

The Cytokine Interleukin-6 and the Chemokines CCL20 and CXCL13 Are Novel Biomarkers of Specific Endogenous Uveitic Entities

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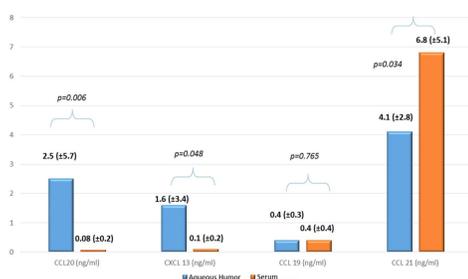
Background:

- Endogenous uveitis is clinically heterogeneous group of potentially blinding intraocular inflammatory diseases.
- They often occur in conjunction with systemic inflammatory diseases, such as HLA-B27-associated uveitis, Behçet's disease, Vogt-Koyanagi-Harada disease and sarcoidosis.
- This study aims to identify novel molecules involved in the pathogenic mechanisms of endogenous uveitis that could serve as potential targets for selective therapy.
- Analysis of the aqueous humor from patients with specific clinical entities of endogenous uveitis may help in generating novel biomarkers for different phenotypes of uveitis.

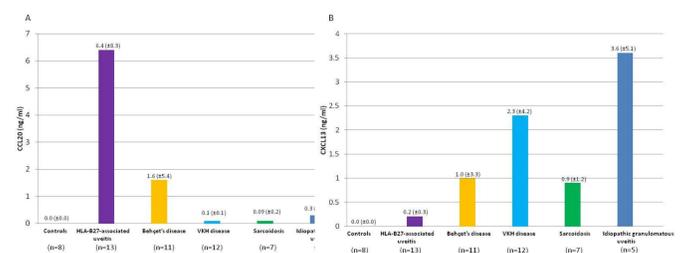
Patients & Methods: Paired serum samples (n=13) and aqueous humor samples (AH)(n=111) from patients with active idiopathic granulomatous uveitis (IGU) or with uveitis associated with HLA-B27-related inflammation. Behçet's disease (BD), Vogt-Koyanagi-Harada (VKH) disease or sarcoidosis and control patients were analyzed in two different multiplex assays.

Results

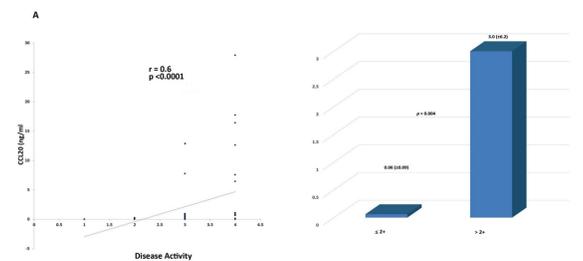
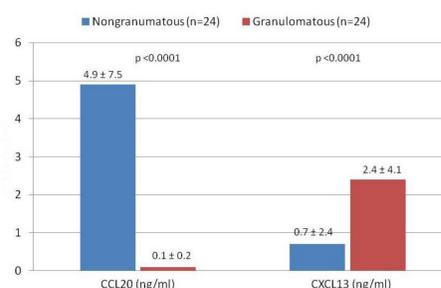
Analysis of aqueous humor from uveitis patients by chemokine Bio-Plex multiplex assay



Disease	No. of samples with detectable levels (%)	Levels detected (ng/ml) (Mean ± SD)	No. of samples with detectable levels (%)	Levels detected (ng/ml) (Mean ± SD)
• HLA-B27-associated uveitis (n=13)	13 (100)	6.4 ± 8.3	8 (61.5)	0.2 ± 0.3
• Behçet's disease (n=11)	8 (72.7)	1.6 ± 5.4	8 (72.7)	1.0 ± 3.3
• VKH disease (n=12)	7 (58.3)	0.1 ± 0.1	11 (91.7)	2.3 ± 4.2
• Sarcoidosis (n=7)	3 (42.9)	0.09 ± 0.2	5 (71.4)	0.9 ± 1.2
• Idiopathic granulomatous uveitis (n=5)	4 (80)	0.3 ± 0.3	5 (100)	3.6 ± 5.1
p-value (Kruskal-Wallis test)		0.001*		0.007*



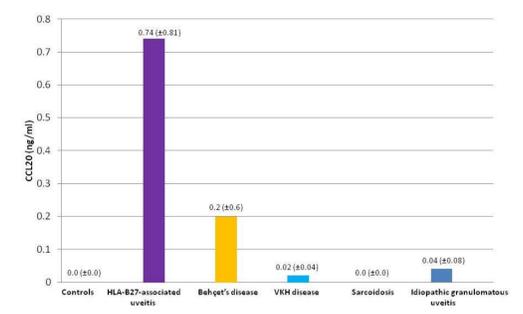
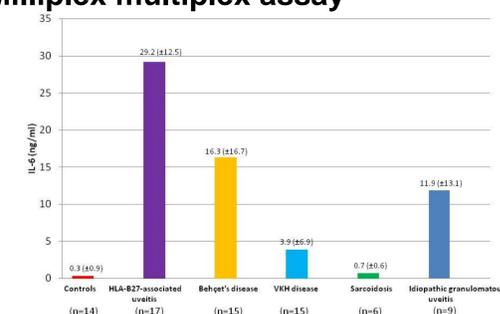
- CCL20 and CXCL13 were not detected in any of the AH samples from the control group.
- The levels of CCL20 in AH samples correlated significantly with the disease activity in all patients ($r=0.6$; $p<0.0001$), in patients with BD ($r=0.88$; $p<0.0001$) and in patients with nongranulomatous uveitis ($r=0.63$; $p=0.001$).
- A significant negative correlation was found between AH levels of CCL20 and CXCL13 in all patients ($r=0.38$; $p=0.008$).



Determination of cytokine and chemokine concentrations in aqueous humor samples from uveitis patients by Milliplex multiplex assay

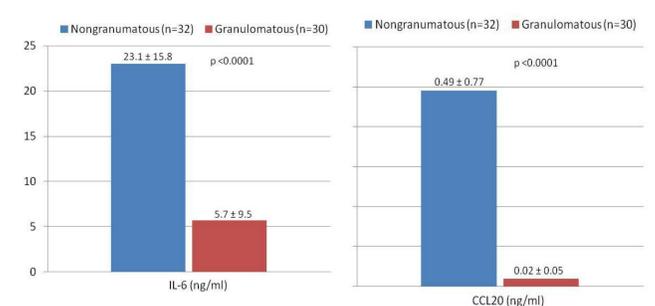
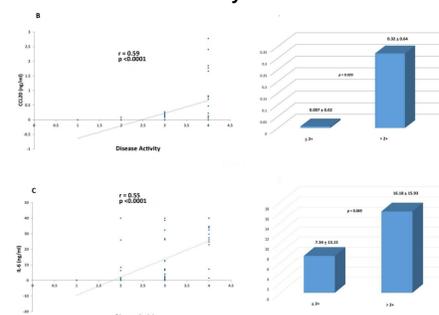
Summary data for CCL20 and IL-6 levels by Milliplex multiplex assay kit

Disease	No. of samples with detectable levels (%)	Levels detected (ng/ml) (Mean ± SD)	No. of samples with detectable levels (%)	Levels detected (ng/ml) (Mean ± SD)
• HLA-B27-associated uveitis (n=17)	13 (88.2)	0.74 ± 0.81	17 (100)	29.2 ± 12.5
• Behçet's disease (n=15)	3 (20)	0.2 ± 0.6	15 (100)	16.3 ± 16.7
• VKH disease (n=15)	4 (26.7)	0.02 ± 0.04	12 (80)	3.9 ± 6.9
• Sarcoidosis (n=6)	ND	ND	6 (100)	0.7 ± 0.6
• Idiopathic granulomatous uveitis (n=9)	2 (22.2)	0.04 ± 0.08	9 (100)	11.9 ± 13.1
p-value (Kruskal-Wallis test)		<0.0001*		<0.0001*



- The levels of IL-6 ($r=0.55$; $p<0.0001$) and CCL20 ($r=0.59$; $p<0.0001$) in AH samples correlated significantly with the disease activity in all patients.
- The levels of IL-6 correlated significantly with disease activity in patients with HLA-B27-associated uveitis ($r=0.58$; $p=0.014$).
- A significant positive correlation was found between aqueous humor levels of IL-6 and CCL20 in all patients ($r=0.69$; $p<0.0001$).

Correlations between IL-6 and CCL20 in aqueous humor levels and disease activity



Conclusions:

- Our findings suggest that the intraocular levels of cytokines and chemokines differ depending on the cause of uveitis.
- In the ocular inflammatory microenvironment of patients with endogenous uveitis, CCL20 might serve as a biomarker of inflammation in HLA-B27-associated uveitis, and the B cell chemoattractant CXCL13 might serve as a biomarker of uveitis associated with VKH disease.
- IL-6-driven immune responses are more potent in HLA-B27-associated uveitis and BD compared with VKH disease and sarcoidosis.
- Our findings also suggest that CCL20, CXCL13 and IL-6 could serve as excellent drug targets for the treatment of specific clinical entities of endogenous uveitis.