

## Microarray Immunoassay Development to Specifically Detect Autoantibodies in Small Intestine Neuroendocrine Tumor (SI-NET) Patients

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**Background:** The majority of small intestine neuroendocrine tumor (SI-NET) patients get late diagnosis and metastatic SI-NETs lack of curative treatments. The unmet demand for primary SI-NETs identification requires novel biomarkers. Our study investigated autoantibodies, towards specific SI-NET-associated-antigens to detect potential novel diagnostic circulating serum biomarkers.

**Methods:** The ImmunoCAP ISAC platform (Phadia-AB, Uppsala, Sweden) is an allergy diagnostic tool. This relies on multiplexing-technology/protein-microarrays for serum or plasma IgE antibodies detection. Our novel assay to measure SI-NETs autoantibodies uses this platform. We spotted 26 SI-NET-associated-antigens on an activated-glass-chip. Fluorescently-labeled secondary-antibodies towards specific human immunoglobulin-isotypes detected SI-NETs autoantibodies.

**Results:** The main finding of this study shows that the ImmunoCAP ISAC is a suitable platform to detect SI-NET patient autoantibodies. The power of this platform is the generation of an immense data amount by using about 30µl of serum for each sample. The pilot test confirmed the assay capacity to detect different presence of SI-NET patients autoantibodies compared to healthy donors, by using 20 samples. In addition, it showed that the majority of autoantibodies were IgG1 and IgG4 isotypes. Thus, a randomized and blinded study privileged the study of these isotypes. The study included 120 samples (30 healthy donors and 90 SI-NET patients, at different stage of disease) confirmed the initial findings. The broader analyses showed the presence of differences between healthy donors and patients regarding the presence of protein component specific autoantibodies of the IgG1 and IgG4 isotypes. Furthermore, chromogranin A, paraneoplastic antigen Ma2 and several tumor associated antigens displayed high levels of IgG4. Moreover, the presence of IgG4 autoantibodies is specifically observed only in the SI-NET patient group.

**Conclusion:** This study investigated whether specific autoantibodies towards SI-NET-associated-antigens are potential circulating biomarkers, by using ImmunoCAP ISAC multiplexing platform. Our findings, support that SI-NET autoantibodies might be developed as novel biomarkers for tumor diagnostics.