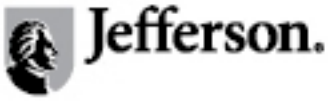

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Quantification of CSF chemokines and cytokines allows for rapid laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens

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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 in great demand. Serious microbial pathogens, especially viruses, are quickly expanding beyond their historic geographic range and may not even be considered in the 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 clinical picture and delay treatment. Bacterial, viral, fungal and protozoan 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 disease 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 25 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). Innate immune analytes quantified included IP-10 (CXCL10), IFNg, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L. Samples were analyzed in duplicate by a FlexMAP 3D (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 receiver operating characteristic (ROC) curve analysis to calculate areas under the ROC curve (AUC) for each analyte to access 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 utilizing Mann-Whitney tests demonstrated that median values (pg/ml) of IP-10 (CXCL10), IFNg, IL-15, MDC (CCL22), 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-viral infection group having higher analyte levels). IP10 (CXCL10) can reliably distinguish between an infectious versus non-infectious CNS process (AUC 0.9778) with an optimal cut-off value of 2023 pg/ml (sensitivity, specificity; 93.0%, 92.0%). In the infectious group, MDC (CCL22) can reliably differentiate between viral and non-viral CNS infection (AUC 0.9545) with an optimal cut-off value of 194 pg/ml (sensitivity, specificity; 91.67%, 87.88%).

Conclusion: CSF levels (pg/ml) of IP-10 (CXCL10) can reliably distinguish infectious versus non-infectious CNS disorders, and in the infectious group, MDC (CCL22) can reliably distinguish between viral and non-viral CNS infections. These results suggest that 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.

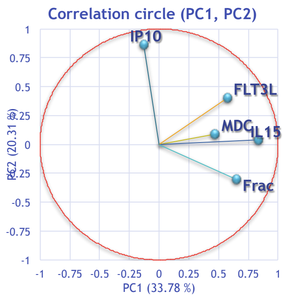
Background Information

Cytokine	Function in the inflammatory response
MCP1/CCL2	marker of non-specific inflammation
IP10/CXCL10	produced by a wide range of CNS cells in response to microbial pathogens; stimulated by multiple pathways
IL15	growth/survival factors for both NK cells and cytotoxic CD8+ T-cells
MDC/CCL22	produced in response to various microbial products; down-regulated by IFNγ
FLT3L	supports maturation of antigen presenting cells
Fractalkine/CX3CL1	support monocyte adhesion to endothelium
IFNγ	major chemokine product of NK cells; stimulates phagocytosis and pathogen killing in macrophages

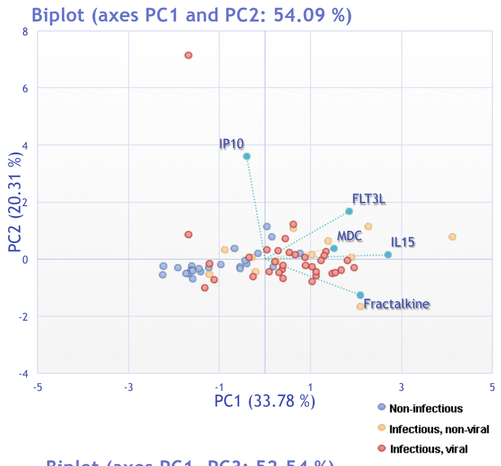
Non-infectious cases	Infectious cases
Headache, idiopathic intracranial hypertension (IIH), subarachnoid hemorrhage, possible autoimmune disease	**Viral**: Enterovirus, Human parechovirus, West Nile Virus, JC Virus, HHV6
	Non-viral: S.epidermidis, S.pneumoniae, M.tuberculosis, B.burgdorferi, Toxoplasmosis, Cryptococcus neoformans

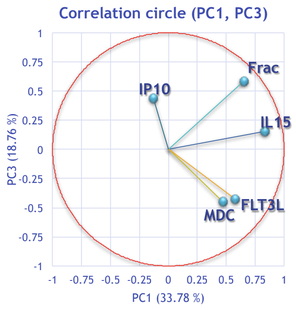
Results: Principal Component Analysis (PCA)

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

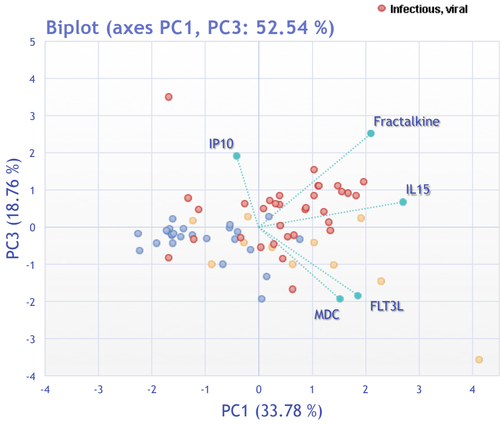


IL15, Fractalkine, and FLT3L influence PC1.
IP10 influences PC2.





IL15, Fractalkine, FLT3L, and MDC influence PC1.
IP10 influences PC3.



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: Mann-Whitney test of significance

	Non-infectious vs Infectious	Viral vs Non-viral
MCP1	* (p=0.0494)	
IFNγ	** (p=0.0035)	
IP10	**** (p<0.0001)	NS
IL15	**** (p<0.0001)	NS
MDC	** (p=0.0028)	**** (p<0.0001)
FLT3L	** (p=0.0057)	NS
Fractalkine	**** (p<0.0001)	NS

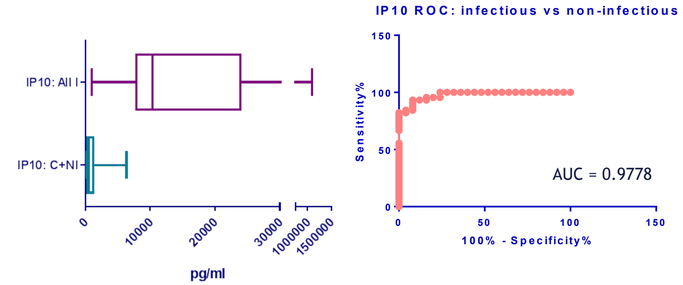
Mann-Whitney tests of significance for non-parametric data

Levels of all studied cytokines (MCP1, IFNγ, IP10, IL15, MDC, FLT3L, and Fractalkine) were **significantly higher in the infectious** compared to the non-infectious group.

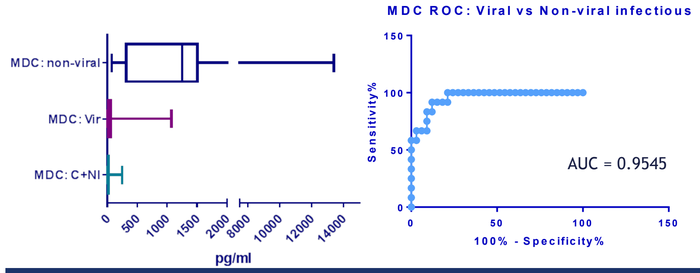
* indicates significant difference between two groups. Number of * represents p-value summary. "NS" indicates no significant difference.

Results: Descriptive statistics and Receiver Operator Curves (ROC)

IP10/CXCL10 can reliably distinguish between an **infectious (All I) versus non-infectious (C+NI)** CNS process (AUC 0.9778) with an optimal cut-off value of 2023 pg/ml (sensitivity, specificity; 93.0%, 92.0%).



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



Case Study

16 year old woman with history of lupus presented with CNS symptoms (ataxia with progression to altered mental status and paralysis).

CSF studies were normal. *CSF microbiology tests were negative.*

MCP1: 5678.88 pg/ml
IP10: 23247.43 pg/ml
MDC: 46.99 pg/ml
FLT3L: 69.63 pg/ml
Fractalkine: 100.63 pg/ml

The cytokine profile supports infectious, viral-type etiology.

MDC level	Sensitivity	Specificity	Likelihood ratio
> 194.0	91.67	87.88	7.563
> 238.3	83.33	87.88	6.875
> 267.3	83.33	90.91	9.167
> 355.9	75.00	90.91	8.250
> 487.1	66.67	90.91	7.333
> 556.6	66.67	93.94	11.00
> 632.1	66.67	96.97	22.00
> 884.2	58.33	96.97	19.25

Four weeks following admission, admission CSF was positive for human parechovirus (HPeV).

Conclusions

- CSF levels (pg/ml) of **IP-10/CXCL10** can reliably distinguish infectious versus non-infectious CNS disorders
- 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.