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Case Report

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Atypical case of B-cell Chronic Lymphocytic Leukemia presenting with extreme hyperleukocytosis

Hiperleucocitoză extremă într-un caz atipic de leucemie limfatică cronică cu celulă B

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Abstract

Very few cases of chronic lymphocytic leukemia (CLL) presenting with extreme hyperleukocytosis are reported in the literature. We describe the case of a 66 years old woman, with newly diagnosed CLL presenting with extreme hyperleukocytosis of 774.2 x 109/liter, Rai stage III and Binet stage C. The patient has no comorbidities and the CIRS score (cumulative illness rating scale) is well below 6, with normal creatinine clearance. Some other interesting aspects related with this case are the atypical immunophenotype, the expression of Cyclin D1, and the B hepatitis viral infection, which made her diagnosis and treatment challenging. The patient was tested for NOTCH1 mutation and it was positive. There is important evidence that NOTCH1 mutations are associated with rapidly progressive disease and resistance to treatment. The distinction of CLL from mantle cell lymphoma (MCL) is not always easy because some MCLs may mimic CLL clinically, histologically, and/or phenotypically. The hepatitis B prophylaxis for viral reactivation was not available an in the end the patient was treated only with fludarabine and cyclophosphamide, without rituximab. CD200 should be introduced in the routine panel for flow cytometry to distinguish CLL from mantle cell lymphoma and NOTCH1 mutation is associated with poor prognosis and should be evaluated at diagnosis. CLL with extreme hyperleukocytosis presentation is very rare and sometimes an atypical CLL may represent a diagnostic pitfall.

***Keywords**: CLL; extreme hyperleukocytosis; NOTCH1 mutation*

Rezumat

În literatura de specialitate sunt prezentate foarte puține cazuri de leucemie limfocitară cronică (LLC), care debutează cu hiperleucocitoză extremă. Descriem cazul unei femei în vârstă de 66 ani, care se prezintă cu hiperleucocitoză extremă de 774.2 x 109 / litru, stadiul Rai III și stadiul Binet C. Pacienta nu are comorbidități

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și scorul CIRS (scala cumulativă de evaluare a bolii) este cu mult sub 6, cu clearance-ul creatininei normal. Alte aspecte interesante legate de acest caz sunt imunofenotipul atipic, expresia ciclinei D1, infecția cu virusul hepatitei B, care au făcut ca diagnosticul și tratamentul acestei paciente să fie o provocare. Pacienta a fost testată pentru prezența mutației NOTCH1 care a fost pozitivă. Există dovezi că mutațiile NOTCH1 sunt asociate cu boala rapid progresivă și rezistență la tratament. Distincția dintre LLC și limfomul cu celule din manta (LCM) nu este întotdeauna ușoară, deoarece unele cazuri de LCM pot mima LLC din punct de vedere clinic, histologic și/sau fenotipic. Profilaxia hepatitei B pentru reactivarea virală nu a fost disponibilă, iar pacienta a fost tratată numai cu fludarabină și ciclofosfamidă, fără rituximab. Markerul CD200 ar trebui să fie introdus în panelul de rutină pentru citometria în flux pentru a distinge LLC de limfomul de manta iar mutația NOTCH1 este asociată cu un prognostic nefavorabil și ar trebui sa fie evaluată la diagnostic. LLC cu hiperleucocitoză extremă la debut este foarte rară, iar uneori, un LLC atipic poate reprezenta o capcană de diagnostic.

***Cuvinte cheie**: LLC; hiperleucocitoză extremă; mutația NOTCH1*

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults, one of the chronic lymphoproliferative disorders that is predominantly diagnosed in the elderly, with common clinical presentations like lymphadenopathy, splenomegaly, hepatomegaly. Approximately one quarter of patients are asymptomatic at diagnosis and referred to the hospital due to an abnormal white blood cell count (WBC). In the literature, there are very few cases reports about chronic lymphocytic leukemia presenting with extreme hyperleukocytosis.

Case report

Here we describe the case of a 66 years old woman, presenting in May 2014, with newly diagnosed CLL and an extreme hyperleukocytosis of 774.2 x 10^9/liter. At presentation, the patient's complaints were fatigability and a weight loss of 7 kg in 4 months. She reported multiple respiratory tract infections in her recent medical history. The hemoglobin level was 9.1 g/dl and the platelet count was 167 x 10^9/l. Biochemistry showed high lactate dehidrogenase activity (741 U/L). The blood smear examination showed 93% small lymphocytes with mature morphological aspect, 5% prolymphocytes and 12 smudge cells. The clinical examination revealed bilateral cervical adenopathies, right latero-cervical and axillary adenopathy, hepatomegaly and splenomegaly. Ultrasound described subhepatic and pancreatic anterior enlarged lymph nodes. This corresponds to Rai stage III and Binet stage C.

A bone marrow aspirate exam revealed high cellularity, with the predominance of small lymphocytes (90%), and frequent smudge cells. In the bone marrow biopsy, the 12 examined osteo-medullary areas showed increased cellularity, averaging 80%, with a significant reduction of fat cells due to a malignant lymphoproliferative process with nodular and interstitial architecture. Immunohistochemical staining was positive for CD20, CD5, and CD79a and negative for CD23. Approximately half of cells were positive for Cyclin D1. Immunophenotyping performed from peripheral blood, revealed a clonal population expressing kappa chains, CD5, CD19, CD20 (bright), CD22, and CD79b positive, partial expression for CD38, CD23 and FMC7, LAIR1 negative. Testing of CD200 was necessary to distinguish CLL from mantle cell lymphoma (MCL) and the marker was positive (1) (Figure 1). The patient was diagnosed with B-CLL. The patient was tested for B and C hepatitis and for

HIV 1 and 2 infection. HBsAg, anti-HBeAb and anti-HBcAb were positive, but the quantitative PCR DNA HBV was undetectable.

Karyotyping and FISH (fluorescent in situ hybridization) analysis were not available. To investigate the prognosis of the disease, molecular studies were performed on DNA extracted from peripheral blood. Taking into account that deletion of the long arm of chromosome 13 is one of the most frequent chromosomal abnormalities in CLL we performed Quantitative Fluorescence-Polymerase Chain Reaction technique (QF-PCR). The markers used for chromosome 13 were from Elucigen QST*R*plus*v2 kit and were localized as follow: 13q12.2; 13q13.3; 13q21.33; 13q22.1; 13q31.1. We observed two signal for each marker used, so we consider that the patient did not present del(13q).

NOTCH1 point mutation was analyzed by amplification refractory mutation system (ARMS) PCR assays as previously described (2). This mutation occurs almost exclusively in exon 34 and usually lead to a premature stop codon (3). The *NOTCH1* mutation is represented by CT deletion in about 80% of CLL patients positive for *NOTCH1* mutation. The investigated CLL patient presented *NOTCH1* mutation.

The patient had no significant organomegaly (no lymphadenopathy above 3 cm in diameter, liver and spleen slightly enlarged). Although the

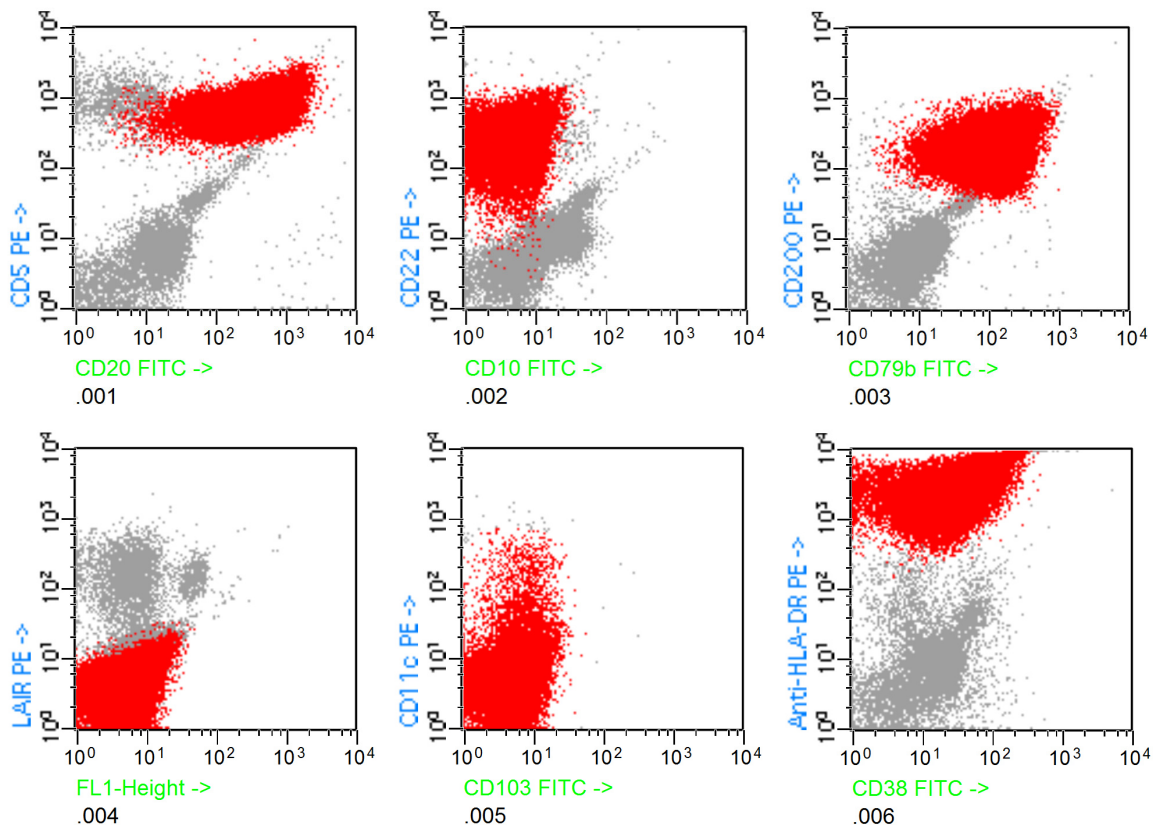

Figure 1. Immunophenotyping showed B cell monoclonal population (kappa chains), with CD19, CD20, CD5, and CD200 positive, CD23 with partial expression and LAIR1 negative, strongly supportive for chronic B cell lymphocytic leukemia.

number of lymphocytes is not an indication for treatment per se, we decided to treat the patient due to highly elevated B cells number, anemia and weight loss. National Comprehensive Cancer Network (NCCN) guidelines recommend starting treatment at a lymphocyte count above 200-300x10^9/liter, unless other indicated (4).

The patient has no comorbidities and the CIRS score (cumulative illness rating scale) is well below 6, with normal creatinine clearance. She has no hepatitis B virus infection history but her serology tested positive.

The therapy of choice was FCR (fludarabine cyclophosphamide rituximab). This treatment is associated in patients above 65 years of age with frequent grade 3 and 4 neutropenia (more than 50% of patients). Tumor lysis was also a concern due to the high number of peripheral lymphocytes. The hepatitis B prophylaxis for viral reactivation was not available and in the end the patient was treated only with FC (fludarabine and cyclophosphamide, without rituximab). We planned 6 cycles at 28 days interval, with G-CSF support if needed. Acyclovir and co-trimoxazol prophilaxy was offered. In September, when the patient came for control, the leukocyte number was normal (5.8x109/liter).

Discussion

The particularity of this case is the onset of the disease with extreme hyperleukocytosis. As we know there are very few cases reported in the literature of this kind. One case was reported in 1988 by Alcalay D. et al, with leukostasis syndrome (5). Other cases with leukostasis syndrome were reported by de Fijter CW in 1996 (6), and by Durzyński T. et al. in 1999 (7). In 1998 Beaubien ER et al. (8) reported a case of sudden death in a patient with CLL, where autopsy revealed pulmonary leukostasis and a large intracardiac mass containing mostly mature lymphocytes and fibrin. We found one case of CLL presenting with extreme hyperleukocytosis and thrombosis of the common femoral vein, reported by Cukierman T. and al. in 2002 (9).

Some interesting aspects related with this case are the atypical immunophenotype, the expression of Cyclin D1, and the B hepatitis viral infection.

Hepatitis viruses are primary hepatotropic viruses and secondary lymphotropic. These viruses are potentially oncogenic and they could be involved in the pathogenesis and outcome of chronic lymphoproliferative disorders. As Bumbea et al. showed in their study, higher expression of B-cell markers CD19, CD20 in CLL with viral infection suggests a change to atypical CLL (10).

Cyclin D1 expression, usually absent in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), has been described in the proliferation centers (PC) of some CLL/SLL. It is important to distinguish CLL/SLL from MCL because, on average, CLL/SLL is considered a low-grade neoplasm even when the disease is nodal, whereas cases of MCL, especially those of nodal type, are clinically aggressive (11).

The distinction of CLL from MCL is not always easy because some MCLs may mimic CLL clinically, histologically, and/or phenotypically and vice versa (12). Some MCLs may be composed of small lymphoid cells with relatively round nuclei and even have foci mimicking PC (13), some present with blood and bone marrow involvement without adenopathy, and some may share even more phenotypic similarities than usual with CLL/SLL such as CD23 expression (13, 14).

In 2005 O'Malley DP et al., reported a case of CLL with cyclin D1 positivity (14). Another study finding suggests that a small subset of CLL overexpresses cyclin D1 in amounts that can be demonstrated by immunohistochemistry (15).

CLL and MCL typically express CD5 and B-cell antigens CD19 and CD20. The typical

CLL also expresses CD23 without FMC7 and shows dim expression of CD20 and dim surface immunoglobulin (sIg) expression. MCL typically expresses bright CD20 and bright to moderate sIg, lacks CD23, and is FMC7+ (16, 17, 18). Atypical-CLL phenotype is the category of clonal B cells that express CD5, but other markers are differentially expressed as compared with CLL: CD5+CD19+, but CD23 weak of negative or CD20, sIg or CD79b bright (19).

Recent evidence shows that CD200 (OX-2 membrane glycoprotein) is strongly expressed in CLL and was revealed to be an excellent marker to distinguish CLL from MCL, even in cases of atypical CLL. The inclusion of this marker in the routine investigation by flow cytometry is strongly recommended (20, 21, 22, 23).

The patient has stage III Rai (anemia) and stage C in Binet clinical staging system which is associated with poor survival. Newer biological prognostic factors are not included in Rai and Binet staging systems and might have value in most patients: DNA sequencing for immunoglobulin heavy-chain variable region (IGHV) gene mutation, flow cytometry for ZAP70 and CD38 and cytogenetic examination or FISH for del(17p) or del(11q).

Our CLL patient presented *NOTCH* mutation, advance phase of the disease (Rai stage III and Binet stage C) and the absence of del(13q).

Ghia et al showed that the presence of *NOTCH1* mutation correlated with resistance to treatment and with the risk of transformation into Richter syndrome (24).

According to Rossi *et al NOTCH1* mutations are an independent predictor of CLL overall survival, are usually mutually exclusive with *TP53* abnormalities, and identify cases with a poor prognosis. *NOTCH1* mutations are associated with rapidly progressive disease (2). A recent report suggested that *NOTCH1*mutations had an adverse impact on outcome and acted as an independent prognostic factor of other clinical and biological features of CLL (for example *IgHV* mutation status and *TP53* disruption) (25).

Sportoletti et al reported that *NOTCH1* mutations in CLL patients at diagnosis is associated with poor clinical outcome (26).

According to the findings of an international, multicenter, randomized, first-line treatment trial (CLL8) which included 817 patients and evaluated first-line therapy with fludarabine and cyclophosphamide (FC) or FC with rituximab (FCR) among untreated CLL patients, *NOTCH1* mutation was identified as a predictive marker for decreased benefit from the addition of rituximab to FC (FCR) (27). Our patient was not tested for del(17p) nor for del(11q). Del(17p) is present in 5% of patients at diagnosis by conventional cytogenetic and in up to 9% by FISH (28). The overall response rate is about 70% and progression free survival is around 11-12 months for these poor risk patients (29).

The expected overall response rate with FCR is 95% (30). 65% of the patients were free of disease at 3 years after randomization in the registration study mentioned above (29), with 77% overall survival and 51% progression free survival at 6 years in MD Anderson single center study (31).

A large cohort of 1160 untreated CLL patients found that *NOTCH1* mutations were associated with *IgHV* unmutated cases (32). According to Jeromin et al *NOTCH1* mutations were less frequent in CLL patients with del(13q).

In the future *NOTCH1* mutation may represent a potentially important therapeutic target in CLL, as it was documented in T-cell acute lymphoblastic leukemia (T-ALL) (33).

In conclusion, CD200 should be introduced in the routine panel for flow cytometry to distinguish CLL from mantle cell lymphoma and *NOTCH1* mutation is associated with poor prognosis and should be evaluated at diagnosis. CLL

with extreme hyperleukocytosis presentation is very rare and sometimes an atypical CLL may represent a diagnostic pitfall.

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Abbreviation list

ARMS - amplification refractory mutation system
CLL - Chronic lymphocytic leukemia
CIRS - cumulative illness rating scale
FCR - fludarabine cyclophosphamide rituximab)
FISH - fluorescent in situ hybridization
MCL - mantle cell lymphoma
QF-PCR - Quantitative Fluorescence-Polymerase Chain Reaction technique
NCCN - National Comprehensive Cancer Network
WBC - white blood cell count

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