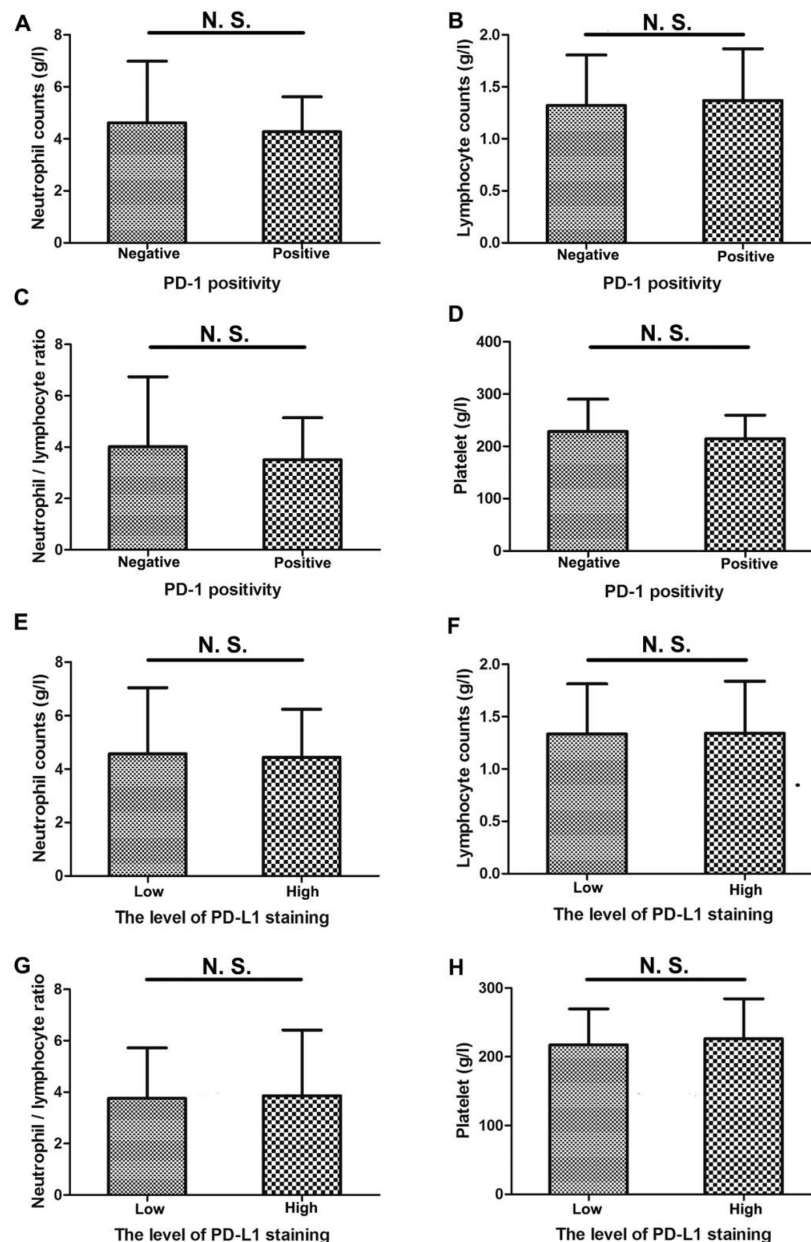


PD-1 and PD-L1 expression in 132 recurrent nasopharyngeal carcinoma: the correlation with anemia and outcomes

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Potential peripheral blood biomarkers in recurrent NPC patients with different PD-1 / PD-L1 level. (A-D) Statistical analysis suggested no significance (N. S.) between PD-1 positivity and pre-retreatment neutrophil counts (A), lymphocyte counts (B), neutrophil / lymphocyte ratio (C) as well as platelet (D) in the 132 patients with recurrent NPC. (E-H) Statistical analysis suggested no significance (N. S.) between PD-L1 level and neutrophil counts (E), lymphocyte counts (F), neutrophil / lymphocyte ratio (G) as well as platelet (H) in this cohort of recurrent patients either. T-test was used to evaluate the association of PD-1 positivity with neutrophil counts and platelet after normality tests and homogeneity of variance test. Nonparametric Mann-Whitney U test was applied to evaluate the association of PD-1 positivity with lymphocyte count and neutrophil / lymphocyte ratio.

Supplementary Table 1: Primary and second antibodies used for immunohistochemistry (IHC) and immunofluorescence (IF) staining

| Antigens | Species antibodies raised in | Methods | Dilution | Suppliers |
|--|-------------------------------------|----------------|-----------------|---|
| PD-1, human | mouse, monoclonal | IHC | 1:100 | Abcam, UK, Cat. #ab52587 |
| PD-L1, human | rabbit, monoclonal | IHC | 1:100 | Cell Signaling Technology, USA, Cat. #13684 |
| PD-L1, human | rabbit, monoclonal | IF | 1:250 | Abcam, UK, Cat. #ab213524 |
| HIF-1α, human | mouse, monoclonal | IF | 1:200 | Abcam, UK, Cat. #ab113642 |

Supplementary Table 2: Second antibodies and counterstaining of nuclei used for immunofluorescence (IF) staining

| Secondary detection system used | Host | Method | Dilution | Supplier |
|--|-------------|---------------|-----------------|--|
| Anti-rabbit IgG (H+L), F(ab') ₂ Fragment (Alexa Fluor® 555 Conjugate) | goat | IF | 1:50 | Cell Signaling Technology, USA, Cat. #4413 |
| Anti-mouse IgG (H+L) F(ab') ₂ Fragment (Alexa Fluor® 488 Conjugate) | goat | IF | 1:50 | Cell Signaling Technology, USA, Cat. #4407 |
| DAPI (nucleic acid staining) | - | IF | 2µg/ml | Sigma, USA, Cat. #D9542 |