Glucocorticoid biomarkers of steroid response and disease activity in Vogt-Koyanagi-Harada disease

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Introduction

- Earlier immunomodulatory treatment is associated with better visual outcomes in refractory VKH patients.
- The evaluation of disease activity may be a challenge due to the known presence of subclinical activity in some clinically inactive VKH patients.
- The mechanisms of glucocorticoid (GC) are mediated by the GC receptor (GR) with two classical transcriptional isoforms: alpha isoform (GRα) and beta isoform (GRβ).
- GR-isoforms have been implicated in the mechanism of GC-refractoriness in other inflammatory diseases.

Objective

- To investigate the role of GR isoforms and MKP1 -a GC inducible gene- in peripheral blood mononuclear cells (PBMC) as a biomarker of GC-refractoriness and disease activity, in patients with VKH.

Patients & Methods

- Prospective cohort study of adult VKH subjects, with no other auto-immune disorders, cancer, pregnancy or systemic anti-inflammatory drugs within 1 month.
- Definition of GC-Refractoriness: Reactivation with an equivalent dose of prednisolone of 10 mg or more, during the first cycle of steroid treatment.
- Definition of reactivation: Anterior chamber cells and/or vitreous haze ≥ 1+ or presence of subretinal fluid/serous retinal detachment or findings compatible with active inflammation on ancillary testing (FFA, OCT, ICG).
- Treatment scheme: Prednisone 1mg/kg/day until controlling inflammation. Azathioprine was added if GC-refractoriness or GC-related side effects.
- After isolation using a Ficoll gradient protocol, PBMCs were cultured in complete RPMI + 10% Fetal Bovine Serum for 3 days.
- PBMCs were stimulated with Dexamethasone 1µM the last 6 of culture.
- PBMCs of healthy donors (HD) were isolated and stimulated with CD3/CD28 (5 µg/ml), LPS (10 ng/ml) and PHA (5 µg/ml) for 24 hrs.
- Biomarkers: Quantitative RT-PCR was used to evaluate mRNA levels of GRα, GRβ, and MKP-1.

Results

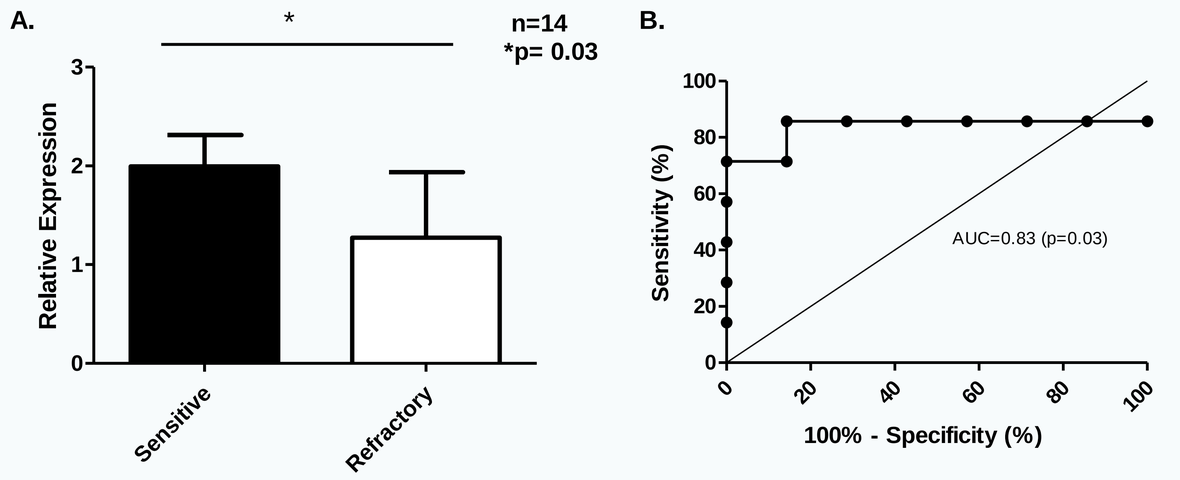
Table 1. Demographics and clinical characteristics of VKH patients (n=20)

Mean age (SD) ,years	35.8±10.7
VKH diagnosis, n (%)	
Probable	6 (30.0)
Incomplete	10 (50.0)
Complete	4 (20.0)
Clinical inflammation, n (%)	
Active	7 (35.0)
Inactive	13 (65.0)
Treatment response, n (%)	
GC-sensitive	10 (50.0)
GC-refractory	9 (45.0)
Unknown*	1 (5.0)

SD= standard deviation; VKH= Vogt-Koyanagi-Harada disease; GC=Glucocorticoid
*Patients who had Immunomodulatory Therapy as a first-line treatment.

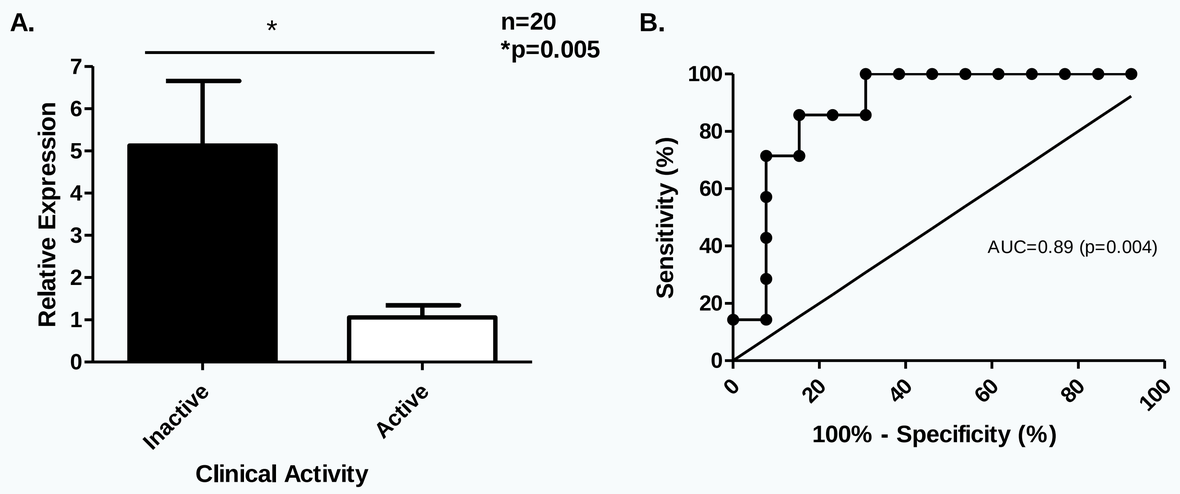
Results (cont'd)

Glucocorticoid Receptor α as a potential biomarker of treatment response



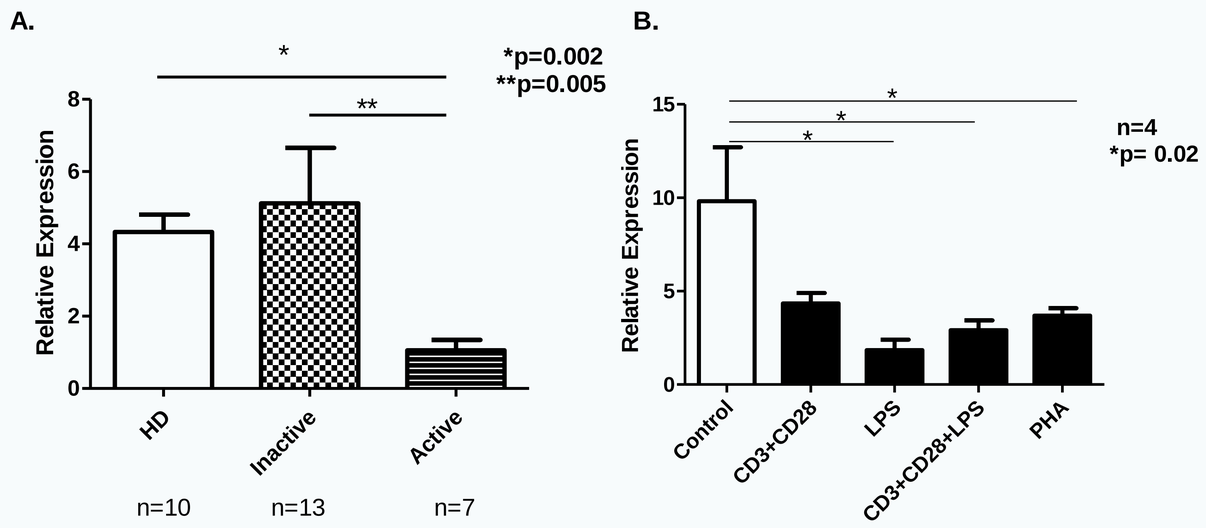
A, Bar graph showing the change in the expression of Glucocorticoid Receptor α (GRα) after 6 hours of *in vitro* stimulation with Dexamethasone 1µM in PBMC of patients with VKH. The cohort was divided in sensitive and refractory patients, considering the clinical response to GC. Data are presented as the mean ± standard deviation (Mann Whitney test, *p=0.03). **B,** Receiver Operating Characteristic curve evaluating the performance of the evaluation of GRα to predict GC response. AUC=Area under the curve.

MKP-1 as a potential biomarker of disease activity



A, Bar graph showing the change in the expression of MKP-1 after 6 hours of *in vitro* stimulation with Dexamethasone 1µM in PBMC of patients with VKH. The cohort was divided in inactive and active patients. Data are presented as the mean ± standard deviation (Mann Whitney test, *p=0.005). **B,** Receiver Operating Characteristic curve evaluating the performance of the evaluation of MKP-1 to determine disease activity. AUC=Area under the curve.

Role of inflammation in the expression of MKP-1 in healthy donors



A, Bar graph showing the change in the expression of MKP-1 after 6 hours of *in vitro* stimulation with Dexamethasone 1µM in PBMC of HD. A comparison with the data regarding active and inactive VKH patients is included. Data are presented as the mean ± standard deviation (Mann Whitney test, *p=0.002 and **p=0.005). **B,** Bar graph showing the change in the expression of MKP-1 after 6 hours of Dexamethasone 1µM in PBMC of HD, pre-stimulated with CD3/CD28, LPS, LPS plus CD3/CD28, and PHA. Data are presented as the mean ± standard deviation (Mann Whitney test, *p=0.02).

Conclusion

- The evaluation of the expression of GR isoforms and MKP1 as potential biomarkers of treatment response and disease activity can contribute to the early identification of GC-refractoriness and subclinical inflammation in VKH patients.

References

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