Characterization of complement system activity in patients with diffuse large B-cell lymphoma (DLBCL)

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a clinically heterogeneous lymphoid malignancy and the most common type of aggressive non-Hodgkin's lymphoma in adults.

One of the treatments used for DLBCL is immunotherapy, which activates complement-mediated-cytotoxicity (CDC) and other mechanisms. CDC activation depends on several factors, including the activity of the complement classical pathway (CP).

HYPOTHESIS AND AIMS

- We hypothesize that the CP in DLBCL is chronically activated by IgG-aggregates as in chronic lymphocytic leukemia (CLL) patients. Thus, the CP activity is decreased due to exhaustion.
- Our aim was to investigate the activity of the complement system and the CDC efficacy in DLBCL patients, and to understand the mechanism of their activity in comparison with normal control (NC) subjects and CLL patients.

METHODS

- Blood samples were collected from 16 normal controls (NC) and 16 naïve DLBCL patients.
- Ig-C5a (a marker of chronic activation) was studied by Western analysis.
- Activities of the CP and Alternative pathway (AP) were followed by C5b-9 levels (the final product of complement activation), and the levels of the Bb and C4d fragments were quantified by ELISA.
- CDC assay was performed using a DLBCL cell line (SU-DHL-4 and SU-DHL-5). The cells were incubated for 12 hr with rituximab (RTX, Mabthera®, 1µg/ml) and 2% human serum as the source of complement. Cell viability was determined by Cell Counting Kit-8, and results were expressed relative to the untreated control cells.

RESULTS

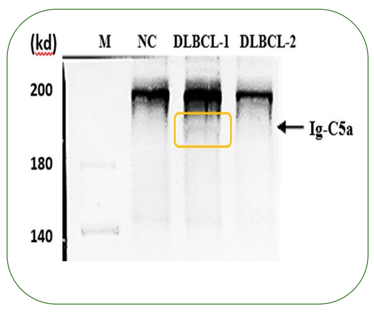

Fig .1: Western analysis showing the Ig-C5a complex. Ig-C5a was found in 66% of DLBCL patients compared to <10% in NC.

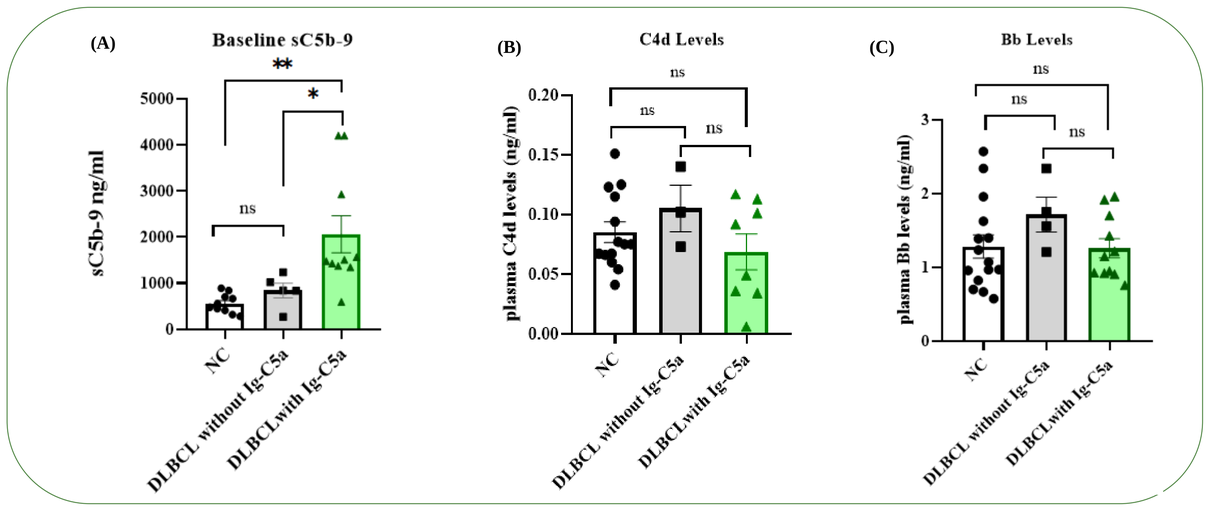

Fig. 2: **The baseline levels of complement activity markers in DLBCL sera.** Compared to NC, the basal levels (without activation) of soluble sC5b-9 were significantly increased in patients with Ig-C5a (A) but there were no differences in the levels of C4d (B) and Bb (C).

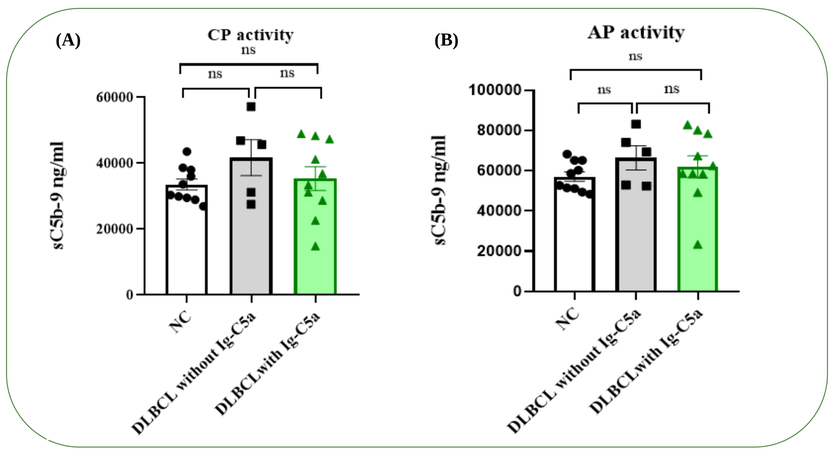

Fig. 3: **Activity of CP and AP.** There were non-significantly different in activity of the CP (A) and AP (B**)** between NC and DLBCL sera.

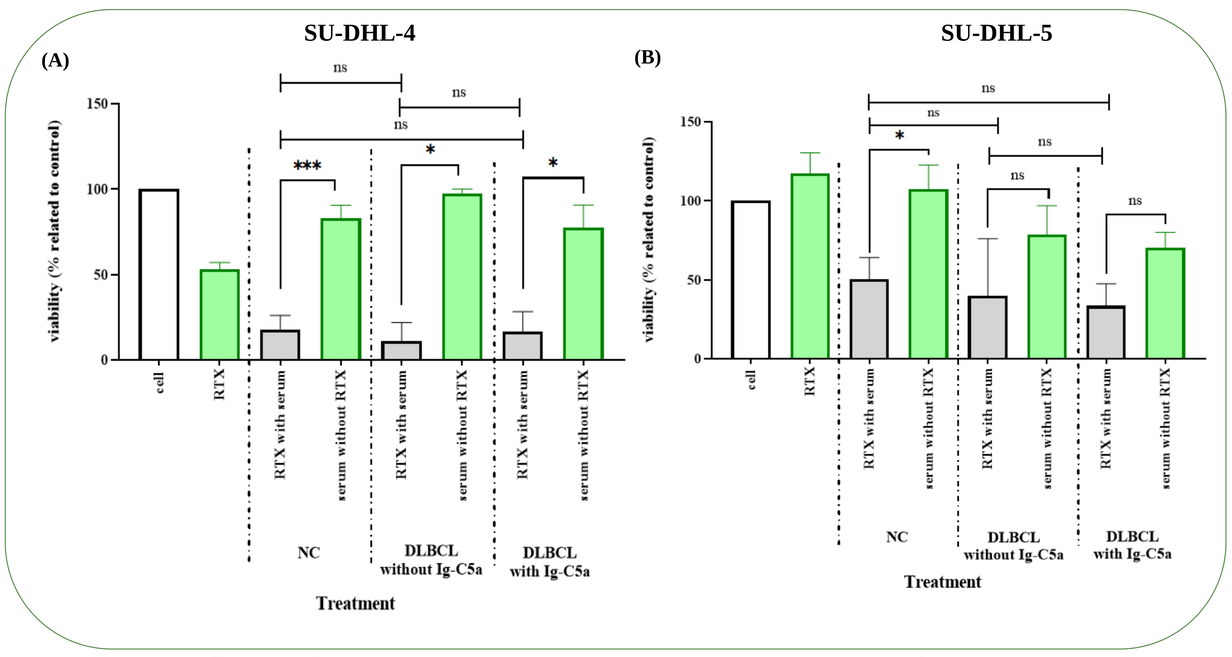

Fig. 4: **CDC efficacy of DLBCL sera**. CDC activity in DLBCL patients before therapy was similar compared to NC in both cell lines. When the DLBCL patients and NC were compared with and without RTX in both cell lines.

SUMMARY AND CONCLUSION

- In 66% of the DLBCL patients Ig-C5a was present and the baseline levels of sC5b-9 were increased, however, this chronic CP activation did not decrease the CP activity. In addition, there were no differences in the baseline of Bb and C4d levels between NC and DLBCL sera.
- CDC activity in DLBCL patients was similar (or superior) to NC, with and without RTX , in the two cell lines that differ in CD20 expression.
- **The chronic activation in DLBCL patients does not cause a decrease in CP activity and does not impair the CDC.**