Correlation Between Cerebrospinal Fluid Beta2-microglobulin and Prognosis of Patients with Acute Myeloid Leukemia Subtype M2 After Chemotherapy

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Primary research
Abstract

Background: The correlation of cerebrospinal fluid beta2-microglobulin (CSF-β2MG) and prognosis of acute myeloid leukemia subtype M2 (AML-M2) receiving chemotherapy is uncertain. We seek to explore the prognostic and predictive value of CSF-β2MG in AML-M2 receiving chemotherapy.

Methods: CSF-β2MG levels were detected by immunoturbidimetry in a cohort comprised of 40 AML-M2 patients, 25 of whom were categorized into the effective chemotherapy group (Complete Remission (CR) & Partial Remission (PR) and 15 patients into the ineffective group (Non-response (NR) & Death). The correlations between changes (%) in CSF-β2MG from baseline (change between the pre- and post-chemotherapy) or CSF-β2MG (the absolute levels of post-chemotherapy) and prognosis classifications of AML-M2 were assessed. The best cut-off points were investigated.

Results: Levels of CSF-β2MG after chemotherapy and percentage change from baseline was variably presented among AML-M2s and progressively more advanced CSF-β2MG levels were associated with worse prognosis and poor therapeutic outcomes. The optimal cut-off value had the potential to be a predictor of treatment failure, hence the value for auxiliary diagnosis and accurate management.

Conclusions: Changes in CSF-β2MG could be used to predict clinical outcomes of chemotherapy in patients with AML-M2. The association between changes in CSF-β2MG and the efficacy of chemotherapy thus becomes clearer but requires further study.

Background

Acute myeloid leukemia (AML) is one of the most common hematological malignancies in adults, with prognosis heterogeneity among patients and a high rate of mortality [1]. Chemotherapy is one of the most important treatment strategies for leukemia [2]. Patients aged 18 to 60 years who can be cured by conventional chemotherapy account for 40–50% [3]. To date, however, many treatments have failed to demonstrate a satisfactory efficacy [4]. Most patients tend to die from recurrence or chemotherapy resistance eventually [5]. Its high mortality rate is partially due to inadequate prognostic assessment of patients [6]. Therefore, evaluating the therapeutic response in hematopoietic diseases is indispensable for disease management [7]. How to carry out subsequent chemotherapy mainly depends on the results of past clinical trials, so looking for a reliable biomarker to facilitate the estimation of chemotherapy response is the core problem to be solved.

In patients with acute leukemia, the assessment of response to early treatment can predict the risk of recurrence and is used for risk-oriented therapy. Residual leukemic cells are traditionally identified by conventional cytomorphologic criteria. Unfortunately, differentiating between normal hematopoietic cells and cancer cells is difficult because of their similarity. Methods to detect remaining leukemia cells based on flow cytometry or polymerase chain reaction (PCR) have a superior sensitivity and accuracy with their ability to recognize leukemic cells present at levels far below those detectable by morphology (ie, minimal...
residual disease [MRD]) [8]. However, nearly 40% of patients with AML have no cytogenetic or molecular markers suitable for PCR monitoring [9].

In AML, central nervous system (CNS) involvement occurs in 2–4% of cases, but it yet represents a challenging clinical problem, because a poor clinical outcome of AML patients is associated with this event [10]. Beta2-microglobulin (β2m) levels in different body fluids like urine, serum and CSF testing after treatment can predict relapse and survival in leukemia. Serum β2m levels reflect tumor burden and cell turnover [11]. β2m is a low molecular weight protein synthesized in all nuclear cells and forming a small invariable light chain subunit of major histocompatibility complex class I (MHC-I) antigens [12]. It is shed from the cell surface or cytoplasm and can be detected in a soluble form in body fluids [13]. Under physiological conditions, β2m is generated at a constant rate. Human leukocyte antigen (HLA)-I molecules are of vital importance in the presentation of antigenic peptides, and promote CD8 + T cells to eliminate tumor cells. However, the expression of HLA-I and/or its component β2m is frequently reduced or lost on the surface of tumor cells, which causes immune escape and survival of tumors [14]. Moreover, serum β2m concentration is independently prognostic for hematopoietic diseases, as well as an independent predictor of total mortality in a general population of older adults [12]. An elevation of CSF-β2MG concentration correlates with a disease progression, and a decline of increased value demonstrates sufficient clinical efficacy [15].

The different trends of β2m in some types of leukemia are significantly associated with chemotherapeutic effects, and many of them have been confirmed. Bo, et al. used serum β2m and Lactic acid (LDH) as indicators to assess the safety and efficacy of treatment of hematological malignancies in older patients. The levels of β2m were determined every 2 weeks during the treatment; and the results indicated a decrease in the concentration of β2m, which occurred in a course-dependent manner [16]. In patients with adult T-cell leukemia, chemotherapy led to a decrease in serum β2m levels in all patients who achieved remission states (patients’ data were gathered at the following time points: 4th, 8th, 12th, 16th, 20th, and 24th week) [17]. So, our aim is to determine whether it holds true for the monitoring effects of chemotherapy in AML-M2s. M2 is a subtype of AML (based on the French-American-British classification system) [18], accounting for 25–30% of all cases [19]. In AML-M2, 30–40% of patients have a characteristic chromosomal translocation t (8; 21) (q22; q22), which is considered to be a genetic marker with a good prognosis [20, 21]. The remaining approximately 70% of patients have large prognosis heterogeneity.

However, it is unclear whether repeated CSF protein assays during chemotherapy can offer information regarding the chemotherapy sensitivity of the leukemic clone. In this study, we examine whether the CSF-β2MG level is a potential chemotherapy prognostic factor in AML-M2 that could be added to known prognostic factors to reduce prognostic variation.

**Methods**

**Patients**
Forty patients with AML-M2, who had complete clinical data and were treated in the Second Affiliated Hospital of Chongqing Medical University from January 2015 to May 2017, were collected as an observation group. These 40 patients with AML-M2 included 23 males and 17 females, with an age range of 6–65 years and a mean age of 36.5 years. The diagnostic criteria for AML-M2 were based on the 2016 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia. Diagnosis was based on characteristic clinical manifestations, morphology, and cytochemistry of tumor cells in the bone marrow and peripheral blood. We recorded the basic laboratory evaluation, response to treatment, and outcome of the whole group. Patients were classified into four subtypes according to the clinical outcome: complete remission (CR), partial remission (PR), non-response (NR), and Death. (Table 1). Measurements of CSF-β2MG were taken in all subjects at baseline (before treatment), and the concentrations at another four time points were detected on the third day after the end of each chemotherapy course. Percentage change in CSF-β2MG levels represents CSF-β2MG up/down-regulation (%) in patients receiving treatment versus baseline.

**Therapeutic Regimens**

Standard induction chemotherapy for patients in our trial consisted of a continuous standard dose of cytarabine infusion (100–200 mg/m^2^) for 7 days combined with either idarubicin (12 mg/m^2^ for 3 days) or daunorubicin (40–60 mg/m^2^ for 3 days). Other cytarabine- and non-cytarabine-based regimens were also included. All patients who received frontline cytotoxic chemotherapy regimens (cytarabine-based and otherwise) were included. Patients who received frontline epigenetic therapy with hypomethylating agents (decitabine or azacitidine) were excluded.

**Collection and analysis of Cerebrospinal fluid**

To investigate the association between CSF-β2MG concentrations and response to chemotherapy, patient's demographics, clinical data, and number of chemotherapy cycles were obtained retrospectively from our hospital medical records. Baseline CSF-β2MG levels were measured before the start of the first day of chemotherapy (The patients included in this study did not receive any adjuvant chemotherapy, surgery, or radiation therapy previous to the sample collection.). During chemotherapy treatment, measured data (CSF-β2MG concentrations: 3 days after each chemo cycle) were collected for at least four cycles. Patients who met all the eligibility criteria and none of the exclusion criteria were enrolled in this study. The eligibility criteria were (1) age 5–65 years; (2) diagnosed as primary or secondary AML-M2 (including AML after myelodysplastic syndrome); (3) no evidence of serious concurrent cardiac, pulmonary, neurological, and metabolic diseases or uncontrolled infections; and (4) adequate liver (serum bilirubin level < 2 × upper normal limit) and renal (serum creatinine < 2 × upper normal limit) function. Exclusion criteria included blast crisis of chronic myeloid leukemia and AML supervening after other chronic myeloproliferative diseases and other progressive malignant diseases. Patients were treated with
three days of daunorubicin and seven days of cytarabine (7 + 3), and each patient received at least four courses of chemotherapy.

CSF samples were collected in sterile collection tubes and centrifuged at 1500 x g for 10 min at 4 °C within 30 min after the lumbar puncture. The CSF aliquots were stored at -80 °C until analyzed. The levels of CSF-β2MG were measured by Hitachi Modular 7600 chemistry analyzer (Hitachi, Tokyo, Japan) with the test kit (Maccura, Sichuan, China).

Criteria of Response and Evaluation of Prognosis

For the determination of outcome, the hospital records of all 40 patients were reviewed. Participants were followed over four chemotherapy cycles in order to dynamically monitor the effect of chemotherapy. Response was evaluated by using the criteria described below after four cycles of therapy [22]. Complete remission (CR) was defined as normal cells with normal bone marrow, normal red blood cell, and medullary composition, less than 5% myeloid cells, peripheral blood count of platelets over $10^5$/ml, and granulocytes over $1.5 \times 10^3$/ml, lasting at least 4 weeks. Patients with regenerative cells in peripheral blood and myeloid cells greater than 5% but less than 25% were defined as partial remission (PR). Patients who met CR bone marrow standards but did not fully recover peripheral blood platelet and/or white blood cell counts were also classified as partial remission (PR). Patients with leukemia cells that persist in the bone marrow or blood or regenerate within 4 weeks of the initial response are considered non-response (NR). The data of the death cases were obtained from the medical record.

Statistical analysis

This study was conducted to evaluate the role played by CSF-β2MG concentration changes according to clinical outcomes. Before statistical analysis, count data were transformed with the Blom method and analyzed for normality. For comparing pre- versus post-treatment, paired t-tests were used. Results are expressed as scatter diagrams (mean ± 95% CI) and boxplots, described as median (P25, P75). Independent-sample t test was used to compare CSF-β2MG in each point among CR and NR or Death group. The Wilcoxon signed-rank test was used to compare percentage changes in CSF-β2MG during treatment among the NR or Death group. Mann–Whitney U test was used compare CSF-β2MG in each point among CR group and NR or Death group; the test was also used to compare CSF-β2MG in each point among the different genders and different prognosis subgroups. We used one-way and two-way analysis of variance (ANOVA) to test the effect of different factors on prognosis. Within-group comparisons were analyzed with paired t-tests or Wilcoxon's signed-rank test. Significance levels were corrected by using conservative Bonferroni's method. The concentration of CSF-β2MG after chemotherapy or changes in CSF-β2MG (expressed as percent change over baseline values) in predicting patients achieving CR was analyzed by using receiver operating characteristic (ROC) curve analysis. Differences among variables (Groupings were based on cut-off values in CR and NR or Death) were evaluated with the use of Pearson's $\chi^2$ for categorical variables. A 2-sided P-value lower than 0.05 was
considered statistically significant. We performed all statistical analyses using the SPSS software (version 25.0; SPSS).

Results

Patient characteristics

Patient characteristics are described in Table 1. In light of treatment response, data of 40 patients diagnosed with AML-M2 were collected in the study, among whom 11 patients with CR, 14 with PR, 12 with NR, and 3 with death. There were 23 (57.5%) males and 17 (42.5%) females and no significant difference in gender was observed ($P = 0.535$). No correlation with prognosis was found for pre-chemo CSF-β2MG levels ($P = 0.532$). As shown in Figure S1, previous research efforts in our group showed that the distributions of CSF-β2MG varied in different types of brain diseases. Mean values ± S.E.M. were compared. Elevation of CSF-β2MG was significantly more common in patients before chemotherapy than in traumatic brain injury (TBI) and brain tumor (BrTm) ($P < 0.001$). The concentration of leukemia before chemotherapy was more discrete than that of the central tendency of brain trauma and brain tumor, which might have a suggestive contribution to the disease progression or treatment effect. In the 40 patients, CSF-β2MG levels varied from patient to patient. The effective rate of disease treatment was 62.5%. Patients in an effective group (namely who achieved CR & PR) were always accompanied by lower CSF-β2MG (before chemotherapy); however, limited by the sample size, the results did not reach statistical significance. ($P = 0.600$).

Table 1
Baseline characteristics of patients

| Characteristics | CR     | PR     | NR     | Death |
|-----------------|--------|--------|--------|-------|
| n (%)           | 11(27.5)| 14(35) | 12(30) | 3(7.5) |
| Age (years)     | 32(6–49)| 42.5(11–65)| 36(17–60)| 27(19–33) |
| Median (Range)  |        |        |        |       |
| Gender: n (%)   | 6(55)  | 4(29)  | 6(50)  | 2(67) |
| Female          | 5(45)  | 10(71) | 6(50)  | 1(33) |
| Male            |        |        |        |       |
| CSFβ2-MG        | 1.50 (0.04–6.90) | 1.60 (0.37–13.80) | 2.74 (0.24–9.20) | 6.83 (5.08–13.70) |
| Median (Range)  |        |        |        |       |

Dynamics of CSF-β2MG concentration in patients receiving chemotherapy
CSF samples of all patients were collected before chemotherapy was initiated; data of effect size were taken from a standardized time point (3 days after each chemo cycle). Due to the small sample size of the death group, the Death was merged with the NR group (The change of concentration in both groups exhibited the same trend throughout the treatment. (Figure S2)). The median CSF-β2MG, mg/L (interquartile range) concentrations in different prognostic subgroups at baseline and at various time points after treatment were detected in all patients (Table 2), and the results indicated different dynamic change trends in the amount of CSF-β2MG, which occurred in a cycle-dependent manner (Fig. 1). The statistical difference of CSF-β2MG levels and percentage changes relative to baseline among four clinical outcome groups during different sampling points were shown in Tables 2 and 3. Notably, during chemotherapy, there was a decreasing trend in the CR group whereas there was an increasing trend in the NR or Death group. There was no apparent change in the PR group from T0 to T4.

**Table 2**

Changes in CSFβ2-MG concentration before and after cycles

| Group       | CSFβ2-MG (mg/L) | Baseline  | T1: After 1st cycle | P value | T2: After 2nd cycle | P value | T3: After 3rd cycle | P value | T4: After 4th cycle | P value |
|-------------|-----------------|-----------|---------------------|---------|---------------------|---------|---------------------|---------|---------------------|---------|
| CR          |                 | 2.40      | (1.23, 4.67)        |         | 1.50                | (0.97, 3.24) | 2.01                | 0.022   | 0.81                | 0.002   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 0.93      | (0.73, 1.16)        |         |                     |         |                     |         |                     |         |
| PR          |                 | 1.65      | (0.80, 2.38)        |         | 1.70                | (1.11, 2.35) | 1.60                | 0.532   | 1.50                | 0.940   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 1.33      | (0.91, 1.88)        |         |                     |         |                     |         |                     |         |
| NR or Death|                 | 1.90      | (1.13, 6.04)        |         | 2.5                 | (1.51, 5.20) | 3.05                | 0.280   | 3.52                | 0.019   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 3.33      | (2.77, 5.90)        |         |                     |         |                     |         |                     |         |

**Table 2**

Changes in CSFβ2-MG concentration before and after cycles

| Group       | CSFβ2-MG (mg/L) | Baseline  | T1: After 1st cycle | P value | T2: After 2nd cycle | P value | T3: After 3rd cycle | P value | T4: After 4th cycle | P value |
|-------------|-----------------|-----------|---------------------|---------|---------------------|---------|---------------------|---------|---------------------|---------|
| CR          |                 | 2.40      | (1.23, 4.67)        |         | 1.50                | (0.97, 3.24) | 2.01                | 0.022   | 0.81                | 0.002   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 0.93      | (0.73, 1.16)        |         |                     |         |                     |         |                     |         |
| PR          |                 | 1.65      | (0.80, 2.38)        |         | 1.70                | (1.11, 2.35) | 1.60                | 0.532   | 1.50                | 0.940   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 1.33      | (0.91, 1.88)        |         |                     |         |                     |         |                     |         |
| NR or Death|                 | 1.90      | (1.13, 6.04)        |         | 2.5                 | (1.51, 5.20) | 3.05                | 0.280   | 3.52                | 0.019   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 3.33      | (2.77, 5.90)        |         |                     |         |                     |         |                     |         |

**Table 2**

Changes in CSFβ2-MG concentration before and after cycles

| Group       | CSFβ2-MG (mg/L) | Baseline  | T1: After 1st cycle | P value | T2: After 2nd cycle | P value | T3: After 3rd cycle | P value | T4: After 4th cycle | P value |
|-------------|-----------------|-----------|---------------------|---------|---------------------|---------|---------------------|---------|---------------------|---------|
| CR          |                 | 2.40      | (1.23, 4.67)        |         | 1.50                | (0.97, 3.24) | 2.01                | 0.022   | 0.81                | 0.002   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 0.93      | (0.73, 1.16)        |         |                     |         |                     |         |                     |         |
| PR          |                 | 1.65      | (0.80, 2.38)        |         | 1.70                | (1.11, 2.35) | 1.60                | 0.532   | 1.50                | 0.940   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 1.33      | (0.91, 1.88)        |         |                     |         |                     |         |                     |         |
| NR or Death|                 | 1.90      | (1.13, 6.04)        |         | 2.5                 | (1.51, 5.20) | 3.05                | 0.280   | 3.52                | 0.019   |
|             |                 |           |                     |         |                     |         |                     |         |                     |         |
|             |                 | 3.33      | (2.77, 5.90)        |         |                     |         |                     |         |                     |         |
Table 3
Percentage change from baseline in CSFβ2-MG

| Group          | T1 After 1st cycle | Difference | P value | T2 After 2nd cycle | Difference | P value | T3 After 3rd cycle | Difference | P value | T4 After 4th cycle | Difference | P value |
|----------------|--------------------|------------|---------|--------------------|------------|---------|--------------------|------------|---------|--------------------|------------|---------|
| CR             | -30.00             | -44.52     | 0.013   | -55.25             | 0.004      | -55.25  | 0.004              | -66.67     | 0.006   |                    |            |         |
|                | (-43.84, -6.69)    | (-53.60, -21.79) |         | (-65.21, -33.82)  |            | (-80.60, -32.00) |         |                     |            |         |
| PR             | -5.88              | -18.75     | 0.600   | -11.76             | 0.730      | -29.41  | 0.530              |            |         |                    |            |         |
|                | (-24.95, 26.59)    | (-30.65, 44.74) |         | (-33.84, 33.71)   |            | (-48.38, 49.90) |         |                     |            |         |
| NR or Death    | -2.09              | 8.33       | 0.691   | 3.45               | 0.096      | 6.90    | 0.125              |            |         |                    |            |         |
|                | (-14.07, 44.58)    | (5.92, 139.05) |         | (9.97, 143.27)     |            | (20.62, 165.49) |         |                     |            |         |

Difference: The value was expressed as percentage change from Baseline

Two-way repeated-measures ANOVA, with prognosis and time as factors, was used to analyze the data (CR and NR or Death). There was a significant difference between both groups (F = 13.881, P = 0.001). A test for interaction showed that the trend with time in CSF-β2MG was significantly different between the three groups (F = 5.896, P = 0.004). Median CSF-β2MG of each point was significantly higher in CR group than in NR or Death group (T1; 1.50 [0.49–5.90] versus 2.5 [0.24-12.00] [P = 0.227], T2; 2.01 [0.24–5.40] versus 3.05 [1.30–13.60] [P = 0.012], T3; 0.81 [0.50–4.20] versus 3.52 [1.40–10.50] [P < 0.001], T4; 0.93 [0.04–2.02] versus 3.33 [0.9–11.90] [P < 0.001]). The same was observed for % change in CSF-β2MG from Baseline (Table S2). One-way repeated-measures ANOVA was used to find the statistical difference among the concentrations obtained at the four different time points, which differed in the two groups (CR; F = 2.022, P = 0.132, NR or Death; F = 4.680, P = 0.040). Paired t-tests demonstrated a modest and not apparent effect of chemotherapy (α < 0.017) (Table S1). In other words, these differences between adjacent and non-adjacent time points were small and did not reach statistical significance. We hope to expand these experiments to include a larger sample size in the future so that we tested whether some specific time point was used to predict the prognosis of AML-M2s.

Percentage change in CSF-β2MG during chemotherapy is shown in Fig. 2A and 2B. In most patients of the chemotherapy group, CSF-β2MG remarkably changed after the second cycle from baseline, due to the therapeutic effect. At T2, in 15 patients with NR or Death was observed 8.33% (5.92%, 139.05%) change and in 11 patients with CR was observed - 44.52% (-53.60%, -21.79%) change in CSF-β2MG from baseline (P = 0.012) (Table S1). Specifically, a 25%, 38%, 50%, and 56% decrease was found 3 days after the first, second, third, and fourth cycle in CR group, respectively. A 15%, 72%, 72%, and 93% increase was found 3 days after the first, second, third, and fourth cycle in CR group, respectively. We further revealed the
differences in CSF-β2MG levels between different prognostic phases, so our results potentially suggested that a downward trend of CSF-β2MG is associated with a better prognosis.

**ROC-curve analysis of CSF-β2MG and % of CSF-β2MG change from baseline**

In both treatment groups (CR and NR or Death), Twenty-six patients were analyzed at four time points, equaling 104 data pairs. Since there is no obvious difference between time points, data were pooled together. In predicting the inefficacy of chemotherapy (NR or Death), according to the ROC curve (ROC: receiver operating characteristic), CSF-β2MG after chemotherapy (ROC area: 0.811, 95% confidence interval [CI]: 0.728–0.894, \( P < 0.001 \)) had a high predictive value for patients with AML-M2. We then assessed the cut-off value of CSF-β2MG levels after chemotherapy by ROC analysis and Youden index, and found 1.505 mg/L as the cut-off value for high vs. low levels of CSF-β2MG. The optimal cut-off point was also the point that maximized the product of sensitivity and specificity, with sensitivity and specificity of 0.883 and 0.614, respectively. The percentage change in CSF-β2MG was expected to have a diagnostic accuracy with an area under the ROC curve of 0.907 (95% CI: 0.841–0.938). When the threshold was set to -25%, the specificity was 84.1% and the sensitivity was 98.3% (Fig. 3). This was a stronger prognostic marker than the absolute level of CSF-β2MG. Other key parameters including positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of this biomarker are shown in Table 4.

### Table 4

Methodological evaluation of CSFβ2-MG in predicting the prognosis of chemotherapy patients.

| Parameter                  | Value       | % change in CSFβ2-MG from baseline |
|----------------------------|-------------|-----------------------------------|
| CSFβ2-MG                   | 0.883       | 0.983                             |
| % change in CSFβ2-MG from baseline | 0.614       | 0.841                             |
| Positive predictive value  | 0.537       | 0.811                             |
| Negative predictive value  | 0.091       | 0.980                             |
| Positive likelihood ratio  | 2.288       | 6.182                             |

**CSF-β2MG and % of CSF-β2MG change from baseline may be two potential biomarkers to predict the prognosis of AML-M2 patients treated with chemotherapy**
To ascertain whether the two optimal cut-off points based on ROC analysis improves predictability, we applied a confusion matrix analysis, and the result is shown in Table 5. Subjects were dichotomized into two groups based on the above cut off values of the risk score. Chi-square tests were used for the analysis of the correlation between CSF-β2MG and clinical prognosis. (CSF-β2MG; $F = 28.492$, $P < 0.001$, % change in CSF-β2MG from baseline; $F = 74.374$, $P < 0.001$). These prediction values had an accuracy of 76.92%, 92.31%, respectively; the latter with a high performance (92.31%) meant 96 out of 104 individuals was correctly classified, whereas only 7.69% (8 out of 104 individuals) was incorrectly classified. In our study, the CSF-β2MG levels and % of CSF-β2MG change from baseline considerably differed between good and poor prognosis patients, and the results indicated a decrease in the amount of CSF-β2MG, which occurred in a clinical feature-dependent manner.

### Table 5
Utility to discriminate patients' clinical outcome based on cut-off value

| Predicted outcomes | Actual outcomes | $P$ value |
|--------------------|----------------|-----------|
|                   | CR (n = 44)    | NR or Death (n = 60) |
| CR ($\leq 1.505$ mg/L) | 27             | 7         | $P < 0.001$ |
| NR or Death (> 1.505 mg/L) | 17             | 53        |
| CR ($\leq -25\%$) | 37             | 1         | $P < 0.001$ |
| NR or Death (> -25\%) | 7              | 59        |

**Discussion**

The combination of an anthracycline with cytarabine is the standard remission induction chemotherapy for patients with AML. This is commonly followed by several cycles of consolidation chemotherapy, in which some high-risk patients subsequently underwent hematopoietic stem cell transplantation (HSCT) [23]. As chemotherapy drugs for the treatment of leukemia (anthracyclines, vinca alkaloids, cyclophosphamide) are some poor penetrators of the blood-brain barrier (BBB), the CNS becomes a 'shelter' for leukemia cells. However, methotrexate and cytarabine with high permeability are strong enough to cross the BBB in the CNS at high doses to reach therapeutic concentrations [24]. Patients found to have CNS leukemia by CSF cytology are treated with intrathecal methotrexate until the cerebrospinal uid is free of leukemic cells [25]. In clinical practice, the assessment of CNS involvement in leukemia is made mainly based on the detailed cytological analysis. CSF cytology has a high degree of specificity, but the literature indicates that the reported sensitivity of a single CSF examination is between 45% and 95% (mean 71%) [26]. It is less useful, though repeated lumbar punctures improve the detection rate of malignant cells [27]. The poor sensitivity limits the diagnostic utility of CSF cytology. And leukemic cells become undetectable after chemotherapy in most patients with AML [28]. Several previous reports have demonstrated that the sensitivity of flow cytometry is superior to conventional cytology for the early identification of CSF infiltration by leukemia or lymphoma cells, but due to the limited number of antibodies, its weak capacity to provide definite diagnostic clues needs to be improved (most likely due to
small sample size and the small number of cells). Apart from this, it is not easy to find the exact phenotype of the neoplastic cells.

β2m is a relatively indicative marker of tumor status. Tumor progression causes the level of this marker to increase, whereas effective therapy results in a decrease in their levels [16]. Elevated CSF-β2MG values could lead to a suspicion of CNS inflammation or malignant infiltration, even if no tumorous cells are present in CSF [15]. Our results showed that CSF-β2MG levels were significantly different before and after chemotherapy aside from the PR group (more validation and comparison of this group are further needed). Percentage change from baseline was also noted. A statistically significant decrease in CSF-β2MG was seen in subjects in CR group compared to baseline. In the CR group, CSF-β2MG was gradually decreased from baseline during treatment, and in the NR or Death group, there was a slight decrease or irregular change in CSF-β2MG.

The association of CSF β2-MG or serum β2m with prognosis in patients with leukemia/lymphoma has been demonstrated through some large clinical cohort study. A prospective analysis of the importance of the plasma levels of β2m in 553 patients with myelodysplastic syndrome (MDS) found that β2m is an independent prognostic variable for survival with weighted significance second only to the karyotype [29]. In patients aged 60 years or older (n = 591), higher levels of serum β2m were found to be an adverse independent factor for the response, survival, relapse-free survival, and event-free survival. In contrast, in younger patients, the situation was different [30]. Leukemia with CNS involvement is not uncommon in clinical practice [31]. Mavligt et al. [32] successfully distinguished leukemia patients with CNS invasion from those without according to whether or not the CSF-β2MG/serum β2m -ratio was greater than 1. However, some scholars obtained several false-positive and false-negative results by using this ratio as the criterion for a positive diagnostic test. The level of β2m in CSF reflects local production, so it does not seem rational to use this ratio to detect CNS disease. Additionally, elevated serum-β2MG values caused by the malignant disorder with rapid cellular turnover and secondary renal insufficiency may be a gradually increased risk of false-negative results [33]. Besides, several biomarkers, such as LDH, fibrinogen, and soluble tumor-related markers in the CSF, might reflect CNS involvement in leukemia patients, but the specificity and sensitivity remain dubious [34–38].

Our study focused on the roles of CSF-β2MG in chemotherapeutic efficacy and predicted the prognosis of patients with AML-M2. The results of our research demonstrate that increased/decreased post-chemotherapy CSF-β2MG levels are associated with different clinical outcomes and efficacy. Moreover, we have shown that patients who had a down-regulated trend of CSF-β2MG may indicate a better prognosis than those who had an elevated CSF-β2MG 3 days after each cycle of chemotherapy. Of note, comparisons between different time points didn’t indicate a time-dependent change of CSF-β2MG levels. This assessment could seriously affect immediate decisions to continue or adjust therapy as well as subsequent options for post-remission therapy and possible bone marrow transplantation.

In several studies, the utility of β2m as a marker for hematologic malignancies have been assessed. The protein is continuously elevated in patients with leukemia and malignant lymphoma; however, β2m is
also elevated in the CSF of patients with multiple sclerosis, sarcoidosis, and human immunodeficiency virus infection [39–41]. Serial studies demonstrated the decrease of CSF-β2MG levels during chemotherapy appeared to be closely related to disease reaction [15]. However, in a study conducted by M. R. Pudek, et al., levels of CSF-β2MG exhibited nonspecific elevation following intrathecal therapy with methotrexate [42]. These above results, together with specificities as low as 0.614, reduce β2m's utility as a single tumor marker but show that it may have some utility as an auxiliary diagnostic index to CSF cytology. To better monitor the in vivo efficacy of chemotherapy drugs that kill leukemia cells, we need markers that dynamically monitor or enable the quantification of the prognostic category. Such markers will desirably be sensitive and specific, and the technique used for the assay will be cheap and simple [43]. Measurement of CSF-β2MG does not need large expenses, and the required amount of CSF is minimal [15]. The diagnostic potential of CSF-β2MG in distinguishing patients achieved CR from those with NR or Death was evaluated with the use of the ROC curve. It showed that the AUC value was a little over 0.8 and 0.9, which indicated that the expression level of CSF-β2MG and % change might be used to predict the chemo-response and prognosis in patients with AML-M2. The CR rate was 80% in patients with CSF-β2MG ≤ 1.505 mg/L compared with 24% in patients with CSF-β2MG > 1.505 mg/L. The NR or Death rate was 3% in patients with % change ≤ -25% compared with 89% in patients with % change > 25%. The data suggests that the optimal cut-off has the potential to separate CR from other prognostic conditions. Because of the small number in Death group, it's impossible to further classify the adverse prognosis and calculate the percentage separately, and we still need more data to confirm this conclusion. Importantly, in the presence of mental or neurological symptoms, cytology and/or flow cytometry testing of CSF obtained from lumbar puncture is required to determine or exclude the presence of central nervous system invasion. Our findings demonstrate sensitivity; however, longer-term survival studies will be needed to show specificity.

The results of these studies mentioned above covered the general orientation of our study and support our conclusions. However, there are some differences in our research. We reported the double effects of CSF-β2MG among patients with AML-M2, addressing a gap in literature. Together with existing predictors, change in CSF-β2MG may enable improved risk stratification regarding which patients will likely obtain improved prognosis. There is a limitation in this study. Since this study was retrospective and unblinded, and our sample size was small. In the future, larger-scale prospective studies are required to confirm which time point is more important for early assessment the outcome of AML-M2 patients.

Conclusions

In summary, this study found that a high concentration of CSF-β2MG was a potential predictor of the outcome of AML-M2 patients. These findings suggest the need for large clinical trials assessing the significance of CSF-β2MG concentrations in patients with AML-M2.

Abbreviations

β2m
Declarations

Ethics declarations

Ethics approval was given by the Research Ethics Committee of the Second Hospital of Chongqing Medical University.

Consent for publication

Authors confirmed that this work can be published. The content of this manuscript is original and it has not been published and accepted for publication or under editorial review for publication elsewhere.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable.

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Author contributions
Pu Li and Tingmei Chen designed and performed experiments and prepared the figures; Jing Shi and Qin Hu performed experiments; Xiaolan Zhou analyzed the data. Weixian Chen, Lijun Zhang, Bo Wang, Xuemei Peng, and Liang Duan contributed to the experimental design and edited the manuscript; Pu Li supervised all the research. Mengli Yao and Pu Li wrote the manuscript. All authors discussed the results and commented on the manuscript.

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**Figures**

**Figure 1**

The dynamic change of CSFβ2-MG at five different time points during following-up time in two groups. The scatter plot and boxplots showing the CSFβ2-MG values found in patients with AML-M2 with different prognosis classifications. (A, B) Comparison of the concentrations of CSFβ2-MG between before and after each cycle of chemotherapy from patients. (A) CR group, (B) NR or Death group. Data were analyzed by paired Student t-tests. Mean and credible intervals (95%) are shown. * indicates statistically significant differences (P < 0.05), ** indicates statistically significant differences (P < 0.01) and n.s. indicates no statistically significant difference.
Figure 2

Percentage change in CSFβ2-MG from baseline in two groups. The Wilcoxon signed-rank test was used to compare changes in CSFβ2-MG during treatment after baseline in the CR group and in NR or Death group (A) CR in the patients receiving chemotherapy; (B) NR or Death in the patients receiving chemotherapy.
Comparison of the sensitivity and specificity of CSF-β2MG/percentage change in CSFβ2-MG in predicting which patients would have a CR by ROC curves. Different ROC curves are represented by curves of different symbol. The AUC of CSF-β2MG was 0.811; the AUC of % change was 0.907 (P < 0.05). ROC, receiver operating characteristic; AUC, area under curve.
Figure 4

Scatterplots showing the levels of CSF-β2MG or % change from baseline and cut-off points for prognostic grouping (1.505, -25% respectively), identified from the ROC curve analysis. N=104 concentrations of CSF-β2MG were classified as “CR” or “NR or Death” according to the GLAGB criteria. Categorical variables were based on cut-off value tested by using Chi-square analysis. All variables were associated with patients’ clinical outcome (left: $P = 0.001$, right: $P < 0.001$). *** $P < 0.001$.

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