Leukemia Research Reports 21 (2024) 100463

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Cup-like nuclei in adult B-cell acute lymphoblastic leukemia with the translocation (4;11)(q21;q23)

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ARTICLE INFO

Keywords:
Cuplike nuclei
Acute lymphoblastic leukemia
Translocation(4;11)(q21;q23)

ABSTRACT

Cuplike Nuclei(CLN) cells, particularly rare in Acute Lymphoblastic Leukemia(ALL), have been documented in only few cases to date. A recent study has revealed a correlation between CLN and IKZF1 deletions in pediatric B-ALL. This study introduces a case of CLN in adult B-ALL with a translocation (4;11)(q21;q23), while discussing the current relevant literature on the subject. Through this examination, several unique characteristics of CLN cells in both ALL and Acute Myeloid Leukemia are highlighted. It is essential to accumulate further data to confirm these distinctive traits, and investigate the potential association between CLN and cytogenetic/molecular abnormalities.

1. Introduction

Acute Lymphoblastic Leukemia (ALL) is identified as a hematologic malignancy, distinguished by the malignant proliferation of lymphoid hematopoietic precursor cells. This malignancy presents cytomorphological distinctions from Acute Myeloid Leukemia (AML), where primitive and immature lymphocytes typically manifest smaller cell bodies, denser chromatin, reduced cytoplasmic volume, and an absence of azurophilic granules, unlike primitive myeloid cells. Notably, blasts exhibiting Cuplike Nuclei (CLN) are prevalent in AML but rare in ALL, leading to the potential misdiagnosis of ALL with CLN as AML based solely on morphological assessment. It underscores the importance of compiling such cases and data to discern subtle distinctions between CLN in ALL and AML, aiding in the diagnosis and evaluation of the clinical significance and prognosis of CLN in ALL and their association with cytogenetic and molecular abnormalities.

Among reported B-ALL cases with CLN, some exhibited the BCR-ABL fusion gene, simultaneously were linked to the IKZF1 deletion, and one recent case involved a DUX4 rearrangement [1–4]. The limited number of documented cases constrains the comprehensive understanding of CLN in ALL. Recent research has highlighted a connection between CLN and IKZF1 deletion in pediatric B-ALL, with these instances predominantly presenting normal karyotypes [5].

Yet, abnormal karyotypes in adult ALL with CLN have not been extensively reported. We describe a case of an adult diagnosed with B-cell ALL featuring CLN, characterized by the atypical chromosomal karyotype translocation t(4;11)(q21;q23).

2. Case report

A 66-year-old female patient presented at the People's Hospital of Wenshan Prefecture, reporting a history of dizziness and weakness lasting five days, accompanied by systemic bone pain. Hematological evaluation revealed a leukocyte count of 235.48×10^9/L, hemoglobin at 57 g/L, and platelets at 66×10^9/L. Examination of the peripheral blood smear identified 89 % blast cells, with 18 % demonstrating cup-like nuclei (Fig. 1). These cells ranged from small to medium in size, featuring round to oval nuclei, fine and loosely arranged chromatin, and one to three prominently visible nucleoli. The cytoplasm appeared scant and pale blue, containing densely distributed small vacuoles, yet devoid of azurophilic granules. A distinctive concave indentation in some nuclei resulted in the characteristic "cup-like" appearance. Bone marrow smear analysis revealed 88.5 % blast cells, mirroring the morphology observed in the blood samples but with a reduced proportion of CLN at 7 % . Cytochemical staining (Fig. 2) indicated negative results for Peroxidase (POX) and Naphthol AS-D Chloroacetate Esterase (NAS-DCE), while PAS staining showed 87 % positivity, displaying a granular scattered distribution of polysaccharides. Flow cytometry analysis revealed approximately 92.01 % naive B lymphocytes among the total nucleated cells, with co-expression of CD19, CD22, cCD79a, and partial expression of CD34, CD38, HLA-DR, but without expression of CD10, CD20, CD3, CD5, CD7, CD13, CD33, CD11b, CD15, CD16, CD56, CD117, CD371,

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https://doi.org/10.1016/j.lrr.2024.100463
Received 21 March 2024; Accepted 14 May 2024
Available online 18 May 2024

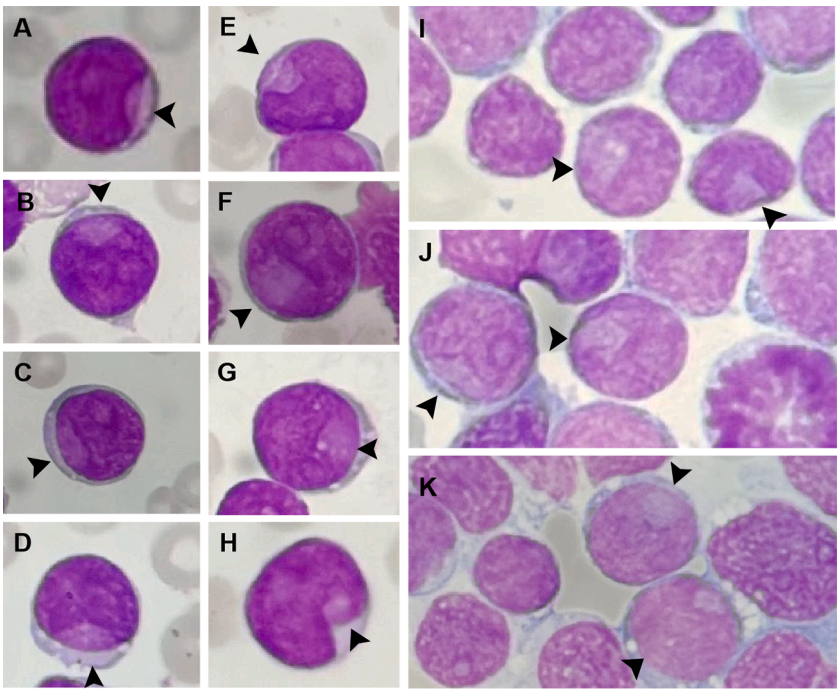

Fig. 1. Peripheral blood smear showing blast with cup-like nuclei (A–H; arrowheads; May–Grünwald–Giemsa stain, 100X in oil immersion). A distinctive concave indentation in some nuclei resulted in the characteristic "cup-like" appearance. Bone marrow smear showing blast cells with cup-like nuclei (I–K; arrowheads; May–Grünwald–Giemsa stain, 100X in oil immersion).

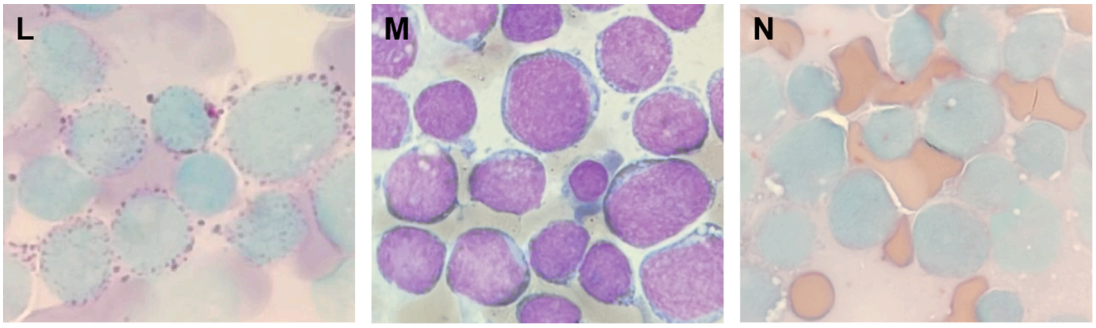

Fig. 2. Periodic acid-Schiff staining (L; 100X in oil immersion) showed 87 % positivity for polysaccharides with a granular scattered distribution. Peroxidase staining (M; 100X in oil immersion) and naphthol AS-D chloroacetate esterase staining(N; 100X in oil immersion) were negative.

aligning with the diagnosis of acute B-cell Lymphoblastic Leukemia (B-ALL). Karyotypic analysis showed irregularities; examination of 10 mid-stage dividing cells disclosed that 7 cells had reciprocal translocations between 4q21 and 11q23, suggesting the presence of acquired somatic genetic alterations potentially related to the AF4-MLL fusion. Despite the lack of further testing for common fusion genes, gene mutations, and other relevant examinations in lymphoma diagnostics, the final diagnosis was acute B-cell lymphoblastic leukemia with translocation (4;11)(q21;q23). Treatment commenced with the COP regimen on November 3rd (cyclophosphamide 1.1 g D1, vinorelbine 30 mg D1, 8, 15, 22; prednisone 50 mg D1–14, tapering starting D15). The patient experienced a recurrent fever and cough during chemotherapy. Sputum cultures and smears identified infections with Gram-positive cocci, Gram-positive bacilli, Gram-negative bacilli, and fungi. A combined treatment of meropenem and voriconazole yielded improvement. The second chemotherapy cycle began on January 2nd with the CAM regimen (cyclophosphamide 0.8 g D1, cytarabine 100 mg D1–3, D8–10; 6-mercaptopurine 100 mg D1–7), leading to the patient's discharge after improvement.

3. Discussion

Cup-like nuclei cells, also known as " fishmouth," are distinguished by nuclei indented more than 25 % of their diameter. Approximately 20 % of all AML cases exhibit blast cells with CLN, typically associated with a normal karyotype, absence of CD34 and HLA-DR, and mutations in NPM1 and/or FLT3-ITD. Conversely, this feature is rare in ALL, with the initial case of an ALL patient exhibiting CLN reported by Özgür Mehtap et al. [1]. in 2011, characterized as an adult ALL with positive BCR-ABL and negative FLT3 ITD. Such occurrences remain uncommon, potentially leading to misdiagnosis if solely based on CLN morphology. Nonetheless, Periodic acid-Schiff (PAS) staining serves as a method for differential diagnosis. In our case, PAS staining demonstrated a positive granular distribution, consistent with the staining characteristics of ALL, an increase in lymphoblasts presents primarily as coarse, granular PAS with scattered distribution, which may appear bead-like or chunk-like. In contrast, in AML subtypes M1, M2, M3, M4, and M5, PAS typically displays a fine, granular, and diffuse distribution. Furthermore, it was observed that CLN in ALL cells often possess more prominent nucleoli, a distinction from AML cells, which commonly lack nucleoli. This

difference may stem from the association of CLN morphology in AML with Nucleophosmin 1(NPM1), a crucial protein molecule located in the nucleolar granular region on chromosome 5 (5q35). NPM1 is known to shuttle between the nucleolus, nucleus, and cytoplasm under physiological conditions. Mutation in the NPM1 gene results in abnorma intracellular distribution of nucleolar phosphoproteins, leading to characteristic cup-like nuclei and indistinct nucleoli. However, CLN in B-ALL is likely not associated with FLT3 or NPM1 mutations [5]. Additionally, the cytoplasm of cup-like nuclei cells in ALL frequently contains numerous small vacuoles but lacks azurophilic granules, mirroring the morphological traits of primitive and immature lymphocytes. This feature may further distinguish cup-like nuclei in ALL cells from those in AML.

Recently, Weijie Li et al. [5] confirmed that CLN are uncommon in B-ALL and revealed a significant association between CLN and IKZF1 deletion in pediatric B-ALL. The IKZF1 gene, encoding the transcription factor Ikaros, plays a pivotal role in lymphoid differentiation. IKZF1 deletions have been found in 70–80 % of BCR-ABL11 B-ALL cases. And abnormalities in the IKZF1 gene can occur as an independent genetic event, not necessarily initiated by the BCR-ABL1 fusion oncogene [6]. The presence of the IKZF1 deletion serves as a poor prognostic indicator, suggesting that the prognosis for CLN may be worse. A notable finding is the occurrence of CLN in B-cell ALL with DUX4 rearrangement, also marked by IKZF1 deletions [4]. Hence, the identification of CLN is clinically valuable, particularly in the absence of cytogenetic results, as it may indicate the presence of the prognostically significant IKZF1 deletion, exhibiting a high positive predictive value. These CLN instances tend to exhibit an early B-precursor phenotype, a normal karyotype, or nonrecurrent cytogenetic abnormalities. Notably, abnormal karyotypes have not been documented in adult ALL with CLN. Unlike previous cases, ours displayed an abnormal chromosomal karyotype: 46, XX, t(4;11)(q21;q23), suggesting potential involvement in AF4-MLL fusion.

The translocation (4;11)(q21;q23) is the most frequent translocation observed in pro-B ALL, accounting for approximately 57 % of all cases. ALL characterized by this translocation exhibits a high malignancy level, a low remission rate, and a propensity for relapse after the initial remission, coupled with a brief survival period and a likelihood of Central Nervous System (CNS) invasion, particularly in adults over 40 years. This translocation (4;11)(q2l;q23) typically results in the formation of the MLL-AF4 fusion gene. The MLL-AF4 fusion gene correlates with a poor prognosis in children diagnosed with ALL, consistent with other MLL rearrangements. A recent study has shown that patients harboring both fusion genes fare better than those with only the MLL-AF4 fusion protein [7]. Another novel study revealed that the prognosis for patients with noninfant 11q23/KMT2A-rearranged ALL has improved, yet allo-HSCT has not influenced outcomes [8]. Unfortunately, further MLL gene rearrangement probes for FISH were not conducted by the patient. While most cases of t(4;11) are diagnosed as ALL, instances of AML have also been documented. Given that the 11q23/KMT2A (MLL) rearrangement occurs in multipotent Hematopoietic Progenitor Cells (HPC) and is linked to both myeloid and lymphoid malignancies, it raises speculation about the association of CLN with both AML and ALL with t(4;11), a chromosomal abnormality closely associated with the MLL/AF4 fusion gene. Sufana Shikdar et al. [9]. reported on a patient with therapy-related ALL who received a topoisomerase II inhibitor for induction chemotherapy. A higher frequency of MLL gene rearrangements, especially t(4;11), was observed in cases of therapy-related ALL compared to therapy-related AML.A recent novel study concludes that the RS4;11 cell line serves as an appropriate in vitro model for studying leukemia with t(4;11), potentially aiding in advancing research and achieving breakthroughs in understanding the relationship between CLN and chromosomal aberrations such as t(4;11) [10].

This paper delves into the nuanced morphology of CLN between ALL and AML. We particularly examine their association with chromosomal karyotype anomalies, such as t(4;11), and genetic aberrations, like IKZF1 deletion. This prompts a call for additional case studies and extended research to verify whether the morphological distinctions can indeed serve as a discriminative factor between the two conditions and if the presence of CLN is linked to specific chromosomal or genetic alterations.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Informed consent

The patient provided informed consent for the inclusion of her data in this report. The patient understood that the results will be fully anonymized, and she cannot be identified via this report.

CRediT authorship contribution statement

Yuyang Lu: Methodology, Writing – original draft. **Xinran Feng:** Data curation, Formal analysis. **Fengyu Chen:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Xiuchun Deng, Chengwei Zhang for their providing the relevant examination results, as well as the patient's medical records.

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