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ORIGINAL RESEARCH

Diagnostic Use of High-Frequency Ultrasound in Differentiating Benign and Malignant Pigmented Skin Lesions

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Objective: This study aims to assess the clinical utility of high-frequency ultrasound in the preoperative differentiation of benign and malignant pigmented skin lesions.

Methods: A cohort of 126 patients with pigmented skin lesions who underwent high-frequency ultrasound examination and subsequent surgical treatment at the First Affiliated Hospital of Henan University of Science and Technology between October 2022 and July 2024 were included in this prospective study. Pathological findings served as the reference standard, and the clinical and ultrasonographic features of benign and malignant pigmented skin lesions were compared. Key ultrasonographic indicators for preoperative differentiation were identified.

Results: Among the 126 pigmented skin lesions, 50 were malignant, and 76 were benign. High-frequency ultrasound correctly identified 41 of the 50 malignant lesions and 68 of the 76 benign lesions. The diagnostic accuracy, sensitivity, and specificity were 86.5%, 94.0%, and 93.4%, respectively. Strong punctate echogenic foci within the lesion (χ^2= 45.390, P < 0.001), the resistance index of the feeding artery (Z= −6.057, P=0.021), and rapid lesion progression (χ^2= 46.140, P < 0.001) were identified as effective indicators for distinguishing benign from malignant pigmented skin lesions.

Conclusion: The combination of high-frequency ultrasound and clinical data aids in the preoperative differential diagnosis of benign and malignant pigmented skin lesions. The type of disease progression, the resistance index of the responsible artery and the presence of intranodular hyperechoic foci are independent risk factors for evaluation.

Keywords: high frequency ultrasound, pigmented skin lesions, skin diseases, skin tumors, skin ultrasound

Introduction

Pigmented skin lesions encompass a broad range of conditions characterized by visible pigmentation changes, with diverse pathological subtypes, atypical clinical presentations, and visually similar appearances.[1] These factors contribute to a high rate of clinical misdiagnosis among lesions with comparable features. Misdiagnosis or missed diagnosis of malignant pigmented skin lesions can delay appropriate treatment, potentially leading to disease progression, cosmetic impairment, and even life-threatening consequences. Therefore, early and accurate differentiation between benign and malignant pigmented skin lesions is essential for guiding appropriate treatment strategies, improving patient quality of life, and ensuring optimal prognostic outcomes.

Skin biopsy remains the "gold standard" for preoperative diagnosis of pigmented skin lesions; however, as an invasive procedure, it frequently results in localized scarring, particularly in cosmetically sensitive areas such as the face.[2] Consequently, many patients are reluctant to undergo this procedure. Currently, dermoscopy is the primary imaging modality used in the evaluation of skin diseases.[3] However, due to its limited penetration depth, it is unable to provide clear visualization of deeper structures, highlighting the need for an effective non-invasive diagnostic method capable of distinguishing common benign and malignant pigmented skin lesions.

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Ultrasound, as a widely utilized non-invasive imaging tool, has become the preferred modality for screening, diagnosis, and follow-up of superficial diseases. Advances in ultrasonic probe technology, particularly in frequency resolution, have expanded its applications in dermatologic diagnostics.[4] High-frequency ultrasound, a relatively novel technique, has been underexplored in the context of pigmented skin lesions. Exploring rapid and effective evaluation indicators is of great significance for improving the detection rate of malignant pigmented skin lesions and avoiding overtreatment. This study leverages the diagnostic advantages of ultrasound in terms of lesion two-dimensional morphology and hemodynamics, combined with clinical data, to explore the independent risk factors for malignant pigmented skin lesions, with satisfactory results.

Data and Methods

Study Participants

This prospective study included 126 patients with pigmented skin lesions who were admitted to the Department of Dermatology or the Department of Plastic Surgery between October 2022 and July 2024. All participants underwent high-frequency color Doppler ultrasound examination followed by surgical treatment. The cohort comprised 52 males and 74 females, with a mean age of 52.93 ± 15.56 years.

Inclusion criteria: 1) No history of other autoimmune skin diseases. 2) Provided informed consent and underwent surgical treatment. 3) Availability of pathological examination results. 4) Presence of a single pigmented skin lesion.

Exclusion criteria: 1) History of prior destructive treatment, including photodynamic therapy, surgery, or radiotherapy before undergoing ultrasound examination. 2) Inability to cooperate with study procedures. 3) Lack of available pathological examination results.

The sample size was determined based on an a priori power analysis. The anticipated effect size for the mean difference between the malignant and benign groups was estimated to be $d = 0.6$ (a medium effect). Under the conditions of $\alpha = 0.05$ (two-tailed test) and a statistical power of 80%, a minimum of 45 participants per group was required. Ultimately, 76 individuals were recruited for the benign group and 50 for the malignant group (total $N = 126$). The actual data analysis revealed that the study achieved a power of 87.8%. The results of the power analysis were presented in Supplementary Table 1.

Data and Methods

Clinical Data Collection

Patient data were recorded, including age (teenager: ≥ 14 years, older adults: ≥ 60 years, and youth/middle-aged: > 14 and < 60), sex, disease duration (the period from initial lesion detection to the time of ultrasound examination), short-term lesion progression (classified as progressive if a lesion was newly developed or had increased in size by ≥ 1 mm within the past month; otherwise, classified as stable), lesion location (with the head, face, neck, and dorsum of the hand considered exposed areas, while all other locations were classified as non-exposed), lesion pigmentation (classified as dark if darker than the surrounding normal skin and light otherwise), and apparent morphology (categorized as regular or irregular). These parameters were assessed at the bedside three days prior to surgery.

High Frequency Ultrasound Examination

Based on lesion location, patients were positioned appropriately to ensure full exposure of the affected area. A disinfection and sterilization coupling agent of appropriate thickness was applied to the site of the pigmented skin lesions High-frequency color ultrasound (Paragon XHD) equipped with dual high-frequency probes (L38-22, central frequency: 30 MHz; L22-10, central frequency: 16 MHz) was used for two-dimensional grayscale ultrasound, color Doppler imaging of blood flow, and Doppler spectral analysis of blood flow pulse within the lesions.

During the examination, probes were maintained in a suspended position to prevent distortion or obscured visualization of the lesion contour due to compression. Maintain a coupling agent thickness greater than 1 mm between the probe and the lesion or skin, and ensure that no air is present. Initially, grayscale ultrasound was used to measure the maximum transverse diameter of the lesion (The maximum diameter parallel to the skin), maximum lesion thickness (distance from the lesion surface to the deepest subcutaneous point perpendicular to the epidermis); The layers of skin involved by the

lesion was recorded (epidermis, dermis, or subcutaneous tissue), lesion shape (nodular, creeping, or irregular), lesion boundary clarity (clear or unclear), internal echogenicity (hypoechoic, isoechoic, hyperechoic, or mixed echogenicity), stratum corneum condition (intact, absent, or thickened), and presence of strong internal punctate echoes.

Subsequently, color Doppler flow imaging was used to assess blood supply within and surrounding the lesion, with identification of the primary artery responsible for vascularization. The blood flow scale was set within the range of 1–2 cm/s to ensure that the blood flow signals do not overflow or disappear. Blood flow was classified using Alder blood flow grading method:[5]

Level 0: No detectable blood flow signal. Level I: Minimal blood flow signals, accounting for < 20% of the lesion. Level II: Moderate blood flow signals, comprising 20%–50% of the lesion. Level III: Abundant blood flow signals, exceeding 50% of the lesion.

Finally, pulsed spectral Doppler was used to record the peak systolic velocity (PSV), pulsatility index (PI), and resistance index (RI) of the responsible artery. The sampling volume should be set at 0.5 mm, the angle between the ultrasound beam and the direction of blood flow should be less than 20°, and a reasonable maximum velocity scale should be set to ensure that the spectral height occupies 1/2 to 2/3 of the range. All examinations were conducted by two or more professionally trained ultrasound diagnosticians.

Grouping Method

Surgical resection was performed for all patients to remove the respective lesions, and pathological examination results were obtained within 7–10 days postoperatively. Based on the pathological findings, patients were classified into either the benign or malignant group. The flowchart of the study was shown in the Figure 1.

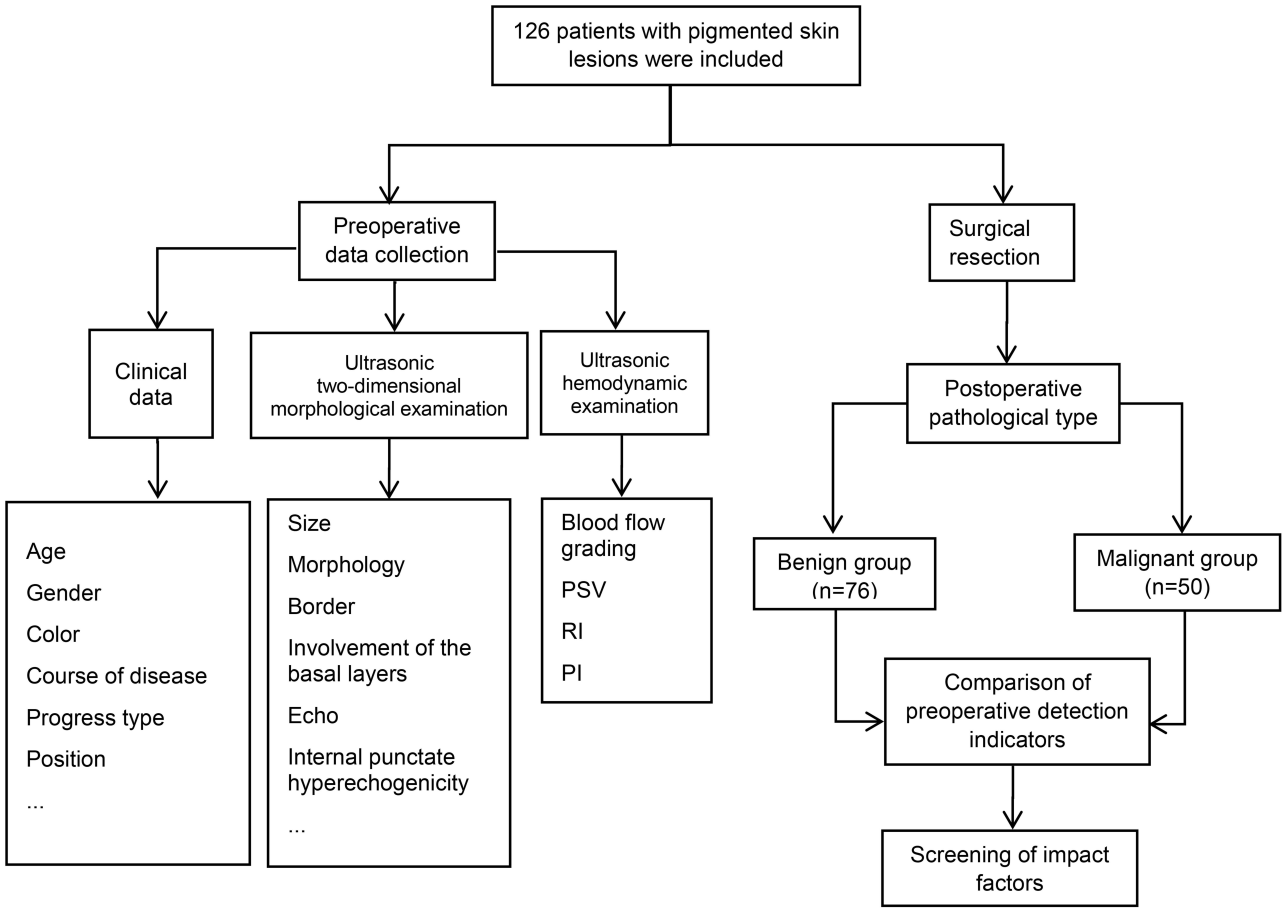

Figure 1 The flowchart of the study.

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Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software. The Kolmogorov–Smirnov test was applied to assess the normality of the data. Normally distributed continuous variables were expressed as mean ± standard deviation (SD), while non-normally distributed continuous variables were reported as median and interquartile range (IQR, 25%–75%). Categorical variables were presented as counts and percentages.

Univariate analysis was conducted to examine the difference between all collected variables and the benign and malignant lesion group. Categorical data were analyzed using the chi-square test or Fisher's exact test. For continuous data that did not follow a normal distribution and ordinal data, nonparametric rank-sum tests were used. Variables found to be significant in univariate analysis were further analyzed using multivariate binary logistic regression to identify independent risk factors for malignant pigmented skin lesions. Receiver operating characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) was used to assess the diagnostic performance of each independent risk factor in differentiating benign from malignant pigmented skin lesions. A significance level of $\alpha = 0.05$ was applied for all statistical analyses.

Results

Clinical Data and Pathological Results of 126 Patients with Pigmented Skin Lesions

Among the 126 patients included in the study, 2 were juveniles, 71 were classified as young to middle-aged, and 53 were older adults. The group comprised of 52 males and 74 females. Of the 126 pigmented skin lesions, 88 (69.8%) were located in exposed areas, while 38 (30.2%) were in non-exposed regions. Lesions were distributed as follows: 70 (55.6%) on the head and face, 22 (17.5%) on the trunk, 17 (13.5%) on the neck, 13 (10.4%) on the limbs, and 4 (3.2%) in the perineal region.

Postoperative pathological examination classified 50 lesions as malignant, comprising 29 cases of basal cell carcinoma (58.0%), 8 cases of squamous cell carcinoma (16.0%), 6 cases of melanoma (12.0%), 4 cases of Paget's disease in the perineum (8.0%), and 4 cases of dermatofibrosarcoma protuberans (8.0%). The remaining 76 lesions were classified as benign, including 51 cases of pigmented nevus (67.1%), 11 cases of seborrheic keratosis (14.5%), 8 cases of hemangioma (10.5%), 4 cases of pilomatrixoma (5.2%), and 2 cases of keratoacanthoma (2.6%).

A comparison of clinical characteristics between patients with benign and malignant pigmented skin lesions revealed statistically significant differences in disease duration, lesion location, and short-term lesion progression. However, no significant differences were observed in age, sex, lesion pigmentation, or morphology as assessed by visual inspection. Detailed comparative data are presented in Table 1.

Table 1 Univariate Analysis of Clinical Data of Patients Diagnosed with Benign and Malignant Pigmented Skin Lesions

	Malignant (n=50)	Benign (n=76)	Z/χ^2	P
Age			5.699	0.058
Juvenile	0 (0.0%)	2 (2.6%)		
Young-middle-aged	23 (46.0%)	48 (63.2%)		
Elderly	27 (54.0%)	26 (34.2%)		
Gender			0.765	0.382
Male	23 (46.0%)	29 (38.2%)		
Female	27 (54.0%)	47 (61.8%)		
Disease course (year)	2 (1.25,3.00)	9 (3.00,25.00)	−0.479	0.000
Growth position			5.818	0.016
Easily exposed parts	41 (82.0%)	47 (61.8%)		
Non-exposed parts	9 (18.0%)	29 (38.2%)		
Depth of color			0.613	0.434
Dark	47 (94.0%)	67 (88.2%)		
Light	3 (6.0%)	9 (11.8%)		

(*Continued*)

Table 1 (Continued).

	Malignant (n=50)	Benign (n=76)	Z/χ^2	P
Apparent morphology			1.999	0.157
Regular (quasi-circle)	46 (92.0%)	75 (98.7%)		
Irregular	4 (8.0%)	1 (1.3%)		
Short-term lesion progress			46.140	0.000
Progressive	39 (78.0%)	13 (17.1%)		
Stable	11 (22.0%)	63 (82.9%)		

High Frequency Ultrasound Manifestations of 126 Patients with Pigmented Skin Lesions

The mean maximum transverse diameter of the 126 pigmented skin lesions was 9.00 mm (range: 2.00–30.10 mm), while the mean maximum thickness was 4.21 mm (range: 0.40–25.00 mm). Most lesions exhibited a moderate-to-low echogenicity with varying sizes, involving the epidermis and dermis. The majority presented as nodular formations or as lesions spreading along the epidermis, while a smaller proportion extended to the dermal-subcutaneous tissue interface. Internal blood flow signals were predominantly sparse, classified as Adler grade 0–1. Additionally, punctate hyperechoic foci were observed in some lesions (Figures 2–4).

A comparison between the benign and malignant lesion groups demonstrated statistically significant differences in maximum thickness ($p < 0.001$), sectional morphology ($p = 0.009$), basal involvement ($p = 0.013$), presence of strong internal punctate echoes ($p < 0.001$), blood flow classification ($p < 0.001$), and RI of the responsible artery ($p = 0.000$). However, no significant differences were observed in maximum transverse diameter ($p = 0.096$), internal echogenicity ($p = 0.614$), stratum corneum condition ($p = 0.114$), PI of the responsible artery ($p = 0.063$), or PSV ($p = 0.086$) between the two groups. Detailed statistical comparisons are presented in Table 2.

Identification of Independent Risk Factors for Malignant Pigmented Skin Lesions

Multivariate binary logistic regression analysis was conducted on the clinical data and the statistically significant indicators were identified in the univariate analysis of high-frequency ultrasound findings. After accounting for collinearity effects, short-term lesion progression, the presence of strong internal punctate echoes, and the resistance index of the responsible artery were identified as independent factors associated with the differentiation between benign and malignant pigmented skin lesions (Table 3).

ROC curve analysis was conducted for short-term lesion progression, strong internal punctate echoes, the responsible artery resistance index, and their combined diagnostic value (Figure 5). The respective area under the ROC curve values were 0.804 (95% CI: 0.722–0.887), 0.777 (95% CI: 0.687–0.868), 0.815 (95% CI: 0.742–0.888), with an RI value greater than 0.54 as the optimal cutoff, the sensitivity and specificity for diagnosing a lesion as malignant were 72.0% and 51.7%, respectively.

Discussion

As the largest organ of the human body, the skin serves as the primary barrier against various external damages.[6] The incidence of skin diseases is high due to the influence of both intrinsic and extrinsic factors, including individual susceptibility, environmental exposures, and variations in lesion location. The complexity of skin diseases presents a diagnostic challenge, as similar conditions may exhibit distinct clinical appearances, while different diseases may present with similar features. Although histopathological examination remains the gold standard for diagnosing skin diseases, its invasive nature, limited sampling capability, and the potential for scarring, particularly in cosmetically sensitive areas, make it less acceptable to many patients. Consequently, there is an urgent need for a diagnostic approach that is non-invasive, rapid, and accurate.

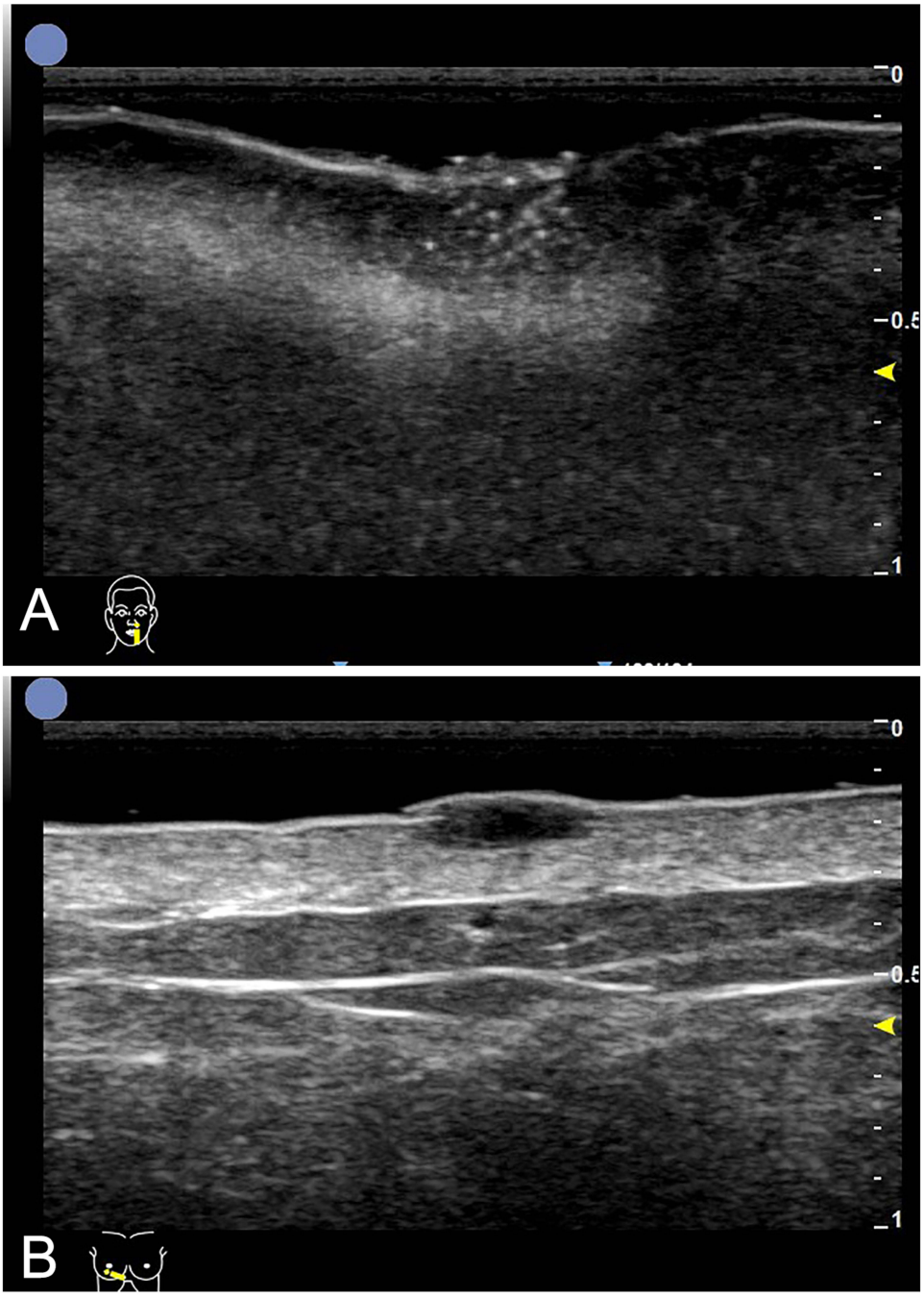

Figure 2 (**A**) Malignant pigmented skin lesion (basal cell carcinoma): The lesion has an ill-defined border, exhibits a creeping growth pattern, and contains hyperechoic foci that are clustered in distribution. (**B**) Benign pigmented skin lesion (nevus): The lesion has a well-defined border, regular shape, and homogeneous internal echoes.

In recent years, non-invasive imaging techniques, such as dermoscopy, reflectance confocal microscopy, and optical coherence tomography, have been widely used in the evaluation and management of skin diseases. However, these methods have limited penetration depth, restricting their ability to assess the extent of lesion infiltration.[7] Ultrasound, as a non-invasive and practical imaging method, offers both high resolution and sufficient penetration depth. Advances in computer technology and high-frequency ultrasound have enabled probe frequencies to reach 20–150 MHz, allowing for detailed visualization of the complete anatomical structure of the skin, including the epidermis, dermis, and subcutaneous tissue. High-frequency ultrasound facilitates the assessment of skin layers and adnexal structures while providing

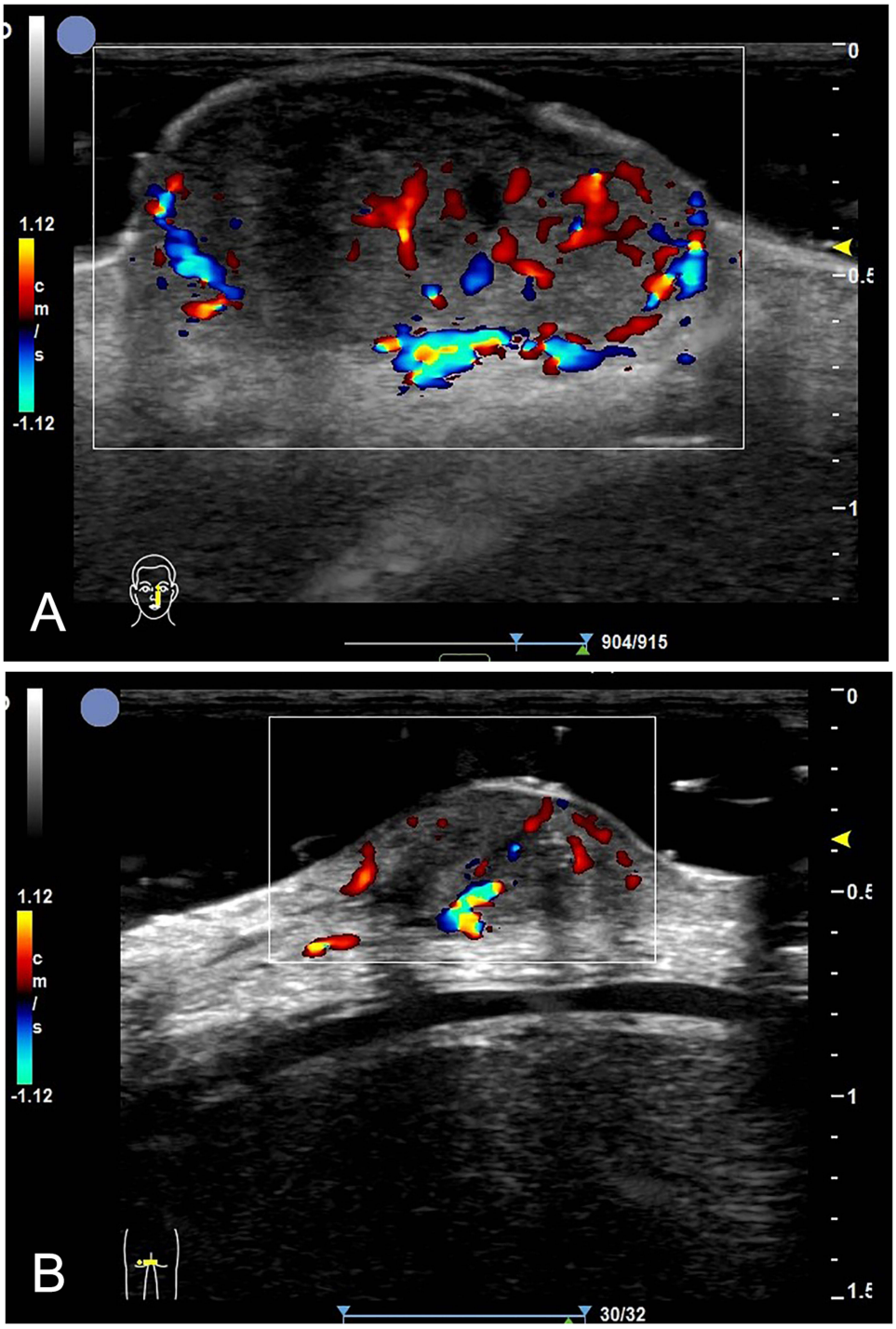

Figure 3 (**A**) Malignant pigmented skin lesion (basal cell carcinoma): The lesion shows abundant blood flow signals in every part, with a tree-branch-like distribution. Alder grading: Grade II. (**B**) Benign pigmented skin lesion (epidermoid cyst): The lesion shows few a blood flow signals internally. Alder grading: Grade I.

morphological details of skin lesions, such as lesion thickness, boundary characteristics, internal echogenicity, and adjacent tissue involvement. Additionally, it enables dynamic visualization of lesion vascularity in a timely manner.[8]

Clinically, determining lesion infiltration depth, breach of the skin layers, and lesion classification (benign or malignant) is key for guiding treatment strategies and prognostication. Pigmented skin lesions are predominantly benign, with malignant variants being relatively rare. However, certain malignant lesions may develop progressively from pre-existing benign lesions, often undergoing a prolonged course before metastasis or significant progression occurs, leading

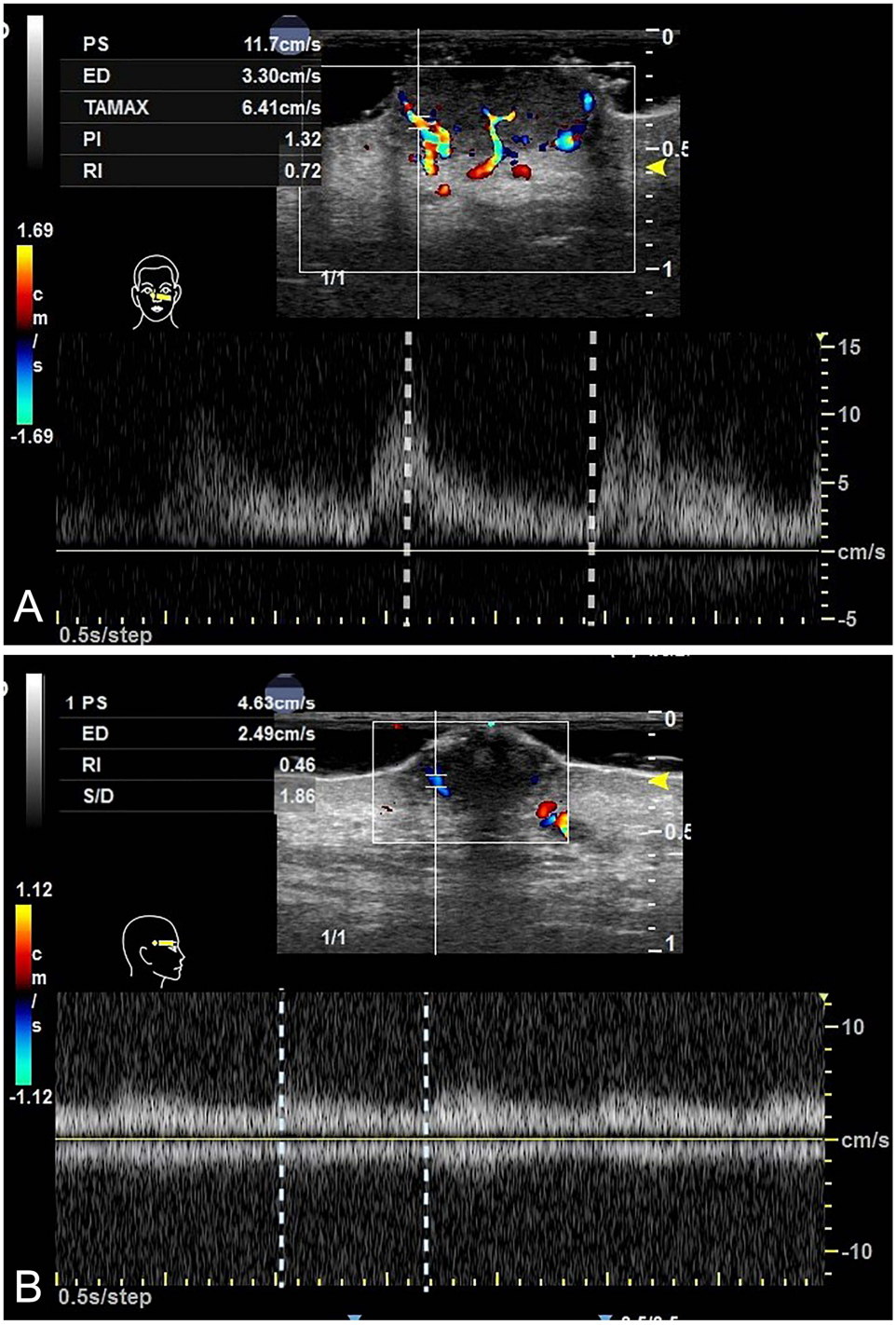

Figure 4 (**A**) Malignant pigmented skin lesion (basal cell carcinoma): Spectral Doppler shows high-resistance blood flow, with a resistance index (RI) of 0.72. (**B**) Benign pigmented skin lesion (keratoacanthoma): Spectral Doppler shows low-resistance blood flow, with a resistance index (RI) of 0.46.

to delayed recognition. Once advanced or metastatic, these conditions pose a serious threat to patient survival. The findings of this study indicate that lesion progression is strongly associated with malignancy, and a rapid increase in lesion size over a short period often indicates a poor prognosis, necessitating early intervention.

A significant correlation has been identified between the nature of pigmented skin lesions and the presence of internal punctate high echoes. Malignant lesions frequently exhibit punctate hyperechoic signals, with basal cell carcinoma often

Table 2 Univariate Analysis of High Frequency Ultrasound Parameters of Benign and Malignant Pigmented Skin Lesions

	Malignant (n=50)	Benign (n=76)	Z/χ^2	P
Maximum transverse diameter (mm)	8.10 (6.58, 9.65)	7.05 (5.43, 11.96)	−1.666	0.096
Maximum thickness (mm)	6.60 (5.48, 7.38)	1.70 (1.20, 3.18)	−7.300	0.000
Resistance index (RI)	0.66 (0.61, 0.72)	0.22 (0.00, 061)	−6.057	0.000
Pulse index (PI)	0.76 (0.59, 0.88)	0.33 (0.00, 0.92)	−1.861	0.063
Peak systolic velocity (PSV) (cm/s)	6.20 (5.50, 6.63)	2.95 (0.00, 7.10)	−1.714	0.086
Strong internal punctate echo			45.390	0.000
Yes	31 (62.0%)	5 (6.6%)		
No	19 (38.0%)	71 (93.4%)		
Blood flow classification			27.219	0.000
0	3 (6.0%)	38 (50.0%)		
I	29 (58.0%)	21 (27.6%)		
II	11 (22.0%)	9 (11.8%)		
III	7 (14.0%)	8 (10.5%)		
Morphology			/	0.009
Nodular	45 (90.0%)	76 (100.0%)		
Creeping	5 (10.0%)	0 (0.0%)		
Irregular	0 (0.0%)	0 (0.0%)		
Echo			/	0.476
No	2 (4.0%)	6 (7.9%)		
Low	48 (96.0%)	70 (92.1%)		
Middle	0 (0.0%)	0 (0.0%)		
High	0 (0.0%)	0 (0.0%)		
Mixed	0 (0.0%)	0 (0.0%)		
Basal level involved			8.669	0.013
Epidermis layer	1 (2.0%)	8 (10.5%)		
Dermis layer	34 (68.0%)	59 (77.6%)		
Subcutaneous tissue layer	15 (30.0%)	9 (11.8%)		
Corneal layer state			4.348	0.114
Missing	1 (2.0%)	0 (0.0%)		
Normal	47 (94.0%)	66 (86.8%)		
Thickened	2 (4.0%)	10 (13.2%)		

Note: / indicates Fisher's exact test.

Table 3 Multivariate Analysis of Comprehensive Indexes of Benign and Malignant Pigmented Skin Lesions

	Malignant (n=50)	Benign (n=76)	Z/χ^2	B	P	OR
Short-term lesion progress			46.140	2.398	0.000	11.002
Progressive	39 (78.0%)	13 (17.1%)				
Stable	11 (22.0%)	63 (82.9%)				
Strong internal punctate echo			45.390	2.573	0.001	13.103
Yes	31 (62.0%)	5 (6.6%)				
No	19 (38.0%)	71 (93.4%)				
Resistance index (RI)	0.66 (0.61, 0.72)	0.22 (0.00, 0.61)	−6.057	3.861	0.021	47.530

demonstrating multiple such foci as a characteristic ultrasonographic feature. Their formation may be associated with specific histopathological structures, including keratin cysts, microcalcifications, apoptosis, and necrosis.[9]

A study by Wortsman et al indicated that the number of punctate hyperechoic foci is prognostically relevant and may help in predicting the risk of disease recurrence. Specifically, lesions with more than seven such high echoes were

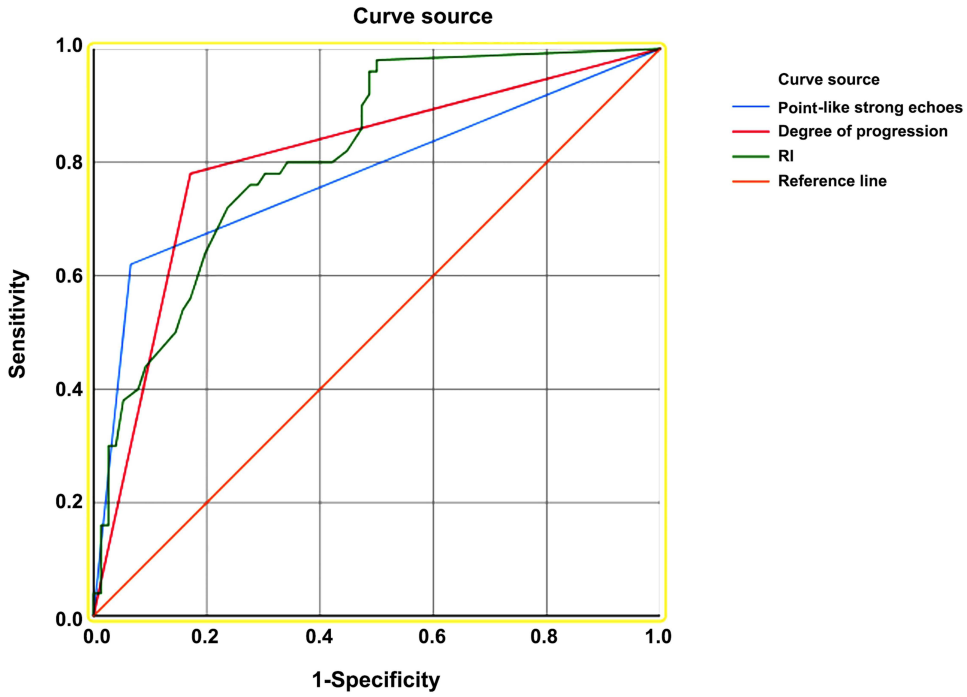

Figure 5 The ROC curves of each risk factor.

associated with an increased likelihood of recurrence.[10] In this study, 62.0% (31/50) of malignant lesions exhibited punctate high echoes.

Color Doppler ultrasound has demonstrated its use in identifying features indicative of malignancy, including increased vascularization within the lesion, elevated blood flow velocity, and the presence of multiple vascular pedicles at the tumor base.[11] The findings of this study indicate that high-frequency dynamic Doppler ultrasound can effectively monitor blood flow within and surrounding tumors, as well as its relationship with adjacent vasculature. Malignant pigmented skin lesions predominantly exhibited blood flow patterns classified as Grades II and III, whereas benign lesions were mostly classified as Grades 0 and I. This disparity may be attributed to the release of tumor angiogenesis factors by malignant tumor cells, which stimulate capillary proliferation and contribute to rapid lesion growth. Notably, with RI > 0.54 strongly indicative of malignancy, thereby necessitating early clinical intervention.

Along with facilitating the preoperative differentiation between benign and malignant lesions, high-frequency ultrasound provides valuable data regarding lesion margins in malignant pigmented skin lesions. A study by Parashar et al confirmed that high-frequency ultrasound can accurately delineate the morphology of basal cell carcinoma preoperatively, with tumor volume measurements obtained via ultrasound demonstrating strong concordance with post-operative histopathological measurements.[12] Furthermore, Jambusaria-Pahlajani et al explored the role of high-frequency ultrasound in improving the accuracy of Mohs microsurgery and found that 20 MHz ultrasound exhibited greater sensitivity in detecting subclinical invasion in larger tumors (diameter > 1.74 cm).[13] Based on current evidence, 20 MHz high-frequency ultrasound is considered more suitable for planning excisional surgery in larger tumors, whereas Mohs microsurgery requires higher-frequency imaging for detailed lesion assessment.

The innovations of this paper were as follows: Firstly, this study utilized spectral Doppler technology to analyze the hemodynamic parameters of the arteries responsible for pigmented skin tumors, both benign and malignant, which has rarely been reported in previous literature. Secondly, this study innovatively combined the use of 22–38 MHz ultra-high-frequency probes to analyze the internal and surrounding tissue conditions of pigmented skin tumors, benign and malignant. This approach compensates for the insufficient resolution of single 15 MHz or lower high-frequency probes used in previous studies, and more clearly reveals the detailed characteristics of the lesions. Thirdly, this study innovatively revealed the independent risk factors for preoperative evaluation of the benign and malignant nature of

pigmented skin lesions using a combination of ultrasound and clinical data. However, this study also has certain limitations, primarily due to the relatively small sample size, which may introduce potential bias in the findings. In addition, due to equipment limitations, the ultra-high-frequency probe used in this study did not have elastography capabilities, which restricted the study's investigation into the hardness of pigmented skin lesions. Future research would require larger sample sizes to further validate these results and enhance the reliability of the conclusions. When conditions permit in the future, elastography parameters could be incorporated into multimodal ultrasonography research. And we also intend to store the expanded dataset in a public database to facilitate verification by other researchers.

Conclusion

In summary, high-frequency ultrasound provides detailed visualization of the internal structure of lesions as well as adjacent anatomical features. This imaging modality offers key data regarding lesion size, margins, vascularization, and surrounding tissue involvement in a non-invasive manner, facilitating optimal preoperative surgical planning. The progression of pigmented skin lesions, the presence of strong internal punctate hyperechoic foci, and the resistance index of the primary supplying artery are significantly associated with the pathological characteristics of the lesions. The diagnostic accuracy, sensitivity, and specificity were 86.5%, 94.0%, and 93.4%, respectively. Among these parameters, a resistance index exceeding 0.54 is highly indicative of malignancy and warrants careful clinical consideration. High-frequency ultrasound demonstrates substantial diagnostic and clinical utility in distinguishing between benign and malignant pigmented skin lesions and is recommended for broader clinical application.

Abbreviations

PSV, peak systolic velocity; PI, pulsatility index; RI, resistance index; ROC, Receiver operating characteristic; AUC, area under the curve.

Data Sharing Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). The study was approved by Ethics Committee of the First Affiliated Hospital of Henan University of Science & Technology. All participants provided written informed consent for their involvement in the study. In cases where participants were under the age of 18, informed consent was additionally obtained from their parents or legal guardians.

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Consent to Publish

The authors affirm that human research participants provided informed consent for publication of the Figures.

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Disclosure

The authors declare that they have no competing interests in this work.

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