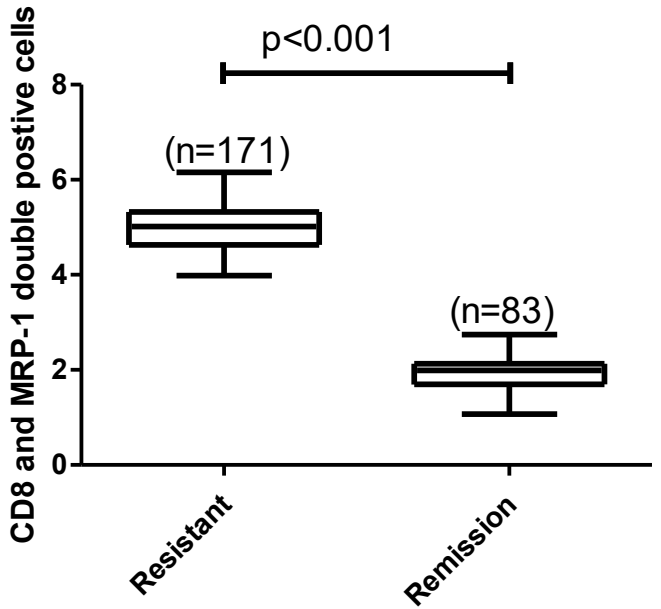
Results


p<0.001
(n=171)
(n=83)
CD4 and P-gp double postive cells
0
2
4
6
8
10
Resistant
Remission


p<0.001
(n=171)
(n=83)
CD8 and P-gp double postive cells
0
2
4
6
8
10
Resistant
Remission


p<0.001
(n=171)
(n=83)
CD4 and MRP-1 double postive cells
0
5
10
15
20
Resistant
Remission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CD8 and MRP-1 double postive cells
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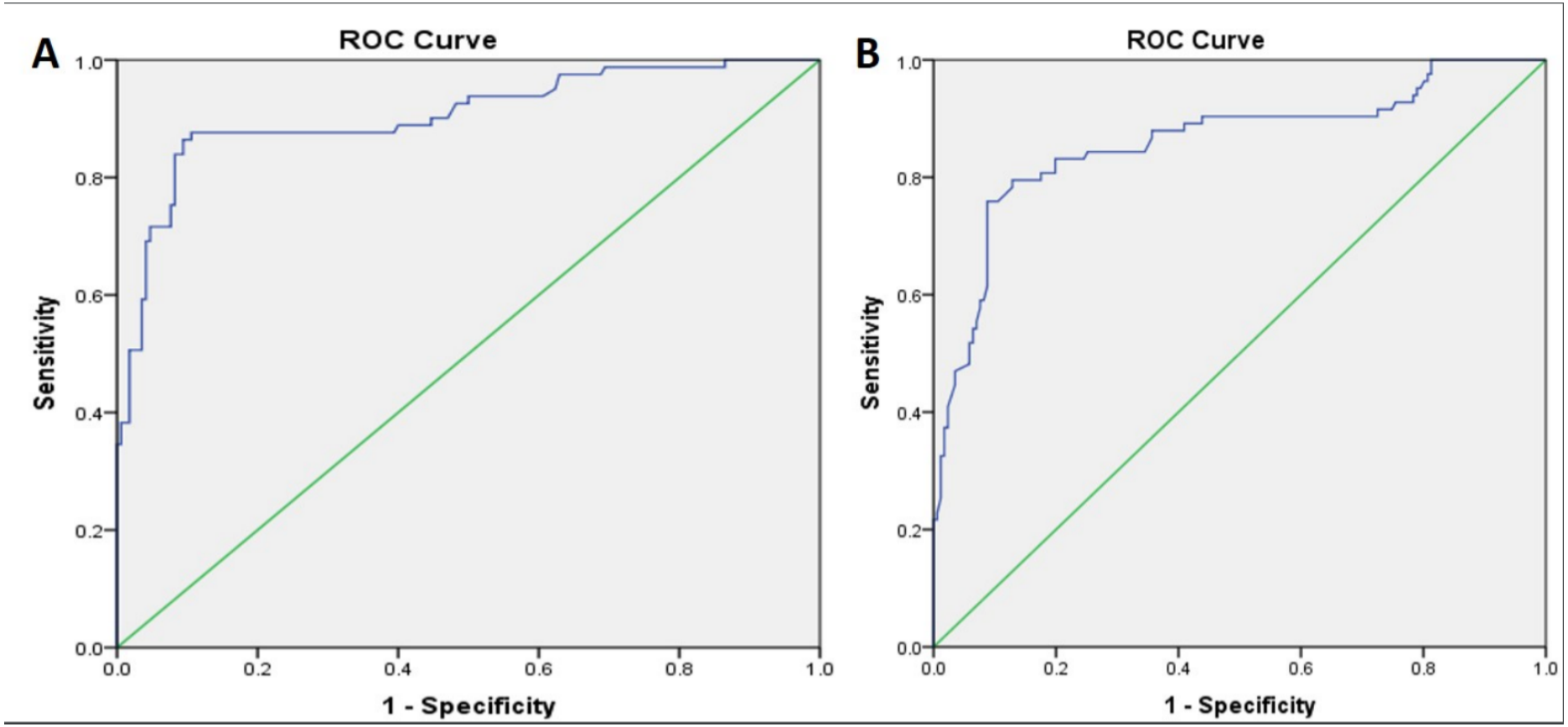

Area Under -Receiver Operating Characteristic (AUC-ROC) curve analysis:
AUC-ROC curve showed P-gp expression on PBMC with a cutoff value of 7.13% predicted steroid resistance with a sensitivity of 86.4% and specificity 90% (A). Similarly, the MRP-1 percentage of 9.62% predicted steroid resistance with a sensitivity of 80.7% and specificity of 80% (B).

Harshit et al, The Pharmacogenomics journal,December 2020, in press

Conclusion

- P-gp and MRP-1 was over expressed on immune cells in steroid resistant nephrotic syndrome patients.
- Functionality of P-gp as well as MRP-1 was increased in steroid resistant nephrotic syndrome patients.
- P-gp and MRP-1 might serve as a potential biomarkers for steroid resistance in nephrotic Syndrome patients.
- P-gp and MRP-1 might be a potential interventional targets for steroid resistance in nephrotic Syndrome patients



Thank you for your patience



Prof. Narayan Prasad Lab