Measurement of CXCL10 in the Management of COVID-19

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Introduction

- CXCL10 is a pro-inflammatory chemokine
  - Secreted in response to IFN-γ by a variety of cells [1]
  - Binds to CXCR3
  - Promotes migration of T cells & monocytes [2]
  - Regulates immune response
- CXCL10 raised in many inflammatory diseases due to immune system dysfunction
  - Type 1 diabetes [3], Rheumatoid arthritis [2], Cryoglobulinaemia [4], SLE [5], Sjögren's syndrome [5], Behçet's Disease [6], COVID-19 [7]
- SARS-CoV-2 enters pulmonary and neuronal cells via upper respiratory tract, triggering
  - CXCL10 production
  - Recruitment of CXCR3-expressing cells
  - Demyelination in CNS
  - Cytokine storm & Acute Respiratory Distress Syndrome (ARDS) [7,8]
- Raised serum CXCL10 in COVID-19 suggests T cell activation [7]
- CXCL10 has been suggested as a biomarker of COVID-19 severity and outcome [9]

Objective

- Verify suitability of commercially available ELISA kit for CXCL10 in diagnostic laboratory

Method

- Serum CXCL10 measured using R&D CXCL10 (IP-10) ELISA kit & Dynex DS2 automated ELISA processor
- Evaluation included precision testing, stability, linearity, recovery, interference, sensitivity, and limit of detection
- 32 samples from 26 patients with COVID-19

Keywords

Chemokine, CXCL10, COVID-19, ARDS

Results

- Validation of kit performance against set criteria (table 1)
  - Acceptable performance

Evaluation Criteria As listed in Validation Plan	Acceptance Criteria As listed in Validation Plan	Acceptable / Not Acceptable
Assay performance compared to manufacturer's claims	• Intra-assay precision <5% • Inter-assay precision <10% • Recovery >88% • Linearity >90% • Sensitivity >80%	• 3.52%: Acceptable • 18.45% • 64.9%. Measured CXCL10 higher than expected. Assay intended to detect raised CXCL10, not deficiency: Acceptable • 311%, r^2 0.99 Measured CXCL10 higher than expected. Assay intended to detect raised CXCL10, not deficiency: Acceptable • 100%: Acceptable
	• Specificity: healthy controls within reference range • Determine limit of detection • Stability: ○ Control aliquots stable at -20^{0}C ○ Comparable results for samples stored at -20^{0}C and 4^{0}C	• 100%: Acceptable • LOD=0.087pg/ml • Acceptable ○ To prepare QC aliquots ○ Samples stable up to 4hours after centrifugation
QC performance	• RnD QCs ○ Within expected ranges • Plotted on Levey-Jennings chart	• Acceptable when freshly reconstituted
	• In-house IQC created • Plotted on Levey-Jennings chart	• To prepare in-house IQC ○ Healthy control ○ Inflammatory disease
Technical validation of assay performance	• CV of calibrator, controls and samples <10%	• Acceptable duplicate CVs
	• Raised CXCL10 levels in predicted patient groups ○ Type 1 DM, RA, SLE, SS, Behcet's	• Acceptable • Raised in: ○ RA, SLE, COVID-19

Table 1: Summary of validation data.

- CXCL10 raised in all samples from patient with COVID-19 (fig. 1)

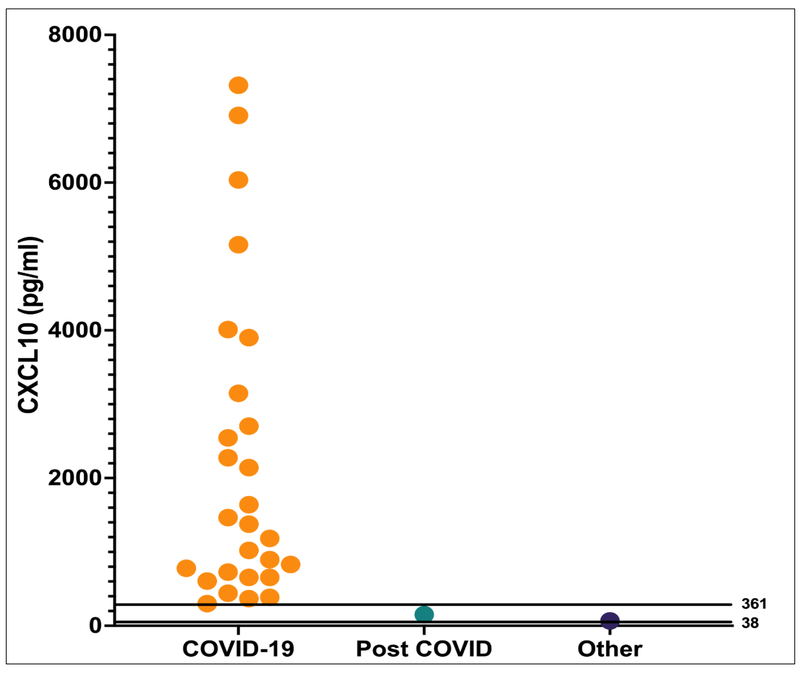

Figure 1: Raised CXCL10 compared to reference range (38-361 pg/ml) in 29/32 samples

Discussion

- Results confirm CXCL10 is raised in COVID-19
  - Provides information on disease severity, informing patient treatment
- Validation data demonstrated that CXCL10 also raised in RA and SLE patients
  - Measurement may be relevant to patient management
- Have not yet determined if CXCL10 is raised in other viral illnesses

Conclusion

- Serum CXCL10 can be accurately and reliably measured in a diagnostic laboratory under real-life conditions

References

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