

Differential alteration in peripheral T-regulatory and T-effector cells with change in P-glycoprotein expression in Childhood Nephrotic Syndrome: A longitudinal Study

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Introduction

Minimal Change Disease (MCD) is one of the most common glomerular disease causing idiopathic Nephrotic Syndrome (INS) in children.

Idiopathic Nephrotic Syndrome responds to glucocorticoid therapy, however 60-80% of patients relapse.

Nasi M et al. Pediatric Kidney Disease 1992

MCD may be associated with T-cell disorder,

Shalhoub RJ, Lancet 1974;2:556-603

increase in inflammatory cytokines levels,

Giuliana L et al. Am J of Kid Diseases 2002; 39: 958-65

podocyte injury with effacement of foot process of podocytes and/or

Barisoni L et al. Clin J Am Soc Nephrol 2007;2:529-42

Steroid non responsiveness may be because of disturbances in T cell subset population and/ or,

One of the factors of the acquired steroid resistance that could be modulate the disease response to pharmacological interventions, such as P-gp expression on immune cells and podocytes, suggest that P-gp may influence cell mediated immune response.

Klimecki et al Blood 1994;83:2451-245

Patients of NS carrying homozygous mutants of single nucleotide polymorphism (SNP) G2677T/A are prone to develop steroid resistance.

Narayan Prasad et al NDT 2011;26:3968-74

P-gp appears to be a double edged sword which protects the cells against the accumulation of xenobiotics and toxins in the cytoplasm

Simultaneously by limiting cytosolic concentration, P-gp is capable of limiting the ability of steroids to bind to their receptors resulting in corticosteroid resistance.

Arthritis Rheum 2005; 52:1676-1683

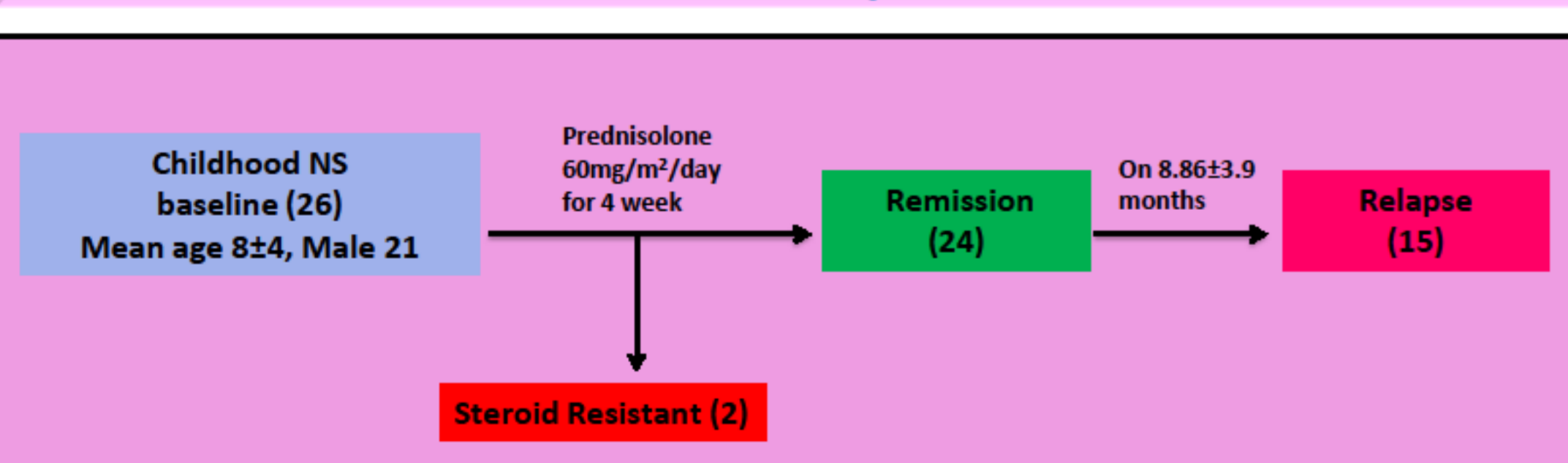
Objectives

To study the frequency of peripheral Treg, Th1 and Th2 lymphocytes and their respective cytokine level at baseline, when achieved remission and during relapse..

To analyze the expression of P-gp and correlate with cytokine levels at baseline, when achieved remission and during relapse.

Materials and Methods

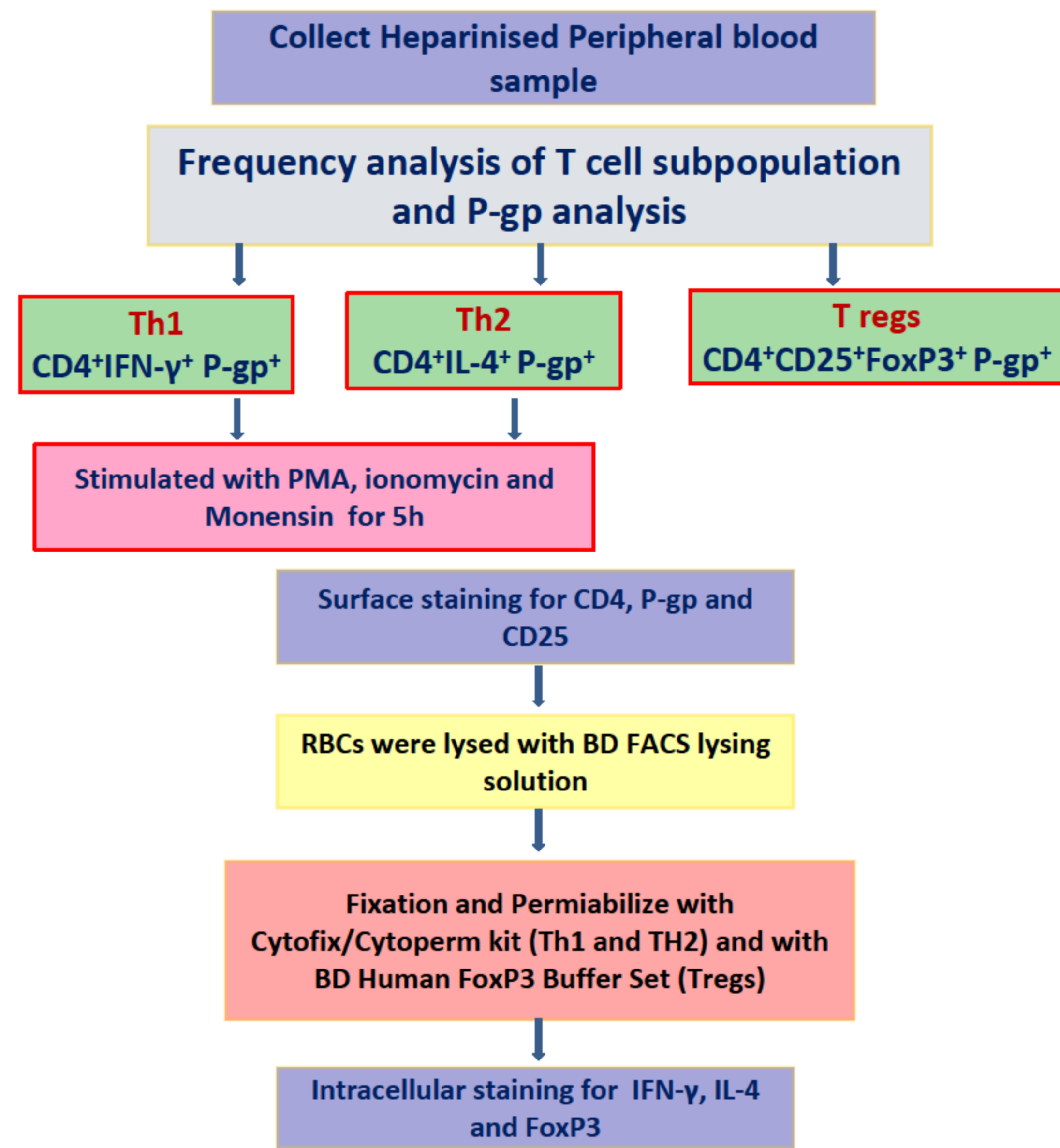
Flowchart of the Study



All patients were recruited as per criteria of ISKDC
Prednisolone dose- 60 mg/m²/d prednisone for 6 weeks followed by 40 mg/m²/ alternate day
Remission- protein excretion <4 mg/m²/h or urine dipstix nil/trace for three consecutive days.
Relapse- protein excretion >40 mg/m²/h or urine dipstix ++ or more for three consecutive days
Steroid resistance- Non responsiveness to 60 mg/m²/d prednisone for 4 weeks
Patients did not have underlying secondary causes
 • negative for hepatitis B surface antigen, anti hepatitis C virus, human immunodeficiency virus seropositivity
 • normal serum complement (C3 and C4) levels

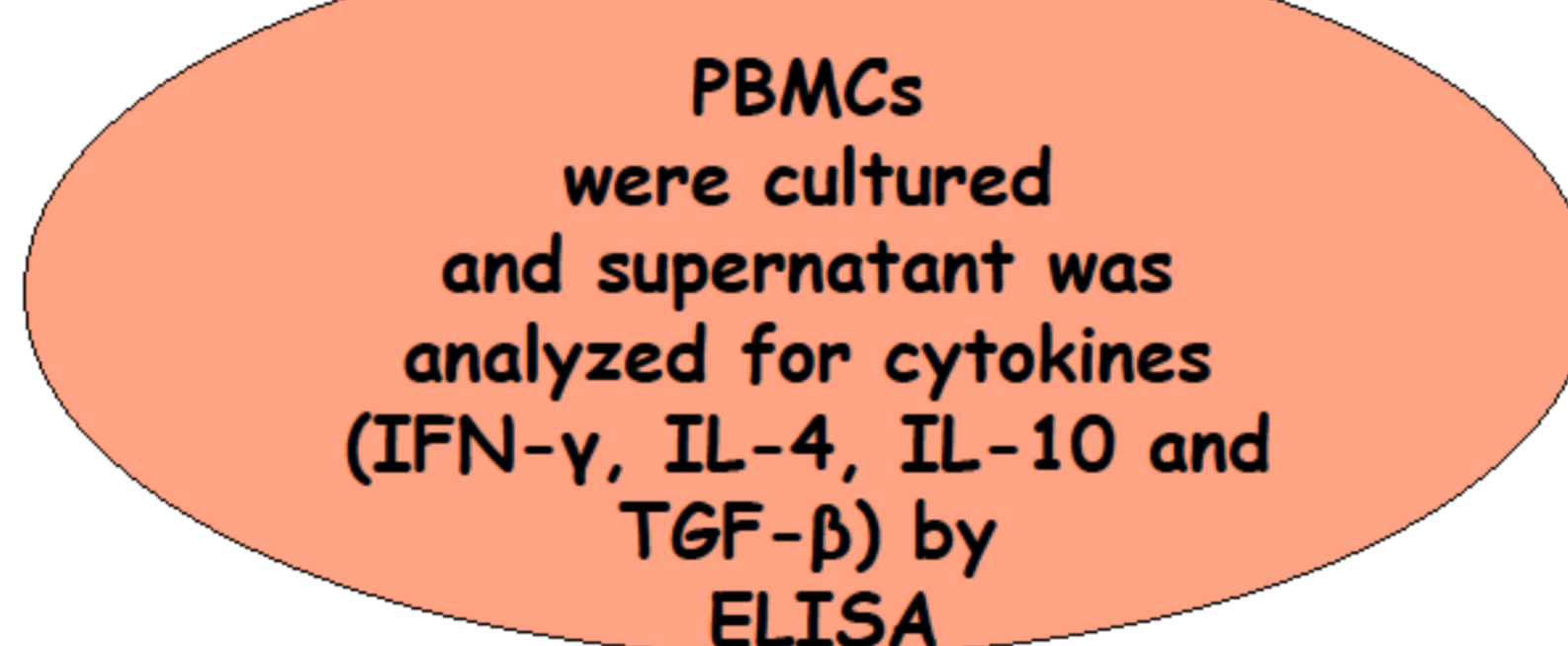
Materials and Methods

T cell phenotype and P-gp expression analysis



Acquired on Flow Cytometry (BD FACS Calibur)

Analysis of In vitro production of cytokines



Results

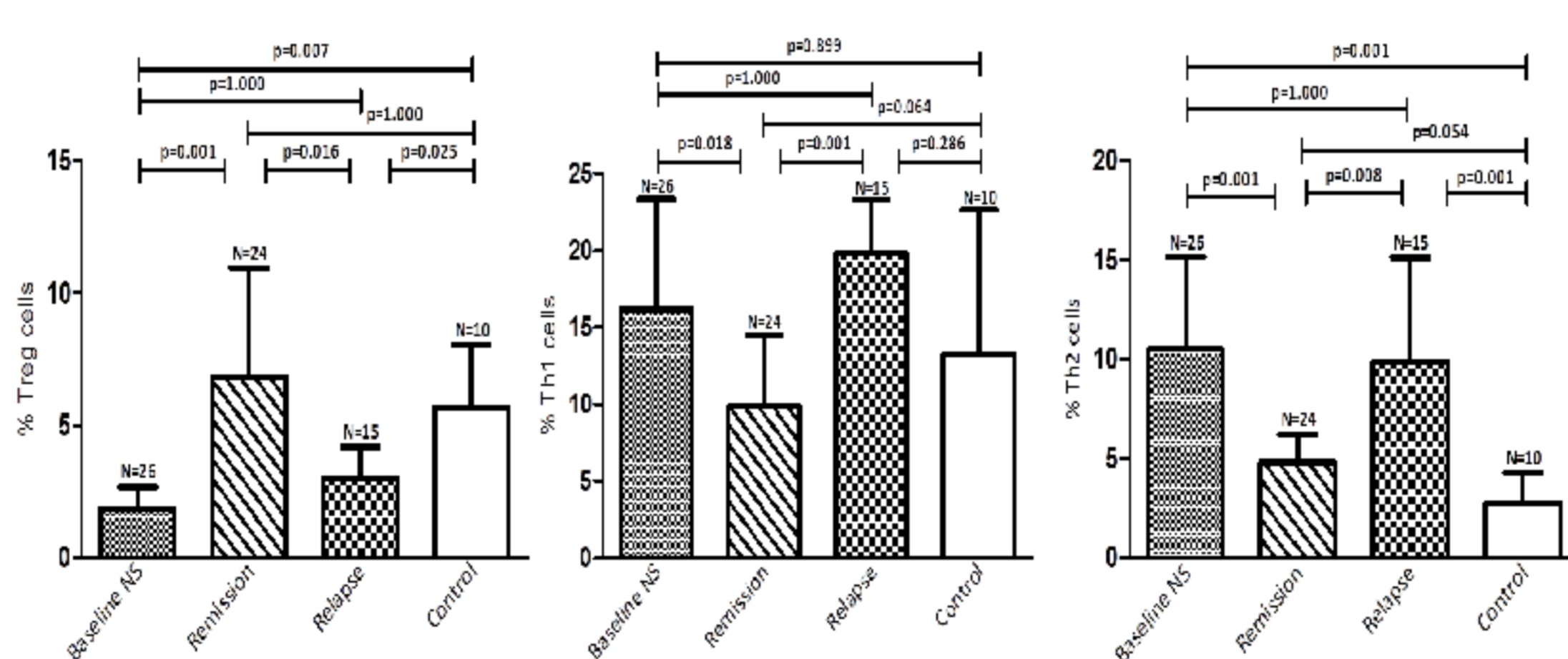


Figure1| Results are expressed as the percentage of CD4⁺CD25⁺FoxP3⁺ Treg, CD4⁺IFN-γ⁺ Th1 and CD4⁺IL-4⁺ Th2 cells in CD4⁺ lymphocyte in blood.

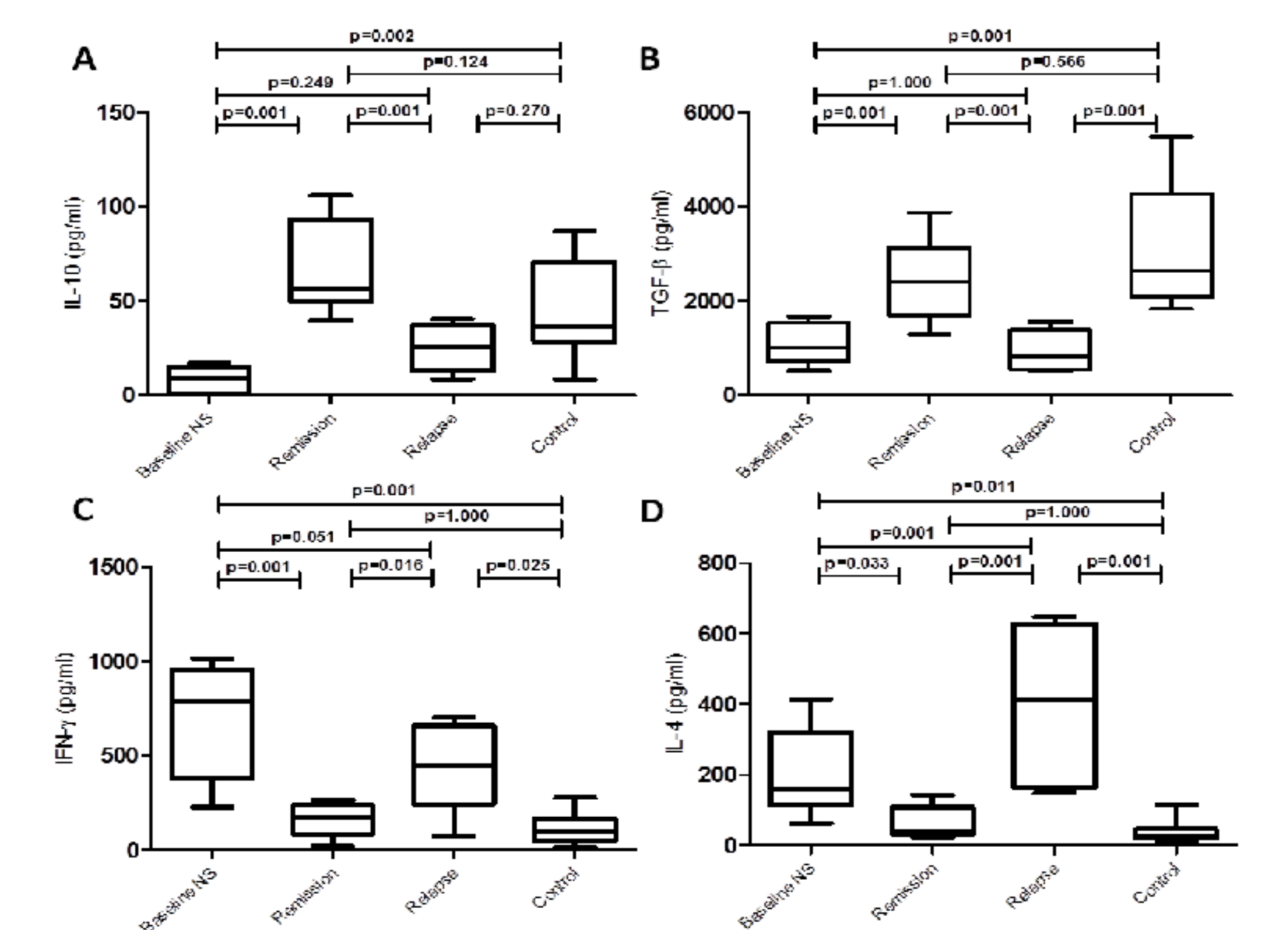


Figure2| Cytokine levels in PBMC culture supernatants during follow up.

Characteristics	Baseline	Remission	Relapse	Control
Pgp % positive cells	8.69±3.62	5.46±3.14	10.02±5.2	4.89±1.73
RFI	8.22±1.82	6.72±1.88	8.65±2.96	7.13±5.26
RFI ×% positive cells	66.59±21.13	35.84±22.26	80.22±28.24	30.41±16.15

Table | P-glycoprotein, RFI and absolute P-gp expression on lymphocytes in different group of patients.

Results

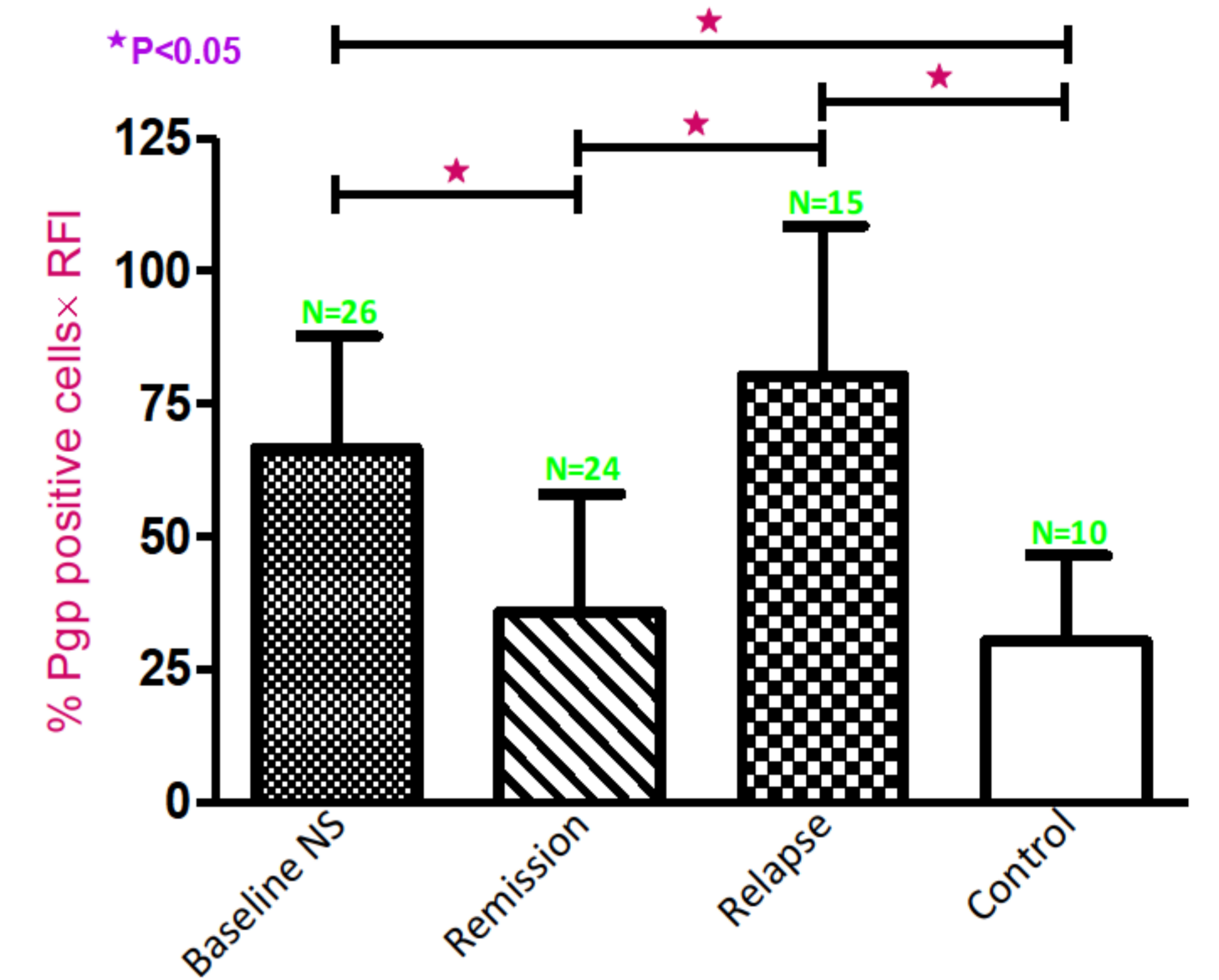


Figure3| Absolute P-gp expressions at baseline, during remission, relapse in INS patients and control.

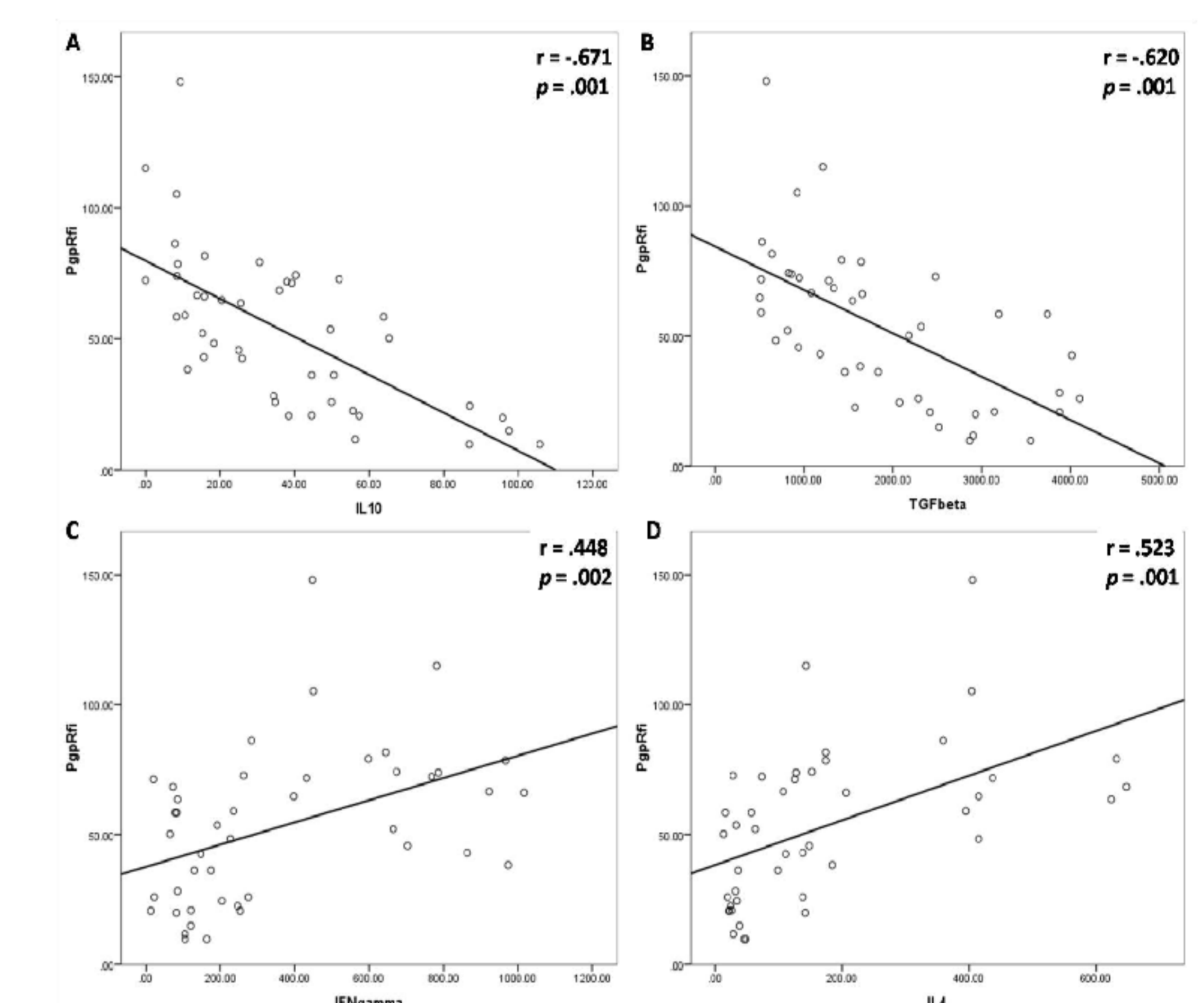


Figure 4| Correlation of absolute P-gp expression on peripheral blood lymphocytes with cytokines IL-10, TGF-β, IFN-γ and IL-4.

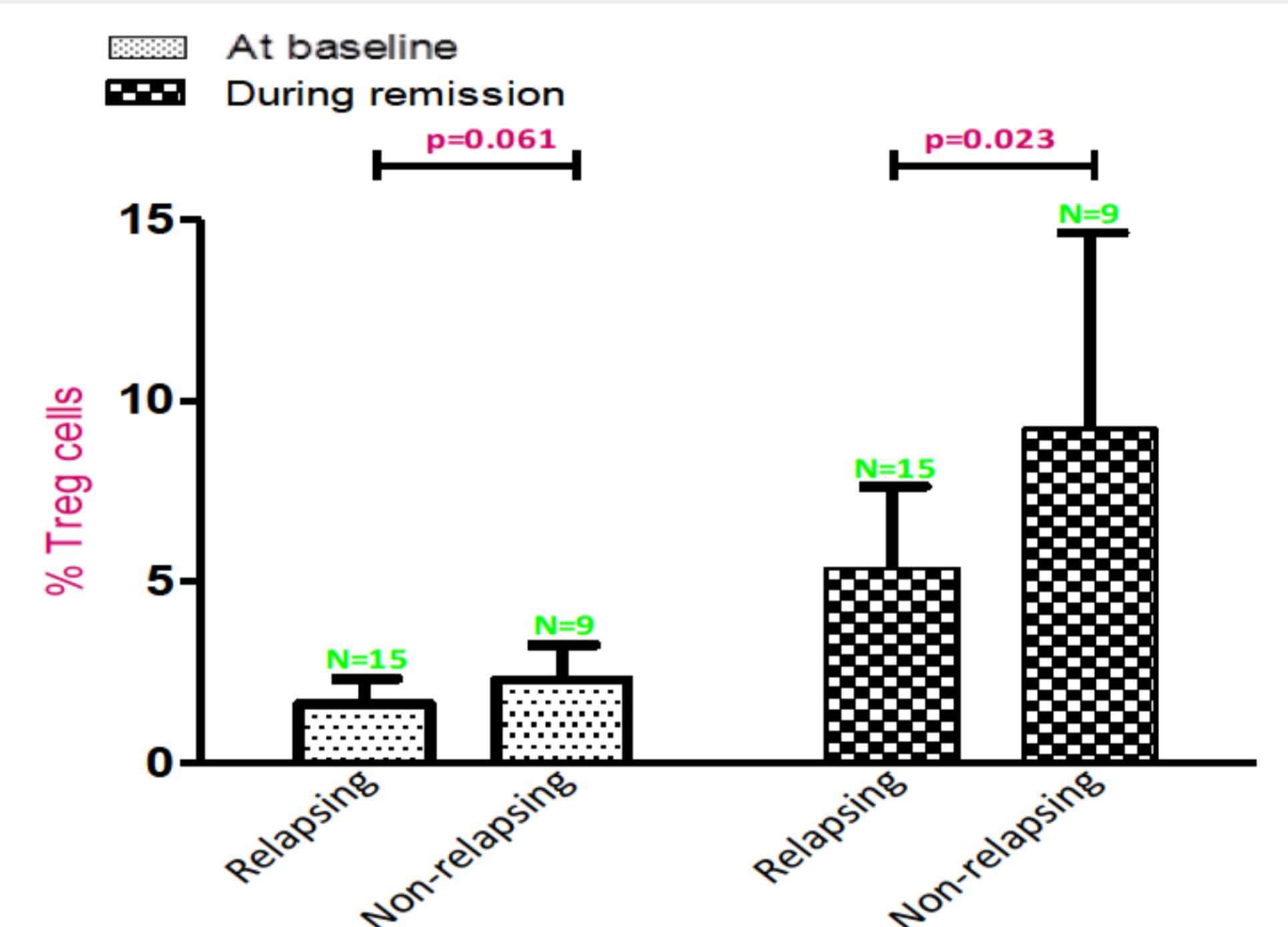


Figure5| Tregs frequency at baseline shows a trend towards significant and a significant difference at remission between relapsing and non-relapsing

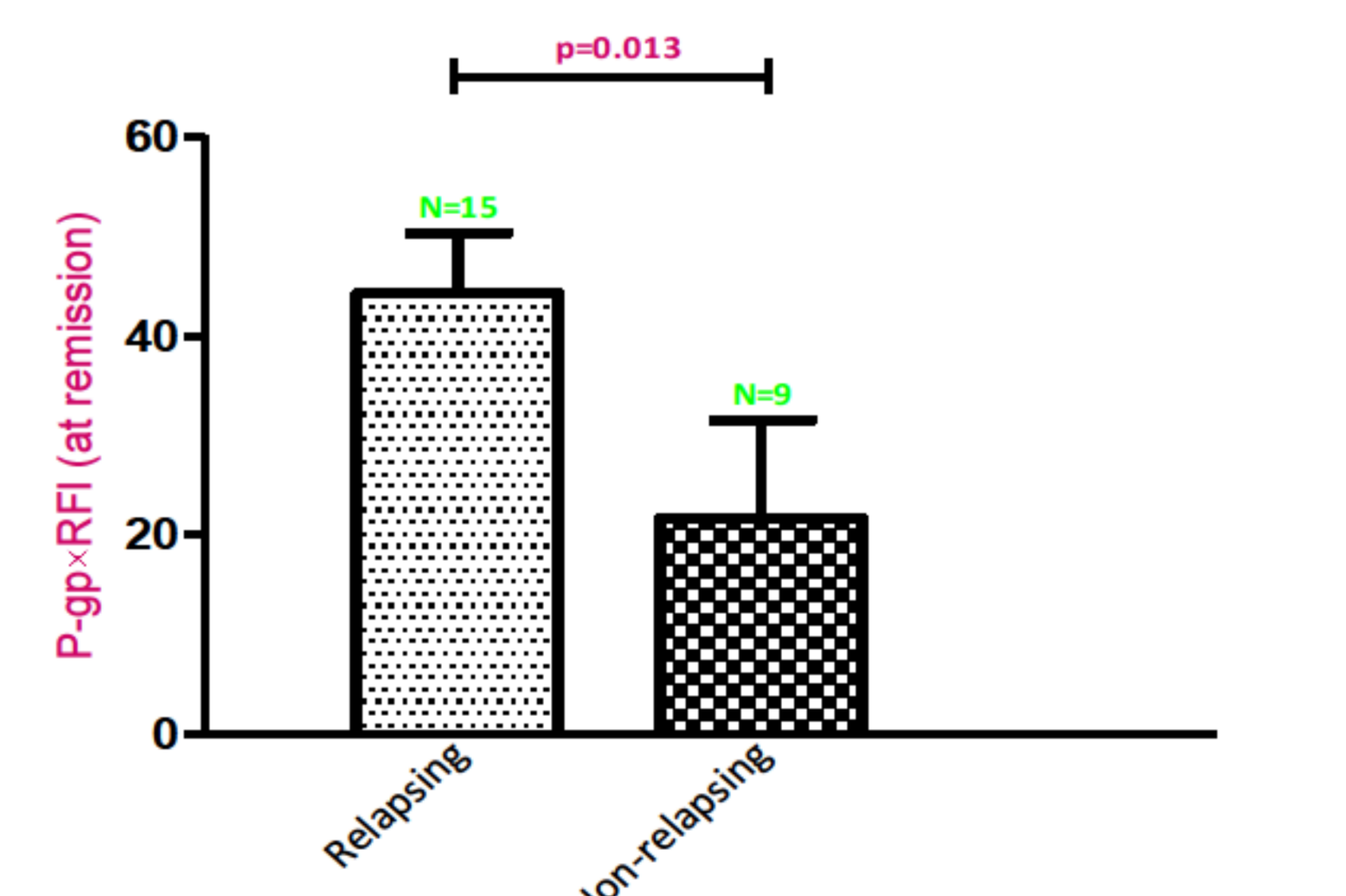


Figure6| Absolute P-gp at remission was significantly higher in relapsing patients.

Conclusions

- ✓ Glucocorticoid therapy in INS induces decreased P-gp expression along with increased frequency and cytokine response of T-regulatory cells.
- ✓ Regulatory cytokines negatively correlates with P-gp expression may have a role in pharmacological interventions in NS patients.
- ✓ Monitoring of P-gp may help in the management of INS patients.

