Development of a Novel Laboratory Test for the Detection of GM-CSF Antibodies to Aid in the Diagnosis of Individuals at-risk for Autoimmune Pulmonary Alveolar Proteinosis (aPAP)

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INTRODUCTION

Detection of antibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF) in human serum plays a pivotal role in the diagnosis and monitoring of individuals with autoimmune pulmonary alveolar proteinosis (aPAP). aPAP is a rare disease characterized by the presence of neutralizing antibodies to GM-CSF which is required by the alveolar macrophage to maintain balance of surfactant [1]. In this study, we present the development and performance characteristics of a novel laboratory test for the detection of GM-CSF antibodies in human serum to diagnose aPAP.

METHOD

A particle-based flow cytometry immunoassay was designed utilizing GM-CSF-coated particles. In brief, the detection of the GM-CSF antibody in serum was achieved using a biotin-labeled secondary antibody specific for human IgG conjugated to streptavidin phycoerythrin substrate (PE). The excitation and detection of the PE bound to the detection antibody is performed using the Luminex 200 instrument. Data acquisition was performed using xPONENT v4.3 software and reported as median fluorescence intensity. To determine the concentration of GM-CSF antibodies, the patient samples were plotted against a custom 8-point standard curve and reported as µg/mL. The assay's precision and sensitivity were assessed through intra- and inter-run evaluation using 40 human serum samples. Clinical accuracy was determined by testing a cohort of 40 samples collected from patients with known GM-CSF antibody status (positive aPAP samples provided by Savara Inc.) and utilizing a cut-off value of 8.0 µg/mL in assessment of at-risk individuals [2].

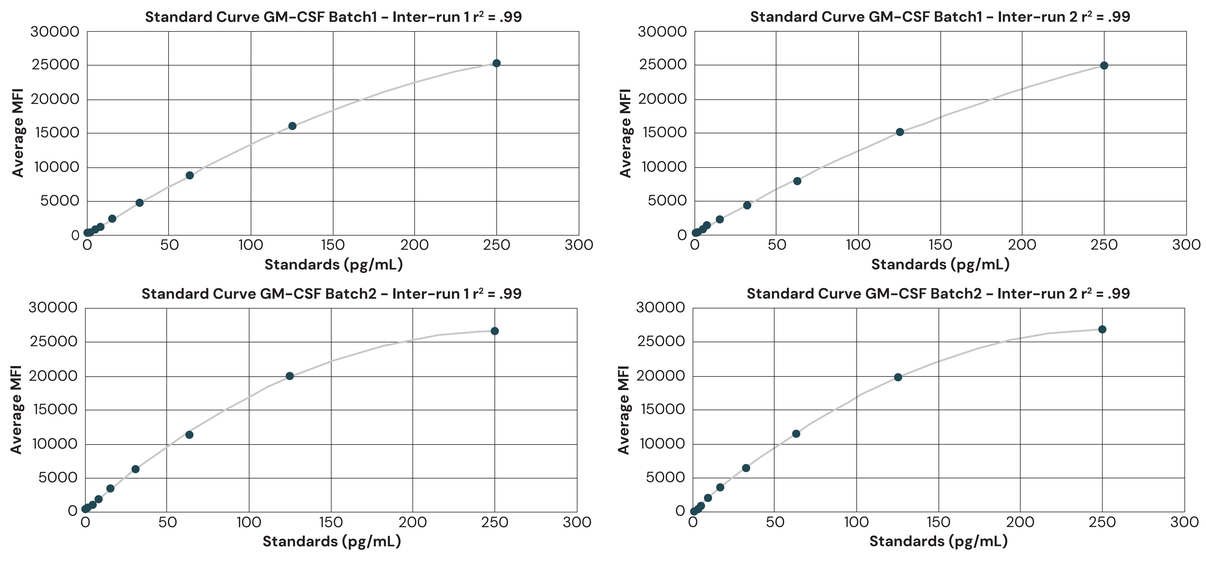

Figure 1. AMR study - Quadratic standard curve analysis for GM-CSF Batch 1 and GM-CSF Batch 2.

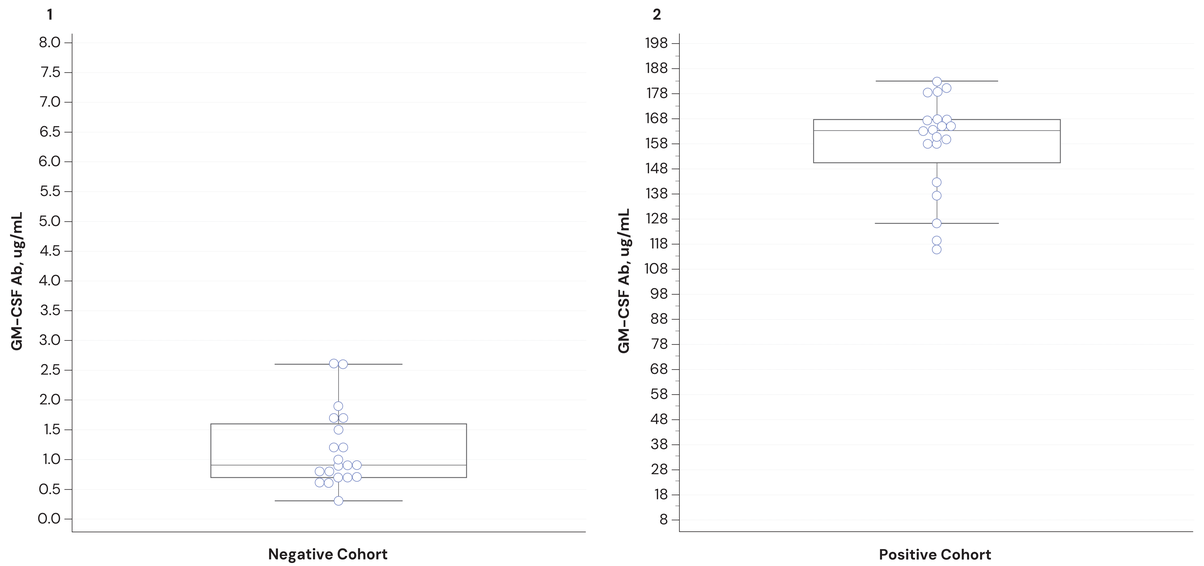

Figure 2. Box and whisker plot representing the negative (1) and positive (2) cohort.

RESULTS

The assay demonstrated high precision, with an inter-run precision of 7.9% and an intra-run precision of 2.7%. The custom quadratic standard curve exhibited an r-square value of 0.99, which ensured precise antibody quantification across a wide range of concentrations (Figure 1) and across different batches of GM-CSF particle beads. The calculated lower limit of quantitation for GM-CSF antibody detection was determined to be 1.95 pg/mL, which indicated high sensitivity in detecting low antibody concentrations. Clinical accuracy achieved 100% in a cohort of 40 subjects that utilized a cut-off value of 8.0 µg/mL (negative cohort: n= 20, GM-CSF antibody concentration range 0.3 µg/mL - 2.6 µg/mL; positive cohort: n=20, GM-CSF antibody concentration range 116.0 µg/mL - 183.2 µg/mL) (Figure 2).

CONCLUSION

Our particle-based flow cytometry immunoassay test, utilizing a custom standard curve, offers a highly precise and sensitive laboratory test for the determination of GM-CSF antibody levels in human serum and can be successfully used as a diagnostic tool to aid in the diagnosis of aPAP.

REFERENCES

1. Trapnell BC, Nakata K, Bonella F, Campo I, Griese M, Hamilton J, Wang T, Morgan C, Cottin V, McCarthy C. Pulmonary alveolar proteinosis. *Nat Rev Dis Primers*. 2019 Mar 7;5(1):16. doi: 10.1038/s41572-019-0066-3. PMID: 30846703.
2. Uchida K, Nakata K, Suzuki T, Luisetti M, Watanabe M, Koch DE, Stevens CA, Beck DC, Denson LA, Carey BC, Keicho N, Krischer JP, Yamada Y, Trapnell BC. Granulocyte/macrophage-colony-stimulating factor autoantibodies and myeloid cell immune functions in healthy subjects. *Blood*. 2009 Mar 12;113(11):2547-56. doi: 10.1182/blood-2009-05-155689. PMID: 19282464; PMCID: PMC2656275.

DISCLOSURE

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