Check for updates

6. Obaji S, Alikhan R, Rayment R, Carter P, Macartney N, Collins P. Unclassified bleeding disorders: outcome of haemostatic challenges following tranexamic acid and/or desmopressin. *Haemophilia*. 2016;22(2):285-291.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Received: 20 May 2020 | Revised: 18 June 2020 | Accepted: 2 July 2020

DOI: 10.1002/ajh.25927

Clonal hematopoiesis in patients with high-grade B-cell lymphoma is associated with inferior outcome

To the Editor:

Clonal hematopoiesis of indeterminate potential (CHIP) is defined as presence of somatically mutated genes, commonly associated with myeloid neoplasm (MN) in the blood of healthy individuals with normal blood values and lack of morphological indications of MN in the bone marrow.[1] Clonal hematopoiesis of indeterminate potential is an age-related phenomenon associated with an elevated risk for the development of MN as well as cardiovascular diseases.[2] The current threshold of the variant allele frequency (VAF) of these CHIP-clones is 2%. However, this threshold is arbitrary, based on the sensitivity limit of next generation sequencing (NGS) technology used in the first CHIP-studies.

Limited numbers of reports are available on the prevalence and the clinical significance of CHIP among lymphoma patients, especially at the time of diagnosis. A few studies have reported that CHIP among lymphoma patients is associated with increased risk for the development of therapy-related MN (TMN) after chemotherapy and/or autologous stem-cell transplantation (ASCT).[3]

In the present study, we investigate the prevalence of CHIP and its impact on treatment outcome in an unselected series of prospectively included, homogeneously treated and clinically well characterized patients with high-grade B-cell lymphoma (HGBCL).

Seventy-four patients diagnosed with HGBCL between 2010-2015 were included in the study. All patients were recruited from the U-CAN consortium,[4] re-evaluated and classified according to the updated World Health Organization (WHO) classification of Tumors of Hematopoietic and Lymphoid Tissues of 2017.[5] Patients were treated according to National treatment guidelines with R-CHOP or R-CHOP like regimens.

Immunostainings were performed to classify tumors of diffuse large B-cell lymphomas (DLBCL) as germinal-center derived (GCB) or non-GCB. Details on immunohistochemistry and fluorescence in situ hybridization (FISH) are provided in the Supplementary material.

Sequencing DNA with NGS was performed on genomic DNA (gDNA) extracted from blood, using the Trusight Myeloid Sequencing Panel (Illumina, San Diego, California) (Supplementary material).

The VAF cut-off for the detection of the variants was set to 5% in order to avoid reporting of potential technical artifacts. Details of the sequencing protocol followed and the variant calling are provided in the Supplementary material. In short, variants had to meet the following conditions to be included in downstream analysis: (a) located within an exonic or splicing region; (b) be non-synonymous; and (c) not listed in the gnomAD database if not also recurrently reported in Cosmic v85.

Overall survival (OS) was calculated from the date of diagnosis to the date of death from any cause. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of lymphoma progression or death due to any cause. Lymphoma specific survival (LSS) was calculated from the date of diagnosis to the date of death of lymphoma (patients who died of other causes were censored). Survival curves and univariate analyses were performed using the Kaplan-Meier method, the log-rank test and Cox proportional hazards regression. The chi-square, the Fisher's exact test or Student's *t* test were used to compare differences between groups. The proportional hazards assumption was tested and was not violated. A *P* value of <.05 was considered to be statistically significant. Statistical analyses were performed using RStudio 1.1.383 and R 4.0.0 software (www.r-project.org).

Clonal hematopoiesis of indeterminate potential was detected in nine of 74 (12%) patients with no statistical difference observed in any of the available clinical features, or number of administered cycles of therapy between patients with CHIP, compared to the remaining ones (Table S2). The CHIP variants were found in the following genes: *DNMT3A* (n = 4), *TET2* (n = 3), *TP53* (n = 2), *KIT*, *EZH2* (n = 1) with a median VAF of 11.7% (range 6.4%-28.2%) (Table S3). In two cases, a *MYD88* and a *NOTCH1* variant were detected. These two cases were included in the non-CHIP group as mutations in these genes are frequently seen in patients with B-cell malignancies, while they are not recurrently mutated in MN. Unfortunately, lack of available material did not allow for targeted re-sequencing of tumor tissue or sorted cells.

Cause of death for patients with CHIP was lymphoma (n = 4) and metastatic colon carcinoma (n = 1). In one patient the cause of death was unknown (Table S3). No death due to cardiovascular disease was recorded.

Patients with CHIP demonstrated significantly inferior PFS, OS and LSS (Figure 1). In particular, the two-year and five-year PFS was 67% and 56% among patients with CHIP, vs 83% and 73% for non-CHIP cases (HR = 2.78 [CI:1.11-6.98], *P* value = .03), the two-year and 5-year OS for CHIP was 66% and 56%, respectively, vs 88% and 79% for non-CHIP (HR = 3.37 [CI:1.33-8.58], *P* value .01). Similarly, the two-year and five-year LSS was 62% and 50% among patients with CHIP, vs 91% and 87% for non-CHIP cases (HR = 4.66 [CI: 1.43-15.21], *P* value = .01) (Figure 1 and Table S4).

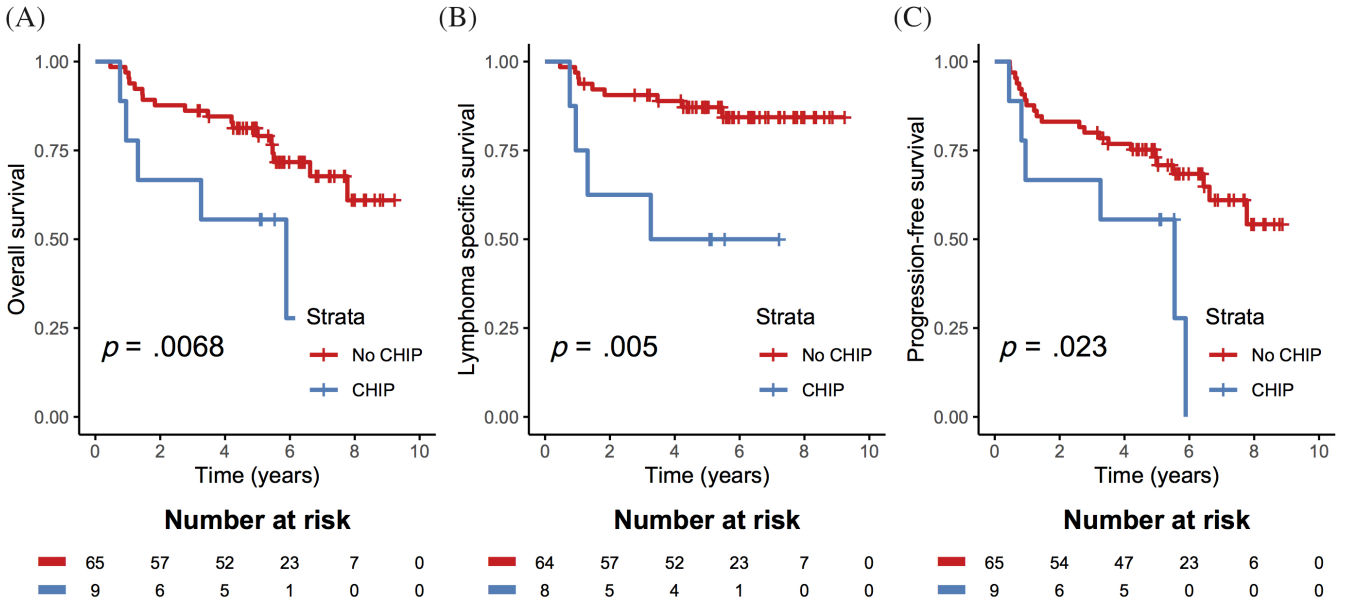

FIGURE 1 Kaplan Meier curves for overall survival (OS), Lymphoma specific survival (LSS) and progression-free survival (PFS) (A, B and C respectively) for patients with CHIP (blue line) and without CHIP (red line)

In multivariate analysis for LSS the presence of CHIP was the only parameter (evaluated features: age >60, male sex, stage III-IV, B-symptoms, elevated lactate dehydrogenase) that retained independent statistical significance, being associated with unfavorable outcome (HR: 3.75 [CI:1.05-13.41], *P* = .04), while a similar trend was observed in multivariate analysis for PFS and OS (HR:2.43 [CI: 0.92-6.39], *P* value = .07) and (HR:2.38 [CI: 0.90-6.31], *P* = .08), respectively] (Table S4).

In the present study, we evaluated for the first time the prevalence and clinical impact of CHIP at the time of diagnosis in a general population-based cohort with HGBCL treated with R-CHOP. We report a prevalence of 12% with *DNMT3A* and *TET2* being the most commonly mutated genes accounting for 64% similarly to previous reports[6] .

The prevalence of CHIP seen in our cohort is in accordance with that reported in age-matched healthy individuals,[1] but lower compared to lymphoma patients treated with ASCT.[3] This discrepancy can be attributed to different compositions of patients among the various cohorts or methodological differences regarding variant interpretation or inclusion of different genes analyzed.

Interestingly, in our cohort, we observed no differences between the CHIP and the non-CHIP cases regarding any of the available clinical or tumor parameters (Table S2). Note, CHIP was equally distributed independent of age, contrasting previous reports. This discrepancy between our and the other studies could be explained by the inclusion of patients with various diagnoses, being analyzed in different disease phases and with markedly different treatment exposures.

We report an unfavorable outcome for patients with HGBCL and CHIP for both OS and PFS, which is in line with previous reports of mixed patients with non-Hodgkin lymphoma. Moreover, CHIP was also associated with worse LSS, with lymphoma being the main cause of death among patients with CHIP. The possible association of CHIP with adverse clinical outcome in our cohort cannot easily be explained. It has been suggested that CHIP promotes the survival of cancer cells. A possible explanation may be the fact that CHIP is associated with increased inflammatory activity as well as immune dysregulation,[6] which could either advocate cancer cell survival or attenuate the effect of chemotherapy. In case this hypothesis is proven, one might argue for less intensive chemo based regimens in lymphoma patients with CHIP. However, little is known regarding the potential impact of CHIP among patients with lymphoma treated with less intensive chemotherapy.

A major limitation of our study is the application of a targeted analysis of 47 recurrently mutated genes in MNs, as well as the use of a 5% VAF as cut-off. Our approach may have led to the underestimation of CHIP due to the use of a higher threshold than usual. Moreover, using whole-exome-sequencing more CHIP variants might have been detected. However, our threshold is closer to the one used in the clinical setting, thus reflecting the prevalence of CHIP in everyday clinical praxis. Moreover, all the most commonly reported CHIP genes are included in the NGS panel that was used in our study. In addition, the number of patients included in our study is limited and therefore our findings should be interpreted with caution.

In three CHIP cases with bone-marrow involvement (Table S3), we detected mutations in *TET2*, *TP53* and *DNMT3A* genes. Despite normal blood values in these patients, the possibility that the detected variants may belong to the lymphoma population rather than the normal hematopoietic tissue cannot be excluded. However, with the exception of *TP53*, variants in *TET2* and *DNMT3A* genes are highly reported in CHIP studies,[6] while not reported as somatic in patients with HGBCL. Unfortunately, lack of available material did not allow further investigation of these cases.

In conclusion, we report that the presence of CHIP is associated with an impaired clinical outcome for patients with HGBCL treated with standard up-front chemo-immunotherapy, a finding with obvious clinical implications, especially in the era of targeted agents. Our study

highlights the need for further prospective validation of the role of CHIP in lymphomas in larger cohorts.

ACKNOWLEDGMENTS

The authors would like to acknowledge Clinical Genomics Uppsala, the Science for Life Laboratory, Dept. of Immunology, Genetics and Pathology, Uppsala University, Sweden for performing the experiments. The material collection was supported by U-CAN, through Uppsala Biobank and the Department of Clinical Pathology, Uppsala University Hospital.

FUNDING INFORMATION

This study was supported by the Swedish Cancer Foundation and Lions Cancer Research Fund, Uppsala, Sweden covering the costs of the sequencing reagents.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Rose-Marie Amini and Panagiotis Baliakas designed the study and wrote the manuscript. Gunilla Enblad, Maysaa Abdulla and Rose-Marie Amini performed data collection. Maysaa Abdulla and Rose-Marie Amini reevaluated the diagnoses. PB, Rose-Marie Amini, Maysaa Abdulla, Gunilla Enblad, Viktor Ljungström, Lucia Cavelier, Tatjana Pandzic, Peter Hollander performed analysis and interpretation. All authors read and approved the manuscript.

ETHICS STATEMENT

This study was conducted in accordance with the Declaration of Helsinki and written informed consent has been obtained from each patient. This study was approved by the Regional Ethical Committee in Uppsala (Dnr 2010/198 and Dnr 2014/233).

Rose-Marie Amini[1,2], Viktor Ljungström[2,3], Maysaa Abdulla[1], Lucia Cavelier[2,3], Tatjana Pandzic[2,3], Peter Hollander[1], Gunilla Enblad[4], Panagiotis Baliakas[2,3]

[1]*Clinical and Experimental Pathology, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
[2]*Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*
[3]*Department of Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden*
[4]*Experimental and Clinical Oncology, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*

Correspondence
Rose-Marie Amini, Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, entrance C5, Uppsala University and Uppsala University Hospital, SE-75185 Uppsala, Sweden.
Email: rose-marie.amini@igp.uu.se
DOI 10.1002/ajh.25927

ORCID

Rose-Marie Amini https://orcid.org/0000-0003-0901-5252
Maysaa Abdulla https://orcid.org/0000-0002-0766-0656
Peter Hollander https://orcid.org/0000-0002-0226-5681
Panagiotis Baliakas https://orcid.org/0000-0002-5634-7156

REFERENCES

1. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
2. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377(2):111-121.
3. Husby S, Favero F, Nielsen C, et al. Clinical impact of clonal hematopoiesis in patients with lymphoma undergoing ASCT: a national population-based cohort study. *Leukemia*. 2020. https://doi.org/10.1038/s41375-020-0795-z. [Epub ahead of print].
4. Glimelius B, Melin B, Enblad G, et al. U-CAN: a prospective longitudinal collection of biomaterials and clinical information from adult cancer patients in Sweden. *Acta Oncol*. 2018;57(2):187-194.
5. Swerdlow SH. In: Bosman FT, ed. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. International Agency for Research on Cancer: International Agency for Research on Cancer (IARC); 2016.
6. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. *Science*. 2019;366(6465):eaan4673.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Received: 18 June 2020 | Revised: 2 July 2020 | Accepted: 6 July 2020

DOI: 10.1002/ajh.25931

Upper airway microbiome changes in children with sickle cell disease during vaso-occlusive and acute chest syndrome episodes

To the Editor:

Sickle cell disease (SCD) results in numerous complications, including acute chest syndrome (ACS) and vaso-occlusive crises (VOC). Beginning in infancy, patients with SCD are exposed to prophylactic antibiotics to prevent invasive pneumococcal disease from functional asplenia. Furthermore, these children commonly receive empiric broad-spectrum antibiotics when they present with febrile illnesses. Note, ACS is a leading cause of morbidity and premature death in people with SCD. Treatment with broad-spectrum systemic antibiotics is recommended for all occurrences, despite limited studies on the true occurrence of bacterial infections in ACS. VOC episodes are the most common complication in children with SCD.