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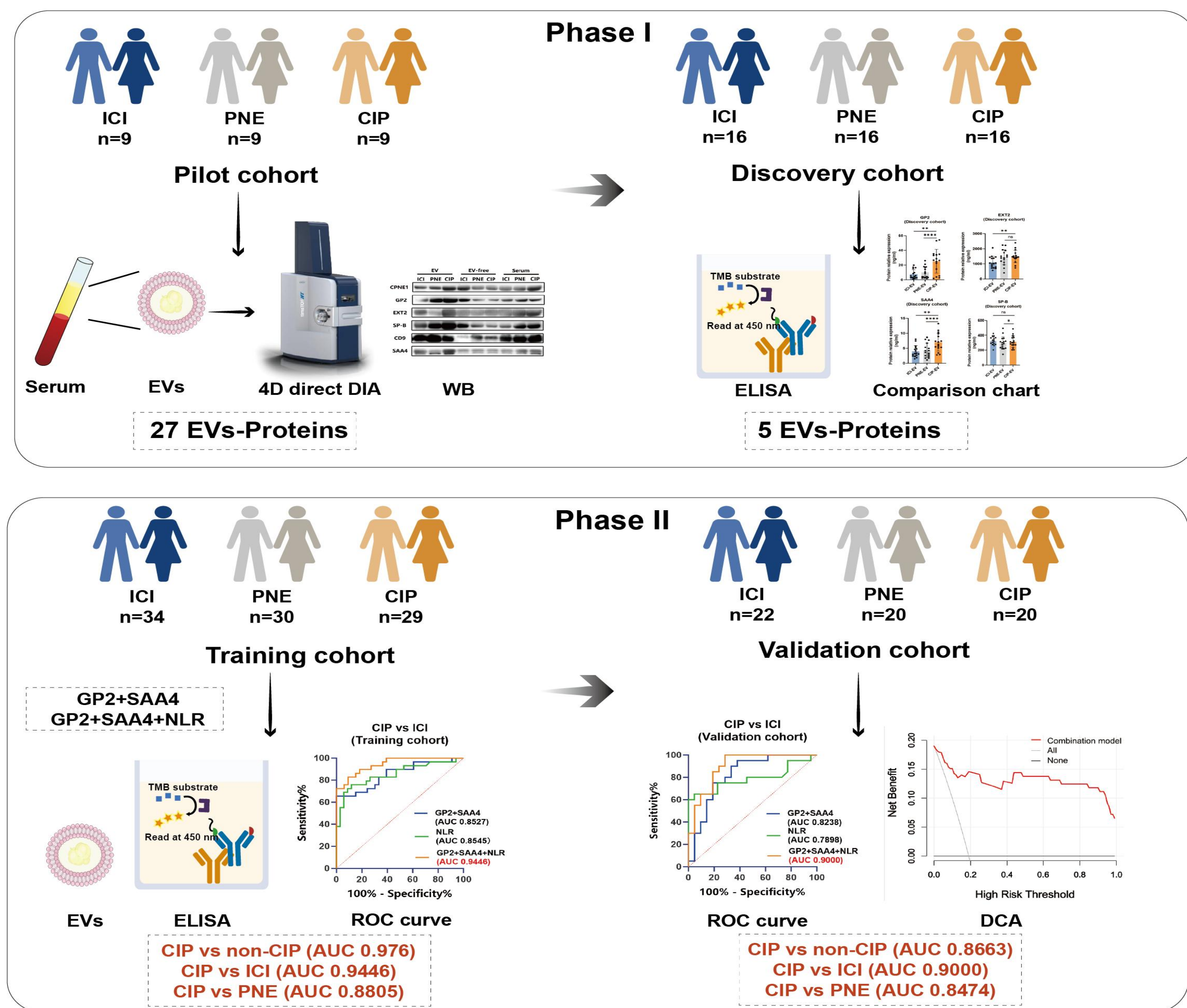
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INTRODUCTION

- Checkpoint inhibitor-related pneumonitis (CIP) is a potentially life-threatening immune-related adverse event (irAE).
- Current non-invasive, clinically translatable approaches for the diagnosis and differential diagnosis of CIP remain limited.
- In this study, we evaluated the diagnostic performance of circulating EV-derived proteins as a liquid biopsy strategy for CIP.

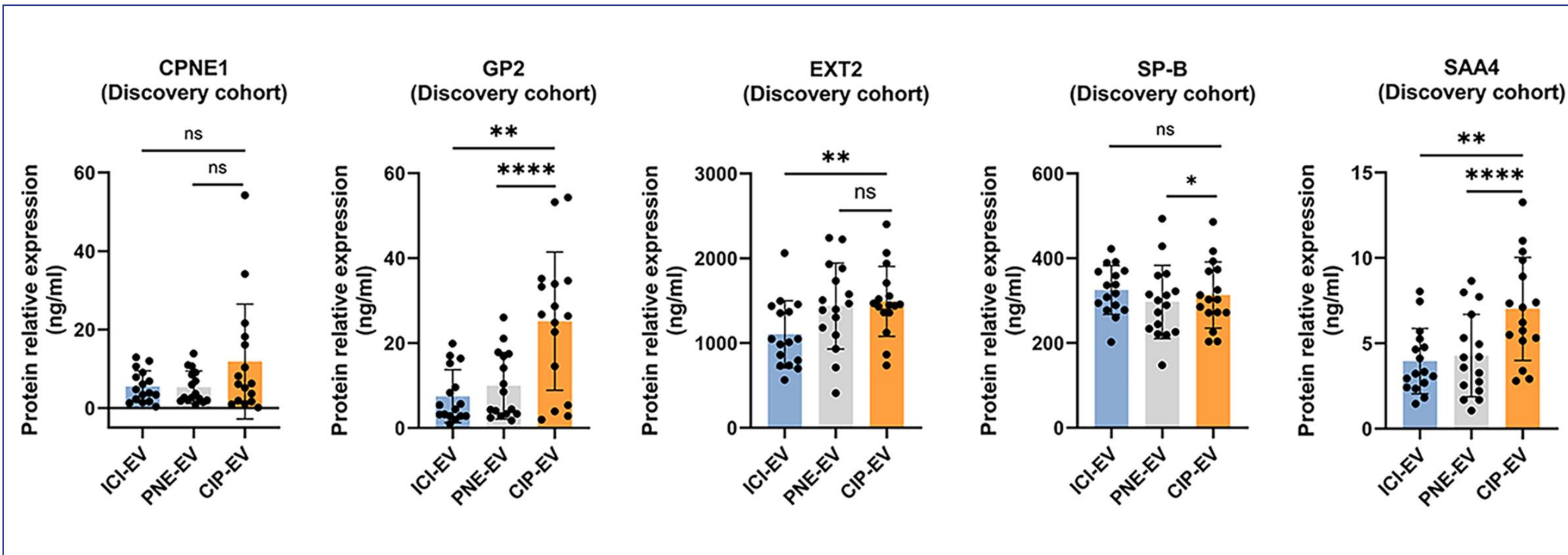
METHODS



A multi-step screening and validation pipeline was employed to identify and refine a diagnostic biomarker panel for CIP.

- (1) Unbiased proteomic screening of serum EVs from a pilot cohort (n=9 per group: ICI, PNE, CIP) using 4D direct DIA mass spectrometry, identifying 27 consistently upregulated proteins in CIP;
- (2) Prioritization and orthogonal validation of the top 7 candidates by western blot (excluding one due to antibody unavailability); ELISA screening of the remaining candidates in a discovery cohort (n=16 per group), which confirmed GP2, EXT2, SAA4, and SP-B as differentially expressed;
- (3) Focused ELISA in a larger training cohort (ICI: n=34, PNE: n=30, CIP: n=29), establishing GP2 and SAA4 as the most robust markers and forming a combined diagnostic model (GP2+SAA4);
- (4) Independent validation of the GP2+SAA4 model in a separate cohort (ICI: n=22, PNE: n=20, CIP: n=20);
- (5) Final integration of the peripheral blood index NLR with the EV protein panel.

Figure 1. GP2 and SAA4 elevated in the CIP group



RESULTS

- Two proteins (GP2 and SAA4) significantly elevated in the CIP group compared to the ICI group and PNE group (**Figure 1**).
- In CIP identification (CIP vs. non-CIP), GP2+SAA4 reached an AUC of 0.8276 in the training cohort and 0.8115 in the validation cohort (**Figure 2A, 2B**).
- In CIP diagnosis (CIP vs. ICI), GP2+SAA4 achieved high diagnostic accuracy in the training cohort (AUC = 0.8527), and was validated in the independent cohort (AUC = 0.8238) (**Figure 2A, 2B**).
- For differentiating CIP from PNE (CIP vs. PNE), GP2+SAA4 yielded an AUC of 0.8149 in the training cohort and 0.8053 in the validation cohort (**Figure 2A, 2B**).
- The efficacy of GP2+SAA4+NLR model was confirmed as the best combination for CIP diagnosis (CIP vs. ICI) with AUCs of 0.9446 (training cohort) and 0.9000 (validation cohort), and effectively distinguished CIP from PNE with AUCs of 0.8805 and 0.8474 (**Figure 3A, 3B**).

CONCLUSION

The EV-based GP2-SAA4 signature, especially when combined with NLR, provides a robust liquid biopsy approach for the early and differential diagnosis of CIP.

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Figure 2. Diagnosis efficacy of the serum EV-proteins in training and validation cohort

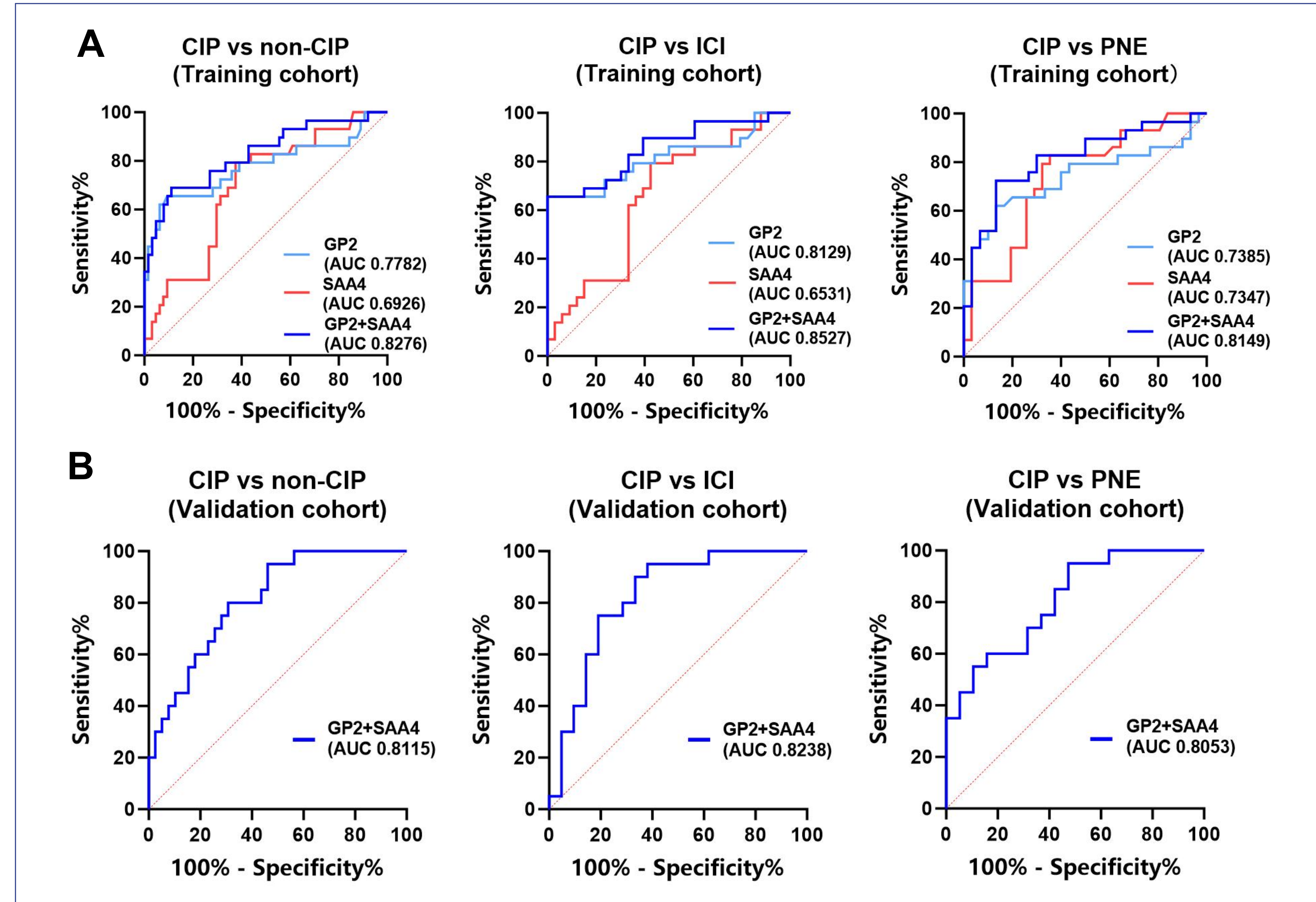


Figure 3. Diagnostic efficacy of the serum EV-proteins combined with routine peripheral blood parameters

