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The proportion of activated B-cell like subtype among de novo diffuse large B-cell lymphoma increases with age

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The prognosis for elderly patients with diffuse large B-cell lymphomas (DLBCL) remains particularly poor. The most common explanation involves co-morbidities related to advanced age, which strongly impact chemotherapy feasibility and tolerance\(^1\). Despite a generally poor prognosis, a recent clinical trial dedicated to patients older than 80 years demonstrated that a significant proportion of DLBCL patients could be cured using rituximab (R) and dose reduced-intensity chemotherapy (R-miniCHOP)\(^2\). In addition to well-known clinical conditions related to ageing, the poor prognosis of DLBCL in elderly patients may also be related to intrinsic biological features of the tumour. The germinal centre B-cell like (GCB)/activated B-cell-like (ABC) signature is considered as a major biological determinant of prognosis, independent of the international prognostic index (IPI), remaining predictive of the outcome in patients treated by immuno-chemotherapy\(^3\). However, the relationship between ageing and the distribution of these two main gene expression profiles (GEP) has not been studied specifically, even though a trend for a higher proportion of ABC patients was reported by Rosenwald and colleagues in patients older than 60 years of age\(^4\). To address this question, we retrospectively determined the GEP of a series of 131 primary de novo DLBCL patients older than 50 years (median age 68 y, range 50-91 y), selected on the basis of histopathological diagnosis and available tumor RNA. The GCB/ABC signature was determined by DASL technology with RNA extracted from fresh frozen material as previously reported \(^5,6\). By this reproducible and robust method, we observed a concordance rate of 70-95% with immunohistochemistry\(^5,6\). A direct comparison with microarray-based technology using RNA extracted from formalin-fixed paraffin-embedded tissues is ongoing.

Using this approach, 51 cases (39%) were classified in the GCB group, 57 cases (44%) in the ABC group and 23 (17%) in an unclassified (intermediate) group (figure 1A). To determine the distribution of the GCB/ABC phenotype according to age, the overall population was divided in four age-related categories: Group 1: 50-60y (n = 36); Group 2: 60-70y (n = 41); Group 3: 70-80y (n= 36) and Group 4: ≥ 80 y (n = 18). According to these categories, the rates of ABC/GCB/unclassified DLBCL are indicated in figure 1B, with a significant increase in the ABC subtype proportion with increasing age. The ABC/non-ABC (including GCB and unclassified) distribution differed significantly between Group 1 and Group 4 (28% of ABC versus 67%, p = 0.01, Fisher exact test). To consolidate our results, we next determined if the same ageing effect was also observed in an independent published series of DLBCL (data accessible from the GEO databank, NCBI, GSE10846) \(^3\). Despite technological differences in the determination of GEP (Affymetrix array versus DASL), a similar increase in the ABC DLBCL proportion with age was observed (figure 1C). In this

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series of patients older than 50 y (median age = 68 y, range = 50-92 y), the percentage of ABC DLBCL increases with age, with a differential distribution between patients between 50-60 y and older than 80 y (33% of ABC versus 54%, p = 0.04, Fisher exact test, figure 1C).

We observed an average increase of 13.7% in ABC DLBCL per 10 years of ageing after the age of 50 y in our series and 7.5% in the Lenz series. A relationship between GCB/ABC distribution and age has been suggested in the context of paediatric lymphoma. Immunohistochemistry analysis indicated that, in this setting, DLBCL is associated most strongly with the GCB subgroup, which reflects the opposite pattern observed in the elderly 7. Whether a continuous increase in the ABC DLBCL proportion with age can be observed remains to be determined.

Attempts to explain such an ABC skewing distribution during ageing remain speculative. The normal repertoire of B/plasma cells becomes less diverse with age, and this loss of diversity is characterised by clonal expansions of B cells in vivo 8. For instance, an increase in the number of B-cells expressing the VH4-34 IgH gene is noted during ageing 9. We have previously shown that VH4-34 + DLBCL expressed frequently IgM, displayed frequently the t(3;14)(q27;q32) translocation and are typically classified in the ABC subtype 5,10. This suggests that the increase in the proportion of ABC DLBCL with age may reflect a change in the B-cell population during ageing. Another hypothesis is related to the putative pathological specificity of DLBCL occurring in elderly patients. EBV-related DLBCL are almost exclusively reported in elderly or very old patients, but this provisional WHO entity seems mostly limited to Asian countries and is rare in Western countries 11.

Age is an obvious and major prognosis factor which impacts directly on treatment strategies, chemotherapy feasibility and tolerance, precluding therefore any survival comparison between young and elderly patients with ABC DLBCL. To confirm the prognostic value of the ABC/GCB signature regardless of age, we compared OS according to molecular classification by producing random pairs of age-matched patients from the publicly available data published by Lenz et al. 12 For each patient randomly selected in the GCB subtype, an age-matched patient was randomly selected from the ABC subtype, in order to form 80 GCB-ABC pairs of patients. In the 50 tested random paired combinations, the ABC subtype remains constantly correlated to an unfavourable outcome indicating that the unfavourable prognostic value of the molecular signature is not related to the ABC skewing distribution during ageing (supplementary figure).

To conclude, our results indicate that in addition to constitutive factors related to advanced age, the prognosis of DLBCL is also conditioned by intrinsic biological features of the tumour.
cells. Despite promising results obtained using conventional immuno-chemotherapy such as R mini-CHOP, new therapeutic strategies in geriatric populations should include molecules able to target oncogenic pathways related to the ABC phenotype, such as the NFκB pathway.
References


Figure 1. GCB/ABC distribution with ageing in DLBCL patients older than 50 years.

A. **Hierarchical clustering.**
DLBCL were clustered according to gene expression as assessed by DASL. Genes fitting the ABC signature and genes fitting the GCB signature are indicated on the right. Patients are identified by their unique patient numbers. A colour code related to age category is indicated (upper part of the HeatMap). Unsupervised hierarchical clustering was performed using ‘COR’ distances (the opposite of Pearson’s r) after Cubic Spline normalisation (GenomeStudio V2009.1 software, Illumina). DLBCL diagnosis was performed according to WHO criteria. Primary mediastinal B-cell lymphomas and T-cell-rich B-cell lymphomas were excluded.

B. **ABC and non-ABC proportions according to age categories in a monocentric series of DLBCL.**
Number of patients by age category are indicated in brackets.

C. **ABC and non-ABC proportions according to age in an independent series of DLBCL**
Number of patients by age category are indicated in brackets.
Supplementary figure. Impact of the GCB/ABC molecular classification in age-matched patients
Random combinations of patients were produced from the series published by Lenz et al. (n = 350). For each patient randomly selected in the GCB group, a patient with the same age (in years) was randomly selected in ABC group, in order to form 80 GCB/ABC pairs of age-matched patients. In the 50 subseries produced, 91% of the 350 samples were selected at least one time, and all LogRank p-values were significative (max = 0.002). A. Overall Survival in the overall population. B Example of survival curves obtained in a series of 80 age-matched patients.