M23 isoform is superior to M1 for detecting AQP4-IgG seroreversion during NMOSD

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Abstract

Antibodies against AQP4 (AQP4-IgG) are present in more than 80% of patients with Neuromyelitis Optica spectrum disorder (NMOSD) and play an important role in disease pathophysiology. Lesions in AQP4-positive NMOSD patients are characterized by mixed immune cell infiltrates as well as IgG and complement deposition around the blood vessels[1,2,3].

Importantly, and described in detail in recent papers, some patients with AQP4-positive NMOSD may experience fluctuations in serum AQP4-IgG levels, including seroreversion (loss of detectable antibodies) and eventual reconversion (the reappearance of detectable antibodies) during the disease course. The clinical relevance of these processes is still under debate[4,5].

Here, we investigated the potential differences in defining seropositivity, seroreversion and reconversion based on the detection of AQP4-IgG to the M23- and M1- AQP4 isoforms in NMOSD patients.

Methods

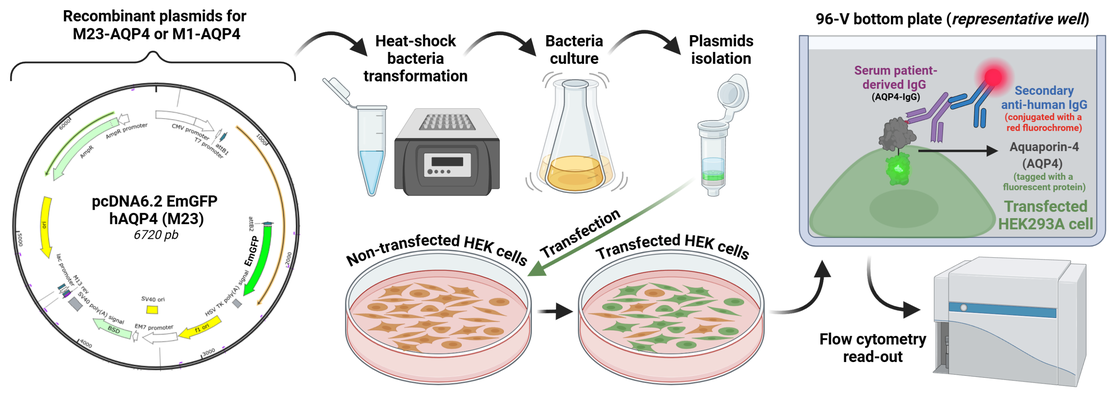

Figure 1. Methods for obtaining M23-AQP4 and M1-AQP4 transfected cells for the investigation of AQP4-IgG in serum samples using the live cell-based assay (CBA).

Human embryonic kidney cells (HEK293A) were used for the transient transfection with the plasmids pcDNA6.2 EmGFP hAQP4 (M23) and pcDNA6.2 EmGFP hAQP4 (M1). Serum samples collected longitudinally from 3 AQP4-positive NMOSD donors (below) were diluted 1:50 and incubated with the transfected cells, followed by anti-human IgG secondary antibody for detecting cell-bound AQP4-IgG. Samples were acquired through flow cytometry (FACS-CBA).

Results

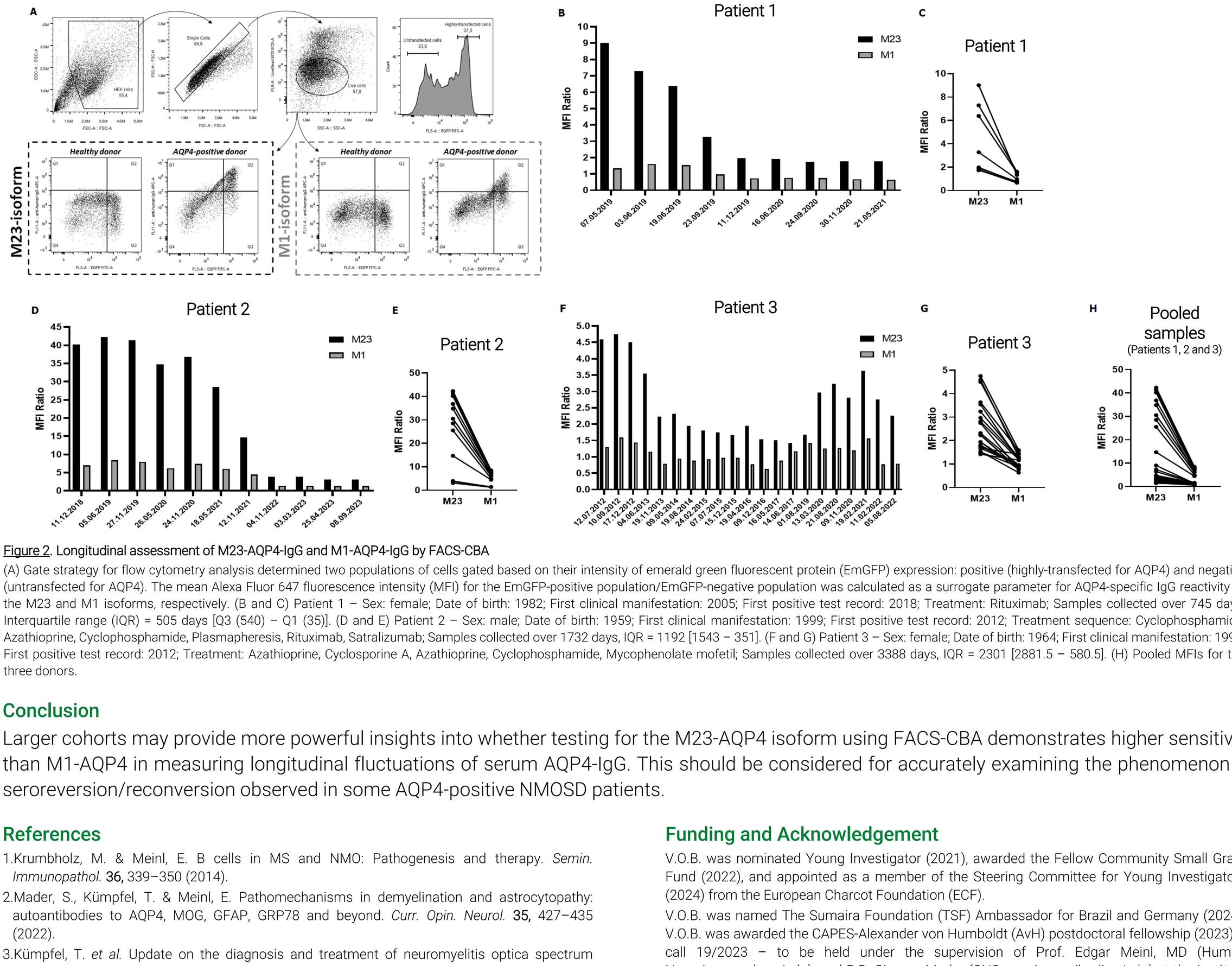

Figure 2. Longitudinal assessment of M23-AQP4-IgG and M1-AQP4-IgG by FACS-CBA

(A) Gate strategy for flow cytometry analysis determined two populations of cells gated based on their intensity of emerald green fluorescent protein (EmGFP) expression: positive (highly-transfected for AQP4) and negative (untransfected for AQP4). The mean Alexa Fluor 647 fluorescence intensity (MFI) for the EmGFP-positive population/EmGFP-negative population was calculated as a surrogate parameter for AQP4-specific IgG reactivity to the M23 and M1 isoforms, respectively. (B and C) Patient 1 – Sex: female; Date of birth: 1982; First clinical manifestation: 2005; First positive test record: 2018; Treatment: Rituximab; Samples collected over 745 days, Interquartile range (IQR) = 505 days [Q3 (540) – Q1 (35)]. (D and E) Patient 2 – Sex: male; Date of birth: 1959; First clinical manifestation: 1999; First positive test record: 2012; Treatment sequence: Cyclophosphamide, Azathioprine, Cyclophosphamide, Plasmapheresis, Rituximab, Satralizumab; Samples collected over 1732 days, IQR = 1192 [1543 – 351]. (F and G) Patient 3 – Sex: female; Date of birth: 1964; First clinical manifestation: 1996; First positive test record: 2012; Treatment: Azathioprine, Cyclosporine A, Azathioprine, Cyclophosphamide, Mycophenolate mofetil; Samples collected over 3388 days, IQR = 2301 [2881.5 – 580.5]. (H) Pooled MFIs for the three donors.

Conclusion

Larger cohorts may provide more powerful insights into whether testing for the M23-AQP4 isoform using FACS-CBA demonstrates higher sensitivity than M1-AQP4 in measuring longitudinal fluctuations of serum AQP4-IgG. This should be considered for accurately examining the phenomenon of seroreversion/reconversion observed in some AQP4-positive NMOSD patients.

References

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