FLOW CYTOMETRIC SCREENING OF HLA-B17 Ag IN HIV+ PATIENTS UNDERGOING ABACAVIR THERAPY

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BACKGROUND

The Abacavir Hypersensitivity Syndrome (AHS) is a life-threatening side effect that can occur in HLA-B*5701+ HIV positive patients treated with Abacavir. Every HIV positive patient eligible for Abacavir therapy must be therefore screened for the presence of the HLA-B*5701 allele and treated only if negative. The B*5701 allele is a member of the HLA-B17 family. HLA-B *5701 typing is mostly based on molecular methods, that are expensive and require a median of 21 days for processing.

In this study we have developed a rapid dual-color Flow Cytometric (FC) assay, including anti-B17 monoclonal antibody that provides a cheap and sensitive screening for putative HLA-B*5701+ patients (**Figure 1).**

METHODS

21 HIV positive patients already SSO-typed for HLA-B*5701 served as positive (6) or negative (15) controls, respectively. Other 437 HIV positive patients were prospectively evaluated for HLA-B17 by FC and their outcome during Abacavir treatment was monitored.

Briefly, 50 μL of EDTA blood were stained with 10 μL of unconjugated IgM monoclonal anti-B17 antigen (OneLambda) in a stain-lyse-and-wash procedure. A secondary PE- anti-mouse IgM was used for indirect immunofluorescence, with anti-CD3 FITC counterstaining. Isotype cold IgM and secondary PE conjugate (RPE-GAM) were used as negative controls. The staining intensity of anti-B17 PE expression on T cells was considered to calculate the reaction cutoff, which was used to discriminate positive and negative cases (**figures 2 and 3**)

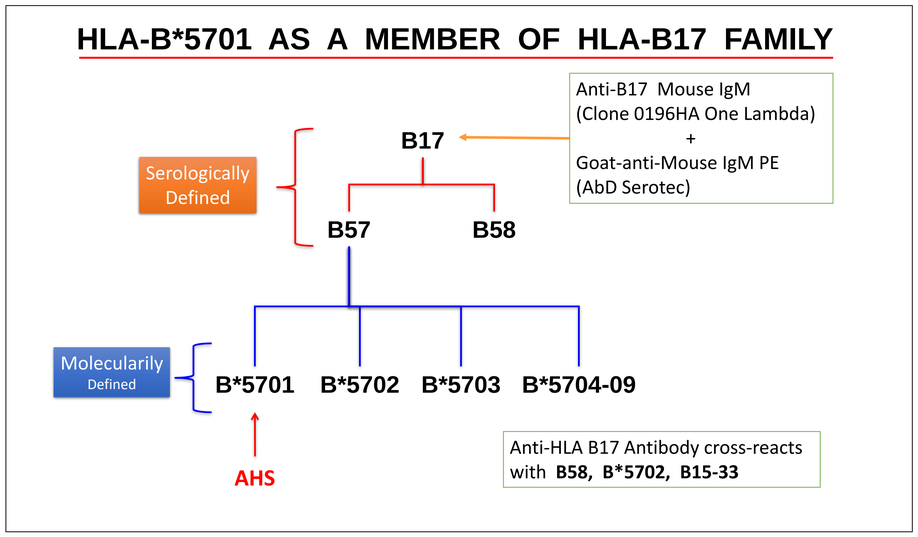

Figure 1: Schematic representation of HLA-B17 family. The Abacavir Hypersensitivity Syndrome (**AHS**) can occur only in HLA-B*5701+ HIV positive patients treated with Abacavir.

RESULTS

The agreement between SSO typing and FC assay in the controls was 6/6 for double-positives; one false-positive FC case was due to the cross-reacting antigen B*5702; whereas 14/14 cases were double negatives (**Table1**). Of the prospective 437 cases, 43 (10%) resulted positive for anti-B17, as expected. In 28/43 cases a confirmatory molecular test for HLA-B*5701 allele was performed, which disclosed the B*5701 in 11 patients. In the other 17 cases different alleles of the B17 family were detected, that did not prevent Abacavir therapy (**Table 2**). None of the 394 FC B17-negative patients developed AHS during Abacavir administration.

CONCLUSIONS

Since this test may be performed in less than two hours, FC assay is a rapid method to evaluate the HLA-B17 phenotype in HIV positive subjects eligible to Abacavir therapy. FC assay proved reliable to safely screen out HLAB*5701-negative subjects, that as reported in literature and as confirmed by our data, represent the majority of cases. Its prospective use allows significant saving of time and money, since it can restrict the confirmative molecular HLAB*5701 typing to the small group of FC positive individuals, that represent only the 10% of tested cases.

References

[1] Small CB, Margolis DA, Shaefer MS and Ross LL. HLA-B*57:01 allele prevalence in HIV infected North American subjects and the impact of allele testing on the incidence of Abacavir-associated hypersensitivity reaction in HLA-B*57:01-negative subjects. BMC Infectious Diseases 2017; 17(1): 256.

[2] Negrini S, Becquemont L. HLA-associated drug hypersensitivty and the prediction of adverse drug reactions. Pharmacogenomics 2017; 18(15): 1441-1457.

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Figure 2: HLA-B*5701 positive case. (**a**) T CD3 positive cells were analysed. (**b**) Isotype cold IgM and secondary PE conjugate (RPE-GAM) were used as negative controls. (**c**) High staining intensity of anti –B17 PE expression identified positive case

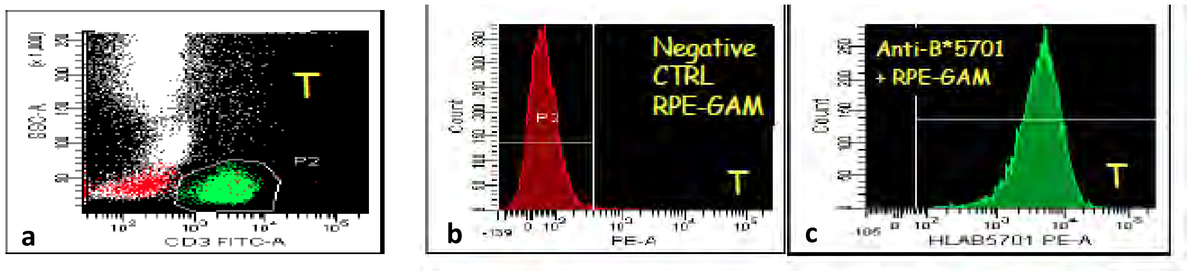

Figure 3:HLA-B*5701 negative case.
(d) Low staining intensity of anti –B 17 PE expression identified negative case

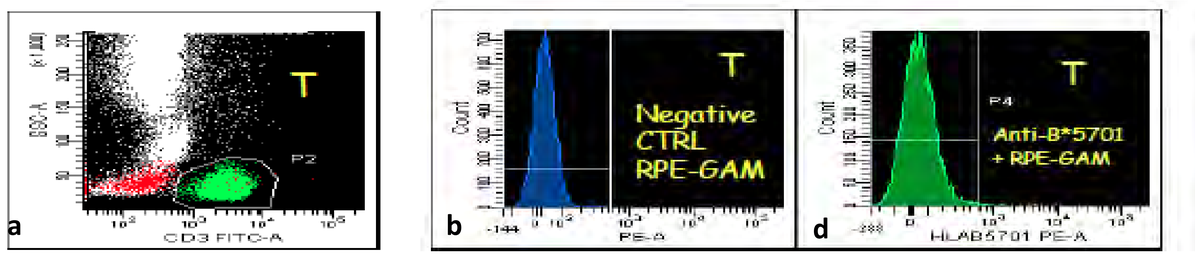

Table 1: Retrospective comparison of HLA-B*5701 SSO typing and FC assay (anti-B17) in a control group of 21 HIV positive patients.

Control Group (21 HIV +)	Positive SSO typed HLA-B*5701	Negative SSO typed HLA-B*5701
Positive anti B 17	6	1
Negative anti B 17	0	14

Table 2: Prospective comparison of SSO typing for HLA-B*5701 and FC assay (anti-B17) in a cohort of 437 HIV positive patients.

437 HIV+	Positive SSO typed HLA-B*5701	Negative SSO typed HLA-B*5701	Not performed SSO typed HLA-B*5701
Positive anti B 17	11	17	15
Negative anti B 17	0	0	394