Analysis of Autoantibody Signatures of Neuromyelitis Optica using Protein Arrays

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Introduction

- Neuromyelitis optica (NMO) and Multiple sclerosis (MS) are inflammatory, demyelinating diseases of the central nervous system. It is important to distinguish NMO from MS, as the course of untreated NMO is more progressive than the course of MS and requires therefore early diagnosis and therapy.
- The diagnosis of both NMO and MS is based on clinical symptoms and imaging techniques. Additionally, a clinically useful blood based biomarker is established for NMO. Autoantibodies directed against the water channel protein aquaporin 4 (AQP4) are present in approximately two thirds of NMO patients, whereas one third of NMO patients are AQP4 sero-negative.
- The goal of this study is to identify novel NMO-specific autoantibodies which can improve the diagnostic accuracy of the anti-AQP4 antibody test. For this purpose we have characterized the autoantibody signatures of AQP4 sero-negative NMO, AQP4 sero-positive NMO and MS patients. In order to identify autoantibodies with differential abundance in serum samples we have compared the autoimmune signatures of NMO and MS patients.

Conclusions

- Using the SeroTag® process we have identified antigen/autoantibody interactions with differential abundance in serum samples of NMO and MS patients. These antigens are candidates for the development of novel diagnostic tests for NMO.
- The autoantibody signatures of AQP4 sero-negative NMO patients were investigated and marker panels were identified allowing the discrimination of AQP4 sero-negative NMO patients from MS patients.
- Additional studies are necessary to further validate the identified marker panels. Therefore, we are currently seeking for partners who can provide an appropriate cohort of AQP4 sero-negative and AQP4 sero-positive NMO patients as well as MS patients.

Technology

The SeroTag® process (Fig. 1) allows the discovery and validation of human autoimmune signatures. SeroTag® utilizes several thousand affinity-purified human antigens to generate bead-based antigen arrays, which are used to identify autoantibodies present in serum samples. A bead-based array comprises up to 500 different antigens immobilized on color-coded microspheres. Consequently, 500 antigen/autoantibody interactions can be screened simultaneously in one measurement. In a study, typically serum samples of two different patient groups are analyzed by comparing autoimmune signatures. This allows the identification of differentially abundant autoantibodies in patient groups. Statistical algorithms are applied to determine the discriminative power of the identified antigen/autoantibody interactions.

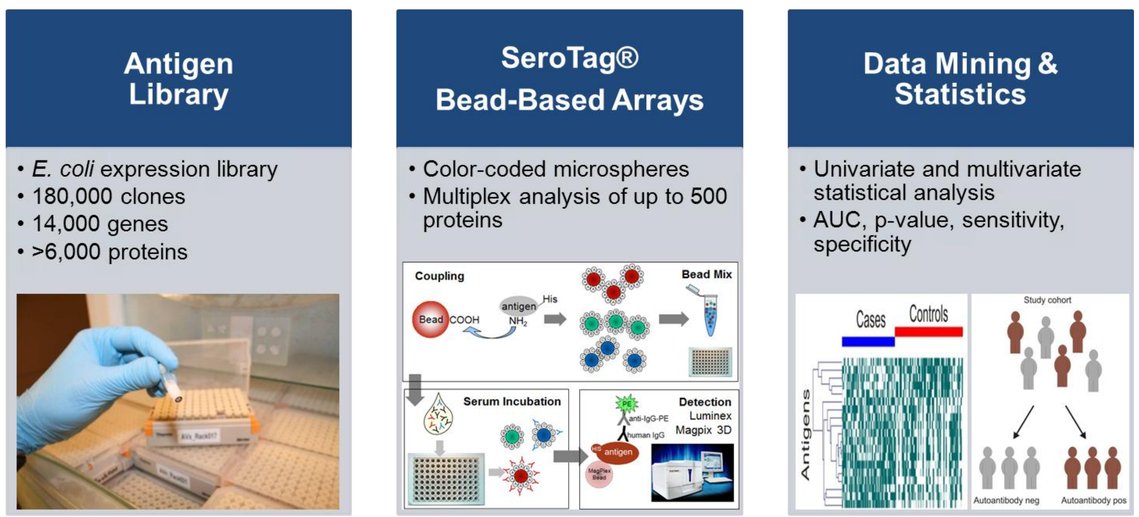


Fig. 1: SeroTag® Process

Study Design

In this study 5969 human antigens were incubated with 63 NMO and 68 MS serum samples to identify antigen/autoantibody interactions. Serum samples were obtained from three medical centers and all serum samples of NMO patients were tested for anti-AQP4 autoantibodies using either indirect immunofluorescence or enzyme immunoassays. Among the NMO group 30 patients were AQP4 sero-positive and 33 patients AQP4 sero-negative.

Table 1: Demographics of NMO and MS patients

Diagnosis group	N/Group	Sex	N/Sex	Age Median	Age Range
NMO	63	F	46	42	23-66
		M	17	52	38-72
MS	68	F	48	44	24-65
		M	20	44	30-64

Results

Selection of Antigens

Univariate and multivariate statistical analysis were performed to distinguish NMO from MS patients. Antigens that show 1.5 fold difference between groups and p-values <0.05 were selected by the univariate approach resulting in a total number of 62 candidates. The best performing single markers are depicted in Fig. 2.

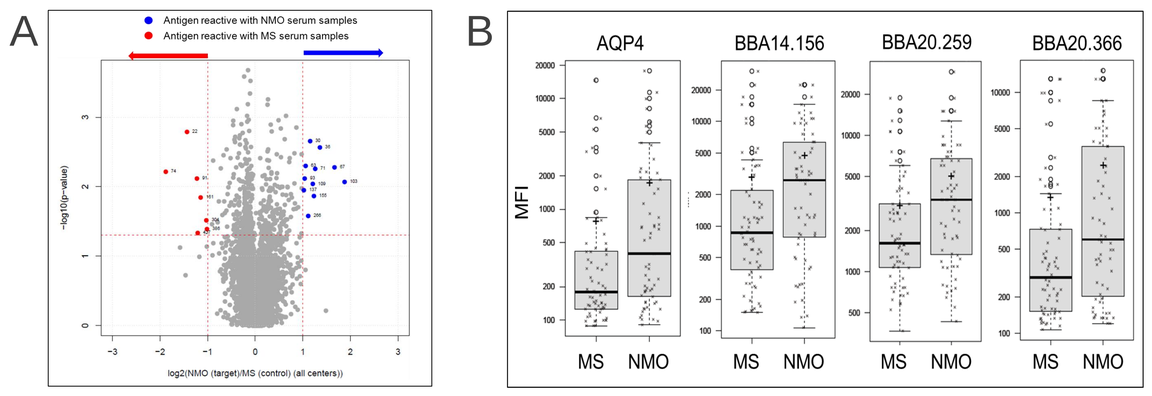


Fig. 2: Visualization of antigen reactivity. (A) In the volcano plot the reactivity of NMO serum samples is compared to MS serum samples for 5969 antigens. The ratio of the antigen reactivity is plotted against the p-value. (B) Box-and-Whisker-plots depicting signal intensities of best performing antigens.

Powered partial least square discriminant analysis (PPLS-DA) and random forest analysis (RF) were applied as multivariate approaches to analyze the data set. Both algorithms were used to rank antigens according to their frequency during feature selection procedure. Antigens that were selected more than 32 times (max. 100 times) by both algorithms were assumed to be suited for the discrimination of NMO and MS patients resulting in a total number of 48 antigens using PPLS-DA and 35 antigens using RF, respectively (Fig. 3A and 3B).
Antigens selected by the three different statistical approaches (univariate approach, PPLS-DA and RF) were merged and a unique set of 105 antigens was obtained. A PPLS-DA model was applied to this antigen set and revealed a clear discrimination of NMO from MS patients (Fig. 3C).

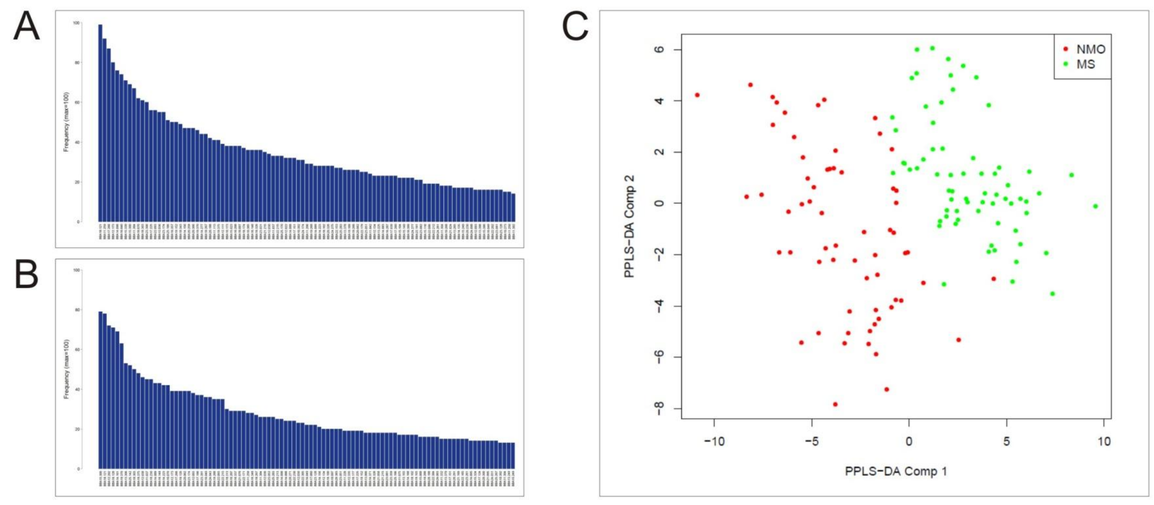
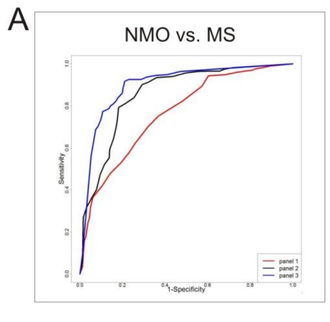


Fig. 3: Selection of antigens. (A) Frequency of selected antigens using PPLS-DA. (B) Frequency of selected antigens using RF. (C) Score plot of PPLS-DA model based on 105 antigens selected by univariate and multivariate approaches.

Definition and Performance of Antigen Panels

For further reduction of the antigen set a logistic regression model with feature selection was used to define smaller antigen panels. Receiver operating characteristics (ROC) analysis were used to calculate the classification performance of the antigen panels to discriminate NMO from MS patients. In addition, antigen panels were determined for the classification of AQP4 sero-negative NMO and MS patients. Thereby, novel marker candidates were identified which may improve the diagnosis of NMO.



C

NMO vs. MS

	AUC	CI(AUC)	Sens.	CI(Sens.)	Spec.	CI(Spec.)
Panel I (N=4)	0.765	[0.714, 0.816]	0.609	[0.440, 0.779]	0.767	[0.709, 0.825]
Panel II (N=7)	0.868	[0.834, 0.902]	0.810	[0.636, 0.983]	0.831	[0.730, 0.933]
Panel III (N=13)	0.928	[0.873, 0.983]	0.840	[0.785, 0.896]	0.800	[0.662, 0.939]

AQP4 sero-negative vs. MS

	AUC	CI(AUC)	Sens.	CI(Sens.)	Spec.	CI(Spec.)
Panel I (N=5)	0.863	[0.805, 0.921]	0.631	[0.339, 0.924]	0.912	[0.810, 1.000]
Panel II (N=9)	0.879	[0.619, 1.000]	0.792	[0.490, 1.000]	0.843	[0.589, 1.000]
Panel III (N=13)	0.866	[0.699, 1.000]	0.817	[0.776, 0.859]	0.871	[0.694, 1.000]

Fig. 4: Classification performance of antigen panels. (A) ROC curves for classification of NMO and MS patients. (B) ROC curves for classification of AQP4 sero-negative NMO and MS patients. (C) Calculated classification values for antigen panels.