Original Article

The level and integrity of plasma circulating cell-free DNA in patients with primary multiple myeloma

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Background: To evaluate the clinical research related to the level and integrity of circulating free DNA (cfDNA) in the plasma of patients with multiple myeloma (MM).

Methods: The plasma samples of 56 patients with newly diagnosed MM and 60 healthy volunteers were collected. ALU247 fragment and ALU115 fragment were used as target genes, and quantitative polymerase chain reaction (qPCR) was used to assess the plasma of the patient and healthy control groups. The cfDNA level in MM was analyzed, and the ALU247/ALU115 ratio was used to calculate the integrity of cfDNA. The correlation between the cfDNA level and integrity and the clinical characteristics of patients with primary MM was analyzed, and their value in efficacy monitoring and prognostic evaluation was evaluated.

Results: The plasma concentrations of ALU247 and ALU115 and the integrity of cfDNA in patients with primary MM were significantly higher than those in the healthy controls (P<0.05). The ALU247 fragment concentration was markedly correlated with the Durie-Salmon (D-S), International Staging System (ISS), and Revised-International Staging System (R-ISS) stages (P<0.05). After three courses of induction chemotherapy, the levels of ALU247, ALU115, and cfDNA integrity in both groups were lower than those before chemotherapy (P<0.05). Patients with curative effects of CR, sCR, and VGPR were classified into the ≥ very good partial response (VGPR) group (n=38), while those with curative effects of PR and SD were allocated into the <VGPR group (n=18). In addition, after chemotherapy, the levels of ALU247, ALU115, and cfDNA integrity of patients in the ≥ VGPR group were significantly lower than those in the < VGPR group (P<0.05). The follow-up results showed that the progression-free survival (PFS) of MM patients with low ALU247 expression was considerably longer than that of MM patients with high ALU247 expression (33.59±1.15 *vs.* 27.31±2.16, P<0.05).

Conclusions: CfDNA levels were significantly elevated in MM patients, and the ALU247 fragment concentration was remarkably correlated with multiple clinical features and had important clinical value for efficacy monitoring and prognostic assessment.

Keywords: Primary multiple myeloma (primary MM); circulating free DNA (cfDNA); efficacy monitoring; prognosis assessment

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Introduction

Multiple myeloma (MM) is a malignant clonal tumor of plasma cells that is characterized by the malignant proliferation of clonal plasma cells in the bone marrow, the infiltration of bone marrow and/or extramedullary tissues, and the secretion of monoclonal immunoglobulin (M protein), eventually leading to target organ damage. The clinical manifestations of MM include bone pain, anemia, renal dysfunction, hypercalcemia, and other symptoms (1). MM accounts for about 1% of all malignant tumors and up to 10% of hematological tumors. Its incidence is second only to lymphoma, and it is the second most common hematological malignant tumor. It is estimated that there were 16,500 new cases of MM and 10,300 deaths in China in 2016, and the age-standardized morbidity and mortality rates per 100,000 were 1.03 and 0.67, respectively (2). Globally, there are approximately 588,161 new cases of MM each year, which places a heavy burden on global healthcare and health resources (3).

The clinical manifestations and biological characteristics of MM are highly heterogeneous, and the survival time of patients can range from several months to 10 years or more. With the development of medical technology, MM treatment has achieved good therapeutic effects. However, since MM is still an incurable disease, the risk of repeated recurrence is inevitable. Thus, identifying effective prognostic indicators for accurate risk stratification of patients, formulating individualized chemotherapy regimens according to prognosis, and monitoring the treatment effect in real-time are crucial for the early detection of recurrence, improvement of the curative effect, and enhancement of the survival prognosis.

Circulating free DNA (cfDNA) refers to the free deoxyribonucleic acid in the circulating blood. The level of cfDNA in healthy people is low, while in cancer patients, owing to the high metabolism of the tumor, more cfDNA is shed into the blood, and the level of cfDNA is generally higher than that of healthy people. The released DNA fragments are mostly characterized by a large degree of variability, mainly in the increase of long fragments (4). Existing studies have shown that the relevant indicators of cfDNA in patients with malignant tumors are positively correlated with the tumor burden; that is, the larger the solid tumor volume, the higher the measured cfDNA concentration and the greater the variability. cfDNA may be an effective indicator for the prognostic evaluation of cancer patients (5). At present, in malignant hematological diseases, research on the application value of cfDNA mostly focuses on lymphoma (6,7), and there are few reports on MM. The release of cfDNA in healthy humans is mainly based on apoptosis. Alu sequence is a short repeat sequence of about 300 bp widely distributed in human genome. Two sets of ALU primers were designed in this study. ALU115 results represent total plasma free DNA. ALU247 only represents the free DNA release of non-apoptotic cells. The integrity of gDNA can be evaluated by the ratio of ALU115 to ALU247 results. Integrity detection is mainly based on the fact that the DNA fragments released during the process of tumor cell necrosis are often long (200–400 bp), that is, the DNA integrity is better. Its DNA fragments are relatively small and have poor integrity. In many studies, the ratio of the concentration of long cfDNA fragments to short cfDNA fragments (Alu247/Alu115) was used as the integrity index to indicate the integrity of circulating cfDNA fragments. Therefore, this study selected patients with primary MM, while healthy people were used as the control group to compare the level and integrity of cfDNA between these two groups. We present the following article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2416/rc).

Highlight box

Key findings

- cfDNA levels were significantly elevated in MM patients, and the ALU247 fragment concentration was remarkably correlated with multiple clinical features and had important clinical value for efficacy monitoring and prognostic assessment.

What is known and what is new?

- cfDNA in patients with malignant tumors are positively correlated with the tumor burden; that is, the larger the solid tumor volume, the higher the measured cfDNA concentration and the greater the variability. cfDNA may be an effective indicator for the prognostic evaluation of cancer patients.
- Two sets of ALU primers were designed in this study. ALU115 results represent total plasma free DNA. ALU247 only represents the free DNA release of non-apoptotic cells.

What is the implication, and what should change now?

- The monitoring of cfDNA levels has important clinical value for the prognosis of MM. It may be a potential prognostic biomarker for MM.

Methods

Clinical data

Fifty-six patients with newly diagnosed MM who were treated in the Tumor Hospital Affiliated to Nantong

Table 1 Baseline data of MM patients

Clinical characteristics	n	%
Average age (years)		
<65	27	48.21
≥65	29	51.79
Gender		
Male	34	60.71
Female	22	39.29
D-S stage		
IIA-IB	25	44.64
IIIA-IIB	31	55.36
ISS stage		
I-II	27	48.21
III	29	51.79
R-ISS stage		
I-II	32	57.14
III	24	42.86
Immunoglobulin (Ig)		
Light chain	7	12.50
IgA	12	21.43
IgG	37	66.07

MM, multiple myeloma; D-S, Durie-Salmon; ISS, International Staging System; R-ISS, Revised-International Staging System; IgA, immunoglobulin A; IgG, immunoglobulin G.

Table 2 Preparation of PCR reaction system

Reagent	Volume (μL)
Hieff® qPCR SYBR® Green Master Mix	12.5
Forward primer	1
Reverse primer	1
Sample cfDNA	1
RNase free dH_2O	9.5
Total volume	25

PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; cfDNA, circulating free DNA.

University from June 2019 to June 2022 were included as the research subjects. Another 60 healthy volunteers were selected as the healthy control group. Patients and healthy volunteers with the following conditions were excluded: pregnancy, diabetes, cardiovascular and cerebrovascular diseases, liver and kidney insufficiency, and autoimmune diseases. The baseline data of MM patients is shown in *Table 1*. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Tumor Hospital Affiliated to Nantong University (No. 2020-060) and informed consent was taken from all the participants.

Determination of cfDNA level and integrity

cfDNA extraction

10 mL of peripheral blood was collected from the MM patients before treatment and after three courses of chemotherapy, and from the healthy volunteers, and injected into a special anticoagulant tube for cfDNA. The collected peripheral blood was stored at 4 ℃, and the experiment was carried out within 8 hours. During the experiment, the peripheral blood was centrifuged at 4,000 r/min for 10 min. 1 mL of supernatant was taken and centrifuged for 10 min at 15,000 r. Next, 200 μL of plasma was taken, and cfDNA was then extracted according to the instructions of the plasma cfDNA extraction kit [Thermo Fisher Scientific (China) Co., Ltd.] and stored at −80 ℃ after extraction.

Primer design and real-time Quantitative Polymerase Chain Reaction Detecting System (QPCR) reaction system configuration

QPCR was applied for the quantitative detection of circulating cell-free DNA. The extracted plasma circulating cell-free DNA was used as a template and ALU115 and ALU247 as the target genes. Preparation of PCR reaction system: the reaction mixture was prepared on ice, and the components are shown in *Table 2*. The amplification conditions were as follows: pre-denaturation at 95 ℃ for 5 min, 95 ℃ for 10 s, and 60 ℃ for 30 s, and the melting curve analysis was performed after 28 cycles. Each

Table 3 Primer sequences

Primer	Sequence
Alu115-F	CCTGAGGTCAGGAGTTCGAG
Alu115-R	CCCGAGTAGCTGGGATTACA
Alu247-F	GTGGCTCACGCCTGTAATC
Alu247-R	CAGGCTGGAGTGCAGTGG

Table 4 Comparison of the cfDNA content and integrity between MM patients and healthy controls ($\bar{x}$±SD)

Group	ALU247 (ng/mL)	ALU115 (ng/mL)	ALU247/ALU115
MM group	121.72±25.83	213.71±54.69	0.61±0.22
Health control group	21.31±5.50	52.37±9.41	0.42±0.13
t	29.41	22.50	5.782
P	<0.0001	<0.0001	<0.0001

cfDNA, circulating free DNA; MM, multiple myeloma.

sample was tested twice. *Table 3* shows the upstream and downstream sequences and fragment lengths of the two pairs of primers (ALU115 and ALU247), designed according to the ALU repeat sequence of the human housekeeping gene.

Drawing of the standard curve and calculation of the cfDNA level

The known concentration of human genomic DNA (100 mL) was used as the standard, which was diluted in five gradients to establish a standard curve with concentrations of 10, 2, 0.4, 0.08, and 0.016 ng/μL. The concentration of cfDNA in the samples was calculated based on the amplification curves of DNA standards ALU247 and ALU115. The integrity of cfDNA was calculated as the ratio of ALU247 content to ALU115 content.

Therapeutic evaluation

The evaluation criteria of therapeutic effect in this study refer to the study of Zeng *et al.* (8). Efficacy was evaluated as complete response (CR), strict complete remission (sCR), very good partial response (VGPR), partial response (PR) and stable disease (SD).

Statistical analysis

Statistical analysis of all data was performed using SPSS 22.0 software. The homogeneity of variances was tested by the Levene test and the normality test was performed according to the Shapiro-Wilk method. We verified that all measurement data in this study conformed to a normal distribution. Therefore, the *t*-test was used to compare the measurement data between groups in this study. The C2 test was applied to compare the count data between the groups. A survival curve was drawn using the Kaplan-Meier (K-M) method. P<0.05 was considered to be statistically significant.

Results

cfDNA content and integrity between the MM patients and healthy controls

The differences in the concentration of ALU247 and ALU115 gene fragments and the integrity of cfDNA between the MM and healthy control groups were statistically significant (P<0.05). The concentrations of ALU247 and ALU115 fragments in the MM group were markedly higher than those in the healthy control group (P<0.05). Likewise, the cfDNA integrity in the MM group was significantly higher than that in the healthy control group (P<0.05) (see *Table 4* and *Figure 1*).

cfDNA levels and clinical features

The ALU247 fragment concentration was significantly correlated with the Durie-Salmon (D-S), International Staging System (ISS), and Revised-International Staging System (R-ISS) stages (P<0.05), but not with age, gender, and immunoglobulin type (P>0.05). There was no notable correlation between the ALU115 fragment concentration and D-S stage, ISS stage, R-ISS stage, age, gender, and immunoglobulin type (P>0.05) (see *Tables 5*,*6*).

cfDNA integrity and clinical features

Statistical analysis showed that cfDNA integrity was significantly correlated with bone marrow plasma cell ratio and creatinine level (P<0.05), but not with age, gender, white blood cell (WBC), peripheral plasma cell ratio, D-S stage, and ISS stage (P>0.05) (see *Table 7*).

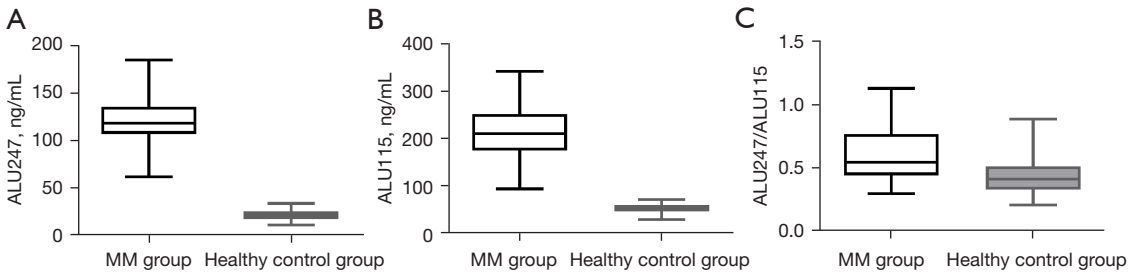

Figure 1 cfDNA content and integrity between MM patients and healthy controls. (A) The concentration of ALU247 in the MM and healthy control groups; (B) the concentration of ALU115 in the MM and healthy control groups; (C) the cfDNA integrity in the MM and healthy control groups. cfDNA, circulating free DNA; MM, multiple myeloma.

Table 5 Correlation between the ALU247 fragment concentration and various clinical characteristics

Clinical characteristics	ALU247 (ng/mL) (mean ± SEM)	T/F	P
Age (years)		1.417	0.1621
<65	116.69±26.92		
≥65	126.40±24.31		
Gender		0.7790	0.4394
Male	114.75±21.35		
Female	125.08±26.19		
D-S stage		2.364	0.0217
IIA-IIB	112.99±26.78		
IIIA-IIIB	128.76±23.13		
ISS stage		2.579	0.0121
I-II	112.88±25.76		
III	129.96±23.43		
R-ISS stage		2.428	0.0185
I-II	114.41±25.73		
III	130.51±22.87		
Immunoglobulin		0.6534	0.6534
Light chain	128.72±19.75		
IgA	119.47±23.54		
IgG	118.53±17.56		

SEM, standard error of the mean; D-S, Durie-Salmon; ISS, International Staging System; R-ISS, Revised-International Staging System; IgA, immunoglobulin A; IgG, immunoglobulin G.

Correlation between cfDNA level, integrity, and efficacy

After receiving three courses of induction chemotherapy, there were seven cases of sCR, nine cases of CR, 22 cases of VGPR, 11 cases of PR, and seven cases of SD, and no patients had disease progression. Changes in the content and integrity of cfDNA in MM patients after chemotherapy were detected, and patients in the MM group were further stratified according to the efficacy evaluation. Patients with curative effects of CR, sCR, and VGPR were classified into

Table 6 Correlation between the ALU115 fragment concentration and various clinical features

Clinical characteristics	ALU247 (ng/mL) (mean ± SEM)	*t*/F	P
Age (years)		1.585	0.1187
<65	225.56±50.91		
≥65	202.68±56.64		
Gender		0.9135	0.3651
Male	222.69±61.65		
Female	205.40±44.77		
D-S stage		0.4942	0.6232
IIA-IIB	209.66±56.79		
IIIA-IIIB	216.97±53.65		
ISS stage		0.2582	0.7972
I-II	211.74±55.34		
III	215.54±54.99		
R-ISS stage		1.916	0.0607
I-II	202.74±53.19		
III	232.26±48.53		
Immunoglobulin		0.9041	0.4110
Light chain	195.36±26.27		
IgA	223.03±66.78		
IgG	222.68±56.47		

SEM, standard error of the mean; D-S, Durie-Salmon; ISS, International Staging System; R-ISS, Revised-International Staging System; IgA, immunoglobulin A; IgG, immunoglobulin G.

the ≥ VGPR group (n=38), while those with curative effects of PR and SD were allocated into the < VGPR group (n=18). The results showed that after chemotherapy, the levels of ALU247, ALU115, and cfDNA integrity in the two groups were lower than those before chemotherapy (P<0.05). Furthermore, after chemotherapy, the levels of ALU247, ALU115, and cfDNA integrity in the ≥ VGPR group were markedly lower than those in the < VGPR group (P<0.05). See *Table 8*.

cfDNA levels, integrity, and progression-free survival (PFS)

A survival curve was drawn using the K-M method. The results showed that the PFS of MM patients with low ALU247 expression was significantly longer than that of MM patients with high ALU247 expression (33.59±1.15 *vs.* 27.31±2.16, P<0.05). Also, the PFS of MM patients with low ALU115 expression was slightly longer than that of MM patients with high ALU115 expression, but the difference was not statistically significant (32.44±1.67 *vs.* 27.95±1.65, P>0.05). Moreover, the PFS of MM patients with different cfDNA integrity was similar (30.45±1.46 *vs.* 31.03±2.26, P>0.05). See *Table 9* and *Figure 2*.

Discussion

cfDNA is a free double-stranded deoxyribonucleic acid that exists in various body fluids, including human peripheral blood, urine, cerebrospinal fluid, pleural effusion, and ascites (4). Its concentration in the peripheral blood of healthy people is very low, about 10–100 ng/mL, but is higher in patients with malignant tumors. In patients with advanced metastatic cancer, the cfDNA concentration can be as high as 5,000 ng/mL (9). In this study, the average concentrations of ALU247 and ALU115 fragments in MM

Table 7 Correlations between cfDNA integrity and various clinical features

Clinical characteristics	ALU247/ALU115 (mean ± SEM)	*t*	P
Age (years)		1.287	0.2035
<65	0.54±0.17		
≥65	0.61±0.23		
Gender		0.6201	0.5378
Male	0.56±0.23		
Female	0.63±0.17		
D-S stage		0.9021	0.3710
IIA-IIB	0.58±0.22		
IIIA-IIIB	0.63±0.21		
ISS stage		0.1249	0.2172
I-II	0.57±0.21		
III	0.64±0.22		
R-ISS stage		0.3623	0.7179
I-II	0.59±0.22		
III	0.57±0.18		
Immunoglobulin		0.7312	0.3149
Light chain	0.67±0.16		
IgA	0.59±0.24		
IgG	0.57±0.19		

SEM, standard error of the mean; D-S, Durie-Salmon; ISS, International Staging System; R-ISS, Revised-International Staging System; IgA, immunoglobulin A; IgG, immunoglobulin G.

Table 8 ALU247, ALU115, and ALU247/ALU115 before and after treatment

Subject	≥ VGPR group (mean ± SEM)	< VGPR group (mean ± SEM)	*t*	P
ALU247 (ng/mL)				
Before chemotherapy	107.01±17.94	117.12±23.71	1.906	0.0615
After chemotherapy	29.90±3.61*	56.01±13.73*	11.45	<0.0001
ALU115 (ng/mL)				
Before chemotherapy	200.17±46.67	215.98±56.55	1.196	0.2363
After chemotherapy	98.68±7.84*	120.15±10.61*	8.307	<0.0001
ALU247/ALU115				
Before chemotherapy	0.58±0.09	0.59±0.23	0.2413	0.8102
After chemotherapy	0.31±0.05*	0.47±0.11*	7.960	<0.0001

*, P<0.05, compared with before treatment. VGRR, very good partial response; SEM, standard error of the mean.

Table 9 cfDNA levels, integrity, and PFS

Subject	PFS (mean ± SEM)	95% CI		χ^2	P
		Upper	Lower		
ALU247				5.806	0.0159
Low expression	33.59±1.15	35.84	33.59		
High expression	27.31±2.16	31.55	27.31		
ALU115				2.848	0.091
Low expression	32.44±1.67	29.16	35.72		
High expression	27.95±1.65	24.72	31.18		
ALU247/ALU115				0.2342	0.6283
Low	30.45±1.46	27.59	33.32		
High	31.03±2.26	26.59	35.46		

cfDNA, circulating free DNA; PFS, progression-free survival; SEM, standard error of the mean.

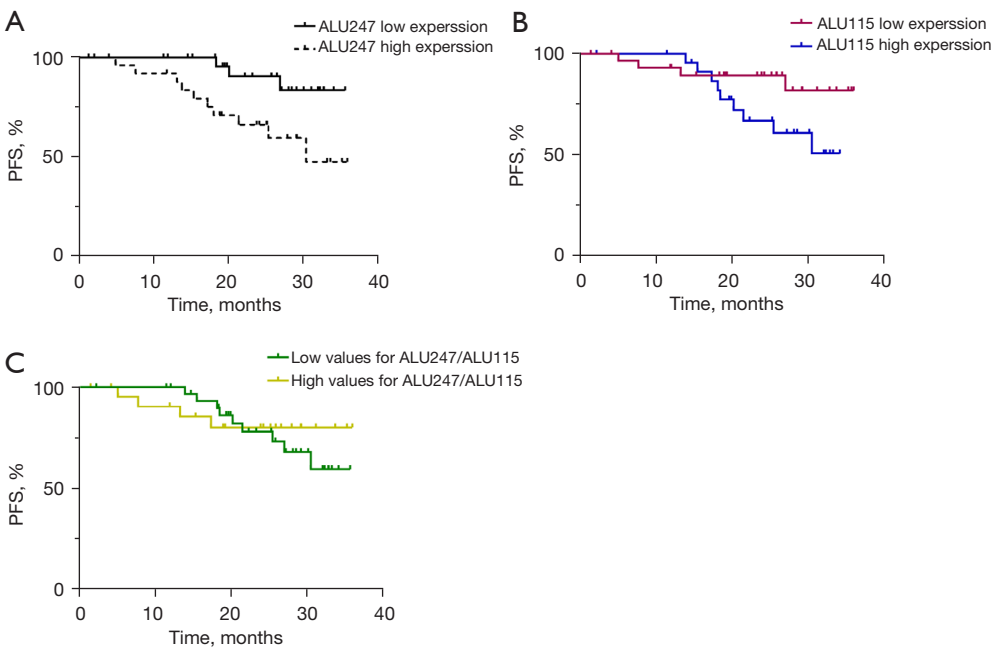

Figure 2 PFS of MM patients with different cfDNA levels and integrity. (A) PFS of MM patients with different ALU247 expression; (B) PFS of MM patients with different ALU115 expression; (C) PFS of MM patients with different cfDNA integrity. PFS, progression-free survival; MM, multiple myeloma; cfDNA, circulating free DNA.

patients were 121.72±25.83 and 213.71±54.69 ng/mL, respectively, and the cfDNA integrity was 0.61±0.22. The level of cfDNA in different studies varies, which may be explained by the fact that there are no unified standards for specimen type, specimen collection process, detection method, and accuracy of test equipment. Generally, when quantitatively detecting cfDNA, each laboratory will choose different reference genes, such as β-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and long interspersed element-1 (LINE-1) (10,11), etc., whose common feature is that they can exist and express stably. However, due to their different copy numbers, it is possible for the test results to

vary. Therefore, in recent years, more laboratories have begun to focus on the use of non-coding DNA, such as ALU tandem repeats.

cfDNA has many advantages as a biomarker for multiple myeloma. For example, bone marrow aspiration and pathology examination are characterized by high trauma, poor repeatability and difficult acceptance by patients. However, cfDNA from tumor cells exists in the patient's blood, synovial fluid and other body fluids, and the samples are easy to obtain without surgery. In addition, cfDNA can overcome the heterogeneity of tumor tissue sample sampling.

ALU tandem repeats are usually 300 nucleotides in length and occupy more than 10% of the human genome. They are the most abundant sequences in the human genome, and because their methylation levels are lower than those of coding genes, they are not easily affected by other factors, which makes them easier to be detected (12,13). The integrity of cfDNA can be assessed by the ratio of long (247 bp, ALU247) and short (115 bp, ALU115) amplicons. This study also compared the differences in ALU247 and LU115 fragment concentrations and cfDNA integrity between the MM patients and healthy volunteers. The results showed that the ALU247 and ALU115 fragment concentrations and cfDNA integrity in the MM group were significantly higher than those in the healthy control group ($P>0.05$). Presumably, the reason for this is that under normal physiological conditions, dead cells are cleared by phagocytosis mediated by macrophages or other clearing cells, and the released DNA fragments are broken down by the liver, spleen, and kidney within about 10–15 minutes, leading to low physiological levels of cfDNA. However, under pathological conditions, the plasma levels of cfDNA are elevated, and actively proliferating tumors may release more cfDNA due to massive cell death coupled with the inability of the liver to adequately break down DNA fragments.

After the initial diagnosis of MM, clinicians need to reasonably evaluate the disease and tumor burden according to D-S, ISS, R-ISS staging to formulate an individualized plan. In recent years, many researchers have found that cfDNA levels are significantly correlated with some clinical features. For example, Deshpande *et al.* (14) reported cfDNA levels were correlated with clinical markers such as lactate dehydrogenase, β2-microglobulin, and ISS stage in MM patients. The results of the present study showed that ALU247 fragment concentration was markedly correlated with the D-S, ISS, and R-ISS stages ($P<0.05$), but no notable correlation between ALU115 and cfDNA integrity and any clinical features was observed, further suggesting that plasma cfDNA levels, especially the ALU247 fragment, may reflect the tumor burden in MM patients.

During the treatment of MM, some biomarkers are often needed to monitor the curative effect, so that the treatment plan can be continuously adjusted accordingly. In recent years, several scholars have reported the feasibility of cfDNA for monitoring the efficacy of MM. Deshpande *et al.* (14) found that the concentration of cfDNA increased significantly 3–5 days after chemotherapy, and then fell back to the baseline level. However, further research was not conducted to explore whether the cfDNA level differed among patients with different curative effects. To this end, the present study compared cfDNA levels in MM patients with different therapeutic effects. It was found that the levels of ALU247, ALU115, and cfDNA integrity in the two groups after chemotherapy were lower than those before chemotherapy ($P<0.05$). Furthermore, after chemotherapy, the levels of ALU247, ALU115, and cfDNA integrity in the ≥ VGPR group were significantly lower than those in the < VGPR group ($P<0.05$). From this, we infer that with the progress of regular chemotherapy, the tumor burden of MM patients gradually decreased, and the plasma cfDNA also showed a downward trend. Due to individual differences, the efficacy of each patient is different, and MM patients in the ≥ VGPR group had deeper remission than those in the < VGPR group, and thus, their plasma cfDNA levels after chemotherapy were lower. Previous study has shown that this phenomenon also exists in lymphoma, suggesting that the treatment effect can be further evaluated by the degree of decrease in plasma cfDNA content before and after chemotherapy (15).

CfDNA levels are also closely related to prognosis and survival. Mithraprabhu *et al.* (16) found that patients with higher cfDNA levels had significantly poorer overall survival, and that reductions in cfDNA levels were associated with improvement after several days of chemotherapy cycles. Waldschmidt *et al.* (17) prospectively enrolled 86 blood samples from 45 MM patients treated with drugs such as pomalidomide, bortezomib, and dexamethasone in a phase II clinical trial. After two cycles of treatment, higher levels of cfDNA were found to be associated with worse PFS (1.6 *vs.* 17.6 months). The results of the presents study showed that the PFS of MM patients with low ALU247 expression was significantly longer than that of MM patients with high ALU247 expression (33.59 ± 1.15 *vs.* 27.31 ± 2.16, $P<0.05$), which showed that the

monitoring of cfDNA levels has important clinical value for the prognosis of MM.

Conclusions

In conclusion, cfDNA levels were markedly elevated in MM patients, and the ALU247 fragment concentration was also significantly correlated with multiple clinical features and had important clinical value for efficacy monitoring and prognostic evaluation.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2416/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2416/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2416/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Tumor Hospital Affiliated to Nantong University (No. 2020-060) and informed consent was taken from all the participants.

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