Biomarkers Challenges: SiMoA Makes the Tiny but Significant Difference

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Introduction

- Biomarkers become increasingly important secondary endpoint for monitoring 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-α, IL-12p70 and IL-13 cytokines 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 edge single molecule array (SiMoA) SR-X™ instrument (Quanterix®).

The biomarkers selected were:

- IL-6, which stimulates the synthesis of acute phase proteins and the growth of antibody-producing B lymphocytes.
- TNF-α, which stimulates vascular endothelial cells to express adhesion molecules and induces macrophages and endothelial cells to secrete chemokines.
- IL-12, a critical cytokine regulating 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-12, 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 electrochemiluminescence based assay, which became the industry standard for biomarkers, offering improved dynamic range in comparison with enzyme-linked immunosorbent assays.
- A dual-laser 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 (5).

Analytical Methods

Platforms and Kits

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

Table 1. Platforms and kits.

MESO QuickPlex SQ 120 (Meso Scale Discovery)	
Capture	Carbon electrodes in the bottom of microplates allow for attachment of biological reagents
Detection	SULFO-TAG electrochemiluminescent labels conjugated to detection antibodies
Quantification	A voltage applied to the plate electrodes lead to light emission by SULFO-TAG labels
Kits used	V-plex Human kit (Meso Scale Discovery)

Luminex Bio-Plex (Bio Rad)	
Capture	Pre-coated with analyte-specific capture antibodies
Detection	Biotinylated detection antibodies specific to the analytes of interest and Phycoerythrin-conjugated streptavidin complete the immune complex
Quantification	Dual-laser flow-based detection instrument
Kits used	Magnetic Luminex performance assay, Human high sensitivity Cytokine premixed kit A (Biotechne®)

SR-X (Quanterix)	
Capture	Antibody capture agents attached to the surface of paramagnetic beads concentrate the analyte of interest present in samples
Detection	Biotinylated detection antibody and β-galactosidase–labeled streptavidin complete the immune complex (Figure 1)
Quantification	Digital and analog quantification (Figure 2)
Kits used	SiMoA bead-based advantage assays-Human (Quanterix)

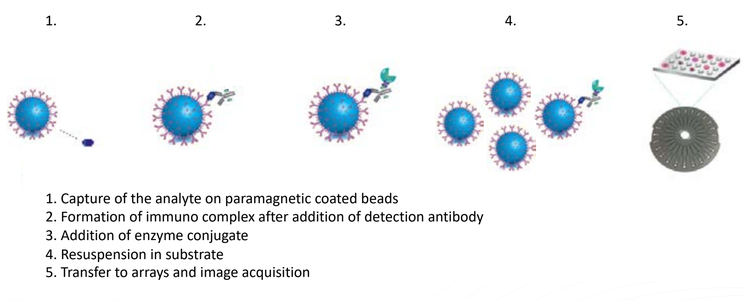
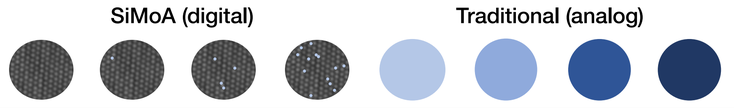


Figure 1. SiMoA bead-based approach – adapted from (6).



Reaction volume ≈ 50 x 10^{-15} L (2 billion times smaller than analog)	Reaction volume = 100 x 10^{-6} L
Diffusion defeated = single molecule resolution = ultimate sensitivity	Diffusion = dilution = low sensitivity
One molecule needed to reach detection limit	Millions of molecules needed to reach detection limit

Figure 2. Digital and analog quantifications – adapted from (7).

Biological Matrices

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

Bland-Altman analyses were conducted to assess the agreement between platforms (8).

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.
- TNF-α measured concentrations are shown in Figure 4.
- IL-12p70 measured concentrations are shown in Figure 5.
- IL-13 measured concentrations are shown in Figure 6.
- Bland-Altman analyses for IL-6 are shown in Figure 7.
- Bland-Altman analyses for TNF-α are shown in Figure 8.

Table 2. Dynamic ranges (pg/mL).

	SR-X (Quanterix)	MESO QuickPlex SQ 120 (Meso Scale Discovery)	Luminex Bio-Plex (BioRad)
IL-6	0.164 - 120	0.366 - 1496	1.660 - 6800
TNF-α	0.276 - 200	0.158 - 646	1.514 - 6200
IL-12p70	0.055 - 40	0.27 - 1102	10.8 - 8860
IL-13	0.0074 - 30	0.246 - 1006	N/AP

Table 3. Below limit of quantitation samples (from 74 samples).

	SR-X (Quanterix)	MESO QuickPlex SQ 120 (Meso Scale Discovery)	Luminex Bio-Plex (BioRad)
IL-6	0	8	43
TNF-α	0	2	3
IL-12p70	0	57	74
IL-13	0	74	N/AP

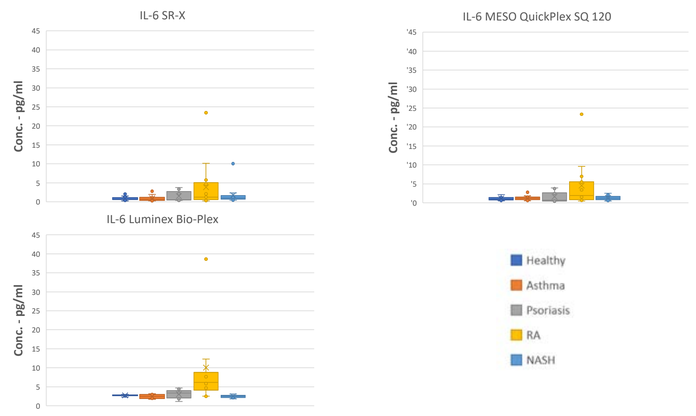


Figure 3. IL-6 measured concentrations.

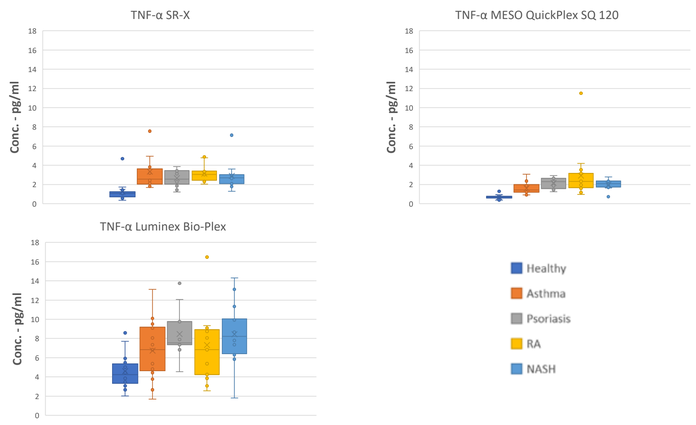


Figure 4. TNF-α measured concentrations.

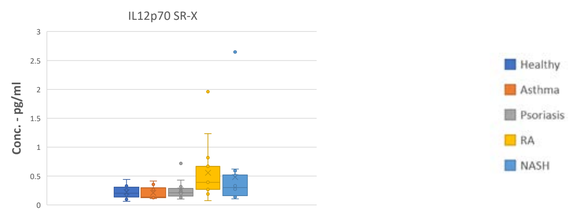


Figure 5. IL-12p70 measured concentrations.

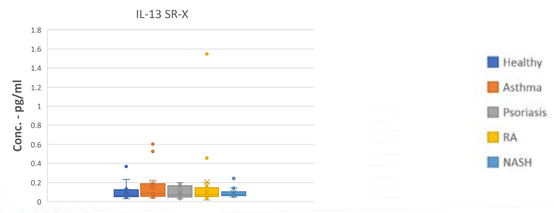


Figure 6. IL-13 measured concentrations.

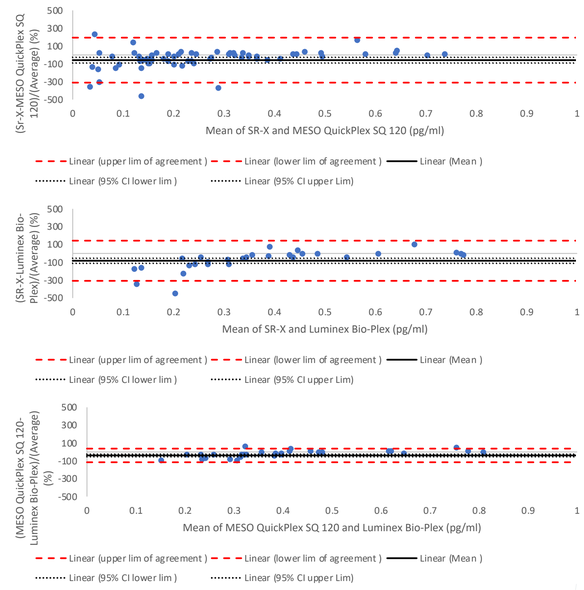


Figure 7. Bland-Altman analyses for IL-6.

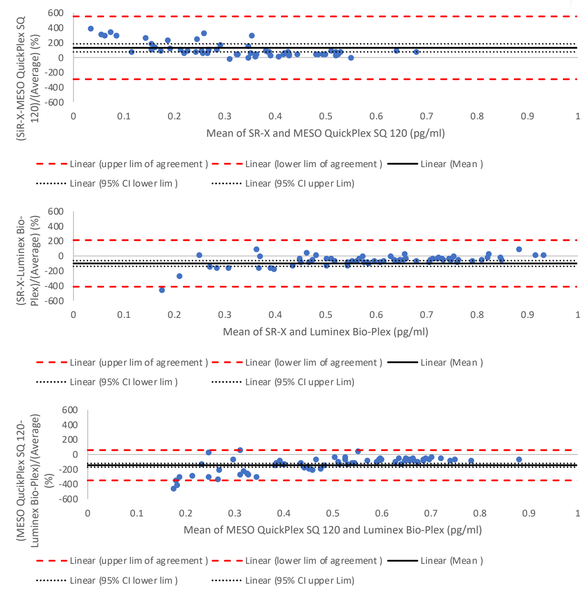


Figure 8. Bland-Altman analyses for TNF-α.

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 CRO 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-α between healthy and diseased groups with the three platforms ($p<0.001$, except with Luminex Bio-Plex for asthma and RA groups: $p<0.05$). 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-α, agreements between platforms were assessed.
- Clear 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 – as 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. Interestingly, all samples were BLOQ with MESO QuickPlex SQ 120, instrument considered as a gold standard for biomarkers analysis.

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.

References

1. CDC.gov. (2018) CDC-Asthma- Data and Surveillance – Asthma Surveillance Data. http://www.cdc.gov/asthma/asthmadata.htm
2. National Psoriasis Fundation. https://www.psoriasis.org/content/statistics
3. Helmick C.G., Felson D.T. et al. (2008), Estimates of the prevalence of arthritis and other rheumatic conditions in the United States- Part I. Arthritis & Rheum.: 58(1):15-25
4. Estes, C., Razavi, H. et al (2018), Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology (Baltimore, Md.), 67(1), 123–133
5. Rissin D.M., Kan C.W. et al (2010), Single-Molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 28(6): 595–599
6. SIMOA Bead Technology, Quanterix website
7. SIMOA, Scientific Principle of SimoaTM (Single Molecule Array) Technology. Whitepaper 1.0. Quanterix Corp. 2013: 1-2
8. Bland J.M., Altman D.G. (1986), Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet, 327 (8476): 307-310

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