# Clonal hematopoiesis is common in bone marrow of patients with classical Hodgkin lymphoma

The term 'clonal hematopoiesis of indeterminate potential' (CHIP) was first introduced by Steensma *et al*. for individuals carrying somatic leukemia-associated mutations with a variant allele frequency (VAF) ≥2%.[1] Aging is strongly associated with the prevalence of CHIP. In the young age group (<45 years), mutations have been found in <1% of cases.[2,3] In elderly people (>60 years), the phenomenon of clonal hematopoiesis is present in 10-15% of people.[4] The clonal expansions most frequently involve somatic mutations in genes that have previously been implicated in hematologic cancers (*DNMT3A, ASXL1, TET2*, etc.),[5] especially myelodysplastic syndromes and myeloid leukemias. It is now widely accepted that mutations can also be detected in genes that are recurrently mutated in lymphoid malignancies, thus CHIP should be distinguished into myeloid CHIP (M-CHIP) and lymphoid CHIP (L-CHIP).[6]

Several studies have described the prevalence and patterns of M-CHIP mutations across several types of hematologic neoplasm, mostly of myeloid or T-cell origin.[5] In non-Hodgkin lymphoma and myeloma, M-CHIP has also been associated with adverse outcomes after autologous stem-cell transplantation,[7-9] and in patients with aggressive lymphomas undergoing CD19-directed chimeric antigen receptor T-cell treatment.[10]

Only limited information is available in classic Hodgkin lymphoma (cHL). A large exploratory study that included all types of lymphomas detected clonal hematopoiesis in hematopoietic stem cells (HSC) harvested from nine of 64 (14%) cHL-relapsed patients undergoing autologous transplantation.[8] More recently, Venanzi *et al*. analyzed clonal hematopoiesis in 40 cHL cases by sequencing microdissected tumor cells and matched normal cells from blood and/or lymph nodes.[11] This study detected M-CHIP in five of the 40 cases (blood and/or tissue clonal hematopoiesis). In three of the five patients (in all of whom first-line therapy failed), clonal hematopoiesis was detected extensively throughout the tissue microenvironment, with very high VAF: mutant *DNMT3A, KRAS,* and *DNMT3A +TET2* in 33%, 92% and 60% of non-neoplastic cells, respectively.

Our aims in the present work were: (i) to define the prevalence of M-CHIP in the HSC niche of bone marrow (BM) tissues, using a targeted deep-sequencing approach; (ii) to analyze potential relationships of M-CHIP with the neoplastic components of the cHL-affected tissues, and (iii) to assess the ultimate relevance of these findings to clinical parameters.

First, we analyzed a cohort of 40 non-tumor BM aspirates and BM biopsies from 37 patients diagnosed with cHL in peripheral lymph node biopsies. Patients who underwent BM aspiration and biopsies for cHL between 2010 and 2022, and for whom residual smear samples were available, were retrospectively identified at MD Anderson Cancer Center Madrid Biobank. These non-tumor BM samples were originally obtained for routine staging procedures. Anonymous clinical data and the tissue samples were provided by the Biobank, after having been collected in accordance with the technical and ethical procedures of the Spanish National Biobank Network and obtained with written informed consent according to the Helsinki Declaration. The study was approved by the institutional review board (CEIm H. Ramón y Cajal, reference 445/22). The clinical and pathological characteristics of the series are summarized in *Online Supplementary Table S1A, B*.

The presence of M-CHIP mutations was analyzed by next-generation sequencing using a custom gene panel for DNA-targeted sequencing (*Online Supplementary Table S2*), on an Ion Torrent S5 sequencer (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, DNA was extracted from BM smears using standard protocols and quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific). Twenty nanograms of genomic DNA were used per sample to prepare the DNA library, which was generated using the Ion AmpliSeq™ Library 2.0 Kit, following the manufacturer's instructions. To amplify target regions, two pools of primers were used to ensure more complete and uniform coverage, to reduce biases, and to improve the sensitivity and specificity of the sequencing process. The resulting amplicons were partially digested with FUPA reagent. Specific barcode adapters were ligated to each sample. DNA products were purified using an Ion Library Equalizer™ kit (Thermo Fisher Scientific) and quantified using a Qubit™ high-sensitivity dsDNA assay kit (Thermo Scientific). A total of 988 amplicons were obtained, each with a size of 125-275 bp.

For bioinformatic analysis and variant filtering, we used Ion Reporter and Alamut software, with ClinVar, Varsome, cBioportal, COSMIC, and genomeAD databases as references. Variants with fewer than 100 reads, or that had previously been described as polymorphisms were excluded. The mean average base coverage depth was 1,210 (range, 801-1,619). The following inclusion criteria were applied: (i) variants with VAF ≥2% and <30% (higher VAF were considered likely to represent germ-line variants) and (ii) mutations that had previously been reported as pathogenic/likely pathogenic or of uncertain significance in public databases (i.e., benign/likely benign variants were discarded); conversely, the presence of synonymous/silent mutations and mutations in intronic regions was a criterion for exclusion.

This approach identified 36 variants in 18/37 BM aspirate samples (46.9%) from 15/32 patients (48.6%) (Table 1, *Online Supplementary Table S3A*). As expected, these mutations occurred in M-CHIP-associated genes, such as *KRAS, TET2,*

**Table 1.** Myeloid clonal hematopoiesis of indeterminate potential variants detected from bone marrow samples and somatic variants from primary lymph node tumors.

| Case# | BM# | CHIP | HRS cells, primary tumors |
|---|---|---|---|
| 1 | 1770 | *KRAS* c.64C>A | - |
| 1 | 1910 | *KRAS* c.64C>A | - |
| 2 | 1580 | - | - |
| 3 | 1589 | - | - |
| 3 | 1593 | - | - |
| 4 | 1600 | *BCOR* c.3436G>A; *JAK2* c.1255C>T | - |
| 5 | 1605 | - | - |
| 6 | 1677 | NA | - |
| 7 | 1902 | - | - |
| 8 | 1901 | - | - |
| 9 | 2087 | *CBL* c.1380_1382delTGA; *KRAS* c.252A>G | - |
| 10 | 2468 | - | *CD38; NOTCH1; LCP1; SOCS1* |
| 11 | 2411 | - | *NOTCH1; CREBBP; SMARCA4* |
| 12 | 2386 | - | *MYB; CARD11* |
| 13 | 2726 | - | *NFKBIA; CSF2RB* |
| 14 | 2745 | - | *STAT6; CYLD* |
| 15 | 2765 | - | *TNFAIP3; CSF1R* |
| 16 | 2781 | - | - |
| 17 | 526 | NA | - |
| 18 | 549 | *TET2* c.3009G>A; *TET2* c.3782G>A | - |
| 19 | 334 | *DNMT3A* c.176C>T; *TET2* c.3454G>A | - |
| 20 | 368 | *RUNX1* ‘c.1085C>T; *ZRSR2* c.-14G>A; *DNMT3A* c.176delC | - |
| 21 | 374 | - | - |
| 22 | 418 | *CBL* ‘c.1380_1382delTGA | - |
| 23 | 443 | *TET2* c.2596C>T; *CDKN2A* c.253G>A; *CDKN2A* c.202G>A; ZRSR2 c.217G>A | *NFKB2* |
| 24 | 457 | *CDKN2A* c.301G>T; *KDM6A* c.1693C>T; *CALR* c.1139A>G | - |
| 24 | 1629 | *NRAS* c.143G>A | - |
| 25 | 518 | *TET2* c.4624C>T; *SETBP1* c.2753G>A | - |
| 26 | 524 | *NPM1* c.38G>A | *NFKB2* |
| 27 | 525 | - | - |
| 28 | 543 | *ZRSR2* c.1282G>A | *TNFRSF14; STAT6; BCL10; CSF1R* |
| 29 | 617 | NA | *CARD11; CSF1R; BCL10* |
| 30 | 722 | - | *CREBBP, NFKBIA* |
| 31 | 657 | NA | - |
| 32 | 671 | *TP53* c.566C>T; *DNMT3A* c.893G>A | - |
| 33 | 746 | - | *CARD11; STAT6; BTK; NFKB2* |
| 34 | 753 | *ZRSR2* c.652G>A; *CUX1* c.3597G>A; *EZH2* c.1265C>T; *MPL* c.473C>T; *DNMT3A* c.506G>A; *TET2* c.4643_4645delAGC | - |
| 34 | 767 | *TET2* c.2965C>T; *DNMT3A* c.506G>A | - |
| 35 | 810 | - | - |
| 35 | 930 | - | - |
| 36 | 975 | *TET2* c.4636C>T; *TET2* c.4954C>T; *CUX1* c.2345delC | - |

Additional data available in *Online Supplementary Table S3*. BM: bone marrow; CHIP: clonal hematopoiesis of indeterminate potential; HRS: Hodgkin and Reed-Sternberg cells; NA: not available.

*CDKN2A, DNMT3A, CALR, MPL, RUNX1,* and *CBL*, among others. The mean VAF was 4.69% (range, 2.12-28.4%).

To explore the possible existence of a clonal relationship with lymphoma cells, we also analyzed somatic mutations from the Hodgkin and Reed-Sternberg (HRS) cells of the 12 patients for whom formalin-fixed, paraffin-embedded primary tumor samples were available. Variants were analyzed by next-generation sequencing using an M-CHIP-specific panel and a cHL-specific targeted custom panel, following established protocols.[12] Confirming previous findings, we were able to detect somatic mutations in the HRS clone in all primary tumors, but we did not find any common variants in comparison with the variants that apparently affect HSC (Table 1). Finally, to verify whether HSC might harbor any mutations indicative of L-CHIP we also repeated the cHL-specific targeted custom panel on available BM samples. We found only three variants from one patient that met the selection criteria (*Online Supplementary Table S3B*), although none of them coincided with the tumor clone.

As expected, a significant association between the presence of M-CHIP and the age of the patients was also confirmed in this series (Figure 1A). We did not find any other association with clinical or pathological variables (sex, stage, cHL subtype). Clonal hematopoiesis has also been associated with inflammation, and inflammation indicators are common in cHL, especially raised values of C-reactive protein and erythrocyte sedimentation rate. Abnormal levels of both indicators were also frequent in this series (*Online Supplementary Table S1B*), but we did not find a significant relationship with the presence of M-CHIP. We noted that cHL patients bearing M-CHIP mutations seemed to have more clinically aggressive tumors and worse clinical outcome (Figure 1B), although the differences were too small to reach statistical significance for such a small sample size. The adverse prognostic impact of M-CHIP was not due to therapy-related myeloid neoplasms, which were not present in our cohort.

The cytological and histological features of the smear samples from BM aspirates and biopsies were reviewed again for all the patients in whom we found M-CHIP variants. Four of these samples exhibited subtle anomalies in the maturation

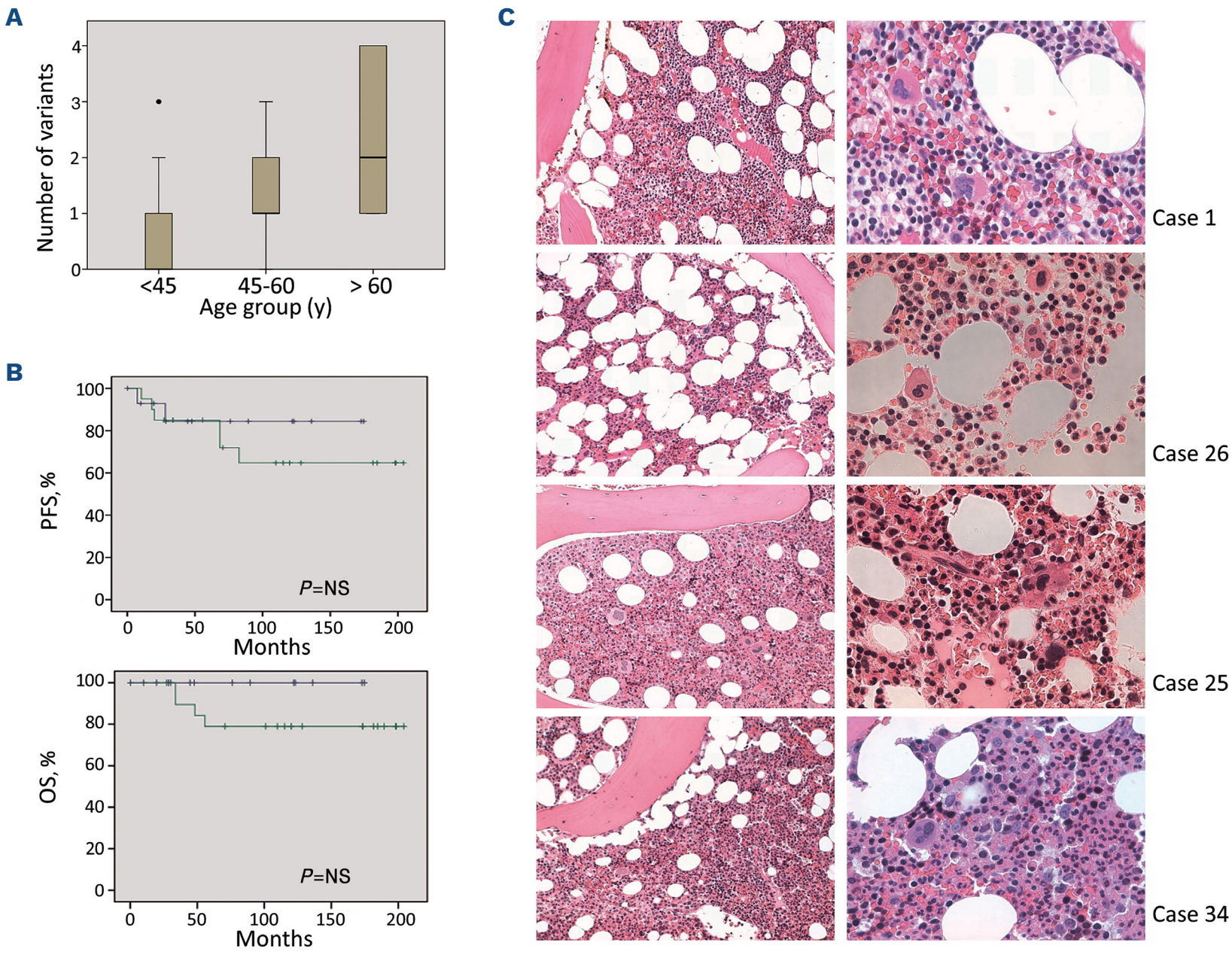


**Figure 1. Clinical relationships and histological characteristics of the samples.** (A) Clonal hematopoiesis of indeterminate potential is associated with age ($P$=0.027, Pearson $\chi^2$-square). (B) Kaplan-Meier curves for progression-free survival and overall survival. (C) Representative photomicrographs of bone marrow biopsies. Original magnification: left panels: x100; right panels: x630; hematoxylin-eosin stain. PFS: progression-free survival; OS: overall survival; NS: not statistically significant.

gradient and even some abnormal cytological features, but no case met the diagnostic requirements for concluding myelodysplastic syndrome (Figure 1C). Case 1, with a *KRAS* mutation detected in two independent BM samples, presented mild hypercellularity with age- and left-shifted maturation, but no other myelodysplastic features. Case 26 (*NMP1*-mutated) was moderately hypocellular and had mild eosinophilia. Case 25 (*TET2/SETBP1*-mutated) was hypercellular with focal monocytosis and very occasional megakaryocytes with subtle dysplastic features. Case 34 (with several variants detected) only showed occasional anomalies in the nuclear segmentation of megakaryocytes.

Overall, we have been able to verify that M-CHIP is a frequent finding in patients with cHL, and that this phenomenon is evolutionarily unrelated to the tumor clone. The presence of such mutations may instead be related to an abnormal immunity and, subsequently, an increased risk of malignant transformation of unrelated B-cell clones and of eventual progression. Similar adverse prognostic implications of clonal hematopoiesis were found in a large series of lymphoma patients undergoing autologous stem cell transplantation; clonal hematopoiesis was detected in 43.1% of patients and was most common in those with T-cell lymphoma (72.2%).[13] However, our results contrast with the link described between follicular helper T-cell lymphomas and clonal hematopoiesis, in which several cases have documented divergent evolution of myeloid neoplasms and angioimmunoblastic T-cell lymphomas from shared clonal mutations.[14]

We found a higher frequency of M-CHIP than reported in a previous study that focused on microenvironmental cells isolated from tumor tissues.[11] Furthermore, the VAF described in that study were significantly greater than what we found in the BM niche. These differences may be related to the different experimental methodologies, a real active dynamic of recruitment of abnormal immune populations towards the environment of HRS cells, or the presence of tumor clones that are not recognizable as such in the non-tumor fraction. Interestingly, we did not find a high preponderance of DAT mutations (considering only *DNMT3A, TET2*, and/or *ASXL1*), and we did see mutations in more rarely reported gene such as *CDKN2, KRAS, NPM1, BCOR,* and *EZH2*. In fact, only eight of 32 patients had DAT mutations, for a prevalence of 25%, which is similar to what has been previously reported. Analyzing the presence of DAT mutations with respect to age (<45 years *vs*. >45 years), the difference did not reach statistical significance. As has been proposed, HSC bearing newly evolved variants can increase in number due to their proliferative and/or survival advantage, leading to clonal expansions. Such clonally expanded HSC can result in an increased risk of hematologic neoplasms, in particular myeloid (myelodysplasia and acute myeloid leukemia) and, probably, T-cell lymphoproliferative disorders. This pattern is often associated with driver mutations in some genetic loci, such as *DNMT3A* and *TET2*, and a clonal relationship with the progressed tumors may be expected.

Cases of cHL, and probably other B-cell lymphomas and myeloma, present a different scenario. The somatic mutations that drive clonal expansion of HSC and progenitor cells can alter the function of terminally differentiated blood cells, including decreased immunosurveillance and the release of abnormal levels of inflammatory cytokines. This milieu may then contribute to the development of lymphoproliferative disorders, including cHL.

Our results confirm that clonal hematopoiesis is associated with cHL, among other hematologic neoplasms.[15] Formal demonstration that M-CHIP represents a predisposing factor for the development of the disease would require different methodological approaches, probably with more extensive next-generation sequencing analyses in longitudinal series of cases. M-CHIP has been associated with aging, chronic inflammation, death from cardiovascular disease, elevated risk of hematologic malignancies, therapy-related myeloid malignancies,[16] and overall mortality.[4] Therefore, it seems reasonable to propose routine early detection of CHIP in multiple clinical contexts.

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**Disclosures**

No conflicts of interest to disclose.

**Contributions**

ED, SF, MM, MDRT and MLD performed the research and analyzed the data. JLS, ME, RO, AMB, CM and AdlF contributed samples/patients' data and interpreted the data. RI and JFG designed the study, supervised the research, and wrote the paper. All authors contributed

to the preparation of the manuscript, revised the draft, and approved the final version for submission.

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**Data-sharing statement**

Access to Fastq files generated during this study will be granted for health, medical, or biomedical purposes, and in accordance with good practice recommendations. Requests for data access should be addressed to the corresponding author.

# References

1. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9-16.
2. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20(12):1472-1478.
3. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559(7714):400-404.
4. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-2498.
5. Genovese G, Kähler 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.
6. Niroula A, Sekar A, Murakami MA, et al. Distinction of lymphoid and myeloid clonal hematopoiesis. Nat Med. 2021;27(11):1921-1927.
7. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol. 2017;35(14):1598-1605.
8. 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;34(12):3256-3268.
9. Mouhieddine TH, Sperling AS, Redd R, et al. Clonal hematopoiesis is associated with adverse outcomes in multiple myeloma patients undergoing transplant. Nat Commun. 2020;11(1):2996.
10. Teipel R, Kroschinsky F, Kramer M, et al. Prevalence and variation of CHIP in patients with aggressive lymphomas undergoing CD19-directed CAR T-cell treatment. Blood Adv. 2022;6(6):1941-1946.
11. Venanzi A, Marra A, Schiavoni G, et al. Dissecting clonal hematopoiesis in tissues of classical Hodgkin lymphoma patients. Blood Cancer Discov. 2021;2(3):216-225.
12. Mata E, Fernández S, Astudillo A, et al. Genomic analyses of microdissected Hodgkin and Reed-Sternberg cells: mutations in epigenetic regulators and p53 are frequent in refractory classic Hodgkin lymphoma. Blood Cancer J. 2019;9(3):34.
13. Lackraj T, Ben Barouch S, Medeiros JJF, et al. Clinical significance of clonal hematopoiesis in the setting of autologous stem cell transplantation for lymphoma. Am J Hematol. 2022;97(12):1538-1547.
14. Ondrejka SL, Amador C, Climent F, et al. Follicular helper T-cell lymphomas: disease spectrum, relationship with clonal hematopoiesis, and mimics. A report of the 2022 EA4HP/SH lymphoma workshop. Virchows Arch. 2023;483(3):349-365.
15. Warren JT, Link DC. Clonal hematopoiesis and risk for hematologic malignancy. Blood. 2020;136(14):1599-1605.
16. Gillis NK, Ball M, Zhang Q, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol. 2017;18(1):112-121.