Quantification of CSF chemokines and cytokines allows for rapid laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens

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Quantification of CSF chemokines and cytokines allows for rapid laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens

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ABSTRACT

Background: Prompt diagnosis of central nervous system (CNS) disease is critical to guide intervention and appropriate therapy. Development of novel laboratory approaches to rapidly classify acute-onset CNS disease is of great demand. Serious microbial pathogens, especially viruses, are quickly expanding beyond their historic geographic range and may not even be considered in a clinician’s differential diagnosis. Unlike bacterial cultures, current viral testing targets a limited number of viruses. Additionally, despite diversity in etiology, signs and symptoms of both infectious and non-infectious CNS disorders can be remarkably similar, which can confuse the clinician on time and delay treatment. Bacterial, viral, fungal and protaline CNS pathogens are sensed by pattern recognition receptors of the immune system, stimulating immediate release of measurable levels of chemokines and cytokines into the CSF. Our objective is to use pathogen-specific chemokine/ cytokine profiles to classify CNS diseases as infectious versus non-infectious and further discriminate between viral and non-viral infections.

Methods: Levels (pg/ml) of chemokines and cytokines were determined in the CSF of 45 patients with documented infectious meningitis or meningoencephalitis (mean age 19.2 years) and in the CSF of 45 patients who were negative for CNS infection (mean age 27.4 years). MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panels (Millipore) were used to measure CSF chemokines and cytokines levels (pg/ml). Immune analytes quantified included IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L. Samples were analyzed in duplicate by FlexMAP (Luminex). Standard curves were generated for each cytokine and median fluorescent intensities were transformed into concentrations by 5-point, non-linear regression. For univariate analysis, comparisons between groups were made using the Mann-Whitney test. We utilized principal operating characteristic (ROC) curve analysis to calculate areas under the ROC curve (AUC) for each analyte to represent the utility of chemokine/cytokine levels as discriminating tests. The ROC generated sensitivity and specificity values were then used to determine clinically optimal cutoff values for the informative analytes.

Results: Univariate analysis employing Mann-Whitney tests demonstrated that median values (pg/ml) of IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L were all significantly higher in CSF from patients with infectious brain disorders than in CSF from patients with non-infectious disorders (p-value < 0.05). MDC (CCL22) demonstrated statistical significance, when comparing viral infections versus non-viral infections (with the non-infectious group having higher analyte levels). IP10 (CXCL10) can reliably distinguish between an infectious versus non-infectious CNS process (AUC 0.978) with an optimal cut-off value of 2032 pg/ml (sensitivity, specificity: 93.0%, 92.0%). In the infectious group, MDC (CCL22) can reliably distinguish viral from non-viral infectious-type processes.

Case Study

16 year-old woman with history of lupus presented with CNS symptoms (stains with progression to altered mental status and paralysis). CSF studies were normal. CSF microbiology tests were negative.

Conclusions

• CSF levels (pg/ml) of IP-10/CXCL10 can reliably distinguish between infectious (All I) versus non-infectious (C-N) CNS process (AUC 0.978) with an optimal cut-off value of 2032 pg/ml (sensitivity, specificity: 93.0%, 92.0%).

• In the infectious group, MDC/CCL22 can reliably distinguish between viral and non-viral CNS infections.

• CSF chemokine/cytokine quantification can serve as a useful laboratory tool for the rapid triage of CNS diseases to help guide prompt therapy and further testing.

RESULTS: PRINCIPAL COMPONENT ANALYSIS (PCA)

Principal Component Analysis (PCA) with the variables IP10, IL15, MDC, FLT3L, and Fractalkine

We used PCA to visually represent the underlying structure of our data and examine the variability of specific cytokines/chemokines to help distinguish among CNS disease states (non-infectious and infectious, as well as viral versus non-viral infections).

RESULTS: MANHAN-WHITNEY TEST OF SIGNIFICANCE

Mann-Whitney tests for significance for non-parametric data

Levels of all studied cytokines (KCp1, IFNy, IP10, IL15, MDC, FLT3L, and Fractalkine) were significantly higher in the infectious compared to the non-infectious group.

RESULTS: DESCRIPTIVE STATISTICS AND RECEIVER OPERATOR CURVES (ROC)

IP10/CXCL10 can reliably distinguish between an infectious (All I) versus non-infectious (C-N) CNS process (AUC 0.978) with an optimal cut-off value of 2032 pg/ml (sensitivity, specificity: 93.0%, 92.0%).

Among the infectious cases, MDC distinguishes viral from non-viral infectious-type processes.