Crossmatch Alarm, Liver Charm:
A strong positive crossmatch complemented by more testing

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Case Description

A 54-year-old female with cirrhosis was waitlisted for a liver transplant. Although sensitizing events are not routinely noted for liver transplant candidates at our center, pre-transplant antibody screening showed strong HLA class I and II antibody reactivity (Fig. 1a, b), suggesting this patient was highly sensitized. At the time of transplant, the retrospective flow cytometric crossmatch (FCXM) unsurprisingly detected strong positive reactivity to both T and B donor lymphocytes. However, the number of events within the T and B cell gates were much lower than those captured in the control wells, with many of the $CD3^+$ and $CD19^+$ events appearing below the expected forward scatter (FSC) parameters (Fig. 1C). This finding suggests that a component of the patient's sera may have contributed to *in vitro* cell death and prompted additional investigation into the cause of this phenomenon. Single antigen bead (SAB) testing confirmed the presence of high levels of class I and II donor specific antibodies (DSA) in the immediate pre-transplant serum (Fig. 1d, e). Although there were no immediate immunological concerns from the clinical program, additional FCXM and SAB testing of the one-day post-transplant sample was performed to assess the dynamic of DSA in the patient's sera.

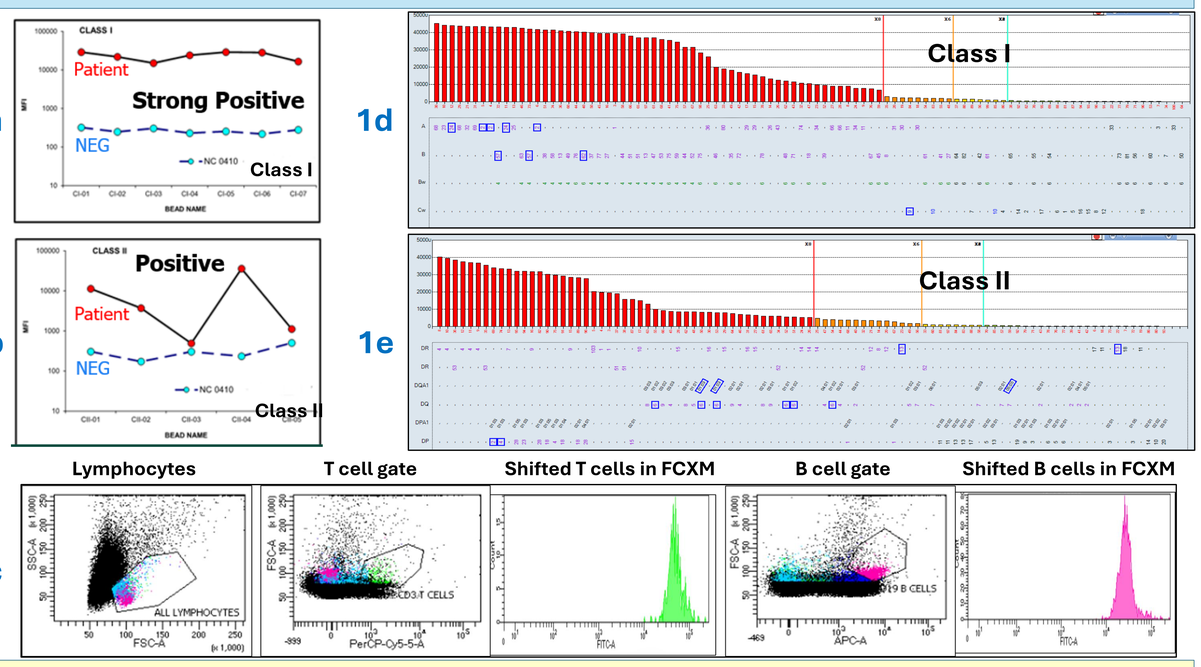

Figure 1. High pre-transplant anti-HLA antibodies levels resulted in a strong positive XM. Antibody screening was (strong) positive for anti-HLA class I (a) and II (b) antibodies. FCXM showed strong positive-T and B cell reactivity, but cell viability was very low (c). SAB detected anti-HLA antibodies including DSA at very high mean fluorescent intensity (MFI) levels (d, e). Blue boxes in "d" and "e" indicate DSA.

Methods

FCXM was performed using the Halifaster Protocol and samples were analyzed on the Beckman Coulter Cytoflex instrument. HLA antibody screening and SAB were performed using LIFECODES® LifeScreen Deluxe Kit (Werfen-Immucor GTI Diagnostics, Inc.) and LabScreen™ (One Lambda, Thermo Fisher Scientific) kits, respectively, per manufacturers' instructions with Luminex Flexmap 3D.

Results

Based on a hypothesis that the likely cell death in the FCXM was due to complement-mediated lysis, the lab requested a one-day post-transplant sample and re-ran the FCXM for pre- and post-transplant sera with and without EDTA treatment. In addition, SAB was performed on 1-day, 1-month, 3-month, and 1-year post-transplant samples to monitor the dynamic of DSA in the patient's sera.

A clear increase in viable cells was observed when pre-transplant serum was treated with EDTA, suggesting that complement might have mediated cell lysis in original FCXM (Fig. 2a). Viable lymphocytes were detected in the one-day post-transplant serum regardless of EDTA treatment. Moreover, the strength of the crossmatch reactivity was reduced for both T and B cells in the one-day post-transplant sample compared to the pre-transplant sample (Fig. 2b).

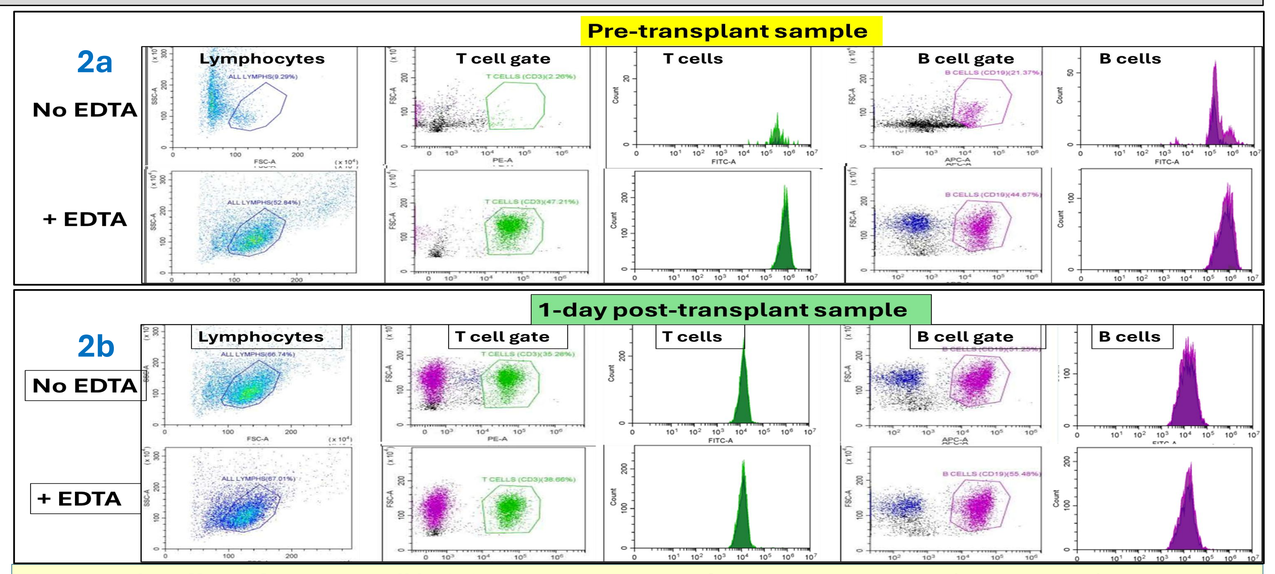

Figure 2. The effect of EDTA on cell lysis in FCXM assay. Use of EDTA in FCXM assay demonstrated an increase in the donor cell viability, suggesting a potential role of complement-mediated lysis (a). The cell viability in the one-day post transplant serum was acceptable regardless of EDTA treatment (b).

Results (Continued)

SAB testing of the one-day post-transplant sample demonstrated a notable drop in anti-HLA antibodies, suggesting the ability of the grafted liver to clear the antibodies (Fig. 3a, b). The level of anti-HLA antibodies including DSA continued to decline at 1-month, 3-month, and 1-year post-transplant (Fig. 4) with no sign of graft dysfunction.

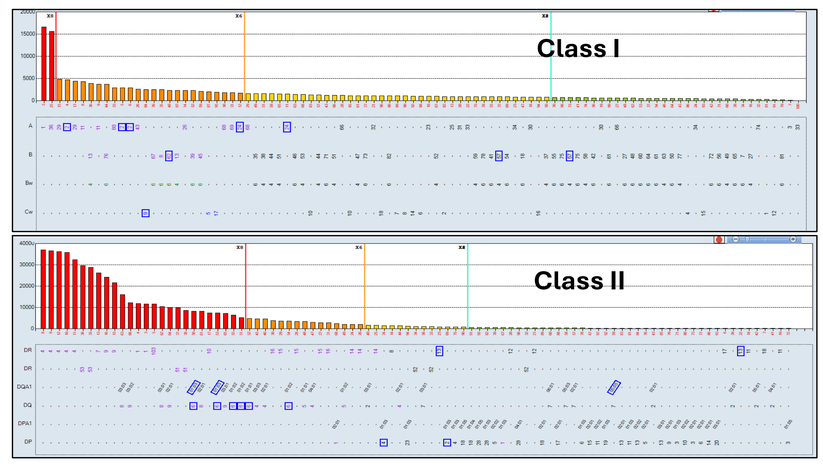

Figure 3. Reduction of anti-HLA antibodies one-day post-transplant. The SAB testing of one-day post-transplant sample showed decreased levels of all anti-HLA class I (a) and class II (b) antibodies including DSA. Blue boxes indicate DSA.

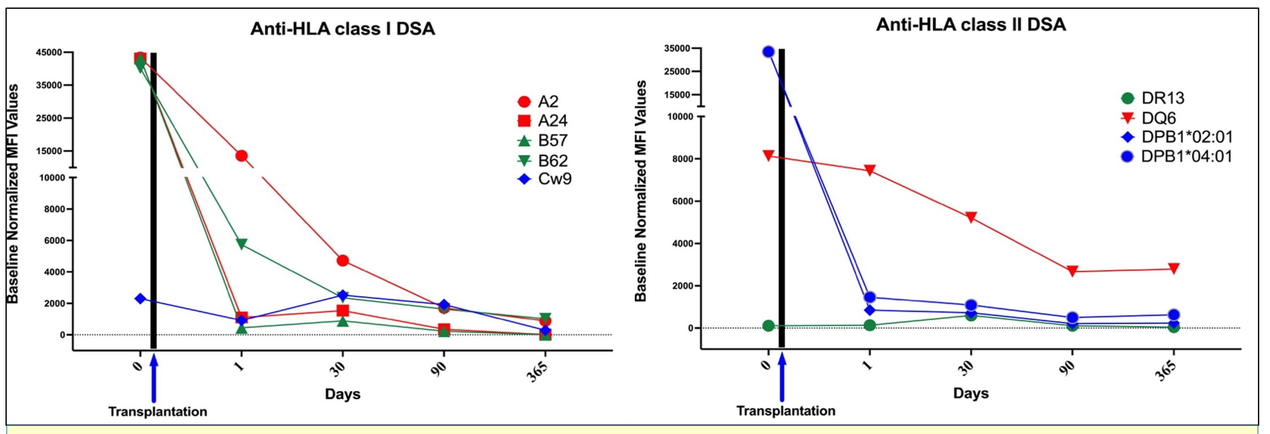

Figure 4. Dynamics of DSA in patient's sera overtime. The MFI is shown for the beads with the most likely donor allele.

Conclusions

This case demonstrates that high DSA levels in pre-transplant samples can reduces donor cell viability in FCXM. This effect can be mitigated by EDTA treatment of the sera.

This case also highlights the liver's unique capability to rapidly reduce all anti-HLA antibodies including DSA post-transplant. However, whether these strong antibody levels might still have a detrimental effect on the liver affecting long-term function is not well understood and further research in the liver protective function towards high DSA levels and effect on clinical outcome is warranted.