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 amphipathic molecules that, along with other proteins, surround oil-soluble fats and cholesterols to form lipoproteins that transport lipids through 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 apolipoprotein levels 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.

Materials and Methods

Human serum samples were purchased from Discovery Life Sciences (Los Osos, CA). The Bio-Plex Pro Human Apolipoprotein Assays 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA) and all kit components were used. Aliquots of serum samples were centrifuged at 1,000 x g for 15 min at 4°C prior to dilution (1:50,000) in sample diluent and stored on ice. Threefold serial dilutions were prepared from a reconstituted apolipoprotein standard using standard diluent. Prior to use, all standards and samples were equilibrated to room temperature for 20 min, and all were run in duplicate. Close attention was paid to incubation times. The Bio-Plex 200 System (Bio-Rad Laboratories, Hercules, CA) was calibrated prior to data acquisition. Data acquisition was performed using Bio-Plex Manager Software and analysis was carried out using Bio-Plex Data Pro Software.

Results

All standard curves performed as anticipated, with 95% 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.

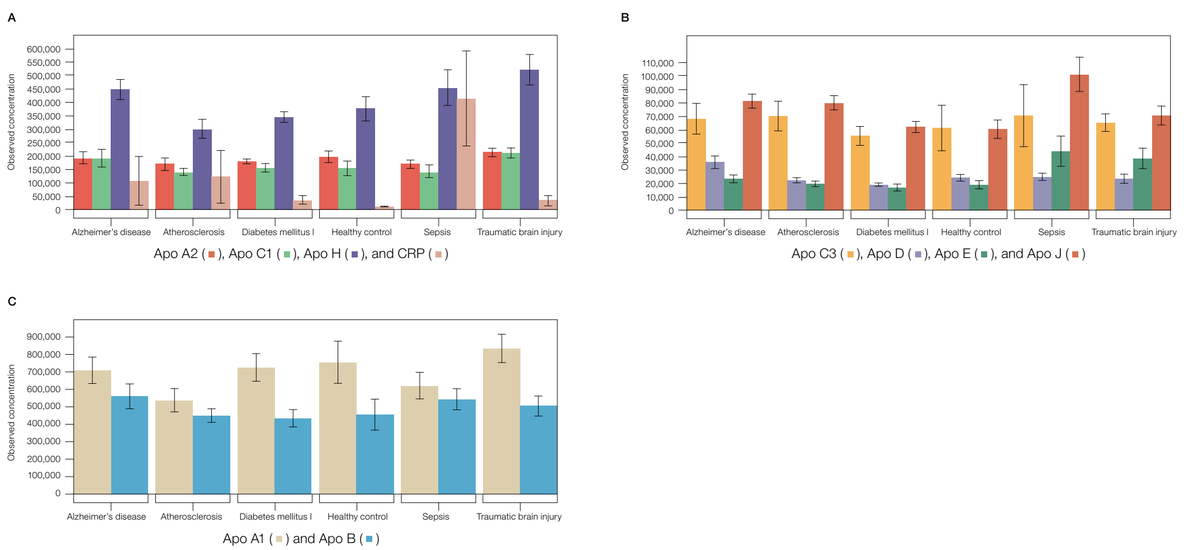

Fig. 1. Average analyte concentrations for diseases tested.

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/clusterin (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).

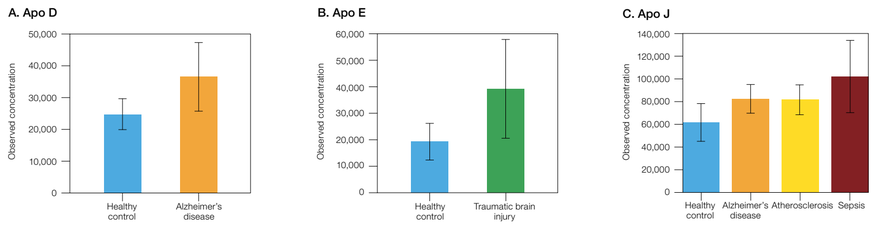

Fig. 2. Observed apolipoprotein concentrations. *P* values are reported in Table 1.

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

	Apo D	Apo E	Apo J
Alzheimer's disease	0.047	–	0.032
Atherosclerosis	–	–	0.041
Sepsis	–	–	0.024
Traumatic brain injury	–	0.047	–

Serum apolipoproteins have been studied as risk factors in coronary health since the 1980s (12). One major finding has been that the ratio of Apo B to Apo A1 can be used as a predictor of myocardial infarction (MI) risk (10, 11, 12). The average (n = 6) apolipoprotein concentrations are shown for healthy (Figure 3A) and atherosclerosis (Figure 3B) donors. We calculated the Apo B/Apo A1 ratios for all female (>18 yr) and male (>18 yr) samples used in this study. Based on these results (Figure 4), we can see that there is an elevated Apo B/Apo A1 ratio in sepsis (2 females and 1 male), indicating a higher risk of MI. Several other donors also appear to have a higher risk of MI, although we do not have any way of corroborating this because we worked with anonymized samples with limited medical information.

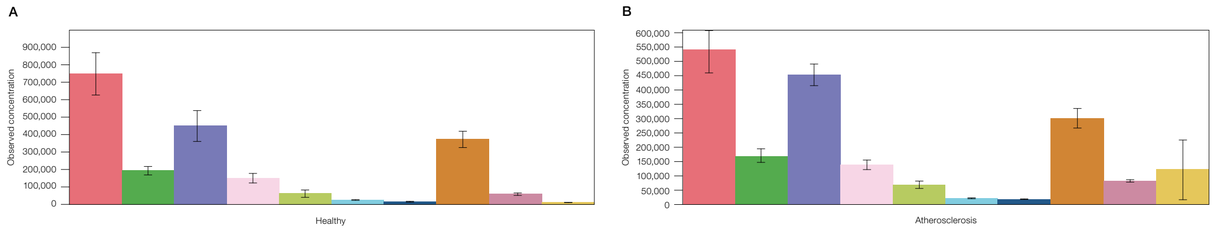

Fig. 3. Average observed concentration of apolipoprotein 10-plex analytes in serum from healthy individuals (A) and atherosclerosis patients (B). Apo A1 ( ), Apo A2 ( ), Apo B ( ), Apo C1 ( ), Apo C3 ( ), Apo D ( ), Apo E ( ), Apo H ( ), Apo J ( ), CRP ( ).

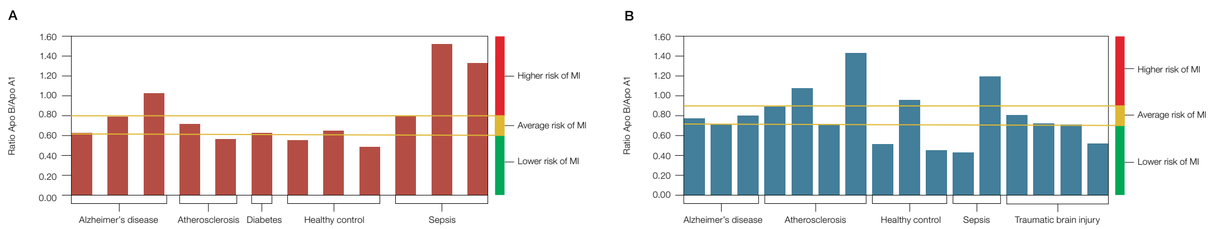

Fig. 4. Calculated Apo B/Apo A1 ratios for females (A) and males (B) in this study. Participants were older than 18 years.

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 was diluted 1:50,000. The apolipoprotein 10-plex panel could be useful in the identification of biomarkers for different disease states.

References

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