Introduction

- Biomarkers become increasingly important secondary endpoints for measuring success of new drugs in the clinic.
- Relevant biomarkers for chronic diseases like inflammatory cytokines/chemokines often show small difference compared to healthy individuals.
- Proper assessment of these differences is pivotal for patient tailored therapy and in order to prevent diseases progression, since particularly smoldering inflammation related to cytokines could lead to very serious diseases like cancer.

The prevalence in United States of the chronic inflammations considered hereafter are:

- Asthma: > 25 million people (1)
- Psoriasis: > 8 million people (2)
- Rheumatoid arthritis (RA): > 1.3 million people (3)
- Nonalcoholic steatohepatitis (NASH): > 15 million people (4)

Aims

The aims of this study were:

- Assess IL-6, TNF-a, IL-12p70 and IL-13 cytokine levels in subjects suffering from chronic inflammations such as asthma, psoriasis, RA and NASH.

- Compare the gold standard MESO™ QuickPlex SQ 120 (Meso Scale Discovery), the Luminex® Bio-Plex® (BioRad) and the latest top single molecule array (SiMoA SR-X™ instrument (Quanterion)).

The biomarkers selected were:

- IL-6, which stimulates the synthesis of acute phase proteins and the growth of antibody-producing B lymphocytes.
- TNF-a, which stimulates vascular endothelial cells to express adhesion molecules and induces macrophages and endothelial cells to secrete chemokines.
- IL-12, a pro-inflammatory cytokine that regulates the balance between Th1 and Th2 cells, as well as enhancing cytotoxic T cell-mediated lysis and natural killer cell activity. IL-12p70 are pro-inflammatory cytokines. The heterodimeric form of IL-12p70, p70, mediates a biological response, whereas a p40 homodimer acts as an IL-12 antagonist.
- IL-13, an anti-inflammatory cytokine that inhibits macrophages activation and suppresses Th1 cell-mediated immunity.

The assay formats compared were:

- An electromulniunonrescence-based assay, which became the industry standard for biomarkers, offering improved dynamic range in comparison with enzyme-linked immunosorbent assays.
- A digital flow-based instrument.

A digital quantification based assay, offering a single molecule detection and therefore opening a new horizon for the detection of critical biomarkers.

Analytical Methods

Platforms and Kits

The platforms and kits considered for this comparison are listed in Table 1.

Table 1. Platforms and kits.

<table>
<thead>
<tr>
<th>Platform/Capture</th>
<th>Capture Antibody</th>
<th>Detection Antibody</th>
<th>Quantification</th>
<th>Kits used</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESO QuickPlex SQ 120 (Meso Scale Discovery)</td>
<td>Carbonic anhydrase in the bottom of microplates allow for attachment of biological magnets</td>
<td>SILFO-TAG electromulniunonrescence labeled conjugate to detection antibodies</td>
<td>A voltage applied to the plates electrodes lead to light emission by SILFO-TAG levels</td>
<td>Vortex Human kit (Meso Scale Discovery)</td>
</tr>
<tr>
<td>Lumine Bio-Plex (Bio Rad)</td>
<td>Pre-coated with analyte-specific capture antibodies</td>
<td>Biotinylated detection antibodies specific to the analysis of interferon and Phycerythrin-conjugated streptavidin complete the immune complex</td>
<td>Dual-laser flow-based detection instrument</td>
<td>Vortex Human kit (Meso Scale Discovery)</td>
</tr>
<tr>
<td>SR-X (Quanterion)</td>
<td>Antibody capture agents attached to the surface of paramagnetic beads connect the analyte of interest present in sample</td>
<td>Biotinylated detection antibody and p-galactosidase-labeled biotinylated streptavidin complete the immune complex (Figure 1)</td>
<td>Digital and analog quantification (Figure 2)</td>
<td>Vortex Human kit (Quanterion)</td>
</tr>
</tbody>
</table>

Results

- Assay dynamic ranges adjusted for sample dilution are indicated in Table 2.
- Samples below limit of quantitation (BLOQ) are reported in Table 3.
- IL-6 measured concentrations are shown in Figure 3.
- IL-12p70 measured concentrations are shown in Figure 5.
- Bland-Altman analyses were conducted to assess the agreement between platforms (8).

Biological Matrices

Human sera were purchased by BioVIT, aliquoted and stored at -80 °C before analysis. For each group, 15 individuals were considered, except for NASH which 14 individuals were tested.

Statistical Analysis

Data were tested for normality (Shapiro-Francia test). Non normal data sets were log transformed. The statistic evaluation was performed using either Student t-test or Mann-Whitney-U test.

Discussion & Conclusions

The study presented here was designed to:

- Assess the modulation of cytokines concentrations in subjects suffering from chronic inflammations.
- Compare the performance of the SR-X instrument -based on the SiMoA approach- with platforms routinely used in CRD for biomarkers quantification such as MESO QuickPlex SQ 120 and Luminex Bio-Plex.

Our findings show that:

- No difference could be observed for IL-6 between healthy and diseased groups. SR-X showed its superiority as it was the only platform where all samples were measured within the range.
- Clear differences could be observed for TNF-a between healthy and diseased groups with SR-X (p<0.05), whereas no assessment was possible neither with MESO QuickPlex SQ 120 nor all the samples from healthy subjects were measured BLOQ- nore with Luminex Bio-Plex for which all samples were measured BLOQ. SR-X showed its superiority as it was the only platform where all samples were measured within the range.
- In both cases, for IL-6 as well as for TNF-a, agreements between platforms were assessed.
- No differences could be observed for IL-12p70 between healthy and RA as well as NASH groups with SR-X (p<0.05), whereas no assessment was possible neither with MESO QuickPlex SQ 120 nor all the samples from healthy subjects were measured BLOQ- nore with Luminex Bio-Plex for which all samples were measured BLOQ. SR-X showed its superiority as it was the only platform where all samples were measured within the range.
- No difference could be observed for IL-13 between healthy and diseased groups with SR-X. SR-X showed its superiority as it was the only platform where all samples were measured within the range.

A proper measurement of low abundant inflammatory cytokines used as secondary endpoint biomarkers requires high sensitivity technology. The single molecule detection system acquired by Celerion Switzerland AG was superior to the competitor technologies. Moreover, this technology provides the flexibility for trained researchers to develop custom assays. Such single molecule detection approach hold promises for patient stratification and to prevent disease progression.