INTRODUCTION

To improve the prognosis of patients with pancreatic cancer improved classes of biomarkers for detection are needed. Analysis of serum cancer antigen 19-9 (CA19-9) is currently used for monitoring and management of pancreatic cancer. Aberrant glycosylation of protein biomarkers has emerged as an indicator of cancer development. As detection of pancreatic cancer by single circulating disease biomarkers has proven inadequate, the idea that a multifaceted pathology may be reflected in simultaneous detection of multiple disease markers has arisen. Biochip Array Technology (BAT) enables the simultaneous detection of multiple biomarkers from a single sample and the aim of this study was to evaluate an enzyme-linked lectin multiplex panel of glycosylated serum biomarkers - CA19-9, Carcinoembryonic Antigen (CEA) and Alpha 1-Acid Glycoprotein (A1AG) - with potential for pancreatic cancer discrimination.

METHODOLOGY

BAT was used for specific capture of glycosylated CA19-9, CEA and A1AG at discrete test regions on a biochip surface. Simultaneous glycosylation-based detection of the biomarkers was achieved using a HRP labelled lectin with fucose specificity as shown below.

The chemiluminescent simultaneous assays were applied to the semi-automated analyser Evidence Investigator.

RESULTS

SAMPLE ASSESSMENT

Serum samples from pancreatic cancer patients (n=20, 35% female, mean age 66.7 years) and normal samples (n=36, 77.8% female, mean age 53.3 years) were assessed. Area under the curve (AUC), sensitivity and specificity of the presented multiplex application were compared with single measurement of CA19-9 and total antigen measurement of these biomarkers.

<table>
<thead>
<tr>
<th>MULTIPLEX GLYCOSYLATION PANEL</th>
<th>ANTIGEN MEASURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full panel</td>
<td>CA19-9</td>
</tr>
<tr>
<td>AUC</td>
<td>0.969</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
</tr>
</tbody>
</table>

Confirmation of alpha 1-acid glycoprotein fucosylation

A: Coomassie stained SDS-PAGE following immunoprecipitation of A1AG from sera of normal and pancreatic cancer patients.

B: AAL-lectin blotting showing fucosylation of A1AG immunoprecipitated from pancreatic cancer sera but not normal.

C: Confirmation of even immunoprecipitation of A1AG from each serum sample.

D: A1AG antigen measures and biochip-based fucosylation signal from the samples analysed in panel A-C showing equivalent total antigen but differential fucosylation.

CONCLUSION

Glycosylation-based multiplex detection improved pancreatic cancer detection when compared with measurement of CA19-9 alone or total antigen measurement. The application of the BAT platform for multiplexed glycosylated biomarker analysis offers accessible, low cost options for cancer screening which can be readily translated to a routine clinical laboratory setting.