LETTER TO THE EDITOR

Presence of copy number aberrations and clinical prognostic factors in patients with acute myeloid leukemia: an analysis of effect modification

To the editor We read with great interest the recent paper by Bănescu et al[1] addressing the association of copy number aberrations (CNAs) with European LeukemiaNet risk category, somatic mutations, clinical features, and overall survival in patients with acute myeloid leukemia (AML). The authors assessed the use of multiplex ligation–dependent probe amplification (MLPA) for analyzing the impact of CNAs on patient outcome. The presence of recurrent somatic mutations in the *FLT3*, *NPM1*, and *DNMT3A* genes was also assessed.[1]

Acute myeloid leukemia is a heterogenous disease characterized by expansion of undifferentiated myeloid precursor cells, leading to impaired hematopoiesis and normal bone marrow failure. Leukemia progenitor cells acquire recurrent genetic abnormalities and/or distinct somatic mutations, known as driver mutations, which is important in disease diagnosis and prognosis as well as impacts the efficacy of chemotherapy.[2] Data on these abnormalities allow risk stratification and further precise classification of leukemia.[3] Deletions, duplications, and other genomic rearrangements result in dosage imbalance of genes. The assessment of these relatively frequent abnormalities may increase our knowledge on the pathogenesis of AML. Copy number aberrations are detected using the gold standard array-based methods: comparative genomic hybridization and single nucleotide polymorphisms, while MLPA is a relatively new, cost-effective, accurate, and reliable technique for CNA detection.

Bănescu et al[1] demonastrated an association between the presence of CNAs and adverse risk category in the European LeukemiaNet classification in patients with AML (P <0.0001). However, in the univariate Cox regression analysis, the presence of CNAs was not found to predict death in the study cohort. There were also no differences in the survival rate in patients with and without CNAs among those with somatic mutations in the *FLT3*, *NPM1*, and *DNMT3A* genes. However, the presence of CNAs was associated with the *FLT3* D835 / FLT3TKD mutation (P = 0.02). A bidirectional interaction between CNAs and the Eastern Cooperative Oncologic Group Scale (ECOG) performance status was also found, which appears to be somewhat surprising. The presence of CNAs in patients with good performance (ECOG ≤2) was found to be associated with better outcome, while in patients in poor clinical condition (ECOG ≥3), it was connected with an increased risk of death. Generally, the presence of CNAs as a genomic instability marker is an important factor adversely affecting survival in cancer patients. Considering the results presented by Bănescu et al,[1] it would be interesting to investigate how many CNAs were found and which genes were most frequently affected. The assessment of CNAs may be interesting in patients both with de novo and with relapsed AML. Furthermore, the longitudinal assessment of CNAs, both at diagnosis and in relapse, might expand our knowledge on AML chemosensitivity and clonal disease evolution.

Nowadays, next-generation sequencing (NGS) technology provides important data for optimization and personalization of AML treatment, and it becomes increasingly widely available. However, MLPA might be considered as a complementary tool to NGS. A recently described MLPA-based NGS technique uses MLPA products to construct a library that can be transferred into the NGS procedure. By using this approach, CNAs of target genes may be detected with improved precision on a large scale.[4]

In the study by Bănescu et al,[1] complete remission was achieved in 15% of patients. According to a recent study on survival in patients with AML treated with standard intensive chemotherapy, remission may be achieved in 70% of patients with de novo AML younger than 60 years and in 50% of older patients, leading to a 5-year overall survival rate of 40% to 50% and 20% to 30% in younger and older patients, respectively.[5] It

is possible that if the authors added more clinical data regarding treatment modalities used in the study cohort, we would be able to better understand the impact of CNAs on risk assessment and outcome in patients with AML.

ARTICLE INFORMATION

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Authors' reply We would like to thank Mensah-Glanowska et al[1] for their interest in our recently published article regarding the multiplex ligation-dependent probe amplification (MLPA) utility in patients with acute myeloid leukemia (AML)[2] and also for their constructive comments.

We agree with the authors that the MLPA-based next-generation sequencing (NGS) technology is a useful method that provides important data for optimization and personalization of AML treatment. We have to mention that for low and middle-income countries, it may be very difficult to support the cost of MLPA-based NGS analysis. The cost of such analysis is lower if the analysis includes a large number of patients, while for 1 to 20 patients, for example, the cost is increased. At the same time, the analysis report for such patients had to be ready as soon as possible, for optimization and personalization of AML treatment. Currently, we are also investigating our AML patients by NGS analysis, trough research grants, but for financial reasons, we perform NGS analysis only when we have more AML samples to be included in each run (Ion PI chip). Unfortunately, in this situation it may be too late for some patients with AML.

Regarding the MLPA technique, we agreed that it had several limitations, but it is useful for the fast investigation of patients with AML at a low cost. In our study, we did not analyze separately our copy number aberrations (CNAs) due to the increased type of different CNAs. As we mentioned, 283 adult patients with AML were evaluated for CNAs and they were found in 90 (31.8%) cases (64 cases with 1 CNA, 11 cases with 2 CNAs, 15 cases with ≥3 CNAs).

Briefly, 6 cases with 17p or 17q deletion (*TP53, IKZF3, UNC13D* genes); 10 with CNAs for 5q, especially 5q33 (*EBF1, MIR145, MIR146A* genes), 12 with CNAs represented by deletions of 7p (*IKZF1*) or 7q (*MET, DPP6, RELN*), 5 with CNAs for 9p21.3 and 9p13.2 (*MTAP, CDKN2A, CDKN2B, PAX5* genes), 6 with CNAs for 2p24.3 and 2p23.2 (*MYCN, ALK* genes), 6 patients with CNAs for 8q24.21 (*MYC* gene), 3 with CNAs for 13q14 (*RB1, MIR15A, DLEU2, DLEU1* genes), 7 with CNAs on X chromosome, 2 with CNAs for 3p26.3 (*GHL1-5* gene), 2 with CNAs for 21q22 (*RUNX1* gene), 2 with CNAs for 21q11(*HSPA13* gene), 1 with CNAs for 10q23.31 (*PTEN* gene), 2 with CNAs for 12p13 (*ETV6, CCND2, MDM2* genes), etc. As it may seem, we identified an increased number of different types of CNAs. Considering the relatively small number of cases for each type of CNA, which was not sufficient to ensure the power of statistical analysis separately on each CNA, we were unable to perform statistical analysis for each CNA. Taking into account that AML is characterized by chromosomal abnormalities (including also translocations) and gene mutations, we recommend the use of MLPA analysis in addition to other cytogenetic and molecular techniques for AML patient investigation.

Our MLPA results presented in the paper are general and must be clinically interpreted with caution. The main objective of the mentioned paper was to assess the utility of a multiplex ligation–dependent probe amplification (MLPA) assay in AML. As it was already mentioned,[1-3] we consider MLPA- and NGS-based techniques useful in the diagnosis of patients with AML due to the clinical impact of the genetic anomalies in AML, but also for precision medicine in AML. Besides, several molecular techniques can be useful in the diagnosis of patients with AML, each of them covering the limits of the other technique.[3]

Regarding the complete remission, it was considered achieved in 15% of our patients while in the study by Medeiros et al,[4] it is estimated that the remission may be achieved in 50% of older AML cases (>60 years). The discrepancies in the results may be explained by the fact that those cases with relapse after complete or partial remission were included in the relapse group. Moreover, we did not subcategorize our patients according to their age. Treatment modalities used in our patients were the standard ones, according to the patient's age and comorbidities, with a low or high dose of so-called 2+3 or 7+3 regimens[5] or a high dose with hematopoietic stem cell transplantation.

ARTICLE INFORMATION

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