Immune cell subset alterations identified by spectral cytometry in APS patients

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I. Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by the presence of antiphospholipid antibodies (aPL). Its clinical manifestations include venous and arterial thrombosis, recurrent pregnancy loss, and thrombocytopenia.
Two major clinical forms are distinguished:

- **Primary APS (APS-I)**, which occurs in the absence of any underlying autoimmune disease
- **Secondary APS (APS-II)**, which develops in association with another autoimmune condition, most commonly systemic lupus erythematosus (SLE).

In our studies, we also included a third group comprising individuals who test positive for antiphospholipid antibodies but display no clinical symptoms. These cases are considered **asymptomatic seropositive (APS-III)** states.

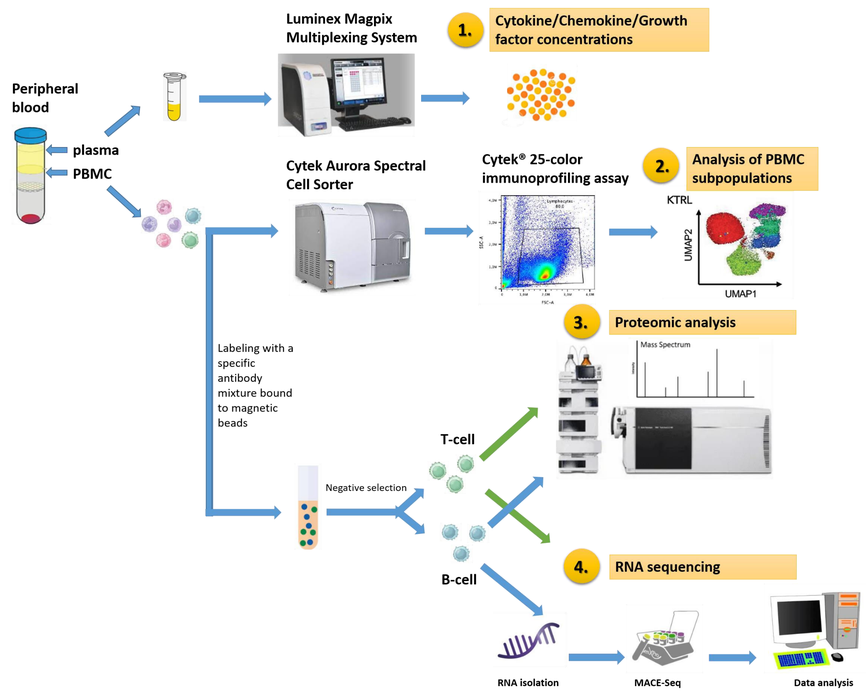
II. Aims

The aim of our study was to identify **cellular immunophenotypic patterns** and **(bio)markers** that may better characterize APS clinical outcomes and therapeutic responses. Understanding such immune signatures could facilitate early **disease recognition** and the identification of **prognostic factors**.

III. Materials and Methods

We analyzed four study groups:

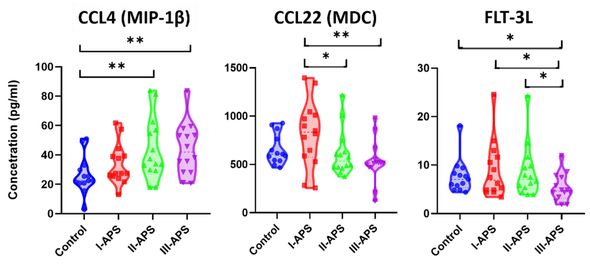
- **APS-I**, **APS-II**, and **APS-III** patient groups, and an age- and sex-matched **healthy control** group.



IV. Results

1. Soluble Marker Analysis

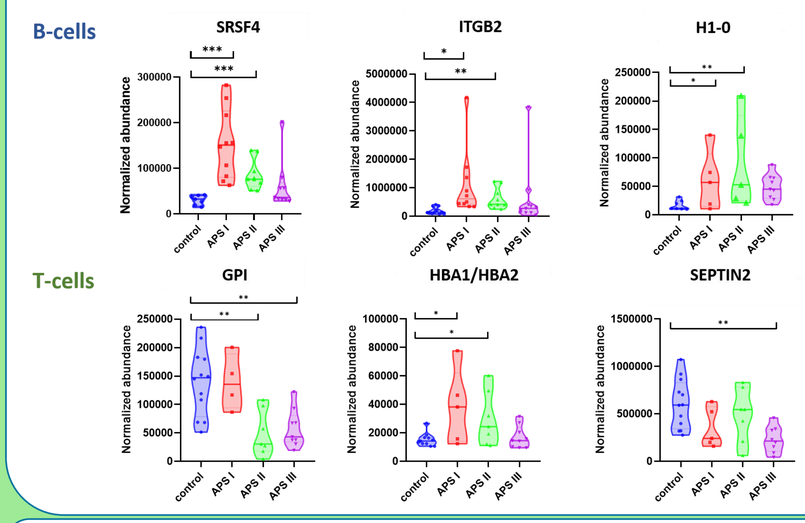
Three soluble proteins – **CCL4 (MIP-1β)**, **CCL22 (MDC)**, and **FLT-3L** – showed significant alterations in APS patients compared with healthy controls. Although none of these proteins have previously been associated with APS, their biological roles suggest potential relevance to disease pathogenesis.



CCL4 is a proinflammatory chemokine involved in vascular inflammation and immune cell migration
CCL22 acts as a Treg-recruiting chemokine, capable of modulating autoimmune responses, but its overexpression can exacerbate vascular inflammation and fibrosis.
FLT-3L regulates dendritic cell and Treg proliferation, influencing immune homeostasis and autoimmune activity.

3. Proteomic analysis

Proteomic analysis identified **29 significantly differentially expressed proteins** across study groups.



Several proteins were characteristic of specific APS subgroups, whereas others were distinctive of the asymptomatic seropositive group, implying that measurable immune alterations might precede or predict the onset of clinical disease.

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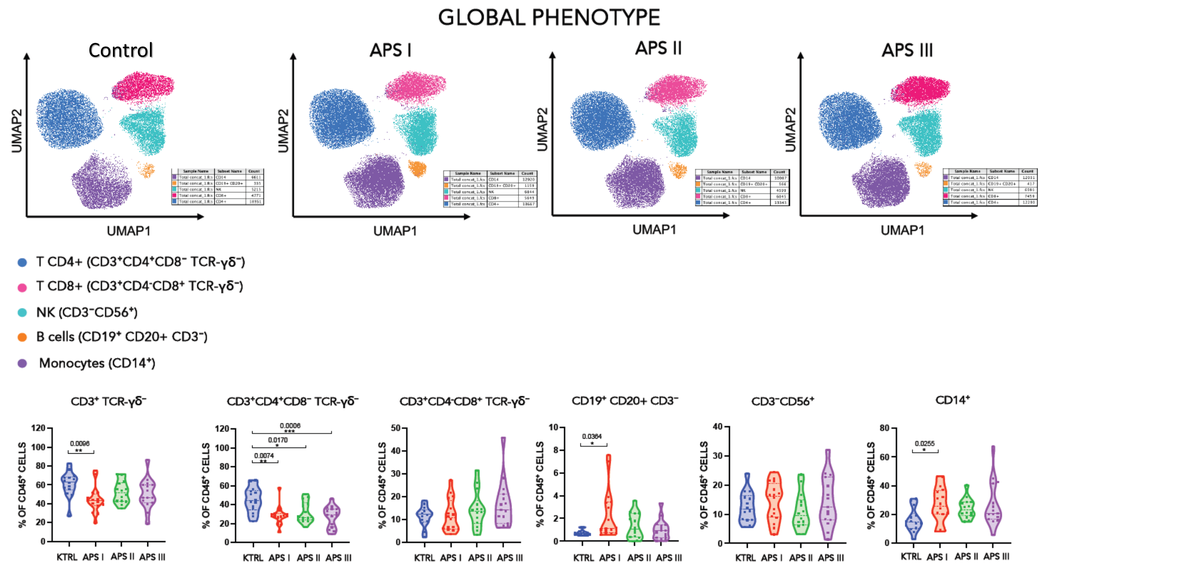
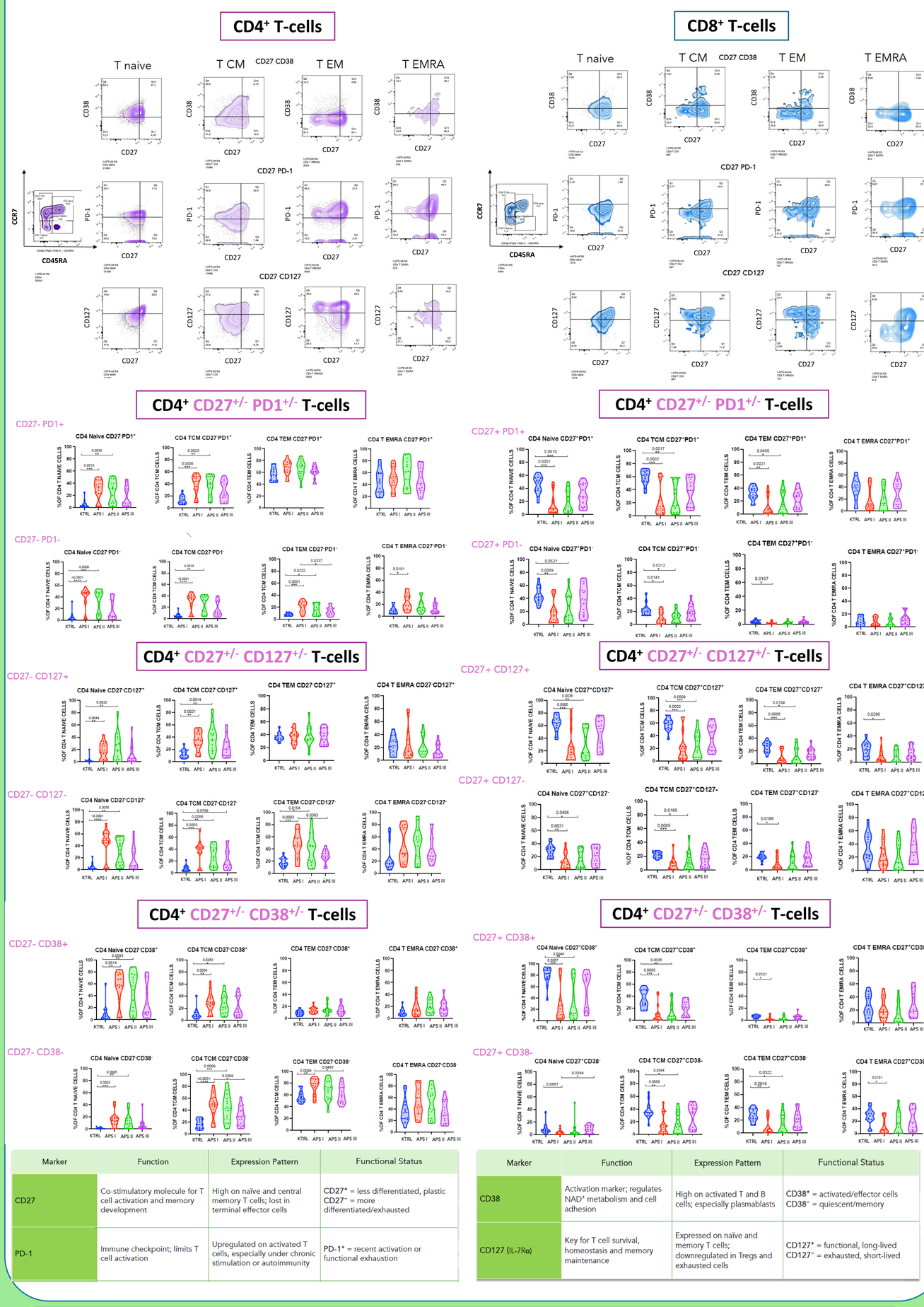
2. Flow Cytometry and Cellular Immune Profiling

Spectral flow cytometry enables quantitative assessment of surface receptor expression and high-dimensional mapping of immune cell subpopulations within PBMCs. Using **25 fluorescently labeled surface markers**, we identified major immune cell types and subpopulations.



Manual gating of CD4+ and CD8+ T-cell markers



Marker	Function	Expression Pattern	Functional Status
CD27	Co-stimulatory molecule for T cell activation and memory development	High on naïve and central memory T cells; lost in terminal effector cells	CD27⁺ = less differentiated, plastic CD27⁻ = more differentiated/exhausted
PD-1	Immune checkpoint; limits T cell activation	Upregulated on activated T cells, especially under chronic stimulation or autoimmunity	PD-1⁺ = recent activation or functional exhaustion

Marker	Function	Expression Pattern	Functional Status
CD38	Activation marker; regulates NAD⁺ metabolism and cell adhesion	High on activated T and B cells; especially plasmablasts	CD38⁺ = activated/effector cells CD38⁻ = quiescent/memory
CD127 (IL-7Rα)	Key for T cell survival, homeostasis and memory maintenance	Expressed on naïve and memory T cells; downregulated in Tregs and exhausted cells	CD127⁺ = functional, long-lived CD127⁻ = exhausted, short-lived

V. Conclusions

Our findings emphasize that the **key immunological hallmarks of APS** include: **B-cell dysregulation**, **aberrant helper T-cell activation**, and **T-cell exhaustion**.
These immune alterations likely underlie the persistent autoantibody production, chronic inflammation, and thrombotic tendency characteristic of APS.
The identification of **immunophenotypic and proteomic marker profiles** holds promise for: improving **diagnostic accuracy**, identifying **prognostic factors**, guiding **personalized therapeutic approaches**, and potentially **preventing thrombotic complications** through early intervention.