

Early adjunctive diagnostic value of contrast-enhanced ultrasound-related quantitative parameter and its relationship with micro-perfusion of nontraumatic necrosis of femoral head

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Abstract. – OBJECTIVE: The aim of this study is to explore the early diagnostic value of contrast-enhanced ultrasound (CEUS)-related quantitative parameter and its relationship with the micro-perfusion of nontraumatic necrosis of the femoral head.

PATIENTS AND METHODS: According to the random and double-blind method, the patients with non-traumatic femoral head necrosis diagnosed and treated in our hospital from July 2019 to January 2022 were selected as the subjects (the research group). According to the staging of the International Society of Bone Circulation for Femoral Head Necrosis, 89 patients with stage II and III A were included (39 patients with stage II and 50 patients with stage III A). 25 patients who conducted physical examination in our hospital during the same time were taken as the control group. Quantitative parameters of CEUS were analyzed. The content of serum vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2) were evaluated. The relationship among the quantitative parameters of CEUS, the expression of VEGF and BMP-2 in serum and the patient's condition, and the value for assisting the early diagnosis of nontraumatic femoral head necrosis were analyzed.

RESULTS: The body mass, body mass index (BMI), blood lipid, and cholesterol levels were much higher in the research group than in the control group ($p < 0.05$). The research group had a markedly higher slope of ascending branch (AS), strength enhancement index (EI), and VEGF and obviously lower decay slope (DS), mean tran-

sit time (MTT), and time to peak (TTP) than the control group ($p < 0.05$). In the research group, compared to stage II, the levels of AS, EI, and VEGF in stage III A patients were memorably higher, and the levels of DS, MTT, TTP and BMP-2 were dramatically lower ($p < 0.05$). Pearson's correlation test showed that AS, EI, and VEGF were positively correlated with the patients' condition, while DS, MTT, TTP and BMP-2 were negatively correlated with the patients' condition ($p < 0.05$). The receiver operating characteristic (ROC) curve analysis showed that the diagnostic area under the curve (AUC) of quantitative parameters of CEUS was 0.961, with sensitivity and specificity of 88.0% and 97.4%, respectively. The AUC of the combined detection of VEGF and BMP-2 was 0.945 with sensitivity and specificity of 82.3% and 87.5%, respectively, and the combined detection had a high diagnostic value ($p < 0.05$).

CONCLUSIONS: The quantitative parameters of CEUS were of great value in the early diagnosis of nontraumatic necrosis of the femoral head with microvascular perfusion and the patients' condition, and provided a reference for the clinical treatment of non-traumatic necrosis of the femoral head. These parameters were expected to be useful indicators for judging the efficacy before and after treatment.

Key Words:

Quantitative parameters of contrast-enhanced ultrasound, Nontraumatic femoral head necrosis, Micro-hematopoietic perfusion, Early aid, Diagnostic value.

Introduction

Femoral head necrosis is a disease caused by the interruption of blood flow of the femoral head, which leads to the death and subsequent repair of bone cells and bone marrow components. Structural changes in the femoral head and femoral collapse are further induced, which leads to joint pain and dysfunction in patients¹. According to the causes, necrosis of the femoral head can be divided into traumatic and non-traumatic necrosis. Femoral head necrosis mainly affects people aged 30 to 50 years and is difficult to cure. There is still no effective treatment method in clinical practice, and most patients must receive total hip replacement^{2,3}. However, the prognosis of patients is still poor even after treatment, which seriously affects the daily life of patients. Therefore, early diagnosis, timely treatment, and maximum preservation of the patients' normal hip function play an important role.

The early onset of nontraumatic necrosis of the femoral head is relatively hidden, and nuclear magnetic resonance technology is currently the main standard for clinical diagnosis⁴. In recent years, with the continuous development of imaging technology, ultrasound technology has been gradually improved. CEUS is a method to obtain blood perfusion information using contrast agents as vascular tracers. Some studies⁵ have found that the blood flow information could be evaluated by CEUS in real-time, which may be an effective alternative method to evaluate the femoral head perfusion during operation. However, the value of quantitative parameters of CEUS in diagnosing non-traumatic necrosis of the femoral head is still unclear.

In this study, the patients with nontraumatic necrosis of the femoral head diagnosed and treated in our hospital from 2019 to 2022 were taken as the subject of this research and were divided into groups according to the stages of the International Society of Bone Circulation. By analyzing the relationship between quantitative parameters of CEUS and micro blood flow perfusion of non-traumatic necrosis of the femoral head, and discussing its early auxiliary diagnostic value, we could provide some theoretical basis for an early clinical treatment.

Patients and Methods

General Materials

This study was approved by the Ethics Committee of Southwest Medical University (number of Ethical approval: BY20-230308). According to the

random and double-blind method, the 89 patients with non-traumatic femoral head necrosis diagnosed and treated in our hospital from July 2019 to January 2022 were selected as the subjects (the research group). Inclusion criteria: (1) All subjects met the diagnosis and treatment criteria for non-traumatic necrosis of the femoral head⁶ and were confirmed by imaging detection. (2) Patients without relevant treatment and history of hip joint trauma before participating in the study. (3) All patients and their families signed the informed consent form. (4) According to the staging criteria of the International Society of Bone Circulation for Femoral Head Necrosis, the patients were classified as stage II or stage IIIA. Exclusion criteria: (1) patients with traumatic necrosis of the femoral head. (2) Patients with severe liver and kidney dysfunction. (3) Patients who could not cooperate effectively with the study. (4) Patients with infectious diseases or hereditary-related diseases. According to the staging of the International Society of Bone Circulation for Femoral Head Necrosis, 89 patients with stage II and III A were selected, including 39 patients with stage II and 50 patients with stage III A. The patients in stage II comprised 27 males and 12 females, with an average age of 51.85 ± 8.65 years. The patients in stage III A were composed of 27 males and 12 females, with an average age of 51.85 ± 8.65 years. 25 patients (14 males and 11 females) who conducted physical examinations in our hospital during the same time were taken as the control group, with an average age of 52.15 ± 9.35 . There was no significant difference in age between groups ($p > 0.05$). The research process is shown in Figure 1.

CEUS

CEUS was conducted using the Color Doppler ultrasound diagnostic instrument (GE Logiq E9, Boston, MA, USA), with the C1-6 convex array probe and a frequency of 2.5-6 MHz. Ultrasound contrast agent Sono Vue (Bracco, Italy) was used here. The patient was placed in the supine position to fully expose the focus area. The probe was placed outside the femoral head and remained stable. When the two-dimensional ultrasound images of the femoral neck, femoral head articular surface, and acetabular lip were clearly displayed, the acetabular lip was placed at the outermost side of the femoral head on the image. The anterolateral side of the femoral head was placed under the probe (the posterolateral wall of the femoral head was the most commonly affected site of necrosis and collapse). The position of the fixed probe remained unchanged, and the ultrasound contrast mode was

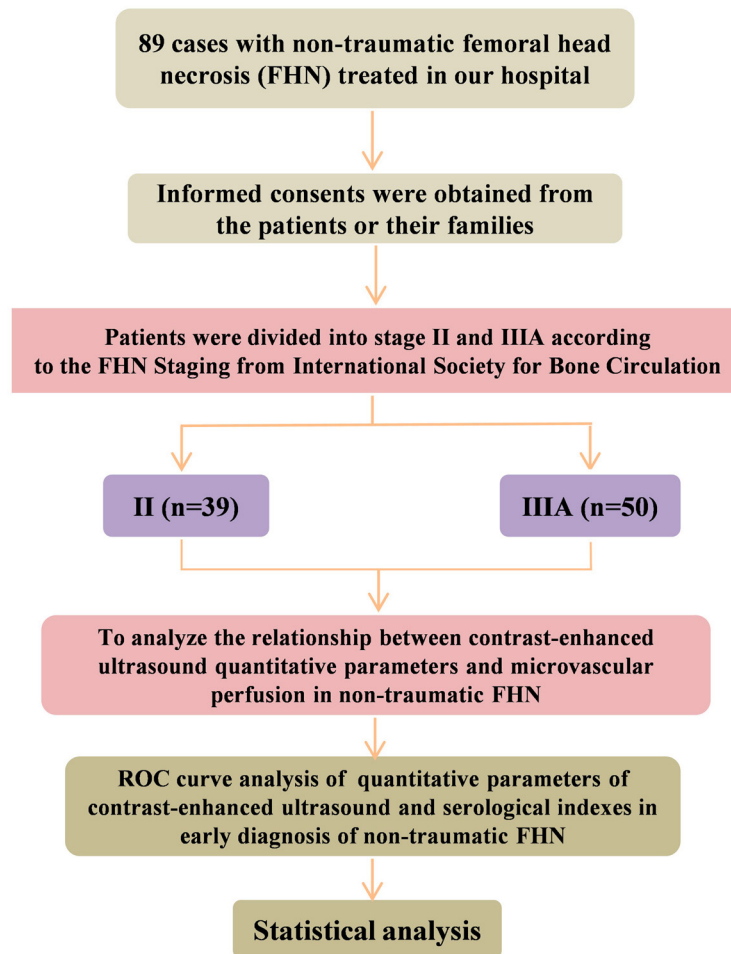


Figure 1. The research process.

entered. A total of 2.4 mL contrast agent suspension was injected through the elbow vein, and the dynamic image acquisition program was started simultaneously. The perfusion and regression process of the contrast agent was observed, dynamic images were collected, and the images were analyzed according to the time-intensity curve. The area of interest in the bone under the surface of the femoral head was delineated, and the quantitative parameters of CEUS were analyzed. If it was necessary to check the other side of the femoral head, it was performed 15 minutes after the end of angiography on one side of the femoral head and after the contrast agent had been completely metabolized. The analysis should be conducted by at least 3 experienced physicians, and the test should be repeated at least 3 times, and the average value should be taken. The injection of all contrast agents should be performed by the same nurse. Two-dimensional ultrasound examination and CEUS examination of

all patients were performed by the same ultrasound examination physician with more than 5 years of work experience.

Outcome Measures

- (1) Quantitative parameters of CEUS were analyzed according to the time-intensity curve, including the slope of ascending branch (AS), decay slope (DS), strength enhancement index (EI), mean transit time (MTT), and time to peak (TTP).
- (2) Detection of the expression of vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2): Enzyme-linked immunosorbent assay was used to detect the changes in the content of serum VEGF and BMP-2.
- (3) Correlation analysis: Pearson's correlation test was adopted to analyze the relationship between quantitative parameters of CEUS, serum VEGF, BMP-2 expression, and the patients' condition.

(4) Diagnostic value: receiver operating characteristic (ROC) curve was established to analyze the value of quantitative parameters of CEUS and VEGF, BMP-2 in the early auxiliary diagnosis of non-invasive femoral head necrosis.

curve. $p < 0.05$ indicated that the difference was statistically significant.

Statistical Analysis

SPSS v. 23.0 (IBM Corp., Armonk, NY, USA) data statistics software was used for data processing. Quantitative parameters of CEUS serum VEGF, BMP-2 levels, and other measurement data were tested by normal distribution, and all were in line with normal distribution. The measurement data were expressed in the form of ($\bar{x} \pm s$). Single-factor analysis of variance of comparisons was used between multiple groups. Those with statistical significance between the two groups were further compared by the least significant difference (LSD)-t-test. The enumeration data, such as the gender, were expressed in [cases (%)] and were compared using χ^2 test. Correlation analysis was conducted by Pearson’s correlation test. The diagnostic value was analyzed through the ROC

Results

Comparison of General Data

There was no significant difference in age, gender, and height between the two groups ($p > 0.05$). The patients in the research group had much higher body mass, body mass index (BMI), blood lipid, and cholesterol levels than patients in the control group ($p < 0.05$, Table I).

Comparison of Quantitative Parameters of CEUS

The research group had a markedly higher content of AS and EI and obviously lower content of DS, MTT and TTP than the control group ($p < 0.05$). In the research group, compared with stage II, the levels of AS and EI in stage III A patients were memorably higher, and the content of DS, MTT and TTP were dramatically lower ($p < 0.05$, Table II).

Table I. Comparison of general data ($\bar{x} \pm s$, %).

General data		The study group (n = 89)	The control group (n = 25)	χ^2/t	p
Gender	Male	58 (65.17)	14 (56.00)	0.705	0.401
	Female	31 (34.83)	11 (44.00)		
Age (year)		51.42 ± 8.79	52.15 ± 9.35	0.362	0.718
Nation		Han nationality	Han nationality		
Pathogeny	Hormone	30 (33.71)	/		
	Alcohol	38 (42.70)	/		
	Special	21 (23.60)	/		
Height (m)		1.72 ± 1.53	1.68 ± 0.39	0.129	0.898
Body mass (kg)		72.22 ± 2.69	63.58 ± 3.14	13.669	< .001
BMI (kg/m ²)		24.41 ± 3.08	22.53 ± 2.18	2.854	0.005
Blood lipid (mmol/L)		1.62 ± 0.72	1.03 ± 0.41	3.915	0.001
Cholesterol (mmol/L)		4.66 ± 0.83	3.91 ± 0.69	4.131	0.001

Body mass index (BMI).

Table II. Comparison of quantitative parameters of CEUS ($\bar{x} \pm s$).

Groups	Cases	AS (dB/s)	DS (dB/s)	EI (dB)	MTT (s)	TTP (s)
The research group	89					
Stage II	39	1.57 ± 0.53	-0.17 ± 0.08	13.19 ± 4.16	57.64 ± 6.49	8.34 ± 1.02
Stage III A	50	2.51 ± 1.03	-0.22 ± 0.09	19.57 ± 5.88	50.16 ± 5.52	6.74 ± 1.12
The control group	25	0.52 ± 0.37	-0.07 ± 0.04	7.04 ± 3.56	78.16 ± 8.03	14.27 ± 2.51
<i>F</i>		57.20	30.67	57.28	157.31	212.64
<i>p</i>		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Ascending branch (AS), strength enhancement index (EI), decay slope (DS), mean transit time (MTT), and time to peak (TTP).

Table III. Expression of serum VEGF and BMP-2 in patients ($\bar{x}\pm s$).

Groups	Cases	VEGF	BMP-2
The research group	89		
Stage II	39	175.47 ± 25.04	20.64 ± 6.59
Stage III A	50	224.54 ± 29.69	14.02 ± 4.26
The control group	25	116.14 ± 21.52	47.69 ± 8.05
<i>F</i>		142.16	263.46
<i>p</i>		< 0.001	< 0.001

Vascular endothelial growth factor (VEGF), bone morphogenetic protein-2 (BMP-2).

Expression of Serum VEGF and BMP-2 in Patients

The research group had a markedly higher content of VEGF and obviously lower content of BMP-2 than the control group ($p < 0.05$). In the research group, compared with stage II, the content of VEGF in stage III A patients were memorably higher, and the content of BMP-2 was dramatically lower ($p < 0.05$, Table III).

The Relationship Between Quantitative Parameters of CEUS, Serum VEGF, BMP-2 Content, and Patients' Condition

Pearson's correlation test showed that AS, EI, and VEGF were positively correlated with the patients' condition, while DS, MTT, TTP and BMP-2 were negatively correlated with the patients' condition ($p < 0.05$, Table IV and Figure 2).

Table IV. The relationship between quantitative parameters of CEUS, serum VEGF, BMP-2 content and patients' condition.

Index	International Society of Bone Circulation staging of femoral head necrosis	
	<i>r</i>	<i>p</i>
AS	0.521	< 0.001
DS	-0.292	0.006
EI	0.524	< 0.001
MTT	-0.540	< 0.001
TTP	-0.593	< 0.001
VEGF	0.662	< 0.001
BMP-2	-0.499	< 0.001

Ascending branch (AS), strength enhancement index (EI), decay slope (DS), mean transit time (MTT), and time to peak (TTP), vascular endothelial growth factor (VEGF), bone morphogenetic protein-2 (BMP-2).

The Value of Quantitative Parameters of CEUS and VEGF, BMP-2 in the Early Auxiliary Diagnosis of Non-Invasive Femoral Head Necrosis

The ROC curve analysis showed that the diagnostic AUC of quantitative parameters of CEUS was 0.961, with sensitivity and specificity of 88.0% and 97.4%, respectively. The AUC of the combined detection of VEGF and BMP-2 was 0.945 with sensitivity and specificity of 82.3% and 87.5%, respectively, and the combined detection had a high diagnostic value ($p < 0.05$, Table V and Figure 3).

Discussion

Femoral head necrosis is a disease induced by insufficient blood supply to meet the needs of bone metabolism, and it is also the main cause of disability⁷. With the change in people's lifestyles, the incidence of femoral head necrosis largely increased. According to relevant statistics⁸, it is estimated that about 10,000 new patients are infected with the disease every year in the United States. In the latest large-scale epidemiological survey⁹ in China, the number of patients with nontraumatic femoral head necrosis in the Chinese population totaled 8.12 million. The pathogenesis of nontraumatic necrosis of the femoral head is complex^{10,11}. Many risk factors are related to the development of the disease, such as trauma, long-term drinking, smoking, and the use of corticosteroids, and most cases are considered idiopathic. If not treated in time, it will gradually progress to femoral head collapse or even joint degeneration with the development of the disease, which will seriously affect the life of patients.

Ultrasound is an important detection method in imaging, which has the characteristics of simple operation and low price.

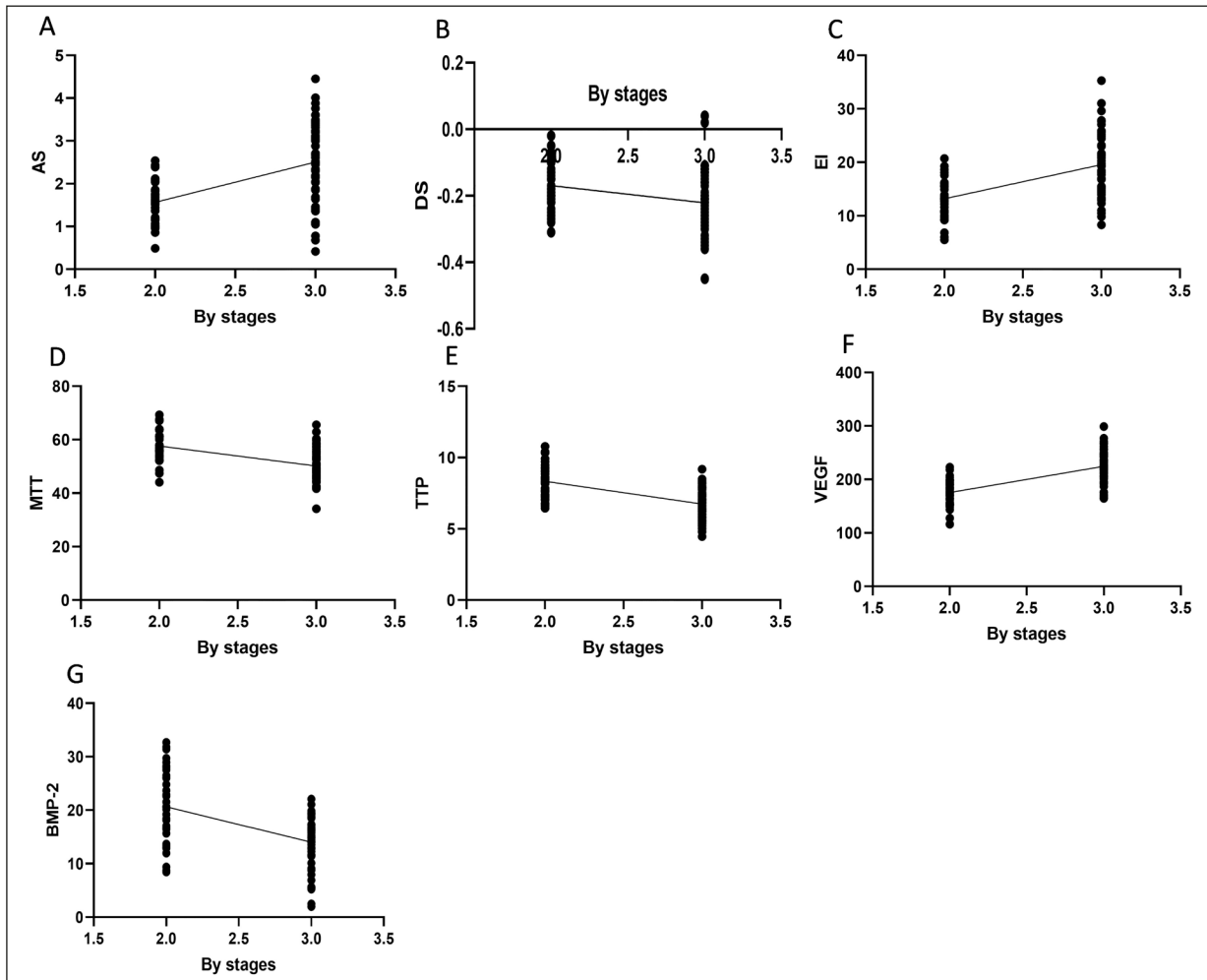


Figure 2. The relationship between quantitative parameters of CEUS, serum VEGF, BMP-2 content and patients' condition. **(A)**, Correlation between AS and disease stage; **(B)**, Correlation between DS and disease stage; **(C)**, Correlation between EI and disease stage; **(D)**, Correlation between MTT and disease stage; **(E)**, Correlation between TTP and disease stage; **(F)**, Correlation between VEGF and disease stage; **(G)**, Correlation between BMP-2 and disease stage.

Table V. The value of quantitative parameters of CEUS and VEGF, BMP-2 in the early auxiliary diagnosis of non-invasive femoral head necrosis.

Index	AUC	Sensitivity (%)	Specificity (%)	p	Cut-off value	95% CI	
						Lower limit	Upper limit
AS	0.805	68.0	92.3	< 0.001	2.16	0.711	0.899
DS	0.685	48.0	84.6	< 0.001	0.25	0.575	0.795
EI	0.809	66.0	84.6	< 0.001	16.52	0.721	0.898
MTT	0.815	65.0	92.3	< 0.001	51.68	0.725	0.905
TTP	0.847	94.0	59.0	< 0.001	8.23	0.769	0.925
VEGF	0.895	78.0	92.3	< 0.001	204.02	0.831	0.959
BMP-2	0.762	96.0	53.8	< 0.001	20.03	0.656	0.868
Combination of ultrasonic quantitative parameters	0.961	88.0	97.4	< 0.001	-	0.922	1.000
VEGF + BMP-2	0.945	82.3	87.5	< 0.001	-	0.904	0.986

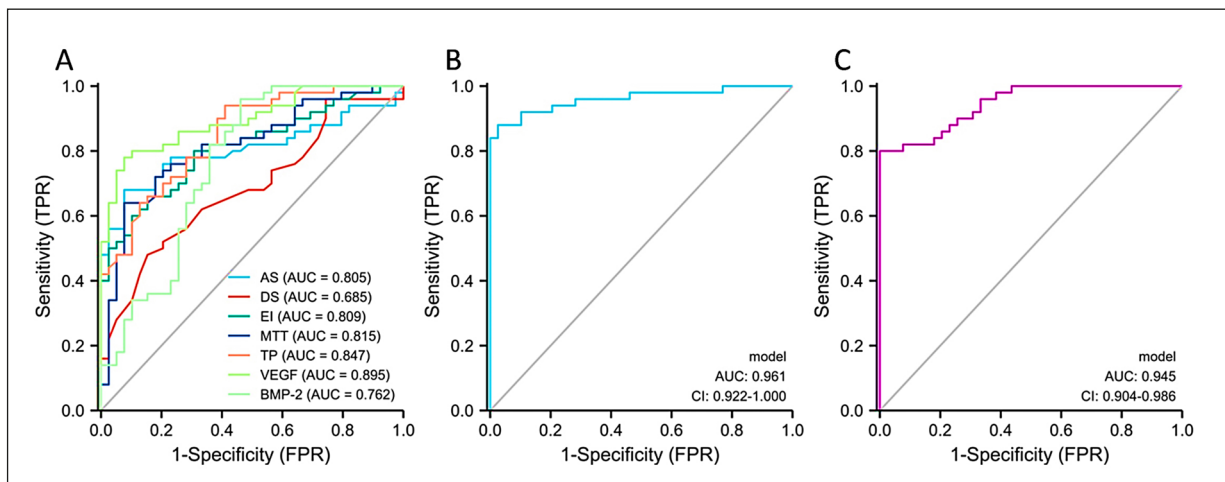


Figure 3. The value of quantitative parameters of CEUS and VEGF, BMP-2 in the early auxiliary diagnosis of non-invasive femoral head necrosis. **A**, Quantitative parameters of contrast-enhanced ultrasound and serological diagnosis; **(B)**, Combined diagnosis of quantitative parameters of CEUS; **(C)**, Combined diagnosis of serological indicators.

With the development of ultrasound technology, CEUS technology is gradually applied to clinical practice, which can directly reflect the micro blood perfusion of the body¹². Since the 20th century, CEUS technology had been applied to the musculoskeletal system, which could effectively exhibit the superficial tissue structure in the sports system. In an animal study¹³, by observing the changes in micro-vessel density and quantitative parameters of CEUS of rabbits in the femoral head necrosis model group and the control group, it was found that the micro-vessel density of rabbits in the model group was largely reduced, and there were significant changes in AS, DS, MTT, and TTP. In addition, the correlation analysis showed that DS was closely correlated with micro-vessel density. CEUS can show the microcirculation of rabbit femoral head necrosis, which may be a useful imaging method for early monitoring and prediction of femoral head necrosis. In a clinical study¹⁴, CEUS was used for detection before and after reset, and it was found that all CEUS could display the blood flow of the femoral epiphysis before and after reset. It is considered that CEUS is a feasible tool to evaluate the adequacy of blood flow after hip replacement surgery. International Society of Bone Circulation staging of femoral head necrosis is an important method to evaluate the condition of patients with non-traumatic femoral head necrosis. In this study, by detecting the changes of quantitative parameters of CEUS in patients at different stages, it was found that the levels of AS and EI in patients were largely increased, while the levels of DS, MTT and

TTP were strongly decreased with the development of the disease. It is suggested that CEUS can effectively evaluate the micro blood perfusion status of patients. The reason is that with the development of the disease, bone tissue is gradually necrotic and decomposed, and a large number of deactivated tissues are absorbed by granulation tissue. When the rate of bone tissue necrosis exceeds the repair rate, the femoral head collapses, and the femoral head blood supply is clearly damaged.

VEGF is an inducer of angiogenesis and lymphangiogenesis, and also a key factor in regulating the balance of the body, which plays an important role in the development and repair of bone. The blood vessels not only serve as the structural templates for bone formation but also provide the necessary cells, growth factors, and minerals for the synthesis, mineralization, and turnover of extracellular matrix in bone. It was found¹⁵ that VEGF could directly control the differentiation and function of osteoblasts and osteoclasts by regulating angiogenesis, contributing to the coupling of osteogenesis and angiogenesis. In animal studies¹⁶, it was found that the expression of VEGF in the femoral head of Wister rats exposed to alcohol was lower, and the number of osteocytes in the femoral head necrosis was larger than that of rats not exposed to alcohol. It is suggested that VEGF might be an important biomarker for diagnosing alcoholic necrosis of the femoral head. BMP-2 is a growth factor with unique bone regeneration characteristics, which belongs to the protein transforming growth factor- β Superfamily and is conducive to acceler-

ating normal bone healing. BMP-2 is considered a second-line treatment for bone nonunion defects due to its strong osteo-inductive properties. The enhanced inflammatory reaction is an important feature of femoral head necrosis. A study¹⁷ found that the content of BMP in patients with femoral head necrosis strongly increased. BMP could not effectively reduce the production of proinflammatory cytokines and inhibit the differentiation of osteoclasts. The reason is that BMP may inhibit inflammatory reactions and osteoclast formation by down-regulating the level of interleukin-34. In addition, BMP-2 induces osteogenesis and chondrogenesis of human mesenchymal stem cells¹⁸. In this study, we found that the level of VEGF strongly increased, and the level of BMP-2 sharply decreased with the development of the disease. In addition, the analysis demonstrated that the quantitative parameters of CEUS, VEGF, BMP-2 content were closely related to the patients' condition, and they had a high early auxiliary diagnosis value for non-invasive necrosis of the femoral head.

Conclusions

The quantitative parameters of CEUS were of great value in the early diagnosis of nontraumatic necrosis of the femoral head with microvascular perfusion and the patients' condition and provide a reference for the clinical treatment of non-traumatic necrosis of the femoral head. They were expected to be useful indicators for judging the efficacy before and after treatment. However, due to a large amount of contrast-enhanced ultrasound-related examination data and the subjective influence of the examiner, certain errors are generated. In addition, due to the limited research time in the present study, the mechanism of femoral head necrosis at different stages still needs further study.

Conflict of Interests

The authors declare that they have no competing interests.

Ethics Approval

This study was approved by the Ethics Committee of Southwest Medical University (number of Ethical approval: BY20-230308).

Informed Consent

The patients and their families signed the informed consent form.

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Authors' Contributions

R. Huo and L. Zheng conceived and designed the experiments, analyzed and interpreted the data. R. Huo and L. Zheng wrote the manuscript. S.-L. Li, S.-K. Wang, C. Ma, H.-Y. Shi, X.-P. Xie, and N.-W. Wang performed the experiments and contributed reagents, materials, analysis tools and data. X.-M. Zhang, B. Liu and L. Peng also performed some experiments. Q.-Z. He and F. Jiang reviewed and revised the article. All authors read and approved the final manuscript.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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