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CASE REPORT

Novel Basophilic Crystals in Acute Myeloid Leukemia with EZH2/DNMT3A Mutations: Aggressive Case with Thrombosis

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SUMMARY

Background: **This study reports a novel case of EZH2/DNMT3A-mutated AML featuring unique basophilic rectangular crystals in blasts, associated with aggressive disease and thrombotic complications.**
Methods: **A 56-year-old male underwent morphological analysis (light microscopy, cytochemistry), clinical monitoring, and molecular profiling.**
Results: **MPO-negative rectangular crystals (3 - 20 µm) persisted despite therapy, demonstrating chemoresistance. These crystals were distinct from Auer rods and associated with an aggressive clinical course, including fatal DVT/PE within 7 months. Molecular analysis revealed EZH2/DNMT3A mutations, suggesting epigenetic dysregulation underlying both crystal formation and thrombotic predisposition.**
Conclusions: **This first-reported association between such crystals and EZH2/DNMT3A-mutated AML highlights their potential as high-risk markers. The findings implicate epigenetic dysregulation in treatment resistance and thrombogenesis, warranting cryo-EM studies to elucidate crystal composition and mechanisms. Clinically, this supports vigilant thromboprophylaxis and exploration of combined epigenetic/anticoagulant therapies for similar high-risk cases.**
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KEYWORDS

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INTRODUCTION

Cytoplasmic inclusions in AML most commonly manifest as Auer rods and faggot cells [1], with rare reports of other inclusions such as Charcot-Leyden crystals [2], Russell bodies [3], and pseudo-Chediak-Higashi granules [4]. We have identified for the first time the presence of MPO-negative rectangular crystals in the bone marrow of an AML patient harboring concurrent EZH2/DNMT3A mutations. These distinctive crystals presented either as single structures, in parallel pairs, as clustered bundles or coexisting with Auer rods within the same cytoplasm. Notably, these rectangular crystals per-

sisted during treatment, demonstrating chemotherapy resistance. The patient subsequently developed multiple thrombotic complications - an exceptionally rare presentation in AML and ultimately succumbed to respiratory failure.

CASE PRESENTATION

A 56-year-old male was incidentally found to have leukopenia (WBC 2.06 x 10^9/L) and anemia (Hb 89 g/L) during routine health screening, with preserved platelets (319 x 10^9/L). Peripheral blood smear examination revealed circulating blasts, prompting immediate hospitalization. Physical examination showed stable vital signs (temperature 36.6℃, heart rate 84 bpm, respiratory rate 20/minute, blood pressure 103/54 mmHg), with pale conjunctivae and anemic facies noted, while no lymphadenopathy, sternal tenderness, or hepatosplenomegaly were observed.

Diagnostic evaluation included bone marrow aspiration demonstrating 90% hypercellularity with dyspoietic features such as pseudo-Pelger cells and multilobated megakaryocytes. Notably, 38% of blasts contained distinctive rectangular crystals (3 - 20 μm) that appeared either as single structures (Figure 1A - B), parallel pairs (Figure 1D), or occasional clusters (3 - 5 per cell) coexisting with Auer rods (Figure 1C, arrows). Cytochemical analysis revealed these rectangular inclusions were MPO-negative (Figure 1E - F, arrow), in striking contrast to the MPO-positive Auer rods (Figure 1F, arrow) and normal granulocytes (Figure 1E). Flow cytometry immunophenotyping showed blast positivity for CD34, CD117, CD13, and CD33. Molecular characterization by next-generation sequencing identified pathogenic mutations in EZH2 (R685C), DNMT3A (R882H), and U2AF1 (S34F), while karyotype analysis demonstrated an abnormal clone (46,XY,+1,der(1;7)(q10;p10)[10]). The clinical course was complicated by thrombotic events despite azacitidine therapy (100 mg SC days 1 - 9), including left calf vein thrombosis (Figure 2B, arrows) (day 8) and pulmonary embolism (Figure 2A, arrows) (day 13), managed with anticoagulation and IVC filter placement. Persistent disease was evidenced by 35.7% residual blasts with ongoing crystal presence post-treatment. Cycle 2 of azacitidine was complicated by hemoptysis (200 mL fresh blood) and active bleeding (PT 15.9 s, FIB 3.8 g/L, aPTT 35.3 s), leading to treatment discontinuation. The disease progressed rapidly to fatal respiratory failure within 7 months, with the characteristic rectangular crystals persisting throughout the clinical course, underscoring their association with chemoresistance and poor prognosis.

DISCUSSION

Intracytoplasmic inclusions in myeloid neoplasms bear significant diagnostic, classificatory, and prognostic implications. The most commonly observed are Auer rods and faggot cells. Auer rods demonstrate positive staining for myeloperoxidase (MPO), periodic acid-Schiff (PAS), Sudan black B, and acid/alkaline phosphatases, while being negative for lipase, glycogen, and DNA/nucleic acid stains [1]. Electron microscopy reveals them to be composed of fused granular masses within the endoplasmic reticulum system. Ultrastructural studies further demonstrate morphological continuity between Auer rods and cytoplasmic granules, supporting their origin from azurophilic granules and being derived from fused lysosomes. The presence of Auer rods in AML typically indicates better prognosis (as a marker of maturation/differentiation), while in MDS and MDS/MPN cases it suggests disease progression. Their persistence in post-treatment AML blasts indicates failure to achieve remission. Other rare crystalline inclusions include: Charcot-Leyden crystals (microcrystals composed of galectin-10 protein found in eosinophils/basophils) observed in NPM1-mutated AML bone marrow [2]; Russell bodies (typically seen in pathological plasma cells) detected in a 65-year-old female AML patient in remission after IA chemotherapy [3]; Pseudo-Chediak-Higashi inclusions identified in therapy-related AML with myelodysplasia-related [4]. We report the first AML case with MPO-negative basophilic rectangular crystals. The distinctive rectangular morphology and basophilic staining of these crystals contrast sharply with the rod-shaped azurophilic Auer rods, suggesting a unique pathogenesis. Clinically, their persistence despite azacitidine therapy implies an association with chemoresistance.

In our case, we identified concurrent EZH2 R685C and DNMT3A R882H mutations. In mammals, DNA methylation patterns are established and maintained by DNA methyltransferases (DNMTs), including DNMT1, DNMT3A, and DNMT3B. Aberrant DNMT activity leading to epigenetic dysregulation contributes to tumorigenesis. Somatic mutations in DNMT3A occur in approximately 22% of de novo AML cases and are associated with poor survival outcomes. EZH2 represents the second histone methyltransferase gene implicated in cancer. The enhancer of zeste homolog 2 (EZH2) gene, located on chromosome 7q35, serves as a core component of polycomb repressive complex 2 (PRC2). Emerging evidence indicates that EZH2 functions as a recruitment platform for DNMTs, demonstrating cooperative activity between EZH2 and DNMT3A [5,6]. In our report, the co-occurrence of EZH2 (R685C) and DNM T3A (R882H) mutations likely contributed to leukemogenesis and disease progression, resulting in rapid clinical deterioration. The patient survived only 7 months from diagnosis to death.

Concurrent arterial and venous thrombosis is exceptionally rare in AML. While the risk of arterial throm-

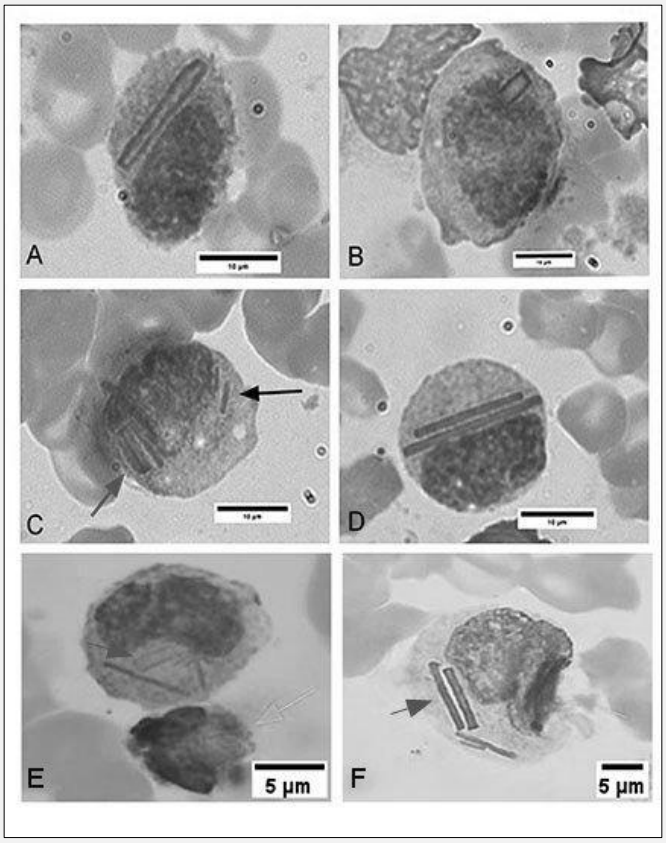

Figure 1. Morphological and cytochemical features of rectangular inclusions in granulocytes.

A - B Single rectangular inclusions observed in granulocytes. C Cluster of 3 - 5 rectangular inclusions (arrow) coexisting Auer rods (arrows) in the same cell. D Parallel pairs of rectangular inclusions. E - F Cytochemical staining for myeloperoxidase (MPO). Rectangular inclusions (arrow, E - F) are MPO-negative, contrasting with strongly MPO-positive Auer rods (arrow, F) and normal granulocytes (arrow, E).

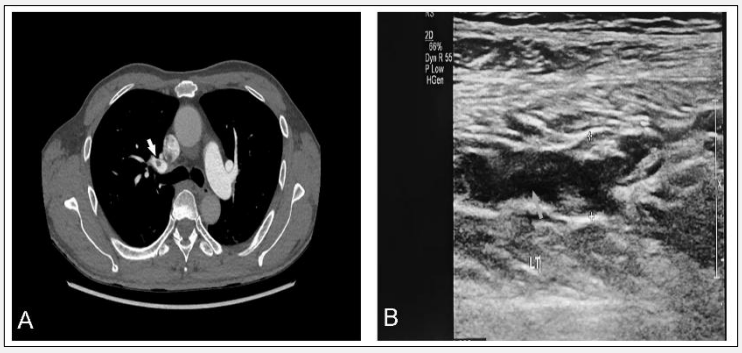

Figure 2. Thrombotic complications in AML with EZH2/DNMT3A mutations.

A Pulmonary embolism (CT angiography, axial view), white arrow: filling defects in right pulmonary artery branches, consistent with acute pulmonary embolism. B Deep vein thrombosis (doppler ultrasound, longitudinal view). Arrow: non-compressible left calf muscular veins with intraluminal thrombus.

bosis typically increases 6 - 12 months after hematologic malignancy diagnosis [7], our patient developed multiple thrombotic complications within this abbreviated clinical course: Day 18: Complete thrombosis in the left calf muscular vein with partial thrombosis in the posterior tibial vein. Day 33: Pulmonary artery thrombosis. Current research confirms that DNMT3A mutations in AML correlate with elevated thrombotic risk [8]. The patient ultimately succumbed to respiratory failure secondary to pulmonary infection.

The limitations of this study include the lack of proteomic analysis of the crystals and the single-case nature of these observations highlight the need for further validation studies.

CONCLUSION

This case highlights the discovery of novel MPO-negative basophilic rectangular crystals in AML with concurrent *EZH2/DNMT3A* mutations, representing a distinct morphological entity differing from classical Auer rods. The crystals' persistence during therapy and association with chemoresistance, rapid disease progression, and unusual thrombotic complications suggest they may serve as a novel prognostic marker for high-risk AML. The co-occurrence of *EZH2/DNMT3A* mutations likely drive both leukemogenesis and thrombotic propensity through epigenetic dysregulation. These findings underscore the need for: 1) comprehensive morphological screening in AML, 2) advanced characterization of crystal composition using cryo-EM/proteomics, and 3) investigation of the mechanistic links between epigenetic mutations, crystalline inclusions, and thrombosis. This case expands our understanding of cytoplasmic inclusions in myeloid malignancies and emphasizes their clinical relevance in risk stratification.

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Ethics Statement:
Informed consent was obtained. The study complied with the Declaration of Helsinki.

Data Availability:
Original data available upon request.

Declaration of Interest:
The authors declare no competing interests.

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