

Case Report

Immunophenotypic Differences in Cerebrospinal Fluid and Peripheral Blood Demonstrating Cancer Heterogeneity in Acute Myeloid Leukemia Patient

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Keywords

Acute myeloid leukemia · Central nervous system leukemia · Leukemic migration · Cancer heterogeneity

Abstract

A diagnosis of acute myeloid leukemia involving the central nervous system (CNS) can be confirmed through cerebrospinal fluid (CSF) and serum flow cytometry. These two detection methods should demonstrate the same immunophenotype due to hematogenous dissemination. Here, we reported a 65-year-old male diagnosed with CNS leukemia with differing immunophenotypes between CSF and peripheral blood. This immunophenotypic shift may suggest leukemic migration within the blood-brain barrier. In addition, the case highlights the concept of leukemic heterogeneity and the importance of considering cancer heterogeneity when analyzing a tumor's genetic profile and selecting therapy for patients.

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Introduction

Acute myeloid leukemia (AML), the most common adult leukemia, is highly heterogeneous in its manifestations. The disease can present with complications from cytopenias such as bleeding and infection as well as complications from an elevated white blood cell count such as leukostasis and organ infiltration [1]. Although recent advancements in the treatment of AML have increased cure rates, the probability of recovery still remains anywhere from 15 to 40% [1] and the 5-year disease-free survival of all patients presenting with AML persists at only 30% [2].

AML can involve various organ systems including the central nervous system (CNS), presenting with symptoms such as headache, nausea and vomiting, visual changes, and cranial nerve palsies. Ultimately, AML cells in the CNS can progress to delirium and other changes in cognition including lethargy and coma. Though rare, the spread of leukemia to the CNS carries a grave prognosis, with an overall 5-year survival of 11% [3]. Thus, early detection of malignancy spread is key to achieving higher survival rates.

The standard protocol for detection of CNS leukemia is through cerebrospinal fluid (CSF) cytology and CSF flow cytometry, which has a detection rate of 86% per lumbar puncture. Flow cytometry identifies light chain restriction, aberrant antigen expression, and pathologic cell morphology. It is useful for identifying tumor lineage to confirm a specific diagnosis, estimate prognosis, and devise a tailored treatment plan for CNS involvement of AML [4].

Theoretically, CSF flow cytometry should mirror serum flow cytometry as a result of the hematogenous spread of the malignancy. However, significant differences between leukemic cells detected in serum and CSF have been uncovered, highlighting the potential role of the blood-brain barrier in leukemic migration [4, 5]. In this case report, a patient presenting with AML exhibits contrasting findings on CSF and serum flow cytometry.

Case Report and Pathology

A 65-year-old man with a past medical history of diabetes mellitus, chronic kidney disease, benign prostatic hyperplasia, and secondary hyperparathyroidism initially presented with confusion and was found to be in diabetic ketoacidosis. His laboratories were notable for a significant leukocytosis of 38,500 with 58% blasts concerning for AML. He was then transferred to our hospital for further management of his hematologic malignancy.

Peripheral blood smear showed numerous circulating blasts (shown in Fig. 1). The blasts were large in size with high nuclear-to-cytoplasmic ratio, fine nuclear chromatin, and prominent nucleoli. Some blasts exhibited nuclear cupping.

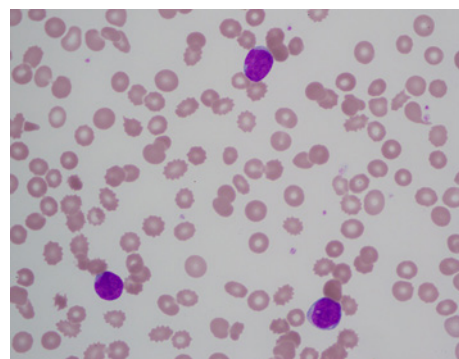


Fig. 1. Peripheral blood smear (Wright-Giemsa stain) showing numerous blasts with typical myeloblast morphology ($\times 100$ magnification).

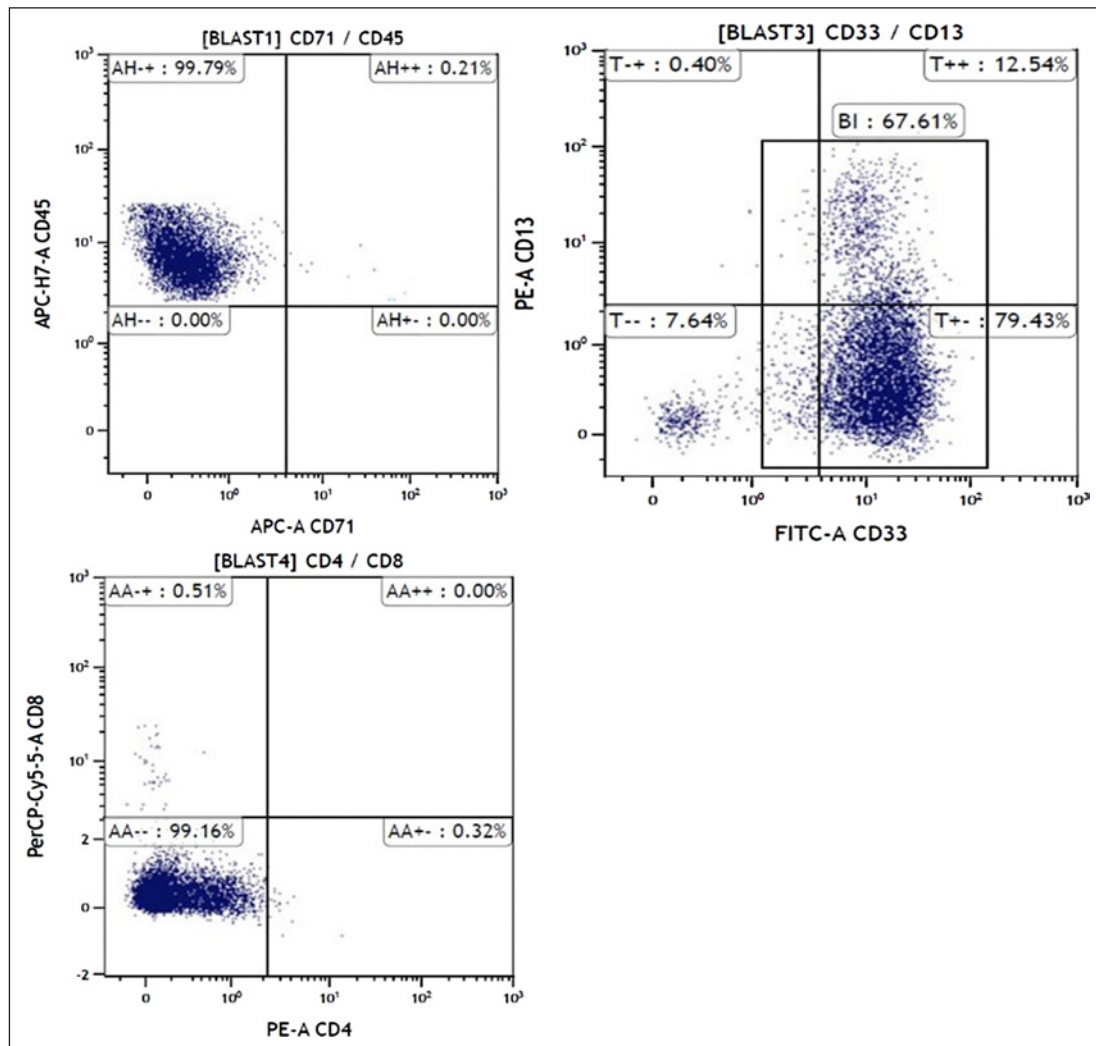


Fig. 2. Peripheral blood flow cytometry showing myeloblasts with the phenotypes CD45+ (dim), CD4-, CD13-.

Peripheral blood flow cytometry (shown in Fig. 2) revealed 68% myeloblasts with the following immunophenotype: CD45+ (dim), CD4-, CD10-, CD11b+ (partial), CD11c+ (dim), CD13-, CD14-, CD16-, CD33+, CD34-, CD38+ (heterogeneous), CD56-, CD64-, CD117+ (partial, dim), CD235a-, HLA-DR-, cCD3-, cCD22-, cCD79a-, MPO+, TdT-, consistent with AML. Next-generation sequencing (NGS) performed on peripheral blood revealed pathogenic mutations in *NPM1* and *IDH2*.

He developed neutropenic fever likely secondary to periodontal disease and/or cellulitis and was started on broad spectrum antibiotics. He was unable to undergo dental extraction due to thrombocytopenia; therefore, this became a barrier to starting chemotherapy.

During this time, he continued to have a waxing and waning mental status without improvement despite being on broad spectrum antibiotics, fungal prophylaxis (posaconazole), and viral prophylaxis (acyclovir). An extensive infectious workup of blood and CSF fluid was negative including blood cultures, fungal cultures, serum Fungitell, aspergillus galactomannan antigen, hepatitis screening, HIV 1/2 antigen and antibody, EBV PCR, CMV PCR, Zika virus PCR, West Nile virus PCR, Lyme antibody, acid fast bacilli stain and culture, and QuantiFERON-TB Gold. His respiratory pathogen panel, COVID swab, MRSA swab, flu/RSV

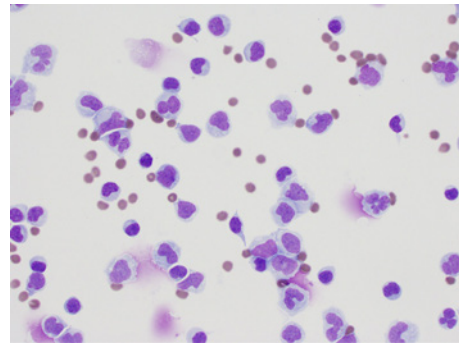


Fig. 3. Cerebrospinal fluid cytology (Diff-Quik stain) showing abnormal cells suspicious for leukemic cells ($\times 50$ magnification).

panel, C. diff toxin, urine streptococcus pneumonia, urine legionella, and urine cultures were also negative. MRI and CT imaging were noncontributory. Acyclovir was discontinued after it was thought to be the offending agent, but the patient's mental status remained poor.

Serial LPs were performed with subsequent CSF cytology specimen which revealed atypical cells (shown in Fig. 3). The cells were large in size and monocytoid with fine nuclear chromatin, prominent nucleoli, and moderate to large amount of cytoplasm.

Concurrent flow cytometry (shown in Fig. 4) revealed a population of abnormal CD117+ cells (12%) which were indeterminate for leukemia. These cells had the following immunophenotype: CD4+, CD10–, CD13+, CD14–, CD16–, CD33+, CD34–, CD38+, CD45+ (bright), CD56–, CD117+, HLA-DR+. The phenotype of these cells was noted to be different from the previously diagnosed AML (CD4–, CD13–, CD45 dim).

In order to confirm that the abnormal cells in the CSF were leukemic cells, NGS was performed on the CSF specimens. The same pathogenic mutations (*NPM1* and *IDH2*) were found in the abnormal cells in the CSF, confirming their leukemic nature with an immunophenotypic shift of uncertain significance.

Ultimately, because of his unchanged mental status despite intrathecal chemotherapy and overall poor performance status, our patient was transitioned to DNR/DNI. He passed within a few hours after being placed on comfort care measures. An autopsy was not performed.

Discussion

Our case highlights differing flow cytometry findings between CSF and peripheral blood samples but with similar mutational changes seen on NGS. CSF samples showed CD45+ bright, CD4+ and CD13+ positive leukemic cells, while peripheral blood showed CD45+ dim, CD4–, and CD13– cells (shown in Table 1). Both showed mutations in *IDH2* and *NPM1*. The significance of this immunophenotypic shift is unclear, but we suspect it may be due to the inherent heterogeneity of leukemia.

Cancer was originally thought to be present as a homogenous entity without variation within tumors. However, data support there is significant heterogeneity within cancer at any given point in time and between points in time. There are two main hypotheses regarding the cause of this, and they are genomic instability and clonal evolution/selection. In an article by Jack and Shaw, the genomic instability hypothesis is described as a range from single base substitutions to whole genome doublings and that these changes are critical to progression of many cancers [6]. This would imply that it is possible to have two or more distinct subpopulations within a single tumor each with their own genetic and molecular profile which evolved independently from a single clone.

The second mechanism by which heterogeneity occurs is through clonal evolution/selection. Tumor initiation occurs in a manner beginning with a “previously nonmalignant cell

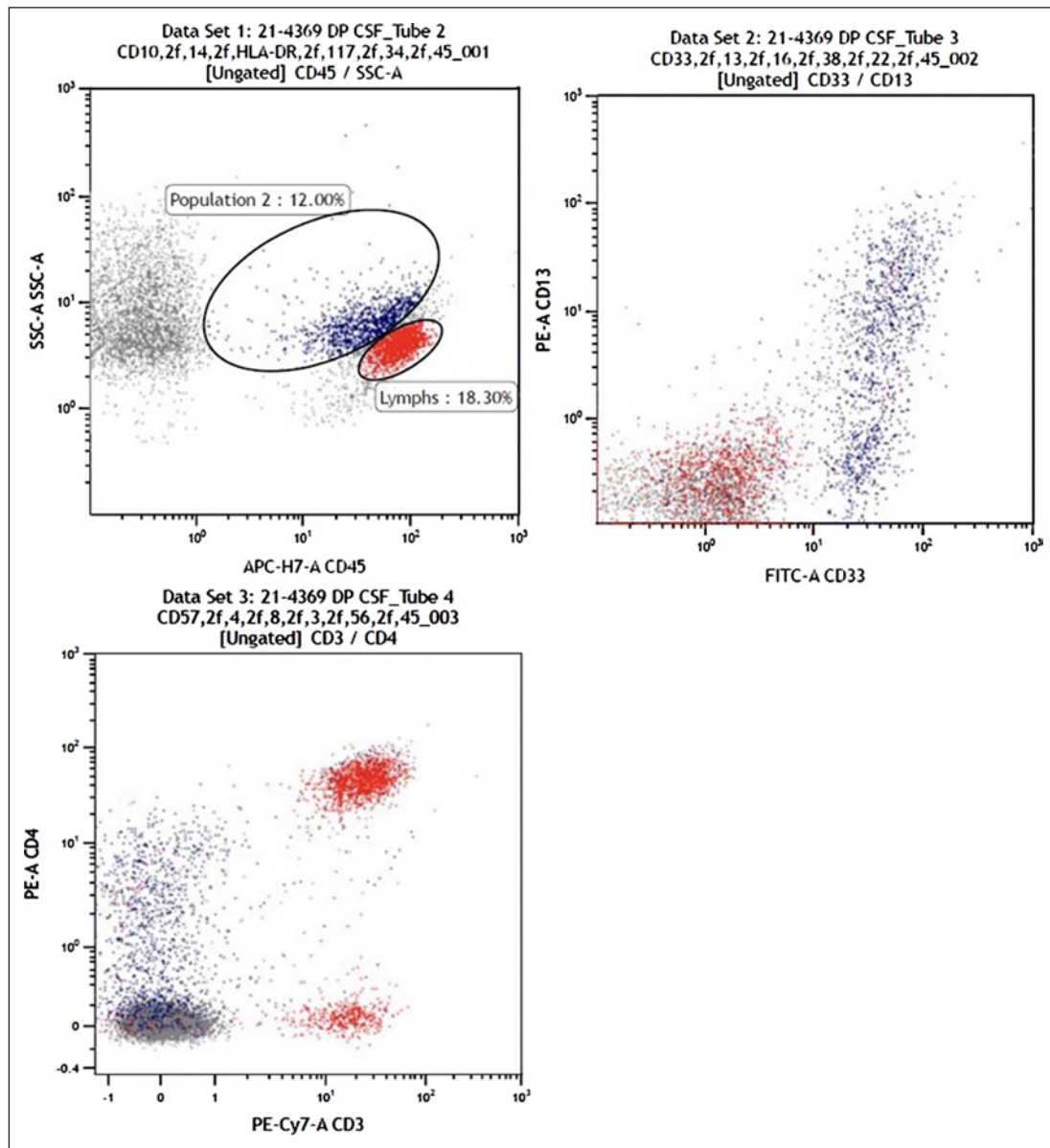


Fig. 4. CSF flow cytometry findings showing percentage of myeloblasts expressing the phenotypes CD45+ (bright), CD4+, CD13+.

Table 1. Comparison of flow cytometry findings between peripheral blood and CSF

Source of specimen	CD45	CD4	CD13
Peripheral blood	Dim	Negative	Negative
CSF	Bright	Positive	Positive

that confers a selective advantage and leads to neoplastic proliferation” [6]. Genomic instability of the expanding population results in genetic diversity that is subject to evolutionary selection pressures leading to an increasingly genetically abnormal and heterogenous tumor. The combination of genomic instability and clonal selection likely led to heterogeneity in our patient, ultimately leading to different immunophenotypes in the CSF and peripheral blood.

In AML specifically, it has been shown that there is significant heterogeneity that often leads to treatment resistance. In a study of 7 patients and their genomic profiles, 6 of those patients exhibited molecular heterogeneity, and though the subpopulations were relatively small, they did exist and proved oligoclonality. This study also showed that when patients relapsed, the originally analyzed smaller subpopulations expanded causing relapse [7].

It is interesting that in our patient, while immunophenotypes were different between the CSF and peripheral blood, the mutations seen on NGS were the same. This would imply that both samples came from the same leukemic precursors with the same driver mutations, but they carried differing CD markers on their cell surfaces. It is impossible to know for sure, however, we hypothesize that different clones evolved inside and outside the CSF due to environmental pressures and microenvironmental differences.

In conclusion, our case highlights an important aspect of leukemia and cancer in general, heterogeneity. Not all cells within a tumor carry the same molecular or genetic profile, and often, there can be vast variations in both. Our case is also the only one in the literature that highlights differing immunophenotypes between CSF and peripheral blood in AML. It is important to recognize heterogeneity as a possibility when it comes to treatment as often cancer heterogeneity leads to treatment resistance. Improved sampling techniques and genetic profiling are needed to combat this aspect of cancer care and to more specifically tailor treatments to patients.

Statement of Ethics

Ethical approval is not required for this study in accordance with local or national guidelines. Written informed consent was obtained from the patient's daughter for the publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Chelsea Edirisuriya, Chetan Jeurkar, and Margaret Kasner treated the patient and contributed to the writing of this case report. Bushra Nazir completed the pathology component and analyses. Mia Belovsky contributed to the writing of this case report.

Data Availability Statement

All data that support the findings of this case report are included in this article, and no additional source data are required. Further inquiries can be directed to corresponding author, Chelsea Edirisuriya.

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