Materials and methods

1 Patients and treatments

This study was a retrospective analysis approved by the Ethics Committee of the First Affiliated Hospital of the Medical School of Zhejiang University, with a total of 157 newly diagnosed multiple myeloma (NDMM) patients enrolled from November 2014 to September 2018. All NDMM patients received first-line treatment with a bortezomib-based regimen in our center, including PD regimen (bortezomib combined with dexamethasone), PCD regimen (bortezomib, dexamethasone combined with cyclophosphamide), PAD regimen (bortezomib, dexamethasone combined with adriamycin) or PTD regimen (bortezomib, dexamethasone combined with thalidomide). Treatment efficacy was evaluated after each cycle according to the IMWG efficacy criteria.

At the same time, 15 healthy volunteers and 96 patients with refractory/relapsed multiple myeloma (RRMM) were enrolled, and their peripheral blood cytokine level were measured. The treatment of RRMM patients was based on the patient's previous treatment efficacy, duration of treatment, remaining adverse reactions, as well as their and their relatives' individual considerations. The treatment drugs included bortezomib, lenalidomide, daratumumab and traditional chemotherapy regimens such as DECP (cyclophosphamide, etoposide, cisplatin and dexamethasone).

2 Data acquisition

All patients were hospitalized during the initial diagnosis and treatment. At the time of diagnosis, the assessment of Durie-Salmon staging (D-S), international

staging system (ISS), revised-ISS staging was performed, and data regarding routine blood examinations, hepatic and renal function indicators, lactate dehydrogenase (LDH) levels, C-reactive protein (CRP) and bone marrow examination results were obtained from the Patients Records System (PRS) of the hospital. The extramedullary extraosseous (EME) involvement was established according to the physical examination, CT, MR and/or PET/CT results of the patients. Fluorescence in situ hybridization (FISH) was performed on the bone marrow cells of most of the newly diagnosed patients to detect specific chromosomal abnormalities, including del (17p13), 1q21 gain, del (13q14), and IgH translocations. A small number of patients with IgH translocations were tested for specific translocations, including t (4; 14), t (11; 14), and t (14; 16). Among them, the 1q amplification was also considered as a genetic abnormality with poor prognosis. The above-mentioned clinical data of patients with RRMM were also obtained through the PRS, but the FISH test of these patients was not repeated when the disease relapsed.

All of the 157 NDMM, 15 healthy volunteers and 96 RRMM were tested for peripheral blood cytokine levels, including IL-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-17A. The serum concentrations of the 7 aforementioned cytokines were quantified using the cytometric bead array (CBA) kit BD™ CBA Human Th1/Th2/Th17 Cytokine Kit (BD Biosciences, San Jose, CA, USA). The minimum and maximum detection limits for all seven cytokines were 0.10 pg/mL and 5000 pg/mL, respectively. The cytokine measurement was performed according to the manufacturer's protocol. Specifically, the detection method was as

follows: draw 3−5 mL of the patient's peripheral blood, collect the blood sample by centrifugation after the blood has coagulated, collect the serum and store it at 2−8 ℃ for further analysis (within 24 h); add the sample to be tested into the capture microsphere mixture containing seven kinds of cytokine specific antibodies, make samples specifically bind to the antibody coated on the capture microsphere surface, and then bind to the PE-labeled fluorescent detection reagent; make the capture microsphere, the samples, and the detection antibody form a double-antibody sandwich complex; analyze the fluorescence intensity of the double-antibody sandwich complex by BD FACScanto™ II flow cytometer (Becton Dickinson, San Jose, CA, USA), and use FCAP Array™ software (BD Biosciences) to generate data in graphical and tabular format, in order to obtain the concentration of cytokines in the sample to be tested.

3 Statistical analysis

The optimal cutoff value of cytokines was based on the receiver operating characteristic (ROC) curve. The correlation between cytokines with various clinical characteristics was assessed by Mann-Whitney U test. Chi-square test or Fisher's exact test were used for the classification data. Progression-free survival (PFS) was calculated from the date of the first course of treatment to the date of disease progression, death, or the final follow-up visit. Overall survival (OS) was measured from the date of the first course of treatment to the date of death or the final follow-up visit. The Kaplan-Meier method was used to generate survival curves, and the diffidence between survival curves were compared using log-rank tests. The Cox

proportional hazards model was used to perform univariate and multivariate analyses. All test result data were bilateral, and *P*-values less than 0.05 were considered to be statistically significant. The statistical analysis was performed using SPSS for Windows 26.0.