# The role of Alpha 2 Macroglobulin in IgG-aggregation and chronic complement activation in patients with chronic lymphocytic leukemia


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## BACKGROUND

- Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the western world.
- In sera of some CLL patients (~40%) the complement (C) system is chronically activated at a low level, via the classical pathway (CP).
- Chronic CP activation involves the formation of IgG-hexamers (IgG-aggregates), occurring only after antigen binding, via specific interactions between the Fc regions of the IgG monomers.

## OBJECTIVES

Our goals were to study:

- The formation of IgG-hexamers in CLL patients and normal controls.
- The hexamers incidence as cell-free and cell-bound forms.
- The C activation capacity of the hexamers.
- The identity of the antigen triggering the hexamerization.
- The levels of the antigen in CLL sera.

## METHODS

- Blood samples were collected from 66 naïve CLL patients and 27 normal controls (NC).
- Biochemical and haematological parameters, and CLL staging were recorded.
- Sera IgG-aggregates were separated, measured and used for assessment of C activation capacity.
- The occurrence of IgG-aggregates on blood cells was studied by flow cytometry.
- The antigen was separated by SDS-PAGE, identified by mass spectrometry and verified by Western blot analysis.
- The serum levels of the antigen were measured by ELISA.


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## RESULTS

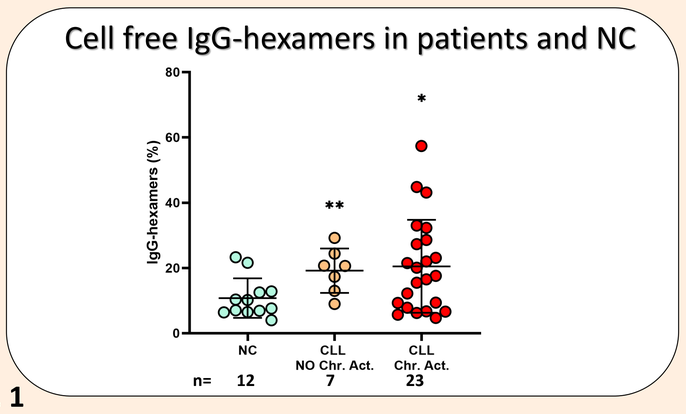


**Fig. 1**
IgG-hexamers were purified and their percentage was calculated in sera/plasma of NCs, patients with chronic C activation (Chr. Act) and patients without chronic C activation (No Chr. Act). * p<0.04; ** p = 0.004, vs NC.

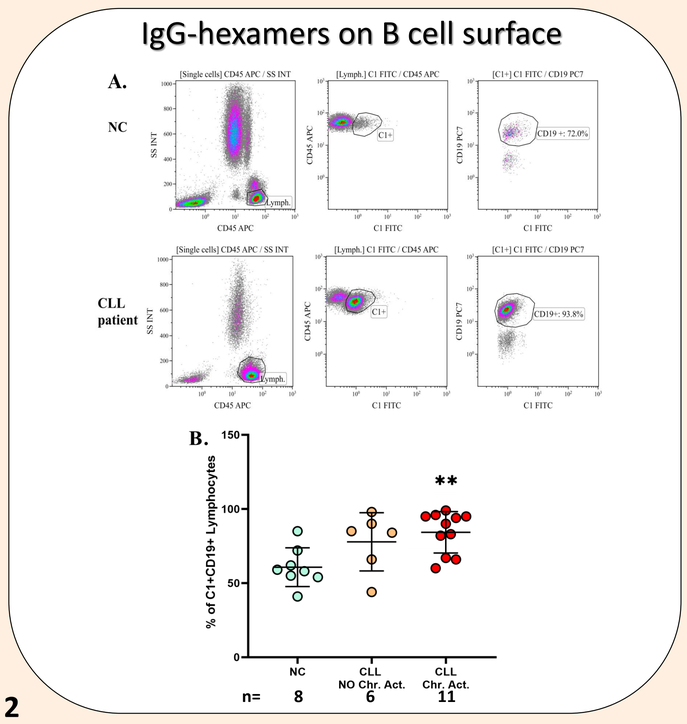


**Fig. 2**
Blood samples were stained with fluorescent antibodies against CD45, CD19 and C1, and analyzed by flow-cytometry, with gating on lymphocytes. Representative results (A) and data summary (B) are shown. ** p = 0.001 vs. NC.

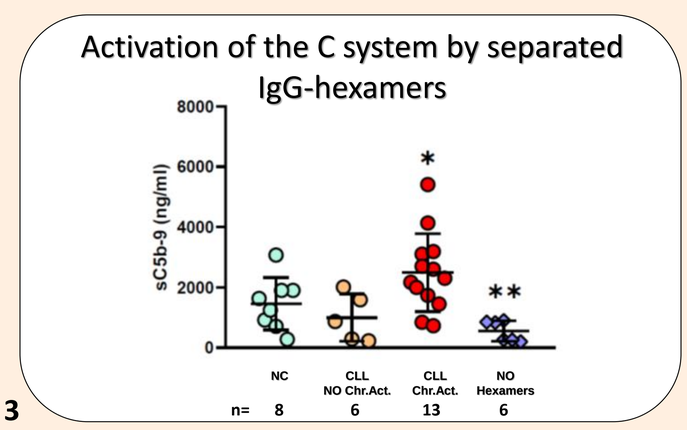


**Fig. 3**
The capability of IgG-hexamers to activate C was studied by incubation with normal serum, followed by measurement of sC5b-9 levels (C activation marker). Sera that were incubated with buffer served as negative control. * p<0.05; ** 0.005, compared to NC and to the negative control.

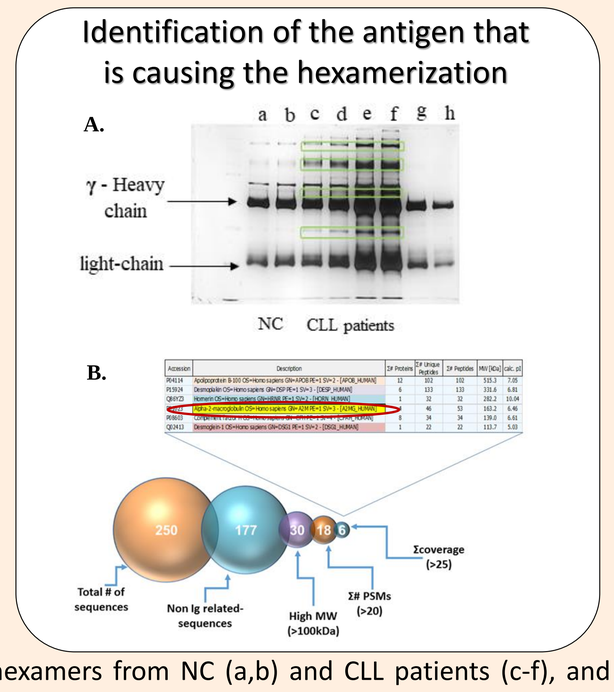


**Fig. 4**
(A) IgG-hexamers from NC (a,b) and CLL patients (c-f), and commercial IgG (g,h) were separated by SDS-PAGE and silver stained. Non-IgG proteins (green frames) were subjected to mass-spectrometry. (B) The selection process of the sequencing data included elimination of all IgG-related sequences, low molecular mass peptides, sequences with a number of identified peptide sequences (peptide spectrum matches-#PSMs)<30, and sequences with coverage<25.

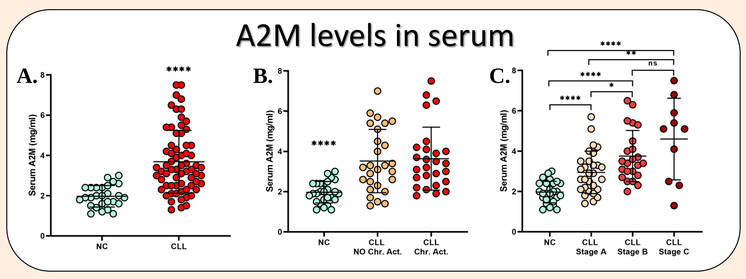


**Fig. 5**
Serum A2M levels were measures by ELISA (A) and correlated with chronic C activation (B) and with the disease stage (C). *, **, ***,**** indicate significant p values of <0.05, <0.01, <0.001, <0.0001, respectively.

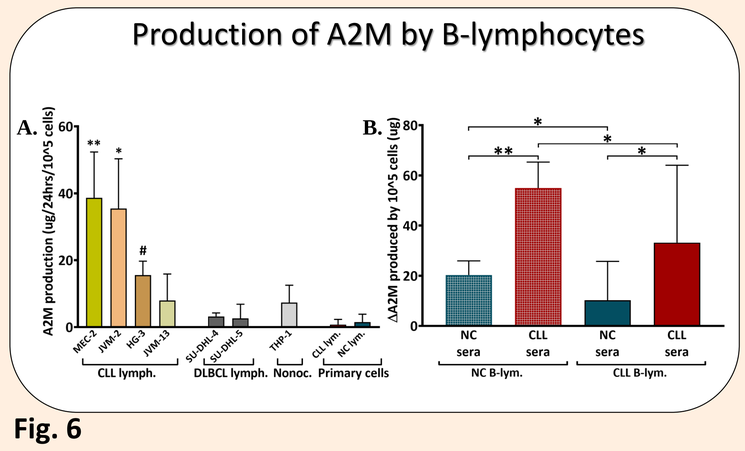


**Fig. 6**
(A) A2M production was studied in CLL (CLL lymph.), lymphoma (BLBCL lymph.), and monocytic (monoc.) cell lines, and in isolated primary B-lymphocytes. (B) Primary B-lymphocytes isolated from blood were incubated with NC or CLL sera. A2M levels were measured and the ΔA2M levels (above controls) were calculated for $10^5$ cells/24 hrs.

## CONCLUSIONS

- Part of the CLL population (44%) show chronic activation of the CP.
- Chronic CP activation is attributed, at least partially, to the cell-free IgG-hexamers in patients serum.
- A2M causes IgG-hexamerization.
- A2M shows increased serum levels in CLL, that are associated with the disease severity.
- Most CLL cell lines, but not primary B-lymphocytes, produce A2M.
- The increased serum A2M levels in CLL may be due to its production by B-lymphocytes.