Assessment of Large Granular Lymphocyte during Dasatinib Therapy and its Relation to Deep Molecular Response in Chronic Myeloid Leukemia

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Abstract: Objective: To compare the differences of deep molecular response (DMR) rate and time of obtaining DMR between dasatinib treated patients with increased and normal levels of large granular lymphocytes (LGLs) in newly diagnosed or imatinib resistant/intolerant chronic myeloid leukemia (CML) patients with positive BCR-ABL fusion gene. Methods: LGLs in peripheral blood were counted by flow cytometry and BCR-ABL fusion gene transcriptional level was detected by real-time quantitative polymerase chain reaction in 25 CML patients before and 1, 3, 6, 9, 12, 15, 18 months after dasatinib treatment. The enrolled patients were classified into LGL+ group and LGL- group according to whether the LGL counts were increased or not. Results: Among the 25 patients investigated, LGLs in 15 cases (5/15, 60%) were increased. Five cases in LGL+ group obtained DMR (33.3%) which was significantly higher than that of LGL- group (33.3% vs 10 %, P < 0.05). The median time of obtaining DMR in LGL+ group and LGL- group were 12 and 15 months respectively. Conclusions: Increased LGLs can be used as an indicator of prognosis in CML patients with positive BCR-ABL fusion gene who treated with dasatinib.

Keywords: Chronic Myeloid Leukemia, Large Granular Lymphocyte, Dasatinib, Deep Molecular Response

Introduction

Chronic myeloid leukemia (CML), represented 15% of leukemia, was a malignant hematopoietic disease characterized by proliferation of immature granulocytes[1]. The occurrence of CML was closely related to the formation of BCR-ABL fusion gene which was produced by translocation of chromosome 9 and 22. BCR-ABL fusion gene was positive in more than 95% CML patients[2]. Moreover, the quantities of BCR-ABL fusion gene expression represented the load of leukemia cells in CML patients. Thus, detection the quantities of BCR-ABL fusion gene could be used to evaluate the effect of treatment in CML patients. Tyrosine kinase inhibitors (TKIs) may prevented proliferation and promote apoptosis of leukemia cells by blocking signal transduction pathways[3]. Recently, a variety of studies have shown that dasatinib, one of the second generation TKI agents, may improve the number of lymphocytes in peripheral blood in CML patients[4,5]. The increased lymphocytes were mainly recognized as LGLs by morphology and immunophenotype analysis showed that the increased LGLs were mainly NK cells and cytotoxic T cells (CTLs) [4,6]. Previous studies have shown that CML patients with increased LGLs might have higher possibility of acquiring deep molecular response (DMR)[6,7]. Thus, increased LGLs were regarded as a predictor of favorable treatment response in CML patients[8,9]. We analyzed the clinical data of 25 CML patients treated with dasatinib to explore the difference of DMR rate and time of obtaining DMR between dasatinib treated patients with increased and normal levels of LGLs in newly diagnosed or imatinib resistant/intolerant CML patients with positive BCR-ABL fusion gene.

Patients and Methods

Patients and treatment

A total of 25 patients diagnosed with CML in Qingdao Municipal Hospital from January 2014 to January 2017 were enrolled in this study and all the enrolled patients were followed up for 18 months. Seven cases were female and 18 cases were male. The median age of these patients was 51 (ranged from 19 to 77) years old. Five of the 25 patients enrolled were newly diagnosed and the other 20 patients were intolerant or resistant to imatinib. Twenty patients were in chronic phase (CP) and the other 5 were in accelerated phase (AP). There was no death or disease progression in all the 25 patients investigated during the follow up period.

All patients enrolled were treated by orally taken the second generation TKI agents-dasatinib. Patients in CP took 100mg once daily and those in AP took 70mg twice daily.

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Assessment of treatment response
Peripheral blood samples of all the enrolled patients were taken before and 1, 3, 6, 9, 12, 15, 18 months after the starting of dasatinib treatment. Lymphocyte subsets, mainly LGLs (NK cells and Cytotoxic T cells) were counted by flow cytometry. The enrolled patients were classified into LGLs+ group and LGLs- group according to whether the LGL counts were increased or not. The quantity of BCR/ABL transcript was detected by real-time quantitative polymerase chain reaction. DMR was defined as less than 0.01% of the International Scale (BCR-ABL<)\(^{13}\).

Statistical analysis
Pearson’s chi-square (\(\chi^2\)) test and Fisher's exact test were used to analyze the data in this study. All the analyses were conducted using SPSS 19.0 software. \(P < 0.05\) was regarded as statistically significant.

Results
DMR in newly diagnosed CML patients
Three out of the 5 newly diagnosed CML patients achieved DMR, including 2 male (1 in CP, 1 in AP) and 1 female in CP. The time of obtaining DMR after dasatinib treatment was 3 and 12 months for the two patients in CP and 15 months for the patient in AP (Table 1).

| Patients | Age (year) | Gender | Phase | DMR (month) |
|----------|------------|--------|-------|-------------|
| 1        | 51         | male   | AP    | + (15)      |
| 2        | 59         | female | CP    | + (3)       |
| 3        | 46         | male   | CP    | + (12)      |
| 4        | 65         | female | CP    | -           |
| 5        | 45         | male   | CP    | -           |

DMR in CML patients resistant or intolerant to imatinib
Twenty CML patients who were resistant or intolerance to imatinib were treated with dasatinib. Three cases (2 cases of male and 1 case of female) achieved DMR and all of them were in CP. The time of obtaining DMR was 6, 12 and 15 months after the treatment of dasatinib respectively. None of the 4 patients in AP achieved DMR (Table 2).

| Patients | Age (year) | Gender | Phase | DMR (month) |
|----------|------------|--------|-------|-------------|
| 1        | 28         | female | CP    | -           |
| 2        | 47         | male   | CP    | + (12)      |
| 3        | 43         | male   | CP    | -           |
| 4        | 66         | female | CP    | + (15)      |
| 5        | 51         | male   | CP    | -           |
| 6        | 70         | male   | CP    | -           |
| 7        | 34         | male   | CP    | + (6)       |
| 8        | 54         | male   | CP    | -           |
| 9        | 57         | male   | CP    | -           |
| 10       | 47         | male   | AP    | -           |
| 11       | 66         | male   | CP    | -           |
| 12       | 52         | male   | AP    | -           |
| 13       | 77         | male   | CP    | -           |
| 14       | 27         | male   | CP    | -           |
| 15       | 72         | female | AP    | -           |
| 16       | 44         | female | AP    | -           |
| 17       | 59         | male   | CP    | -           |
| 18       | 46         | male   | CP    | -           |
| 19       | 41         | male   | CP    | -           |
| 20       | 19         | female | CP    | -           |

Table 1 DMR in newly diagnosed CML patients

Table 2 DMR in CML patients resistant or intolerant to Imatinib
Correlation between LGLs and DMR
Fifteen patients who had increased LGLs after dasatinib treatment were classified as LGL+ group. Five of them (33.3%) achieved DMR. The other 10 patients who had normal LGLs after dasatinib treatment were classified as LGL- group. Only 1 of the 10 patients (10%) achieved DMR. The incidence of DMR between LGL+ group and LGL- group was significantly different (fisher's exact test, P=0.034) (Table 3).

Table 3 DMR in LGL+ and LGL- groups

| Groups | DMR (+) | DMR (-) | Total (n) |
|--------|---------|---------|-----------|
| LGL+   | 5 (33.33%) | 10 (66.66%) | 15 |
| LGL-   | 1 (10%)  | 9 (90%)   | 10 |
| Total  | 6       | 19       | 25 |

The relationship between LGL and BCR-ABL fusion gene transcriptional level in LGL+ group
Fifteen of 25 (60%) patients had increased LGLs after dasatinib treatment. Six and seven of them had increased counts of NK cells and CTLs respectively. Two cases had increased counts of both NK cells and CTLs.

The patients in LGL+ group had increased counts of NK cells and CTLs after dasatinib treatment (Table 4), and both of them were negatively correlated with the level of BCR-ABL

Table 4 The counts of NK cells, CTLs and level of BCR-ABL

| Group | Treatment | NK cells (10^7/L) (CD16+CD56+) | CTLs (10^7/L) (CD8+CD28+) | BCR-ABL
|-------|-----------|-------------------------------|---------------------------|-----------|
| LGL+  | Before    | 0.57±0.06                     | 0.06±0.01                 | 142.2±11.9 |
|       | After     | 1.81±1.03                     | 0.21±0.13                 | 2.41±2.2  |

Median time of achieving DMR after dasatinib treatment
The median time of obtaining DMR in LGL+ group and LGL- group were 12 and 15 months respectively.

Discussion
Studies have shown that the first generation TKIs imatinib could target BCR-ABL fusion gene and significantly improved progression free survival (PFS) and overall survival (OS) in CML patients. Compared to imatinib, the second generation TKI agent dasatinib had an enhanced effect on BCR-ABL fusion gene. It has been reported that the affinity between dasatinib and tyrosine kinase is 100 times higher than that of imatinib.

The defects of T lymphocytes and NK cells play an important role in the occurrence and development of hematological malignant diseases. T lymphocytes take part in the regulation of tumor immunity, while NK cells can nonspecifically kill malignant leukemia cells. Immune deficiency of T cells is common in CML patients, and the immune function of CML patients is closely related to the disease progression of CML. Therefore, observing the changes of T lymphocyte subsets and NK cells in peripheral blood is valuable to monitor the progression of CML patients. The transcriptional level of BCR-ABL fusion gene reflects the severity of CML patients, which is of great importance to the treatment of CML. Therefore, detection BCR-ABL fusion gene transcriptional level is particularly important to evaluate the therapeutic efficacy and prognosis of CML patients.

Powers et al. discovered that 31% (5/16) CML patients showed LGL lymphocytosis (increased LGLs) after dasatinib treatment. Nagata et al.'s study showed that the LGL lymphocytosis in peripheral blood appeared in 9 out of 20 patients with CML who were continuously treated with dasatinib during the follow-up period. They also demonstrated that LGL lymphocytosis might correlate with obtaining molecular response (MR). Tanaka et al. showed that 15 of 25 CML patients who was treated with dasatinib had LGL lymphocytosis in peripheral blood. Of which, the counts of NK cells and CTLs increased in 15 and 11 cases respectively. And LGL lymphocytosis might relate to achieving complete molecular response (CMR). In this study, we showed that increased LGLs stimulated by dasatinib were associated with high rate of DMR and short time of achieving DMR in CML patients. These results were in accordance with the abovementioned studies and suggested that increased LGLs can be used as an indicator of prognosis in CML patients with treated with dasatinib.

It has been reported that dasatinib stimulated immune reaction in the formation of LGL lymphocytosis in peripheral blood of CML patients. The precise mechanism of LGL proliferation during the dasatinib treatment is still unclear. It is presumed that dasatinib could activate or modulate the proliferation and function of NK cells.

In this study, we showed that increased LGLs can be used as an indicator of prognosis in CML patients with positive BCR-ABL fusion gene who treated with dasatinib. Due to the limited number of patients...
enrolled in this study, we expected further studies with larger sample size to verify our results.

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