Profiling Apolipoproteins in Disease

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Abstract

Detecting changes in biomarker levels or even the presence of biomarkers in limited samples can be difficult with traditional ELISA techniques. Multiplexing magnetic bead-based assays not only affords much higher sensitivity but also allows for sample conservation by testing for multiple analytes in a single well. In this study, we used an apolipoprotein multiplex panel to screen for some of the traditional apolipoproteins within the statin pathway (Apo A1, Apo A2, Apo B, Apo C1, Apo C3, and Apo E), along with additional targets important for cardiovascular disease, neurobiology, cancer, and inflammation research (Apo D, Apo H, clusterin (Apo J), and C-reactive protein (CRP)). Purchased serum samples representing several different diseases, including atherosclerosis, diabetes mellitus type I, sepsis, Alzheimer’s, and traumatic brain injury (TBI), were diluted 1:50,000. With the exception of CRP levels in some sepsis samples, all readings were within range. We conclude that this technology, and specifically this Apolipoprotein 10-Plex Panel, can be applied to many areas of disease research and requires minimal serum sample to generate robust data.

Introduction

Apolipoproteins are amphoteric molecules that, along with other proteins, surround oil-soluble fats and cholesterol to form lipoproteins that transport lipids throughout the circulatory and lymphatic systems. Apolipoproteins can also serve as enzyme cofactors, receptor ligands, and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in tissues.

While the role of apolipoproteins in the formation of lipoproteins is well known, apolipoproteins themselves have increasingly been shown to be important biomarkers in many biological conditions such as disease and infection. From heart disease risk factors to protective functions, apolipoproteins are intimately involved in cardiovascular disorders. Lung and respiratory conditions, including pulmonary fibrosis and apnea, have recently piqued interest in studies involving Apo E and Apo A (1). In sepsis, changes in high-density lipoproteins (HDLs) and apolipoproteins may indicate serious systemic conditions.

The objective of this study was to demonstrate that apolipoprotein profiling in multiplex may be a useful tool for biomarker research in various disease states. We surveyed human serum samples from healthy individuals as well as patients with atherosclerosis, Alzheimer’s disease, traumatic brain injury (TBI), sepsis, and diabetes type 1. Our goal was to determine whether the analytes in this 10-plex assay were present in detectable quantities in human serum across many disease states.

Results

All standard curves performed as anticipated, with 99% of all CVs for replicates below 10%. Thirty-six serum samples diluted 1:50,000 had data points on each of the ten assay standard curves. Each sample was assayed in duplicate; the %CV was under 7% for all analytes with one exception. Results are shown in Figure 1, which is separated into three panels based on the observed concentration levels of the analytes.

Fig. 1. Average analyte concentrations for diseases tested.

Table 1. Apolipoprotein 10-plex panel analytes that show statistically significant (P < 0.05) correlation with disease.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Correlation with Disease</th>
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<tbody>
<tr>
<td>Apo A1</td>
<td>Lower risk of MI</td>
</tr>
<tr>
<td>Apo A2</td>
<td>Average risk of MI</td>
</tr>
<tr>
<td>Apo A4</td>
<td>Higher risk of MI</td>
</tr>
<tr>
<td>Apo C1</td>
<td>Lower risk of MI</td>
</tr>
<tr>
<td>Apo C3</td>
<td>Average risk of MI</td>
</tr>
<tr>
<td>Apo E</td>
<td>Higher risk of MI</td>
</tr>
<tr>
<td>Apo J</td>
<td>Lower risk of MI</td>
</tr>
<tr>
<td>CRP</td>
<td>Average risk of MI</td>
</tr>
</tbody>
</table>

Results (continued)

In comparing the observed concentration values of diseased vs. healthy individuals, several apolipoproteins correlated with disease (Figure 2 and Table 1). While this study is quite small, these findings may warrant further investigation. There are reports in the literature indicating a role for Apo J/Beta 2 (2, 3, 4, 5) and Apo D (6) in Alzheimer’s disease. Apo E has been shown to be relevant in TBI (7), while Apo J has also been studied in atherosclerosis (8) and sepsis (9).

Conclusions

We demonstrate that all ten targets in the multiplex assay were detected in serum from individuals with different disease states. Multiplexing magnetic bead-based assays can conserve precious samples for this multiplex assay, serum dilution was 1:50,000. This apolipoprotein 10-plex panel could be useful in the identification of biomarkers for different disease states.

References

5. Thambisetty MM et al. (2010), Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. Arch Gen Psychiatry 67, 744–76;