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Tumor-marker gangliosides (TMGs) have enormous potential as diagnostic biomarkers, particularly in early stage-cancer detection, despite being understudied in this context. Aberrant levels of gangliosides, including GD2 and GD3, have been identified in several cancers such as ovarian, breast, neural and brain, and bone cancers. AOA Dx is focused on the development of multiple platforms aimed at detecting and quantifying gangliosides in serum, with a specific focus on ovarian cancer. In line with these efforts, AOA has successfully developed a mass spectrometry platform (HPLC-MS) for the detection and quantitation of disialo-gangliosides GD2 and GD3 using combination of targeted and untargeted techniques. Additionally, we have developed immunoassays such as TLC immunostaining and ELISA for the detection and quantitation of GD2, with further ongoing work for CD3 immunoassays. These platforms allow for the detection of gangliosides secreted into serum.

Multi-platform development

Mass Spectrometry: Untargeted and targeted ganglioside methods were developed using a qTOF and QqQ to identify and quantify GD2 and GD3 from purified GD2 standard and extracted serum samples. GD2 Competitive ELISA: A single antibody ELISA was developed using the competitive platform. Briefly, the plate was coated with GD2 conjugated to a protein carrier, and a biotinylated antibody is mixed with extracted serum before being added to the plate. The more antibody that binds to native substrate, the less antibody available to bind to the antibody immunoassay. This phenomenon is not observed PBS (negative control). The 5X spike is quantitative (lower concentrations), but lower amounts show lower recovery in serum (higher concentrations). The y-axis shows linear range for purified GD2.

Conclusions and next steps

All three platforms (HPLC-MS, TLC immunostaining, ELISA, and mass spectrometry) are feasible for the detection of GD2. We have also determined that our sample preparation and cleanup procedures are both robust and reproducible. Moving forward, we will begin using these platforms to analyze human samples and will continue to develop the method for analysis of GD3.

Gangliosides: powerful novel biomarkers

- Aberrant ganglioside levels identified in several cancers
- Heterogenous levels/distribution indicate unique disease signature
- Gangliosides relatively low in healthy serum
- Tumor marker gangliosides (TMGs) increased in cancer