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Original Article

Prognostic implication of early minimal residual disease evaluation in patients with chronic myelomonocytic leukemia

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Abstract: To investigate the prognostic implication of minimal residual disease (MRD) evaluation in chronic myelomonocytic leukemia (CMML), we conducted a restropective study included a total of 174 CMML patients in our hospital from January 2010 to March 2021. In which 50/174 (29%) bone marrow samples were conducted by multiparameter flow cytometry (FCM) assessed MRD analysis after the first three cycles of treatment and were included in this study. MRD was detected by six- to eight-colour FCM. The achievement of early MRD negativity had better clinical outcomes in patients with CMML, which fared better prognosis in terms of not only PFS (P=0.006) but also OS (P=0.02) after the first cycle, and PFS (P=0.023 and P=0.041) after the second and third cycles, whereas no significantly influence in OS. In addition, MRD negative after initial treatment remained its independent prognostic value associated with PFS (adjusted hazard ratio [HR] 0.161, 95 CI 0.035-0.738; P=0.019) and OS (adjusted HR 0.136; 95 CI 0.017-1.077; P=0.059), indicating that patients with MRD-negative after the initial treatment alone could obtain the greatest clinical benefit. According to MRD level, the patients were divided into 4 different groups: very low risk (fewer than 10^{-4} cells) in 15 cases, low risk (10^{-4} to 10^{-3} cells) in 6; and 6 were at intermediate risk (fewer than 10^{-3} to 10^{-2} cells). The rest of 23 patients were were assigned to the high-risk grades (more than 10^{-2} residual cells), we find this risk stratification model is significantly associated with better PFS (P=0.002) but marginal significantly associated with OS (P=0.068). Notably, patients with DNMT3A mutation fared a shorter PFS in the MRD positive subgroup (P=0.068). MRD is highly predictive of prognosis, and its combination with molecular profile may help identify patients at increased risk for progression to further improve the management of patients with CMML. Large-scaled investigations are warranted to validate our conclusions and its potential in clinical practice.

Keywords: Chronic myelomonocytic leukemia, minimal residual disease, immunophenotyping, prognosis

Introduction

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic malignancy that combines the characteristics of both myelodysplastic syndromes and myeloproliferative neoplasms (MDS/MPN) [1-3]. The levels of bone marrow (BM) or peripheral blood blasts in newly diagnosed CMML patients were lower than those of acute leukemia, but the prognosis of CMML was not better than that of acute leukemia, with a median overall survival of approximately 2.5 years, and up to 25% of patients transform to acute myeloid leukemia (AML) [1, 4]. Numerous pretreatment prognostic models are well established so far by bone marrow or peripheral blood blast, karyotype, and genetic mutation, which closely associated with clinical prognosis of patients with newly diagnosed CMML and developing adapted treatment strategy [2, 4-9]. Evaluation treatment response by conventional morphologic analysis also provides much important information about the chemotherapy sensitivity of leukemia in an individual that cannot necessarily be estimated prior to medicine administration [10]. In the context of precision medicine, however, further improvement of prognosis may be accomplished through assessment of measurable residual disease (MRD), which refers to a small subpop-

ulation of neoplastic cells that is incapable to detect by conventional morphology analysis [10, 11], whereas they remains intratumoral heterogeneity of the primary tumor, allowing them to develop drug-resistance and evolution under continuous drug environment, and then regrow to become the dominant tumor population [12], subsequently drive disease progression or relapse. The persistence of MRD has been showed to be correlated with worse prognosis in certain malignant hematologies, including chronic myeloid leukemia (CML), acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL) [10, 13, 14]. In the latest published European Leukemia Net MRD guidelines, it defined MRD-negative complete remission in acute leukemia as outcome definition [15-17]. Furthermore, higher levels of MRD before allogeneic stem cell transplant (allo-SCT) was associated with a higher relative risk for relapse and inferior outcome. Therefore, MRD is also useful in determining whether allo-HSCT should be performed [11, 18]. In recent years, MRD has been the strongest indicator for clinical outcome, after either chemotherapy alone or after allo-HSCT [11, 13, 19]. By contrast, little information is currently available concerning MRD profiles in CMML and the relationship between MRD in combination with genetic CMML subtypes and prognosis has not yet taken a hold.

In the current retrospective analysis, we aimed to examine the significance of immunophenotypically MRD levels so as to evaluate relavant prognosis in CMML patients. And it may help improve clinical risk stratification and decision-making in patients with CMML.

Material and methods

Patients

A total of 174 patients who were newly diagnosed with CMML at the First Affiliated Hospital of Zhejiang University from January 2010 to March 2021 were enrolled, in which 50/174 (29%) patients were conducted by multiparameter flow cytometry (FCM) MRD analysis after the first three cycles of treatment and were included in this study. The diagnosis of CMML was established according to the current WHO criteria by a combination of clinical findings, morphologic evaluation of peripheral blood and bone marrow aspirate samples, and conventional cytogenetic and molecular analysis. Measured outcomes were progress-free survival (PFS) and overall survival (OS). PFS was defined as survival with no evidence of relapse or acute transformation. OS was defined as the time from dieases newly diagnosis to death, regardless of the cause. Minimal or measurable residual disease defined as posttherapy neoplastic cells remain at levels which are undetectable from cytomorphologic, whereas can be detected by FCM. MRD negative defines leukemic cells <0.01%, conversely, MRD positive defines leukemic cells higher than 0.01%. All patients had at least one BM aspirate specimens submitted for FCI analysis. The study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhejiang University.

MRD assessments

We used six- to eight-colour FCM for bone marrow samples MRD levels assessement after treatment, which is able to identify cluster differentiation 1a (CD1a), CD2, sCD3, cCD3, CD4, CD5, CD7, CD8, CD34, CD45, CD56, CD99, CD117, HLA-DR and terminal deoxynucleotidyl transferase (TdT). We considered MRD positive when a cluster of >20 cells expressing two or more leukemia-associated immunophenotypic (LAIP) markers was detected at diagnosis. When patients who were lack of LAIP markers expression at diagnosis, MRD was considered as a population of cells that deviated from the normal pattern of antigen expression in a particular cell line at a particular stage of maturation compared to normal or regenerated BM. The MRD detection sensitivity was 0.01%.

At least 200000 samples were acquired for MRD analysis routinely. Homotypic control monoclonal antibodies were applied. Standardized measurements and routine quality control were carried out according to manufacturer recommendations. Samples were collected by a three-laser Navios instrument (Beckman Coulter, Fullerton, CA, USA).

Statistical analysis

The Chi-square test and Fisher's exact test and the t-test were performed for categorical and continuous variables to compare with the population between MRD negative group and MRD positive group. The Kaplan-Meier method and

the log-rank test were used in the survival analysis. Cox's proportional hazard regression model was applied for the multivariate analysis. Patients who were lost to follow-up were censored at the last contact date. A *P*-value of 0.05 or less was considered to be statistically significant. All the analyses mentioned above were performed using the SPSS (version 26; SPSS, IBM).

Results

Baseline characteristics

A total of 174 patients who were newly diagnosed with CMML were enrolled, in which 50/174 (29%) patients had BM aspirate specimen submitted for multiparametric flow cytometry immunophenotyping MRD analysis after treatment and were included in this study. Patients baseline clinical characteristics were summarized in **Table 1**. Which was incorporated follow-up time, patients' gender, age, percentage of BM blast, hemoglobin, platelet count, neutrophil count, numbers of allo-HSCT recipients, population distribution in CMML-specific risk classification and 2016 WHO subtype. The most common mutant genes detected (>4 cases) in 27 samples and karyotype are also included. The meidian follow-up time is 14 months. The median age of the total is 61.5 years old, with male to female ratio of 33:17. The median age is 62.5, which range from 22-81, twenty-two were younger than 60 and the remaining 27 patients were older than 60. We also compared the characteristics of patients with MRD negative group and MRD positive group after initial treatment in this table. Except that the median platelet counts in the MRD positive group were significantly lower than in the MRD negative group (P=0.005), there were no significant differences for follow-up time, patients' gender, age, percentage of BM blast, hemoglobin, platelet count, neutrophil count, numbers of allo-HSCT recipients, mutant genes, karyotype, population distribution of CMML-specific risk classification and WHO subtype between the two group.

The presence of MRD negative after the first cycle treatment are correlated with best prognosis

We investigated the outcomes of CMML patients who had MRD testing at least once after the first 3 cycles of treatment **Figure 1**. The median duration of overall survival was 27.7 months. Patients who achieved MRD negative after the first cycle treatment have superior OS (P=0.02) and PFS (P=0.006) (**Figure 1A** and **1B**) than MRD positive patients. The patients also have superior PFS (P=0.023 and 0.041) (**Figure 1C** and **1D**) if they achieved MRD negative after 2 and 3 cycle treatment, however, they showed no difference in OS. In our study, patients who did not achieve MRD negative untill 3 cycle treatment had worst outcome, they were high at risk for disease progression, and with overall survival similar to those with persistence MRD positive. In addition, Cox's proportional hazard regression model was applied for the multivariate analysis, MRD negative after initial treatment (adjusted for gender) is the independent predictive factor associated with PFS (adjusted HR 0.161, 95 CI 0.035-0.738; P=0.019) and OS (adjusted HR 0.136, 95 CI 0.017-1.077; P=0.059) (data not show). Therefore, patients with MRD-negative after the initial treatment alone could obtain the greatest clinical benefit. Furthermore, patients who achieved MRD-negative after initial treatment favored longer post-transplantation OS (P=0.28) and PFS (P=0.15) than MRD-positive patients, although the difference did not show statistically significant.

In MRD positive subgroup, patients with DNMT3A mutation have a shorter PFS. Based on the complexity of the relationship between genetics and prognosis in chronic myelomonocytic leukemia [20, 21], we went a step further to see if patients with different genetic mutations in MRD positive subgroup after initial treatment had different outcomes. Of interest, patients with DNMT3A mutation fared a shorter PFS in the MRD positive subgroup (P=0.068) (**Figure 1E**), whereas no such difference was discovered in OS. And there was no statistical difference between TET2, ASXL1 and NRAS, even chromosome karyotyping and prognosis.

MRD level after initial treatment better reflects prognosis of PFS than OS

According to MRD level, we divided the patients into 4 different groups: very low risk (fewer than 10^{-4} cells) in 15 cases, two relapsed and 1 died; low risk (10^{-4} to 10^{-3} cells) in 6, among 4 underwent disease progression (relapsed in 2 cases, transformed to AML in 2 cases). The

Table 1. Patients baseline characteristics

Characteristic	Patients (N=50)	MRD negative (N=15)	MRD positive (N=35)	*P* value
Follow-up, median (range), mo	14 (0.7-39.1)	17.5 (2-33.4)	10.4 (0.7-39.1)	.25
Sex, n (%)				.474
Male	33 (66)	11 (22)	22 (44)	
Female	17 (24)	4 (8)	13 (26)	
Age, years				.239
Median, (range)	62.5 (22-81)	59 (22-81)	69 (30-78)	
≤60		5	18	
>60		10	17	
Bone marrow blasts, %				.123
Median, (range)	8.75 (1.5-19.0)	7.5 (1.5-14.5)	9.5 (3.0-19.0)	
Hemoglobin, g/L				.727
Median, (range)	92 (41-140)	99.5 (41-138)	84.5 (43-140)	
Platelets, × 10^9/L				.005
Median, (range)	66 (4-443)	163.5 (23-443)	49.5 (4-358)	
Neutrophils, × 10^9/L				.807
Median, (range)	11.68 (0.5-123.3)	12.04 (2.3-88.1)	11.25 (0.5-123.3)	
CMML-specific risk classification: n (%)				.663
Low	38 (76)	13 (26)	25 (50)	
Median	6 (12)	1 (2)	5 (10)	
High	6 (12)	1 (2)	5 (10)	
2016 WHO subtype, n (%)				.944
dCMML	13 (26)	4 (8)	9 (18)	
pCMML	37 (74)	11 (22)	26 (52)	
Allo-HSCT, n (%)				.705
YES	9 (18)	2 (4)	7 (14)	
NO	41 (82)	13 (26)	28 (56)	
Genetic mutation, n (%)	27 cases aviliable			
TET2				.636
Positive		3 (11)	9 (33)	
Negative		4 (15)	11 (41)	
ASXL1				.633
Positive		3 (11)	5 (19)	
Negative		4 (15)	15 (56)	
NRAS				.580
Positive		2 (7)	3 (11)	
Negative		5 (19)	17 (63)	
DNMT3A				.155
Positive		0	6 (22)	
Negative		7 (26)	14 (52)	
Karyotype, n (%)				.304
Normal	38 (76)	13 (26)	25 (50)	
Abnormal	12 (24)	2 (4)	10 (20)	

remaining two were died; and 6 were at intermediate risk (fewer than 10^{-3} to 10^{-2} cells), two underwent disease progression (relapsed in 1 cases, transformed to AML in 1 cases). One occurred early death after transplantation. The rest of 23 patients were were assigned to the high-risk grades (more than 10^{-2} residual cells), and 7 cases progressed (relapsed in 1 cases,

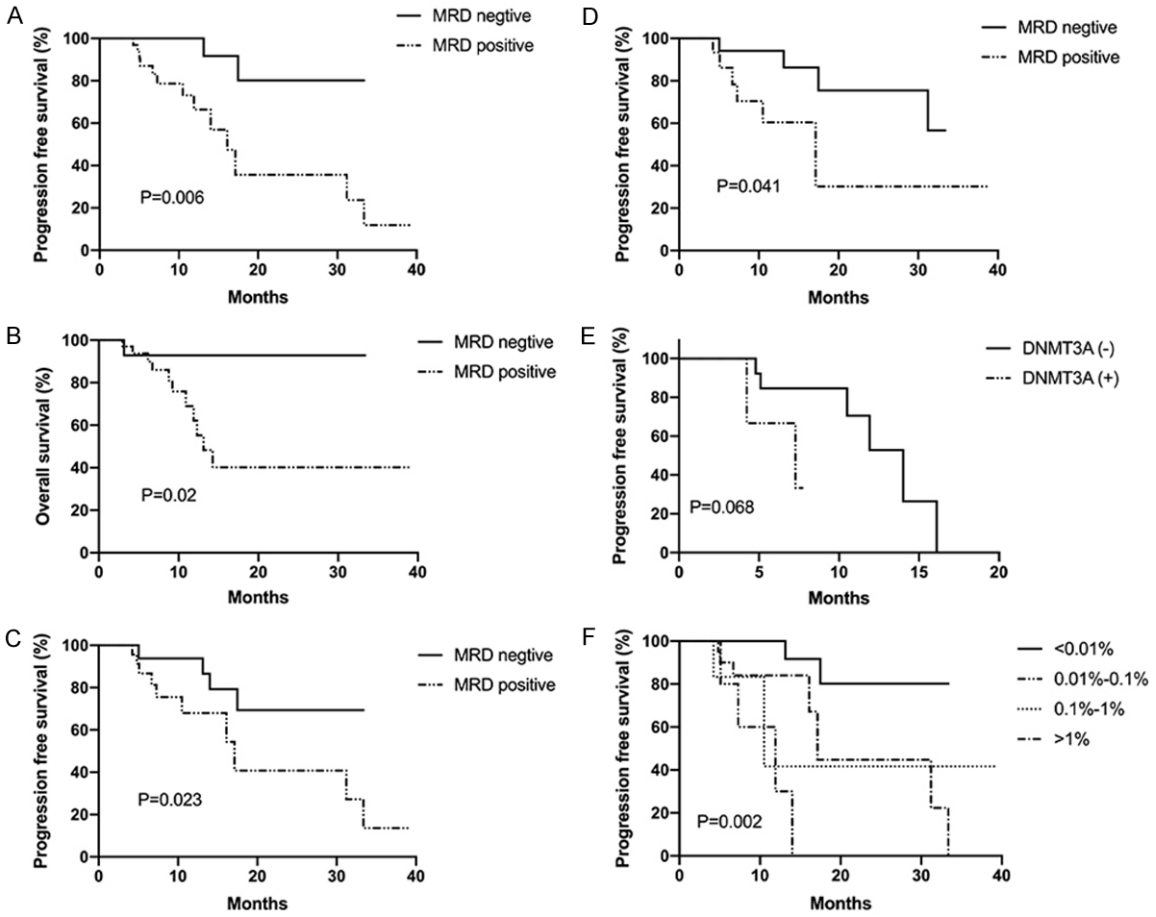

Figure 1. PFS (A) and OS (B) in MRD negative vs MRD positive after first treatment; PFS (C) and PFS (D) in MRD negative vs MRD positive after two and three cycles treatment, respectively; PFS (E) of patients with DNMT3A mutation in MRD positive subgroup; PFS (F) in 4 subgroups according to MRD level after first treatment.

transform to AML in 6 cases) and 7 died. We find this risk stratification model were significantly associated with PFS (P=0.002) but marginal significantly associated with OS (**Figure 1F**). Therefore, risk stratification according to MRD level is a powerful predictor of PFS than OS.

Discussion

The results from this analysis show early MRD negative achieved facilitates prediction outcome of both OS and EFS in CMML. While 2 or more cycles are required to achieve MRD negative is associated with higher risk of progression and poorer survival. In the professor Feller's finding of 72 AML patients, which the percentage of MRD in BM after the first cycle (P=0.002, cutoff level of 1%), second cycle (P=0.0011, cutoff level of 0.14%) and third cycle chemotherapy (P=0.0011, cutoff level of 0.11%) all strongly correlated with relapse-free survival [22]. It may attribute to drugs available now have less impact on this disease that fail to alter the disease course or affect mutation allele burdens [23]. Early response to therapy is an important prognostic factor in leukemia [24], which we have also verified in CMML. However, the propotions of early MRD negative patients in our cohort account for only a small part, even less than a half. Therefore, there is an unmet need for optimizing treatment modalities, such as hypomethylating agents, as well as elucidating possible targets unique to the CMML clone, which could substantially improve survival and quality of life of CMML patients. Clinical trials dedicated specifically to CMML are needed to explore the efficacy and safety of novel treatment modalities [24, 25].

In addition, MRD negative patients should be given full consideration whether to perform

hematopoietic stem cell transplantation, since it is the only therapeutic option that remains the potential for cure intent [2], and earlier transplantation in the course favoured better clinical benefit, especially in high risk group [14, 26-29]. Besides, multiple retrospective investigations have been launched to estimate the efficacy of allo-SCT in this disease. Patients in chronic phase achieved superior 5-year OS over post CMML-blast transformation after HSCT (51% vs 19%), underscoring the urgency of early allo-HSCT intervention [23, 28]. The European Group for Blood and Marrow Transplantation report, a largest to-date study of 513 CMML patients, reported the 4-year estimated RFS and OS of 27% and 33%, respectively. On multivariable analysis, achieving a morphological complete response (CR) when pretransplant was the sole statistically significant prognostic factor [2, 30]. However, morphological assessment is a rough measures method of assessing remission status, with poor sensitivity and significant differences between observers. MRD dectection is a more precise technique, which is measurable clinically relevant amounts of malignant cells (as many as 10^{10} leukemic cells) when standard cytomorphologic analysis is incapable to detect [10, 31]. Although the significance of MRD analysis in pre-transplantation CMML patients has yet not been well established, the evidence of the value of MRD after transplantation is convincing in several other hematology diseases, such as ALL [11, 32], AML, MDS [33, 34]. In ALL, Bader et al. indicated that higher pre-HSCT MRD level was closely related with inferior event-free survival (EFS) [32]. Shen et al. also disclosed that patients who proceed to transplant with MRD positivity had a significantly higher rate of relapse (HR=3.26; P<0.05), lower relapse-free survival (RFS) (HR=2.53; P<0.05), and lower overall survival (OS) (HR=1.98; P< 0.05) than of negative MRD [11, 35]. In the setting of allo-HSCT, those AML patients with MRD-positive morphologic remission have been shown to be high at risk for relapse, and the 3-year overall survival was resemble to those with active AML (>5% marrow blasts by morphology), while MRD-negative remission patients have markly superior clinical outcome [36-38]. The GIMEMA adult AML1310 trial disclosed that, however, patients who in intermediate risk group can avoid perform allo-HSCT if MRD is undetectable, in MRD positive ones allo-SCT can actually acquire clinical benefit such as prolonged OS and DFS similar to those of the MRD-negative category [37, 39, 40]. Thus, MRD status is also applicable for the decision of whether to perform allo-HSCT. In our study, MRD-negative patients after the initial treatment favoured better OS (P=0.28) and EFS (P=0.10) after transplantation when compared to MRD-positive patients. However, these difference did not reach statistical significance, possibly due to the fact that only 9 allo-HSCT patients in our analysis. Larger population studies are urgently needed for confirmation. In addition, MRD monitoring can be used as a promising predictor tool of impending disease progression and should be part of routine follow-up for allo-HSCT recipients of CMML, although retrospective analysis available now is lacking, it has been well applicated into clinical practice in ALL [41, 42] and AML [10, 17, 18].

The present study also disclosed that MRD monitoring derived from initial therapy response is a robust indicator in predicting disease progression. Furthermore, a personalized therapeutic schedule asks for risk stratification. High-dose chemotherapy, combination acute myeloid leukemia-type therapy, earlier allo-HSCT might be applied for higher percentage of MRD patients. Clinical trials is an attractive alternative approach and should be considered if available because the overall outcome of therapeutic interventions are far from optimal [26, 43].

In addition, the understanding of MRD is inseparable from understanding intratumoral heterogeneity-the driving force behind minimal residual disease-vital for the identification of resistance drivers that results from branching evolution. In fact, this has already been well-explored in several solid tumors, such as prostate cancers [44] and lung cancers [12]. More recently, Wilkinson et al. confirmed that nascent prostate cancer heterogeneity drives evolution and resistance to intense hormonal therapy. According to their study, tumor heterogeneity, manifested as tumor genomic and histological diversity at baseline, was positively correlated with residual diseases, which increased the risk of drug resistance due to greater tumor subclones as well as a more complex branching evolutionary path, while patients with mini-

mal residual disease had a lower recurrence rate after treatment [44]. Furthermore, intratumoral heterogeneity and tumor evolution can propelled by multiple factors, such as genome doubling, mutational burden, and somatic copy number alterations [12]. Indeed, When Wilkinson et al. performed whole exome sequencing and immunohistochemistry to identify potential molecular differences between exceptional responder (ER) and incomplete and nonresponder (INR) cases, where hot-spot mutations to TP53 and loss of chromosome 10q were significantly enriched in the INR group (P=0.044 and P=0.023, respectively) [44]. In the present study, we also found patients with DNMT3A mutation in MRD positive population may more likely to suffer from disease progression (P= 0.068), though there was no statistical difference between MRD positive group and MRD positive group. In addition, although clinical researchers have always taken the post-treatment MRD levels as an indicator of therapeutic efficacy in hematological malignancies [45-48], the mechanism of the intratumor heterogeneity driven MRD and drug resistance has not been well-elucidated, which will be the focus of our following research. In the context of multiregion whole genome and whole exome sequencing methods, as well as emerging technologies such as liquid biopsy and single cell methods available, the study of targetable drivers of MRD demonstrates a promising prospect not only in CMML but also in all maligment hematologies.

In conclusion, early MRD assement can offer reliable prognostic imformation in CMML. In future studies, risk stratification should be based not only on risk assessment at diagnosis, but also on MRD as a treatment-dependent prognostic factor. Besides, the relationship between intratumor heterogeneity drivers of MRD and drug resistance seems to be a promising prospect in maligment hematologies. However, this study exists several limitations mainly related to cohort design and its retrospective nature, thereby allowing intrinsic biases that may affect the results. Firstly, a relatively small sample size of 50 CMML patients had MRD data available for analysis, no definitive conclusion can be drawn. Large-scaled studies are warranted to validate our conclusions and its potential in clinical practice. Further, our findings that rely on the clinical manifestation and laboratory test results require external validation.

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Disclosure of conflict of interest

None.

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