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The importance of MRD monitoring in intensive treatment for chronic lymphocytic leukemia – a case report

Znaczenie monitorowania MRD w intensywnym leczeniu przewlekłej białaczki limfocytowej – analiza przypadku klinicznego

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S u m m a r y

Chronic lymphocytic leukemia is the most common leukemia diagnosed in adults. The median age of diagnosis is 65. The goal of treatment especially in younger patients should be putting them in deep remission, without a minimal residual disease (MRD). The standard currently used to monitor response to treatment does not take into account the status of MRD. The present case-study is of a patient with chronic lymphocytic leukemia diagnosed in a 38 year old. The duration of illness as well as the observation of the patient has now been 13 years. In our case, the effects of each treatment were evaluated not only on the basis of hematological parameters and physical examination but also taking into account the condition of the minimal residual disease. Eliminating pathological CLL cell clones was only after intensive chemotherapy supportive allogeneic haematopietic stem cell transplantation from a related donor.

S t r e s z c z e n i e

Przewlekła białaczka limfocytowa jest najczęstszą białaczką rozpoznawaną u dorosłych. Mediana wieku w momencie diagnozy wynosi 65 lat. Celem leczenia, szczególnie chorych w młodszym wieku, powinno być wprowadzenie ich w głęboką remisję, bez minimalnej choroby resztkowej (MRD). W stosowanym obecnie standardowym monitorowaniu odpowiedzi na leczenie nie uwzględnia się stanu MRD. U prezentowanego pacjenta przewlekłą białaczkę limfocytową zdiagnozowano w 38. roku życia. Czas trwania choroby i obserwacji pacjenta wynosi obecnie 13 lat. W opisywanym przypadku efekty poszczególnych terapii były oceniane nie tylko w oparciu o parametry hematologiczne i badanie fizykalne, ale uwzględniały również stan minimalnej choroby resztkowej. Wyeliminowanie patologicznego klonu komórek CLL nastąpiło dopiero po zastosowaniu intensywnej chemioterapii wspomaganej allotransplantacją macierzystych komórek krwiotwórczych od dawcy rodzinnego.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia one may encounter in the Western World (25-30%) (1). The standardized incidence rate is 4/100 000 people per annum and increases with age (2). The median age at diagnosis is 65. Men get sick twice as often as women (2).

Monoclonal CLL is a disease characterized by the accumulation of morphologically mature but not functionally B cells (rare T or NK) in peripheral blood, bone marrow, lymph nodes, spleen and, very rarely, in skin (2, 3). Neoplastic cells express the following surface antigens: CD19+, CD5+, CD23+, CD20+/-, sIg +/- (4). The moment of the initiation of the treatment depends on the severity of the disease and the choice of treatment depends on the correct assessment of the expectancy of patient survival independent of CLL as well as the biological state of the patient and his comorbidities. The goal of treatment, especially in younger patients, should be putting them into deep

remission, without a minimal residual disease (MRD – minimal residual disease). By default, the monitoring response to treatment is carried out in accordance with the criteria of the NCI (National Cancer Institute, 1988) (5), which do not take into account the condition of minimal residual disease.

CASE DESCRIPTION

A 38 year-old man was hospitalized for the first time in the Department of Hematology and Bone Marrow Transplantation, Medical University of Silesia, Katowice in August 2002. In an interview in June 2002 he said that he had noticed a significant reduction in body weight (10% within 3 months), night sweats, and recurrent infections. With comorbidities Gilbert's syndrome diagnosed in adolescence.

On admission he was in a generally good condition, ECOG = 0. On physical examination lymphadenopathy was revealed: bilaterally enlarged cervical lymph nodes, axillary and inguinal to about 3 cm. An abdominal ultrasonography showed hepatosplenomegaly (the longitudinal span of the right hepatic lobe was 16 cm, and that of the spleen 13 cm). In the peripheral blood examination we observed hyperleukocytosis: WBC – 15.2 G/L (N: 4-10 G/L) with 12.7 G/L morphology mature lymphocytes displaying the following phenotypes: CD5+, CD19+, CD20+, CD23+, CD79b+, CD22+, CD38+ (70% of the cells), CD43+, ZAP70+ (50% of the cells). In addition, we did not observe anemia, Hgb – 14 g/dl (N: 11.2-15.8 g/dl) and thrombocytopenia, PLT – 235 G/L (N: 130-400 G/L). The relevant laboratory findings included: alanine aminotransferases; Alat – 19 IU/L (N: 10-40 IU/L), asparate aminotransferases; Aspat – 22 IU/L (N: 10-42 IU/L), lactate dehydrogenase; LDH – 201 IU/L (91-180 IU/L), serum bilirubin level – 38 umol/L (N: 3.4-17.1 umol/l), creatinine – 78 umol/L (N: 55-113 umol/l), beta2-micro-globulin – 1975 ug/l (N: 1500-3000 ug/l), immuno-globulin; IgG – 7.2 g/L (N: 7-16 g/l), the negative direct and the indirect Coombs antiglobulin test. In the study of peripheral blood FISH stated did not reveal: deletion of the short arm of chromosome 17p, deletion of the long arm of chromosome 11q, deletion of the long arm of chromosome 13q and trisomy of the chromosome 12. The trephine biopsy showed: diffuse infiltration of the small lymphoid B cells (CD20+, CD23+, CD5+, cyclin D1 occupying 30% of the bone marrow. Ki67 proliferative index in CLL cells were 2-35%. Hematopoiesis preserved. Islands of granulopoiesis MPO(+) and erythropoiesis poorly and rich-cells. Megacaryocytes numerous and scattered.

Chronic lymphocytic leukemia in stage II – by the Rai classification and B – in accordance with the Binet classification was diagnosed. Because of the presence of these general symptoms the patient was qualified for chemotherapy using cladribine alone. From August 2002 to January 2003, the patient received 6 cycles of this treatment. In February 2003 the patient achieved complete remission 1 (CR1) with a positive

minimal residual disease examined using flow cytometry MRD = 0.705%. After 28 months of complete remission in June 2005 the patient was re-admitted to the Department of Hematology due to a recurrence of lymphadenopathy, splenomegaly, and lymphocytosis. The first relapse of CLL was diagnosed (Rai-II, Binet-B) and the patient received chemotherapy in accordance with the protocol CC (Cladribine, Endoxan). In October 2005, after 3 cycles of chemotherapy, the patient achieved CR2-positive MRD = 1.2%. In May 2008 there was discovered a second recurrence of the disease: Rai-II, Binet-B and we started treatment in accordance with the protocol Flu-Camp (Fludarabine Campath). During treatment, infectious complications were observed (Herpes viruses, CMV) and the patient required the substitution of immune-globulins. In August 2008, after 2 cycles of treatment both CR3 along with a positive MRD = 0.88% was achieved. After 16 months of complete remission in December 2009, there was a third recurrence of CLL, and the stage was estimated to be: Rai-IV, Binet-C. We started treatment in accordance with protocol R-FC (Mabthera, Fludarabine, Endoxan) which the patient received until October 2010. After 6 cycles of this treatment, there was CR4 but without the eradication of the minimal residual disease MRD = 0.29%. After the findings the CR4 patient was recommended for an allogeneic hematopoietic stem cells transplantation from a HLA-matched related donor. The indications for the transplantation were: young age of the patient, recurrent nature of the disease, short time from remission to progression, following adverse prognostic factors (elevated levels of LDH, increased expression of CD38 and ZAP70, general symptoms and diffuse infiltration CLL in trephine-biopsy) during the estimated diagnosis. In October 2011 the patient received an allogeneic haematopoietic stem cells transplantation from a HLA fully-matched sister. Reduced-intensity conditioning (RIC): Empathic, Fludarabine, Melphalan was applied. In the prevention of acute graft versus host disease (aGVHD) methotrexate and cyclosporin was initially used intravenously, then orally at a dose dependent upon the concentration in the blood. On 19-20.10.2011 there was performed a hematopoietic stem cell transplantation. Transplanted peripheral blood apheresis product contained 7.72 x 10e8 nucleated cells/kg, 5.23 x 10e6 CD34+ cells/kg and 22.7 x 10e7 CD3+ cells/kg.

15 days after the transplantation the patient displayed symptoms of acute graft versus host disease (aGVHD) grade I (s-2, G-0, h-0). 28 days after the transplantation graft failure was diagnosed: WBC = 0.1 g/L, dependence of a concentration of red blood cells and platelets transfusion, donor chimerism examined using the RT-PCR method was 0% donor. Immune-suppressive therapy was discontinued and the patient was recommended for the second alloHSCT. The second transplant procedure began in December 2011. The donor was also HLA-matched related donor (sister). In the conditioning regimen we used endoxan and

anti-lymphocyte globulin (ATG). GvHD-prophylaxis consisted of cyclosporin switched to Mycophenolate mofetil because of renal failure. On 14-15.12.2011 there was performed a second hematopoietic stem cell transplantation from the same HLA-matched related donor (sister). Transplanted peripheral blood apheresis product contained 8.78 x 10e8 nucleated cells/kg, 7.6 x 10e6 CD34+ cells/kg and 9.44 x 10e7 CD3+ cells/kg. 25 days after the transplant procedure we observed aGVHD grade I (p-1, G-0, h-0). Regeneration proceeded as follows: WBC > 1.0 G/L was achieved 18 days after the transplant, ANC > 0.5G/L was achieved 22 days after the transplant, PLT > 20G/L was achieved 18 days after the transplant. In controlled examinations performed in the 28 days after alloHSCT we observed regeneration of haematopoiesis in the bone marrow, and for the first time since the beginning of treatment we achieved a negative MRD = 0.003%. A mixed chimerism donor was 87%, while 100 days after alloHSCT the patient achieved 100% donor chimerism with negative MRD. The posttransplant period was complicated by viral infections and hypogammaglobulinemia requiring periodic supplementation of the immune-globulins. At present the patient is regularly checked in The Outpatient Bone Marrow Transplantation Department. During the last visit, control examinations did not reveal: organomegaly, lymphadenopathy and chronic graft versus host disease (cGvHD). The results of biochemical investigations were as follows: WBC = 3.9 g/L (N: 4-10 G/L), Hgb = 13.5 g/dl (N: 11.2-15.8 g/dl), PLT = 137 g/L (N: 130-400 G/L). Alat – 22 IU/L (N: 10-40 IU/L), Aspat – 26 IU/L (N: 10-42 IU/L), LDH – 156 IU/L (91-180 IU/L), total bilirubin – 35.9 umol/L (N: 3.4-17.1 umol/l), creatinine – 85 umol/L (N: 55-113 umol/l), B2-microglobulin – 1775 ug/l (N: 1500-3000 ug/l), IgG – 6.2 g/l (N: 7-16 g/l). We also performed a bone marrow aspiration biopsy which did not reveal pathological infiltration while continuing to find 100% donor chimerism and negative MRD = 0.008%.

DISCUSSION

In the presented case CLL was diagnosed in a relatively young patient. We achieved a complete hematological response due to early chemotherapy but minimal residual disease evaluation performed using immunophenotypic method was positive.

The use of immune-chemotherapy allowed us to obtain remission before alloHSCT. In accordance with the guidelines EBMT alloHSCT there is indicated in patients fulfilling one of the following criteria (high-risk disease):

1. No response or early relapse (within 12 months) after purine analogue-containing therapy.
2. Relapse (within 24 months) after purine analogue combination therapy.
3. p53 deletion/mutation (del17p13) requiring treatment (6).

Transplants should be performed as soon as the EBMT criteria are met and when we achieve complete remission. In these case its effects are the best (7-8). There is no doubt that the crucial therapeutic principle of allo-HSCT in CLL is a graft versus- leukemia (GvL) activity. In addition, the importance of GvL in CLL is indicated by a reduced relapse risk in the presence of chronic graft versus host disease (GvHD). An increase of relapse is associated with the use of T-Cell depletion (9). There appears to be sound evidence that GvL activity represents the main contributor to durable disease control after alloHSCT even in low-risk CLL. In the above-described patient the indications of alloHSCT were: young age of the patient; short periods of remission after chemotherapy; achieving complete remission and the presence of a HLA-matched related donor (sister). After the first and second transplant we observed GvHD with mild severity. In the monitoring of the treatment effects we observed standard hematological criteria but we also monitored minimal residual disease using multicolor flow cytometry. Lymphocyte was characterized by follow antigen: CD19, CD23, CD5, CD79b, CD22, CD43, CD81, CD22, CD38 and CD20. Normally, response to the treatment of CLL is defined by clinical findings (the lack of general symptoms for at least 2 months, size of the liver and spleen), bone marrow and peripheral blood examination (level of platelets, lymphocytes and neutrophils). CR defined by these clinical findings actually includes patients harboring 10e1-10e10 neoplastic cells. Assessment of a minimal residual disease (MRD) as a measurement of a tumor load in hematologic malignancy is becoming a tool for monitoring the depth of disease response and predicting early relapse (10-12). Positivity and negativity of MRD are defined by the threshold for the detection of malignant cells among totally nucleated cells estimated with modern techniques to be around 1:10 000. A minimal residual disease has been monitored using two major methods:

– the ASO-PCR (allele-specific oligonicleotide polymerase chain reaction) method using the polymerase chain reaction allows one to detect specific sequences of nucleotides in the DNA encoding the heavy chain variable region of the immunoglobulin – IgHV (immunoglobulin heavy-chain variable region),
– FLC (flow-cytometry) – flow cytometry which detects cells harboring a specific phenotype and depicts their number among the normal cell population.

The eradication of the minimal residual disease in CLL indicates an improved outcome by prolonged progression-free survival (PFS), MRD-negative CR patients demonstrated a significantly improved PFS compared to those with MRD-positive CR (13-15). Moreover MRD monitoring allows optimization of the treatment process. Strati et al. showed that MRD negativity in bone marrow samples after three cycles of FCR resulted in the same PFS and OS as those found in patients with no detectable MRD after six courses of FCR. Also in patients after allo-HSCT quantitative MRD

monitoring seems to be a valid instrument for preemptive immune intervention directed at disease eradication such as the tapering immune-suppression, the use of DLI, post-transplant immune-therapy (including monoclonal antibodies and alternative B-depleting or immune-modulating agents such as lenalidomide (16, 17).

CONCLUSIONS

Monitoring minimal residual disease may help us (understand the mechanism of) predict relapse in CLL. What is more, it may serve as an independent individualized prognostic indicator predicting PFS, OS in patients with CLL and it may assist in treatment-related decision-making.

BIBLIOGRAPHY

1. Provan D, Singer ChRJ, Baglin T, Dokal I: Oxford Handbook of Clinical Hematology. 3rd edition. Oxford University Press 2011: 150-159.
2. Dmoszyńska A (red.): Hematologia. Seria: Wielka Interna, pod red. A. Antczaka, M Myśliwca, P Pruszczyka. T. 10, Medical Tribune Polska, Warszawa 2011: 446-458.
3. Cerroni L, Zenahlik P, Hofler G et al.: Specific cutaneous infiltrates of B-cell chronic lymphocytic leukemia. A clinic-pathologic and prognostic study of 42 patients. Am J Surg Pathol 1996; 20: 1000-1010.
4. Boguradzki P, Boguradzka A, Jędrzejczak W: Symptomatologia chłoniaków – od teorii do praktyki klinicznej. Medycyna po Dyplomie 2013; 22: 56-65.
5. Moszyńska A: Leczenie rozrostowych chorób hematologicznych. Medycyna Praktyczna, Kraków 2011.
6. Apperley J, Carreras E, Gluckman E, Masszi T: Haematopoietic Stem Cell Transplantation. The EBMT Handbook 6th edition, 2012: 377-382.
7. Sorror ML, Storer BE, Sandmaier BM et al.: Five-year follow-up of patients with advanced chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. J Clin Oncol 2008; 26: 4912-4920.
8. Brown JR, Kim HT, Li S et al.: Predictors of improved progression-free survival after non-myeloablative allogeneic stem cell transplantation for advanced chronic lymphocytic leukemia. Biol Blood Marrow Transplant 2006; 12: 1056-1064.
9. Dreger P, Brand R, Milligan D et al.: Reduced-intensity conditioning lowers treatment-related mortality of allogeneic stem cell transplantation for chronic lymphocytic leukemia: A population – matched analysis. Leukemia 2005; 19: 1029-1033.
10. Cave H, van der Werff ten Bosch J, Suciu S et al.: Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer – Childhood Leukemia Cooperative Group. N Engl J Med 1998; 339: 591-598.
11. Andersen NS, Pedersen LB, Laurell A et al.: Preemptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. J Clin Oncol 2009; 27: 4365-4370.
12. Grimwade D, Lo Coco F: Acute promyelocytic leukemia: a model for the role of molecular diagnosis and residual disease monitoring in directing treatment approach in acute myeloid leukemia. Leukemia 2002; 16: 1959-1973.
13. Robertson LE, Huh YO, Butler JJ et al.: Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: clinical, pathological, immune-phenotypic, and molecular analysis. Blood 1992; 80: 29-36.
14. O'Brien SM, Kantarjian HM, Cortes J et al.: Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 2001; 19: 1414-1420.
15. Bosch F, Ferrer A, Villamor N et al.: Fludarabine, cyclophosphamide, and mitoxantrone as initial therapy of chronic lymphocytic leukemia: high response rate and disease eradication. Clin Cancer Res 2008; 14: 155-161.
16. Strati P, Keating MJ, O'Brien SM et al.: Eradication of bone marrow minimal residual disease may prompt early treatment discontinuation in CLL. Blood 2014; 123: 3727-3732.
17. Delgado J, Milligan DW, Dreger P: Allogeneic hematopoietic cell transplantation for chronic lymphocytic leukemia: Ready for prime-time? Blood 2009; 114: 2581-2588.