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ORIGINAL RESEARCH

Serum CAPN1 and CLEC2 as Biomarkers for Hearing Loss Severity and Internal Auditory Artery Narrowing in Sudden Sensorineural Hearing Loss

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Background: The pathogenesis of sudden sensorineural hearing loss (SSNHL) remains unclear, with inner ear microcirculatory disturbance being an important mechanism. However, serum biomarkers for evaluating this mechanism are inadequately studied. This study investigated the expression of calpain-1 (CAPN1) and C-type lectin-like receptor 2 (CLEC2) and their correlations with hearing loss severity and inner ear artery diameters in SSNHL patients.

Methods: In this prospective study, we consecutively enrolled 150 patients with SSNHL admitted between March 1, 2021, and June 1, 2025, and 150 healthy controls undergoing routine physical examination. Serum CAPN1 and CLEC2 concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Vascular measurements were based on MRI-derived large artery caliber (including the internal auditory artery, basilar artery, and anterior inferior cerebellar artery). Pearson correlation analysis was used to evaluate the relationship between CAPN1, CLEC2 and these arterial diameter parameters. Logistic regression analysis was performed to identify risk factors for SSNHL. Receiver operating characteristic (ROC) curves were used to evaluate the discriminative efficacy of CAPN1 and CLEC2.

Results: CAPN1 and CLEC2 levels were significantly elevated in the SSNHL group and increased with the severity of hearing loss ($P < 0.001$). All three arterial diameters were significantly smaller than those in the healthy group and were negatively correlated with CAPN1 and CLEC2 levels ($P < 0.001$). Multivariable logistic regression analysis showed that elevated WBC count, neutrophil count, CAPN1, and CLEC2 were independent risk factors, whereas increased arterial diameter was a protective factor ($P < 0.05$). The AUCs for CAPN1 alone, CLEC2 alone, and both combined were 0.804, 0.793, and 0.863, respectively, demonstrating good discriminative performance.

Conclusion: Elevated serum CAPN1 and CLEC2 levels correlated with reduced diameters of arteries supplying the inner ear and severity of hearing loss in patients with SSNHL, suggesting their potential value as novel biomarkers for evaluation of inner ear microcirculatory disturbance and prediction of SSNHL. However, external validation and longitudinal outcome studies are warranted to establish their clinical applicability.

Keywords: sudden sensorineural hearing loss, calpain-1, C-type lectin-like receptor 2, Inner ear microcirculatory disturbance

Introduction

Sudden sensorineural hearing loss (SSNHL) is defined as a sensorineural hearing decline of unknown etiology occurring within 72 h, and its pathogenesis remains incompletely understood.[1] A study has demonstrated that SSNHL is associated with adverse perfusion-related parameters (such as microvascular dysfunction) and enhanced oxidative stress; however, conventional inflammatory markers fail to specifically identify critical pathological events including blood-labyrinth barrier disruption or microthrombosis.[2] Therefore, exploring novel biomarkers capable of specifically reflecting the

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pathological processes of inner ear microcirculatory disturbance holds significant implications for early recognition and precision treatment of SSNHL.

Calpain-1 (CAPN1) is a Ca^{2+}-dependent cysteine protease; its overactivation cleaves cytoskeletal proteins, tight-junction proteins, and ion channels, disrupts vascular endothelial barrier integrity, and promotes abnormal platelet adhesion. CAPN1 has been implicated in cerebral ischemia–reperfusion injury and cardiac microvascular damage.[3–6] Single-cell sequencing reveals high Capn1 expression in the murine cochlear lateral wall, with a 1.8- to 2.3-fold up-regulation after hypoxia–reperfusion, indicating that CAPN1 is a stress-responsive enzyme in cochlear microvessels.[7] In addition, CAPN1 inhibitor MDL-28170 significantly reduces hair-cell loss and lowers auditory brainstem response (ABR) thresholds in noise-exposed rats, preserving hearing function.[8] However, systemic data on serum CAPN1 levels in SSNHL patients and their relationship with inner-ear microcirculatory impairment are lacking.

C-type lectin-like receptor 2 (CLEC2) is expressed predominantly on platelet surfaces; binding to its ligand podoplanin triggers platelet activation, microthrombus formation, and reciprocal release of endothelial inflammatory mediators.[9,10] In a $FeCl_3$-induced arterial thrombosis model, platelet-specific CLEC2 deletion reduced thrombus volume by approximately 40% and markedly suppressed fibrin deposition.[11] Deep-vein thrombosis studies further showed that CLEC2 knockout decreased both thrombus weight and length without prolonging bleeding time, indicating that CLEC2 blockade attenuates microthrombosis.[12] Whether CLEC2 participates in or drives inner-ear microcirculatory disturbance in SSNHL remains unexplored.

The diameters of the internal auditory artery, basilar artery, and anterior inferior cerebellar artery measured by MRI serve as proximal imaging indicators of inner ear blood supply. Although these measurements do not represent direct assessments of cochlear capillary microcirculation, stenosis or dilation of proximal vessels can directly affect downstream cochlear blood flow. Previous studies have demonstrated that reduced cochlear blood flow plays a significant role in noise-induced hearing loss, suggesting that indirect evaluation of cochlear perfusion status through proximal vascular parameters holds clinical significance.[13] Accordingly, this study aims to investigate serum CAPN1 and CLEC2 levels in SSNHL patients and to analyze the relationships of these biomarkers with the severity of hearing loss and the diameters of arteries supplying the inner ear, thereby providing novel indicators for evaluating inner ear microcirculatory disturbance in this context.

Materials and Methods

Sample Size Estimation and Statistical Power Analysis

The validity of this study was assessed based on two key indicators (CAPN1 and CLEC2). To ensure adequate statistical power for all indicators, the final sample size was determined according to the core indicator with the greatest demand and the most difficult to achieve statistical significance (CAPN1).

Sample size planning was conducted based on preliminary data from a small-scale pilot study of 12 subjects (Healthy group: 3.45±1.56 ng/mL; SSNHL group: 4.12±1.78 ng/mL). For this core indicator, a two-sample Welch's unequal-variance *t*-test was employed under the setting of $\alpha=0.05$ (two-tailed test), with a target statistical power of 90%, estimating that 133 subjects per group would be required.

The actual total sample size enrolled was 300, with a 1:1 ratio between the two groups (150 subjects per group). Following study completion, post-hoc power analysis was performed to verify the actual statistical power. Calculated using PASS 2025 software (NCSS, USA), the actual power to detect the mean difference in CAPN1 between groups under the current sample size reached 93.26% (exceeding the predetermined 90%). Given that the current sample size of N=300 has already met the high-power requirement for the core indicator, the statistical power for the other key indicator (CLEC2) in this study would necessarily be higher than 93.26%. In conclusion, the sample size employed in this study provides sufficient statistical power to detect true between-group differences for all indicators.

Study Participants

This prospective observational study enrolled 150 consecutive patients diagnosed with SSNHL who were treated at our hospital between March 1, 2021, and June 1, 2025. All patients presented with unilateral hearing loss (left ear: 87

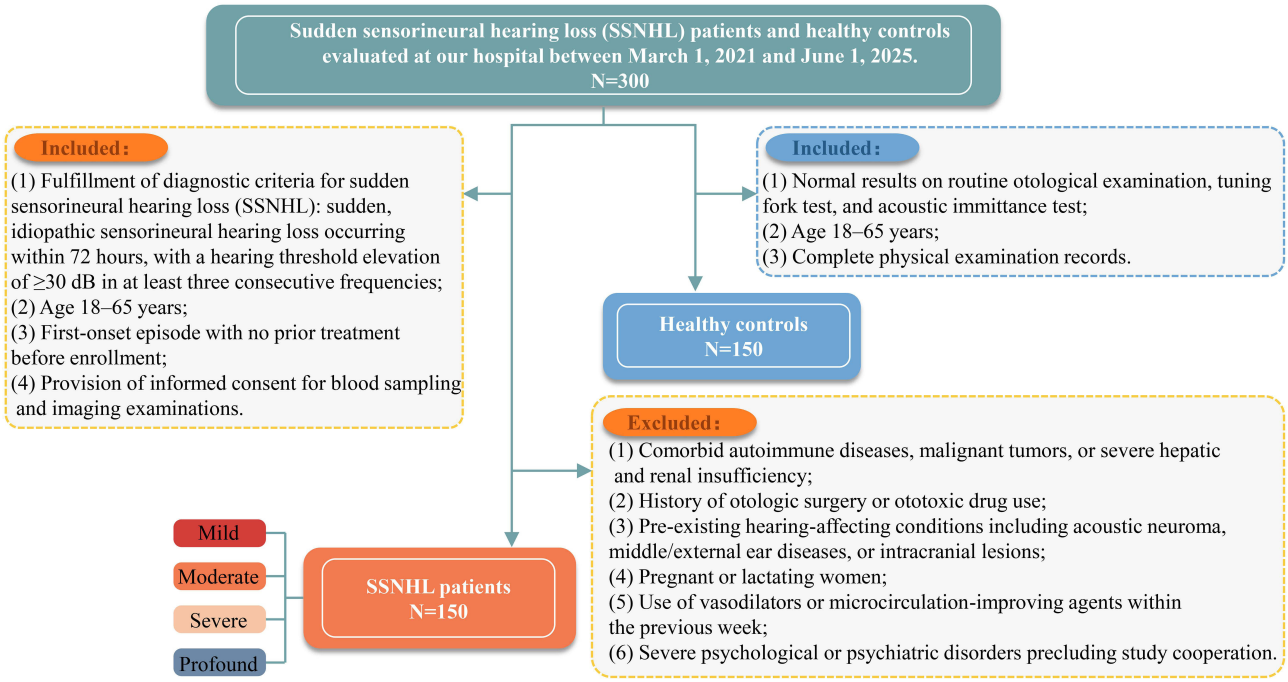

Figure 1 The participant flow diagram.

[58.0%]; right ear: 63 [42.0%]). The median time from symptom onset to presentation was 50 hours (range 4–111, mean ± SD: 48.6 ± 22.5). Vertigo occurred in 79 patients (52.7%) and tinnitus in 103 (68.7%). Additionally, 150 healthy individuals who underwent physical examination during the same period were recruited as the healthy control group (The participant flow diagram is shown in Figure 1). SSNHL inclusion criteria: (1) fulfillment of the diagnostic criteria[14]—acute, idiopathic sensorineural hearing loss developing within 72 h with a threshold shift ≥ 30 dB in at least three contiguous frequencies; (2) age 18–65 years; (3) first episode of SSNHL with no prior treatment (including systemic or intratympanic medications such as glucocorticoids, vasodilators, or antiviral agents; hyperbaric oxygen therapy; intratympanic injections; or hearing aids); (4) provision of written informed consent for blood sampling and imaging. SSNHL exclusion criteria: (1) autoimmune disease, malignancy, or severe hepatic or renal impairment; (2) previous ear surgery or ototoxic drug exposure; (3) history of vestibular schwannoma, external/middle-ear disease, or intracranial pathology affecting hearing; (4) pregnancy or lactation; (5) use of vasodilator or microcirculation-improving drugs within 1 week; (6) severe psychiatric disorders precluding cooperation. Control inclusion criteria: (1) normal findings on otoscopy, tuning-fork tests, and tympanometry; (2) age 18–65 years; (3) complete medical records.

Demographic and Clinical Characteristics

Gender, age, body-mass index (BMI), smoking and alcohol use, and comorbidities were recorded for all participants.

Laboratory Assays

On presentation, 5 mL fasting blood was drawn from an antecubital vein, centrifuged at 2000 × g for 20 min, and serum aliquots stored at −20 °C until analysis.

① Complete blood count (white blood cells [WBC], neutrophils, lymphocytes, platelets) was measured with an automated hematology analyzer (Mindray BC-5130). Platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) were calculated as platelet count ÷ lymphocyte count and neutrophil count ÷ lymphocyte count, respectively.

② Serum CAPN1 and CLEC2 concentrations were determined by enzyme-linked immunosorbent assay (ELISA). The CAPN1 ELISA kit (catalog number: BYHS502945, Nanjing Boyan Biotechnology, China) has a detection range of 0.625–20 ng/mL and a sensitivity of 0.156 ng/mL. The CLEC2 ELISA kit (catalog number: BY-EH118621, Nanjing

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Boyan Biotechnology, China) has a detection range of 0.625–20 ng/mL and a sensitivity of 0.1 ng/mL. All kits were used within their expiration dates, with intra-assay and inter-assay coefficients of variation (CV) both < 10%. Prior to testing, all reagents were equilibrated to room temperature (20–25°C), and subsequent procedures were performed strictly according to the manufacturer's instructions. Absorbance was read at 450 nm on a microplate reader within 15 min. Standard curves were generated by four-parameter logistic fitting, and serum CAPN1 and CLEC2 concentrations were calculated accordingly. All samples and standards were run in duplicate. Samples were arranged by unique identification numbers, and the operator was blinded to group allocation and clinical data.

Measurement of Inner-Ear Feeding Artery Caliber

Patients were positioned in supine. Imaging was performed using a Siemens MAGNETOM Prisma 3.0T MRI scanner with a three-dimensional time-of-flight magnetic resonance angiography (3D TOF-MRA) sequence. The imaging parameters were as follows: repetition time (TR) 20–25 ms, echo time (TE) 3.5–4.5 ms, flip angle 15°–20°, field of view (FOV) 160 mm × 160 mm, matrix 512 × 512, slice thickness 0.5 mm, yielding an isotropic spatial resolution of 0.3–0.5 mm. Vessel diameter measurements were performed on a Syngo.via workstation using multiplanar reconstruction (MPR). The external diameter was measured on cross-sectional planes perpendicular to the long axis of the vessel: the internal auditory artery was measured at 5–8 mm from the internal auditory canal meatus within the internal auditory canal; the basilar artery was measured at the mid-pons within the basilar sulcus; and the anterior inferior cerebellar artery was measured at 5–10 mm from its origin from the basilar artery. All measurements were performed and calculated by a radiologist with more than 5 years of experience, who was blinded to the clinical diagnosis of the subjects. Each parameter was measured three times, and the mean value was calculated.

Hearing-Loss Stratification

All patients with SSNHL underwent pure-tone audiometry on the day of admission. Testing was performed in a standard soundproof booth (ambient noise <30 dB(A)) using a calibrated Conera diagnostic audiometer between 8:00 and 11:00 AM. Subjects were required to remain awake and quiet. The test sequence was air conduction followed by bone conduction. The examiner communicated fully with the subject and observed their responses to ensure accurate behavioral thresholds, with repeated confirmation at each frequency. This study adopted the four-frequency pure-tone average (4fPTA) calculation method (500, 1000, 2000, and 4000 Hz) recommended by the WHO World Report on Hearing.[15] For clinical grouping, patients were classified into four categories: mild (20 to <35 dB), moderate (35 to <65 dB, encompassing the original moderate and moderately severe grades), severe (65 to <80 dB), and profound (≥80 dB, encompassing the original severe-to-profound and total deafness grades), with 35 dB and 65 dB representing the thresholds for disabling hearing loss and severe intervention, respectively. To ensure accuracy, approximately 10% of subjects were randomly selected for retesting by the same technician within 24 h; if any threshold differed by >10 dB, remeasurement was performed and the mean of the two measurements was used.

Statistical Analysis

All analyses were performed with SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY), and graphs were prepared in GraphPad Prism 9.5 (GraphPad Software, San Diego, CA). The primary endpoints were the between-group differences in serum CAPN1 and CLEC2 levels and their correlation with the internal auditory artery diameter. Secondary endpoints included correlations with the basilar artery and anterior inferior cerebellar artery diameters, subgroup comparisons across different degrees of hearing loss, comparisons of inflammatory markers, and ROC curve analysis. The Holm-Bonferroni method was applied to adjust for multiple comparisons in secondary endpoint analyses to control the false-positive rate. Categorical variables were compared using the chi-square test. Normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Normally distributed continuous variables were expressed as mean ± SD and compared using the independent samples *t*-test; non-normally distributed variables were expressed as median (interquartile range [IQR]) and compared using the Mann–Whitney *U*-test. Multiple linear regression analysis was performed with PTA as the dependent variable and serum CAPN1, CLEC2 levels, and inner ear feeding artery diameters as independent variables. Pearson correlation analysis was used to evaluate the associations between serum

CAPN1 and CLEC2 levels and the diameters of arteries supplying the inner ear in SSNHL patients. Multivariable logistic regression was performed using forward stepwise selection (Forward: LR, entry criterion $P < 0.05$) to identify risk factors for SSNHL. Multicollinearity was assessed using the variance inflation factor (VIF), with VIF > 5 indicating multicollinearity. ROC curves were constructed to evaluate the discriminative performance of serum CAPN1, CLEC2, and their combination model. The combination model was a logistic regression incorporating CAPN1 and CLEC2, and ROC curves were generated based on predicted probabilities. Optimal cutoff values were determined according to the maximum Youden index. Bootstrap internal validation (1000 iterations) was performed to correct for optimism. DeLong's test was used to compare differences in AUC among models.

Results

Comparison of Demographic Characteristics and Laboratory Parameters

No significant differences were observed between the two groups with respect to gender, age, BMI, history of smoking and alcohol consumption, and comorbidities, as well as peripheral lymphocyte count, platelet count, and PLR (P > 0.05). Patients with SSNHL exhibited significantly higher peripheral white blood cell and neutrophil counts, as well as an elevated NLR, than healthy controls (P < 0.05) (Table 1).

Comparison of Serum CAPN1 and CLEC2 Levels and Inner Ear Artery Diameters Across Hearing Loss Severities in SSNHL Patients

This study included 150 patients with SSNHL, comprising 32 mild cases (21.33%), 39 moderate cases (26.00%), 50 severe cases (33.33%), and 29 profound cases (19.33%). Serum CAPN1 and CLEC2 levels demonstrated an increasing trend, whereas the diameters of the internal auditory artery, basilar artery, and anterior inferior cerebellar artery showed a decreasing trend with the severity of hearing loss (Table 2).

Multiple linear regression analysis was performed with pure-tone average (PTA) as the dependent variable and CAPN1, CLEC2, internal auditory artery diameter, basilar artery diameter, and anterior inferior cerebellar artery diameter as independent variables. Collinearity diagnostics revealed that all variance inflation factors (VIF) were <2, indicating no multicollinearity. The regression model was statistically significant ($F = 95.205$, $P < 0.001$, $R^2 = 0.768$, adjusted $R^2 = 0.760$). Internal auditory artery diameter ($B = -61.024$, $SE = 10.060$, $\beta = -0.330$, $P < 0.001$), basilar artery diameter ($B = -19.911$, $SE = 3.163$, $\beta = -0.322$, $P < 0.001$), and anterior inferior cerebellar artery diameter

Table 1 Comparison of Demographic Characteristics and Laboratory Parameters

Variable	SSNHL Group (n=150)	Healthy Group (n=150)	$t/\chi^2/Z$	P
Gender (n/%)				
Male	79 (52.67)	82 (54.67)	0.121^	0.728
Female	71 (47.33)	68 (45.33)		
Age	48 (45, 51)	49 (47, 51)	−1.854#	0.064
BMI ($\bar{x} \pm s$, kg/m^2)	22.37±1.86	22.48±2.06	−0.486*	0.627
Smoking history (n/%)	22 (14.67)	18 (12.00)	0.462^	0.497
Alcohol use (n/%)	28 (18.67)	23 (15.33)	0.591^	0.442
Comorbidities				
Hypertension (n/%)	32 (21.33)	39 (26.00)	0.904^	0.342
Diabetes mellitus (n/%)	20 (13.33)	16 (10.67)	0.505^	0.477
WBC ($\bar{x} \pm s$, ×10^9/L)	6.75± 1.30	6.37 ± 1.12	2.685*	0.008
Neutrophil count [M (P_{25}, P_{75}), ×10^9/L]	4.50 (4.40, 4.70)	4.30 (4.20, 4.40)	−11.306#	0.000
Lymphocyte count [M (P_{25}, P_{75}), ×10^9/L]	1.85 (1.50, 2.10)	1.80 (1.50, 2.00)	−0.927#	0.354
Platelet count ($\bar{x} \pm s$, ×10^9/L)	226.69± 6.14	227.55± 6.37	1.194*	0.233
PLR [M (P_{25}, P_{75})]	124.74 (106.85, 150.75)	129.95 (111.38, 151.17)	−1.246#	0.213
NLR [M (P_{25}, P_{75})]	2.59 (2.22, 3.13)	2.39 (2.10, 2.87)	−2.445#	0.014

Notes: ^χ^2 value; #Z value; *t value.
Abbreviations: BMI, body mass index; WBC, white blood cells; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

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Table 2 Comparison of Serum CAPN1 and CLEC2 Levels and Diameters of Arteries Supplying the Inner Ear Among SSNHL Patients with Different Degrees of Hearing Loss

Group	n	CAPN1 ($\bar{x} \pm s$, ng/mL)	CLEC2 ($\bar{x} \pm s$, ng/mL)	Internal Auditory Artery ($\bar{x} \pm s$,mm)	Basilar Artery ($\bar{x} \pm s$, mm)	Anterior Inferior Cerebellar Artery ($\bar{x} \pm s$, mm)
Healthy	150	3.24±1.08	3.80±1.03	0.95±0.12	3.46±0.50	1.45±0.35
Mild	32	3.70±1.17	4.28±1.19	0.86±0.12	3.23±0.33	1.24±0.30
Moderate	39	4.38±0.98	4.89±1.27	0.78±0.08	2.92±0.30	1.10±0.21
Severe	50	4.92±1.33	5.56±1.18	0.70±0.05	2.73±0.34	0.91±0.17
Profound	29	5.53±1.27	6.29±1.25	0.62±0.04	2.42±0.10	0.73±0.11

Abbreviations: CAPN1, Calpain-1; CLEC2, C-type Lectin-like Receptor 2; SSNHL, sudden sensorineural hearing loss.

(B = −21.095, SE = 4.081, β = −0.255, P < 0.001) exhibited significant negative predictive effects on PTA. Conversely, CAPN1 (B = 1.999, SE = 0.705, β = 0.124, P = 0.005) and CLEC2 (B = 2.271, SE = 0.695, β = 0.146, P = 0.001) demonstrated significant positive predictive effects on PTA.

Correlations of Serum CAPN1 and CLEC2 with Arteries Supplying the Inner Ear in the SSNHL Group

Pearson correlation analysis revealed that serum CAPN1 and CLEC2 levels were negatively correlated with the diameters of arteries supplying the inner ear (anterior inferior cerebellar artery, basilar artery, and internal auditory artery) in the SSNHL group (P < 0.001) (Figure 2). Further partial correlation analysis demonstrated that the negative correlations of CAPN1 and CLEC2 with diameters of arteries supplying the inner ear remained significant after adjusting for common confounding factors including age, gender, BMI, smoking, alcohol consumption, and hypertension (P < 0.001).

Multivariable Logistic Regression Analysis of Risk Factors for SSNHL

Multivariable logistic regression analysis was conducted with SSNHL occurrence (SSNHL group = 1, healthy group = 0) as the dependent variable. Independent variables included WBC count, neutrophil count, NLR, CAPN1, CLEC2, and the

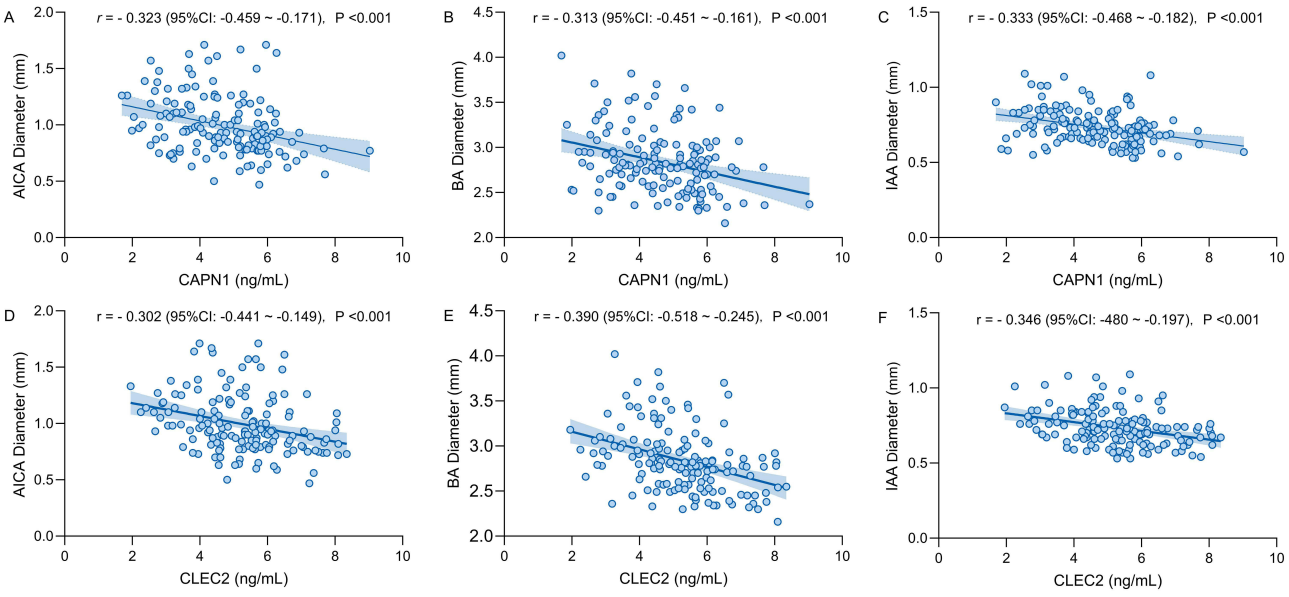

Figure 2 Correlations of serum CAPN1 and CLEC2 with Diameter Indices of Arteries Supplying the Inner Ear. Correlations of CAPN1 with the diameters of the AICA (**A**) BA (**B**) and IAA (**C**); correlations of CLEC2 with the diameters of AICA (**D**) BA (**E**) and IAA (**F**). n = 150, Pearson correlation analysis; r represents the Pearson correlation coefficient (95% CI), and the light blue shaded areas indicate the 95% confidence intervals of the fitted lines.
Abbreviations: AICA, anterior inferior cerebellar artery; BA, basilar artery; IAA, internal auditory artery.

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Table 3 Multivariable Logistic Regression of Factors Associated with SSNHL

Variable	β	S.E	Walds	P	OR	95% CI	VIF
WBC count	0.424	0.213	3.972	0.046	1.527	1.007–2.317	1.031
Neutrophil count	0.257	0.098	6.915	0.009	1.293	1.068–1.566	1.318
NLR	0.030	0.367	0.007	0.935	1.030	0.502–2.114	1.033
Internal auditory artery	−3.380	0.610	30.730	0.000	0.034	0.010–0.113	1.983
Basilar artery	−1.735	0.610	8.098	0.004	0.176	0.053–0.583	1.688
AICA	−2.449	0.864	8.040	0.005	0.086	0.016–0.469	1.633
CAPN1	0.697	0.251	7.698	0.006	2.008	1.227–3.285	1.395
CLEC2	0.818	0.264	9.632	0.002	2.266	1.352–3.798	1.345

Abbreviations: SSNHL, sudden sensorineural hearing loss; VIF, variance inflation factor; WBC, white blood cells; NLR, neutrophil-to-lymphocyte ratio; AICA, anterior inferior cerebellar artery; CAPN1, Calpain-1; CLEC2, C-type Lectin-like Receptor 2.

Table 4 Diagnostic Utility of Serum CAPN1 and CLEC2 for SSNHL

Variable	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)
CAPN1	0.804	0.756–0.853	4.360 ng/mL	60.67	88.00
CLEC2	0.793	0.742–0.844	4.375 ng/mL	76.00	73.33
CAPN1+ CLEC2	0.863	0.821–0.904	–	72.00	87.33

Abbreviations: CAPN1, Calpain-1; CLEC2, C-type Lectin-like Receptor 2; SSNHL, sudden sensorineural hearing loss.

diameter of arteries supplying the inner ear (internal auditory artery, basilar artery, and anterior inferior cerebellar artery). The results demonstrated that elevated WBC count, neutrophil count, CAPN1, and CLEC2 levels were independent risk factors for SSNHL occurrence, whereas increased diameter of arteries supplying the inner ear was a protective factor ($P < 0.05$). All variables had a VIF < 2, indicating no significant multicollinearity (Table 3).

Discriminative Performance of Serum CAPN1 and CLEC2 for SSNHL

ROC curve analysis yielded AUCs of 0.804 for CAPN1 alone, 0.793 for CLEC2 alone, and 0.863 for the combination, indicating favorable discriminative ability when both markers were used jointly (Table 4 and Figure 3). Internal validation was performed using bootstrap resampling with 1000 iterations. The optimism-corrected AUC for the combination model was 0.854 (95% CI: 0.811–0.897), representing a decrease of 0.009 from the original AUC of 0.863, with an optimism of 1.0%, indicating good model stability.

Discussion

SSNHL is an acute hearing loss of unknown etiology, for which inner ear microcirculatory disturbance is considered an important pathogenic mechanism.[2,16] The primary endpoints of this study were predefined as group differences in CAPN1/CLEC2 levels and their associations with internal auditory artery caliber, with all other analyses designated as exploratory secondary endpoints. This prospective observational study enrolled 150 SSNHL patients and 150 healthy controls. The results demonstrated that serum CAPN1 and CLEC2 concentrations were elevated in SSNHL patients, exhibited significant positive predictive effects on PTA, and were negatively correlated with the diameters of the internal auditory artery, basilar artery, and anterior inferior cerebellar artery. Elevated levels of both biomarkers were identified as independent risk factors for SSNHL. Furthermore, CAPN1 and CLEC2, both individually and in combination, showed favorable discriminative performance for SSNHL, suggesting that serum CAPN1 and CLEC2 may serve as a novel serum biomarker panel for assessing inner ear microcirculatory impairment and early identification of SSNHL.

The diameters of the internal auditory artery, basilar artery, and anterior inferior cerebellar artery serve as proximal imaging indicators of inner ear blood supply. Alterations in these vessels can directly affect downstream cochlear perfusion. Previous studies have established that reduced cochlear blood flow is closely associated with hearing loss,

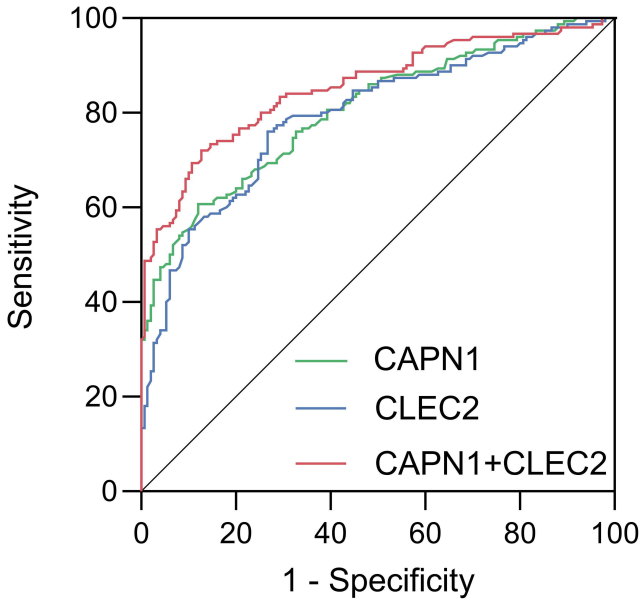

Figure 3 ROC Curves of Serum CAPN1 and CLEC2 for Discriminating SSNHL from Healthy Controls.

suggesting that indirect assessment of cochlear microcirculatory status through proximal vascular parameters holds clinical value.[13] Lee et al found that acute auditory syndrome may serve as a warning sign for anterior inferior cerebellar artery infarction, with approximately 31% of patients experiencing prodromal symptoms characterized by hearing loss and tinnitus 1–10 days prior to infarction. These findings suggest that ischemia in the anterior inferior cerebellar artery territory can involve the inner ear and vestibulocochlear nerve, leading to acute auditory dysfunction. This study supports a direct pathological link between proximal vascular perfusion abnormalities and inner ear microcirculatory disturbance as well as hearing loss.[17] Additionally, another study has demonstrated that MRI-measured cochlear nerve diameter serves as a prognostic indicator for hearing recovery in elderly patients with idiopathic sudden sensorineural hearing loss, confirming that alterations in inner ear structure and function can both be assessed through imaging parameters.[18] The present study measured the diameters of the internal auditory artery, basilar artery, and anterior inferior cerebellar artery as imaging indicators for indirectly assessing inner ear microcirculatory status. The results showed that serum CAPN1 and CLEC2 levels were elevated in patients with SSNHL and increased in parallel with the severity of hearing loss. Furthermore, these levels were negatively correlated with the diameters of these vessels, suggesting that serum CAPN1 and CLEC2 may serve as potential novel biomarkers reflecting inner ear microcirculatory disturbance, providing a potential laboratory basis for early identification of SSNHL.

CAPN1 is a Ca^{2+}-dependent cysteine protease that becomes overactivated under ischemic or oxidative stress conditions.[6,19] Animal studies have demonstrated that intense noise exposure can activate cochlear CAPN1, disrupt gap junction function in the spiral ligament, and lead to hearing loss; CAPN1 inhibitors can block this process, suggesting their potential as therapeutic targets for hearing protection.[20] In the present study, we found that serum CAPN1 levels were elevated in SSNHL patients and negatively correlated with internal auditory artery caliber, indicating that CAPN1 may reflect inner ear microvascular stress and barrier dysfunction, and is closely associated with the pathological process of inner ear microcirculatory disturbance.

CLEC2 is a type II transmembrane receptor expressed on platelet surfaces, and its endogenous ligand podoplanin is widely distributed in vascular endothelial cells and various tissues.[21,22] A study has shown that CLEC-2 expression on platelets is a critical factor driving infection-related hepatic thrombosis and is indispensable for splenic thrombosis, indicating that CLEC-2 serves as a common core mechanism for inflammation-induced multi-organ thrombosis.[23] Furthermore, CLEC-2-mediated platelet activation is a key driver of venous thrombosis, and selective Btk inhibition can effectively block this pathway without increasing bleeding risk, suggesting that targeting CLEC-2 signaling

represents a potential antithrombotic strategy.[24] Based on the aforementioned evidence and our findings, we reasonably speculate that CAPN1 and CLEC2 may participate in inner ear microcirculatory disturbance through different pathological mechanisms: CAPN1 primarily mediates vascular endothelial injury and inflammatory responses, whereas CLEC2 mainly drives platelet activation and microthrombosis. Their synergistic effects aggravate microcirculatory disturbance, which is consistent with our observation that serum levels increased in parallel with hearing loss severity and were negatively correlated with the caliber of multiple vessels.

Multivariable logistic regression identified elevated serum levels of CAPN1 and CLEC2 as independent risk factors for SSNHL, and ROC analysis indicated that these markers, both individually and in combination, exhibited high discriminative value for SSNHL, suggesting their potential as serum biomarkers for SSNHL identification. Moreover, this study identified elevated WBC and neutrophil counts as independent risk factors for SSNHL, findings consistent with previous reports.[25] Therefore, in clinical practice, patients presenting with sudden tinnitus, aural fullness, or mild hearing loss should be evaluated for serum CAPN1 and CLEC2 together with routine inflammatory indices such as WBC and neutrophil counts to enable early detection and intervention for SSNHL and to reduce the incidence of permanent hearing loss.

This study has several limitations. First, the single-center cross-sectional design limits the generalizability of our findings and precludes establishment of a causal relationship between CAPN1, CLEC2, and SSNHL. Second, although common confounders were adjusted for, lack of systematic documentation regarding statin/antiplatelet medication history, precise time window of symptom onset, and characteristics of vertigo/tinnitus may have influenced the observed associations. Third, MRI-measured inner ear-feeding artery diameters serve only as indirect radiological indicators of inner ear microcirculatory function. Fourth, the biomarker cutoff values were derived from a single-center cohort without external validation, and their generalizability as clinical biomarkers remains to be determined. Finally, the cross-sectional design precludes evaluation of the longitudinal relationship between dynamic biomarker changes and hearing outcomes, limiting determination of their incremental prognostic value. Future multi-center prospective cohort studies employing standardized and reproducible vascular imaging protocols are warranted to validate whether this biomarker combination provides incremental value for risk stratification and therapeutic decision-making beyond conventional clinical predictors.

In summary, CAPN1 and CLEC2 were elevated in SSNHL patients and seemed to increase with severity. These markers are closely associated with inner ear microcirculatory disturbance, suggesting that they are promising candidate biomarkers for risk stratification, but not definitive diagnostic tools. The current findings should be regarded as exploratory rather than conclusive evidence for establishing causal relationships. These findings require further validation through multi-center prospective cohort studies and dynamic monitoring of treatment response prior to clinical translation.

Abbreviations

SSNHL, sudden sensorineural hearing loss; CAPN1, Calpain-1; CLEC2, C-type Lectin-like Receptor 2; ELISA, Enzyme-Linked Immunosorbent Assay; MRI,Magnetic Resonance Imaging; AUC, Area Under the Curve; ROC, Receiver Operating Characteristic; WBC, White Blood Cell; NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; BMI, Body Mass Index; WHO, World Health Organization; ABR, Auditory Brainstem Response.

Data Sharing Statement

All data in this study are available from the corresponding author on reasonable request.

Ethical Statement

This study was approved by the Ethics Committee of Hebei Engineering University Affiliated Hospital (Approval No. 2021012) and adheres to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants, ensuring their confidentiality and anonymity throughout the research process.

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Disclosure

The authors report no conflicts of interest in this work.

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