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Autoimmune hemolytic anemia complicating a chronic neutrophilic leukemia: A case report of a rare association

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Key Clinical Message: Chronic neutrophilic leukemia is a rare disease with a poor prognosis. Its diagnosis is challenging in the lack of genetic tools. It can infrequently be associated with autoimmune hemolytic anemia.

Abstract: Chronic neutrophilic leukemia is a rare disease with poor prognosis, characterized by a sustained mature neutrophilic leukocytosis in the absence of monocytosis or basophilia with few or no circulating immature granulocytes, hepatosplenomegaly, and granulocytic hyperplasia of the bone marrow. In addition, no molecular markers for other myeloproliferative neoplasms are detected. The 2016 WHO classification included the presence of the CSF3R mutation as a key diagnostic criterion for this disease. Although anemia may be present at diagnosis, hemolytic one rarely complicates myeloproliferative neoplasms. Treatment is largely based on cytoreductive agents, but bone marrow allograft remains the only curative option. We report the case of a patient with chronic neutrophilic leukemia associated with autoimmune hemolytic anemia. We describe the epidemiological, clinical, prognostic, and therapeutic features of this disease in addition to the difficulties of its diagnosis and management in Tunisia.

KEYWORDS
chronic neutrophilic leukemia, cytoreductive therapy, hemolytic anemia, poor prognosis

JEL CLASSIFICATION
Haematology

1 | BACKGROUND

Chronic neutrophilic leukemia (CNL), a rare myeloproliferative syndrome, is potentially aggressive and has a poor prognosis.[1] CNL is a rare neoplasm whose true incidence is still unknown according to 2019 National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER) data.[2] Median survival varies between 21 and 30 months, with a 5-year survival of 28%.[1]

It is characterized by the presence of oncogenic driver mutations in the factor 3 receptor in most CNL patients, and it usually occurs in the elderly. Autoimmune

hemolytic anemia (AIHA) is rarely seen in chronic my-eloproliferative neoplasms, making the association between CNL and AIHA more unexpected.

The first-line therapy for CNL is based on cytoreductive agents in the absence of matched donors.[3,4] Yet, bone marrow allograft remains the only curative treatment.[1]

We here present the case of a 42-year-old female patient, with no medical history, diagnosed with CNL complicated by AIAH. We also describe the epidemiological, clinical, biological, and diagnostic aspects of this disease and its different therapeutic strategies.

2 | CASE PRESENTATION

A 42-year-old Caucasian woman, with no personal or familial medical history, was referred to our department for persistent hyperleukocytosis of 30×10^9/L and left hypochondrial pain evolving for more than 3 months. No history of severe bleeding, infections, or recent drug intake was reported. Clinical examination showed splenomegaly of 4 cm with no palpable adenopathy. No signs of anemic, infectious, or inflammatory syndromes were noted.

3 | INVESTIGATIONS

Her complete blood count (CBC) showed a normocytic regenerative anemia (Hb: 10.9 g/dL; MCV: 85 fl; Retic: 125×109/L), WBC: 35×109/L, and PLT: 377×109/L. The blood smear revealed as follows: 60% neutrophils (21×109/L), 12% lymphocytes (4×109/L), 2% monocytes (0.7×109/L), 2% eosinophils (0.7×109/L), 4% basophils (1.4×109/L), and a 19% of neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes). A direct Coombs test (DCT) was negative. The haptoglobin level was <.08 g/L. The hemostasis workup (prothrombin ratio, activated partial thromboplastin time, fibrinogen) and the hepatic and renal checkups revealed no abnormalities. The uric acid level was normal at 134 µmoL/L (normal value: 180–400 µmoL/L), and the lactate dehydrogenase (LDH) level was elevated to 885 U/L (normal value: 20–200 UI/L). There was also a high alkaline phosphatase (PAL) level at 177 UI/L (normal value: 50–150 UI/L) in addition to a predominantly unconjugated hyperbilirubinemia at 35 mg/L (normal value <10 g/L).

Folic acid and vitamin B12 dosage as well as ferritin were normal. The serum protein electrophoresis showed no abnormalities.

The first diagnosis suspected was chronic-phase chronic myeloid leukemia (CML). The bone marrow aspiration revealed a granulocytic hyperplasia of the bone marrow (84%) with normal maturation and no signs of

dysplasia. In addition, there were no blasts in the bone marrow.

The karyotype was normal, and the BCR-ABL transcript was not detected in the molecular examination. The analysis of JAK2 mutations was negative. Given the atypical presentation of the disease, we suspected the diagnosis of atypical CML and a bone marrow biopsy was performed. It revealed a hypercellular marrow due to hyperplasia of the granulocytic lineage with many neutrophils with neither dysgranulopoiesis nor signs of myelofibrosis.

A revision of the first blood smear was performed showing a neutrophil count over 80% and a 9% of myelemia. This was explained by the fact of confusing the neutrophils' band forms with myeloid precursors.

Hence, the diagnosis of CNL was retained on the basis of clinical, biological, and histological data according to the WHO 2008 classification given that CSF3R mutation analysis is not available in our country.[5]

4 | TREATMENT

Cytoreduction with hydroxyurea at a dose of 1000 mg/day was initiated resulting in an initial but partial hematological improvement after 3 months: The WBC decreased to 6.7×10^9/L but the hemoglobin level dropped to 7.8 g/dL with a reticulocyte count of 354×10^9/L and a persistent negative DCT. Cold agglutinin titer was negative (≤64).

The LDH level was elevated to 468 U/L, and unconjugated bilirubinemia was high at 44 mg/L.

Viral serologies and antinuclear antibodies were negative. All causes of corpuscular hemolytic anemia were ruled out; for instance, the flow cytometry showed no evidence for paroxysmal nocturnal hemoglobinuria.

The patient was put on prednisolone at a dose of 1 mg/kg/day and Foldin. A check-up done 10 days after treatment showed an improvement of the hemoglobin level to 9 g/dL with a reticulocyte count of 199×10^9/L.

5 | OUTCOME AND FOLLOW-UP

Treatment with hydroxyurea at the same dose was continued. Corticosteroid treatment was maintained at full dose for 2 months and then was tapered over 4 months. The 9-month follow-up showed a reduction in spleen size from 19 cm to 13.8 cm. The CBC revealed a decrease in the WBC count to 9×10^9/L with a hemoglobin level of 12.6 g/dL. The good response to corticosteroids is one more argument in favor of the diagnosis of AIHA complicating this rare myeloproliferative syndrome. We obtained a normal CBC at 14-month follow-up. The spleen size was then 12.5 cm.

6 | DISCUSSION

CNL is a rare myeloproliferative neoplasm characterized by persistent clonal proliferation of neutrophils, granulocytic hyperplasia of the bone marrow, and hepatosplenomegaly. Officially recognized in 2001 as a distinct disease, CNL was added to the WHO classification system in 2008 and the definition was based on clinical, cytological, and molecular criteria.[3] According to that, hepatosplenomegaly is the most important feature on the patient's examination. Cytological results show, on the one hand, persistent clonal proliferation of neutrophils (leukocytosis of $\geq 25 \times 10^9$/L of which $\geq 80\%$ are segmented neutrophils and bands forms, <10% circulating neutrophil precursors, the absence of monocytosis or basophilia), and on the other hand, granulocytic hyperplasia and neither dysplasia nor myeloblasts in the bone marrow.[3,6] In addition, there are no clinical or molecular criteria for other myeloproliferative neoplasms. The diagnosis of CNL in our patient meets all the above criteria.

CNL occurs mainly in the elderly. The median age at diagnosis is 66 years with ranges varying between 15 and 86 years and with a slight male predominance.[3,7,8]

The majority of patients are asymptomatic at diagnosis. They may have signs such as fatigue, hemorrhagic symptoms, or bone pain. Clinical examination may reveal signs of bone marrow insufficiency, hepatomegaly, and/or splenomegaly, which indicate an advanced stage of the disease. Node involvement is relatively rare.

In addition to an abnormally high neutrophil count, the blood count may also reveal monocytosis usually $<1 \times 10^9$/L, mild anemia (the type of which is unspecified in the literature), a normal platelet count, or mild thrombocytopenia.

The particularity of our case resides in the association of this CNL with an AIHA since it rarely complicates myeloproliferative neoplasms.[9] The response to corticosteroids supports the immunological nature of this anemia in the absence of other etiologies.

The bone marrow morphology, an important diagnostic criterion for CNL, shows a hypercellular marrow, granulocyte hyperplasia (without dysgranulopoiesis) with normal maturation and the presence of less than 5% myeloblasts. These criteria are useful to distinguish CNL from other myeloproliferative neoplasms such as atypical CML which is a heterogeneous association between myeloproliferative and myelodysplastic disorders.[1]

The initial karyotype is often normal. Nonspecific cytogenetic abnormalities occur in a minority of patients.[3,9,10] In 2002, a study by Reilly showed that 37% of CNL cases had an abnormal karyotype consisting mainly of trisomy 8, trisomy 21, 11q deletion, and 20q6 deletion and these abnormalities are seen either at the time of diagnosis or at the time of progression to acute leukemia.[11] In our case, the initial karyotype was normal.

The 2016 update of the WHO classification included the existence of the CSF3RT618I mutation or other activating mutations of CSF3R among the diagnostic criteria for CNL (Table A1 in Appendix). These molecular findings found in the majority of CNL patients (90%) allowed a better understanding of this disease.[12–14]

This genetic analysis is not available in Tunisia. We, therefore, referred to the WHO 2008 criteria to establish the diagnosis of CNL.

The overall prognosis of patients with CNL is generally poor, although the clinical course is variable and the median survival time is estimated to be 24 months.[11,15,16] Death is usually caused by cerebral hemorrhage, blast transformation, or fulminant infection.[3,17] Several studies have evaluated the prognostic relevance of several factors, but until recently, no prognostic scoring system was available for risk stratification in CNL.[8,18,19]

Progression of the disease is usually suspected by lack of response to therapy, progressive refractory neutrophilia, increased transfusion requirements, worsening of visceromegaly, the appearance of additional molecular or cytogenetic abnormalities, and eventually blast transformation, which has been reported exclusively as myeloid.[16]

The rarity of this disease makes treatment options very limited. Cytoreductive therapy such as hydroxyurea, interferon-alpha, and hypomethylating agents have been shown to temporarily control the disease, whereas standard intensive induction chemotherapy does not result in complete hematologic remission and confers a significant risk of mortality from infection or bleeding.[3,4,16]

Indeed, hydroxyurea has been the most commonly used treatment as first-line therapy with an initial response in 75% of patients and a median treatment duration of 12 months.[3]

According to the 2018 study by Szuber et al., out of 19 patients predominantly treated with hydroxyurea as first-line therapy, almost half required second-line therapy and one-third required three or more lines of treatment.[8] Other cytoreductive agents can be used, but interferon-alpha is the only agent that has shown potential for durable remissions as published in limited case reports.[4,20] In our case, hydroxyurea was effective for 14 months, and in case of CNL relapse, only interferon-alpha is available as a second-line treatment option in Tunisia.

After the discovery of the CSF3R mutation, this biomarker became a diagnostic and a therapeutic tool. In fact, juxtamembrane mutations in CSF3R are thought to confer sensitivity to JAKI kinase inhibitors as they may inhibit signaling of the oncogenic JAK–STAT pathway.[12,21]

Allogeneic bone marrow transplantation remains the only known curative treatment, although it is rarely performed given the age of the majority of patients with CNL.[1]

7 | CONCLUSION

AIHA rarely complicates myeloproliferative syndromes, which makes the association between CNL and AIAH highly unlikely. The diagnosis of CNL in our case was based on clinical and cytological findings with the absence of criteria for other myeloproliferative syndromes. The discovery of new genetic markers, such as CSF3R, furthers our understanding of CNL and provides new diagnostic tools and therapeutic targets. The future challenge in Tunisia will be to introduce these genetic markers in the diagnostic workup of CNL and consequently facilitate the diagnosis of such a rare disease.

AUTHOR CONTRIBUTIONS
Rim Rakez: Writing – original draft; writing – review and editing. **Ons Charef:** Resources. **Wiem Boufrikha:** Conceptualization. **Syrin Rassas:** Resources. **Sarra Boukhriss:** Validation. **Mohamed Adnene Laatiri:** Supervision; validation.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL
The authors declare that the article follows the Helsinki Declaration guidelines and that informed written consent was obtained from the patient prior to the publication.

CONSENT
Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

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APPENDIX 1

TABLE A1 World Health Organization (WHO) Criteria for CNL diagnosis.

1. Peripheral blood leukocytosis
- Increased number of white blood cells circulating in the peripheral blood $\geq 25 \times 10^9$/L

2. Bone marrow biopsy that shows a greater than normal percentage of bone marrow cells
- Neutrophils increased in percentage and number
- Neutrophil maturation appears normal
- Myeloblasts (immature cells) <5%

3. Does not meet WHO criteria for CML BCR-ABL 1+, PV, ET, or PMF

4. No genetic rearrangement (mutation) of
- PDGFRA—platelet-derived growth factor receptor, alpha polypeptide [as seen in chronic eosinophilic leukemia (CEL)]
- PDGFRB—platelet-derived growth factor receptor, beta polypeptide [as seen in chronic myelomonocytic leukemia (CMML)]
- FGRF1—fibroblast growth factor receptor 1 [as seen in chronic eosinophilic leukemia (CEL) and some subtypes of acute myeloid leukemia (AML)]
- PCM1-JAK2—(as seen in atypical chronic myeloid leukemia (aCML) and erythroid leukemia)

5. Presence of CSF3R T618I or other activating CSF3R mutation OR in the absence of a CSF3R mutation
- Persistent neutrophilia (at least 3 months)
- Enlarged spleen (splenomegaly)
- No identifiable cause of reactive neutrophilia including the absence of a plasma cell neoplasm
- If reactive neutrophilia is present, demonstration of malignant myeloid cells by cytogenetic or molecular studies

Abbreviations: CML, chronic myeloid leukemia; CSF3R, colony-stimulating factor 3 receptor; ET, essential thrombocythemia; PCM1-JAK2, pericentriolar material 1-Janus kinase 2; PMF, primary myelofibrosis; PV, polycythemia vera.