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Lymphatic Vessels in the Inner Ear of Patients With Meniere Disease: A Novel Pathological Finding

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Abstract

Background. Meniere disease, characterized by intermittent episodes of vertigo, fluctuating sensorineural hearing loss, tinnitus, and aural pressure, is a common cause of vertigo in humans. The pathogenesis of Meniere disease remains unknown. The current study aimed to describe a novel pathological change discovered in the inner ears of patients with Meniere disease who underwent labyrinthectomy.

Methods. This retrospective case-control study was conducted with 21 patients with MD who underwent labyrinthectomy. A total of 15 patients diagnosed with acoustic neuroma or glomus jugular tumor were review over the same period of time as control. The clinical information of the patients and the pathological features of the membrane are described.

Results. The new pathological tissue was a morbid membrane structure sealing the round window, characterized by the formation of lymphatic capillaries. Histochemical and immunofluorescent staining was positive for D2-40, LYVE-1, podoplanin, and PROX1, which are the classical markers of the lymphatic vessels. Transmission electron microscopy revealed that the lymph capillaries lacked a typical basement membrane and that their ends were blind, composed of a single layer of endothelial cells with valval connection structures between adjacent capillary epithelial cells.

Conclusion. This is the first report of lymphatic vessels in the human inner ear, and this pathological structure is a completely new discovery. The lymphatic vessels may develop due to inflammation or decompensation of pressure in the inner ear, suggesting that the inner ear can reactively form lymphatic vessels in some inflammation and fluid flow-dependent pathological conditions. The current findings help in improving our understanding of the pathogenesis of Meniere disease.

Keywords

inner ear, labyrinthectomy, lymphatic vessel, Meniere disease, pathology

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Meniere disease (MD) is a complex inner ear disease characterized by spontaneous episodes of vertigo, fluctuating sensorineural hearing loss, tinnitus, and aural fullness.[1,2] The estimated prevalence varies from 17 to 513 cases per 100,000 people depending on the study. Its prevalence is different according to the place where the study is performed: reports range from 1.4% to 2.7% in primary care[3] and reached up 13.8% in an otoneurology-dedicated unit.[4] Recent epidemiological surveys have shown an increase in MD incidence with socioeconomic development.[5–7]

The pathogenesis of MD is still unclear. Endolymphatic hydrops (EH), the ballooning of the endolymphatic fluid space within the inner ear, is the most prominent and consistent histopathological feature of MD.[8] Although EH has been demonstrated in nearly all cases of MD,[9,10] this is not an MD-specific change, as it can also be observed in other otologic diseases, including vestibular migraine, otosclerosis, viral infection, trauma and tumor, as well as in some asymptomatic healthy people.[11,12] Therefore, the definitive pathogenesis of MD remains to be established. Previous studies have revealed lesions involving the cochlear and vestibular components of the inner ear in patients with

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MD[13,14]; however, these findings are relatively nonspecific. We need new information that will eventually lead us to the true pathoetiology of MD.

Given the heterogeneity of MD manifestations, the etiology is suspected to be multifactorial and can include polygenic predisposition, immune factors, endolymphatic sac dysfunction, and viral infection. Among them, immunoinflammation has attracted more and more attention. It has been reported that increased systemic inflammation and local inflammation of the inner ear in patients with MD. Therefore, the effect of chronic inflammation on the inner ear needs further observation.

The lymphatic system is an open-ended route that carries a protein-rich fluid from the peripheral to the cardiovascular circulation and is known to be a trafficking route for immunocompetent cells during immune surveillance. The lymphatic and blood vascular systems function in concert to regulate the tissue fluid homeostasis of the body. Lymphatic capillaries can be observed throughout most of the body's tissues. The central nervous system (CNS), bone marrow, cornea of the eye, and the inner ear were previously thought to lack lymphatic systems.[15,16] Recently, a paradigm shift occurred in this field following the discovery of structures potentially similar to those of the lymphatic system in the meninges of the CNS. These lymphatic structures are considered to be associated with the absorption of cerebrospinal fluid and immune cell circulation. However, the presence of lymphatic vessels in the cochlea or vestibule has not been reported.

Here, we describe a novel pathological finding in the vestibules of patients with MD who underwent labyrinthectomy: a membranous structure independently located under the footplate of the stapes, composed of lymphatic vessels, collagen fibers, and squamous epithelium. To our knowledge, this is the first report of lymphatic vessels forming in the inner ear in a pathological state. Further analysis of its composition and formation process will help us better understand the etiology and pathogenesis of MD.

Materials and Methods

Patients

Between August 2019 and December 2020, a retrospective case-control study was conducted with 21 patients who were diagnosed with definite MD according to the Classification Committee of the Bárány Society[17] and who had consented to labyrinthectomy in Shandong Provincial ENT Hospital. All patients had severe hearing impairment and failed to respond to treatment with betahistine (12 mg, TID) and hydrochlorothiazide (25 mg, BID) for ≥6 months. Among these patients, 2 had accepted sac decompression surgery and 2 had undergone triple-semicircular canal plugging (TSCP) surgery. In summary, since intractable vertigo could not be controlled by pharmaceutical treatment, sac decompression surgery, or TSCP surgery in these patients, labyrinthectomy via a transcanal approach (1 case) or a transmastoid approach (another 20 cases) was performed. The clinical features investigated included age, sex, lesion laterality, duration of disease, presence of Tumarkin drop attacks, hearing loss, vestibular function, and MD-related surgical history. The severity of EH was evaluated by means of delayed Gadolinium-enhanced magnetic resonance imaging (MRI) in all patients.

Before performing a more rigorous assessment, we reviewed 15 patients diagnosed with acoustic neuroma or glomus jugular tumor over the same period of time, 7 of whom had stapes removed as part of the surgical procedure. MRI information showed no enhancement or occupation of the cochlea and vestibule. We compared the findings here reported with results obtained in the 7 patients used as controls. This study was conducted in compliance with the Declaration of Helsinki and was approved by the ethics committee of Shandong Provincial ENT Hospital, Cheeloo College of Medicine, Shandong University (no. XYK2019090813). Written informed consent was obtained from all patients.

Surgery

Case 1 underwent a transcanal labyrinthectomy as previously described.[18] In brief, surgery was performed under general anesthesia. After the outer ear and ear canal were suitably prepared for operation, the ear canal skin was infiltrated with 1% lidocaine with 1:100,000 epinephrine. A tympanomeatal flap was then incised, elevated, and reflected anteriorly. The chorda tympani nerve was preserved in routine fashion. The stapedial tendon was then transected, the incudostapedial joint disarticulated, and then the stapes mobilized and removed. The saccule, utricle, and ampullate ends of the semicircular canals were then removed with the aid of a 3-mm right-angled hook. The tympanomeatal flap was returned to its normal position and held in place with packing. In Case 1, after removal of the footplate of the stapes from the oval window, a pink opaque tissue was noted sealing the oval window and blocking the leakage of perilymph (**Figure 1A**). This membranous structure attaching to the footplate and ring ligament around the oval window was neither a membranous structure of the saccule nor one of the utricle (**Figure 1B**). The soft tissue was then removed from the oval window (Supplemental Video S1, available online) and analyzed with immunochemistry and electron microscopy.

The other 20 cases underwent standard transmastoid labyrinthectomy as described previously.[19] In brief, a postauricular incision was made 1 cm behind the postauricular crease, and the underlying soft tissue was elevated off the mastoid cortex. A simple mastoidectomy was then performed. After exposure and removal of the bony labyrinth, the membranous labyrinth including the saccule, utricle, and ampulla was removed. In order to determine whether the membranous structures under the footplate of the stapes found in Case 1 occur commonly and specifically in MD, we positioned a tympanic mirror

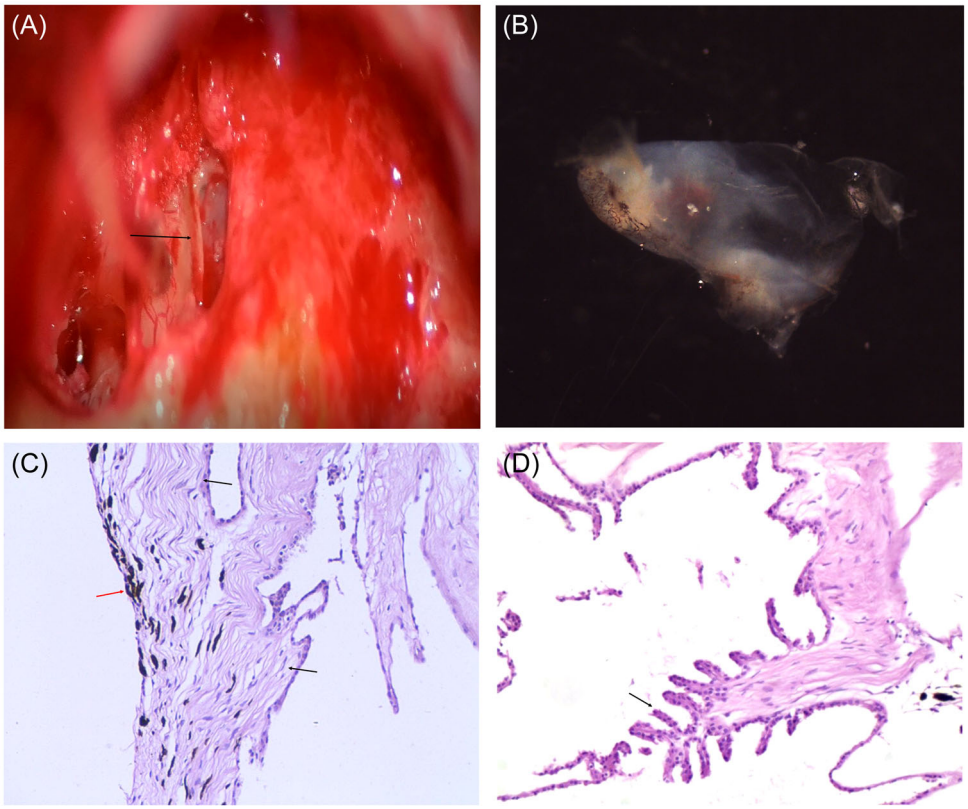

Figure 1. General morphology and hematoxylin and eosin findings. (A) Surgical findings in case 16 who underwent labyrinthectomy. After the footplate of the stapes was removed from the oval window, a pink opaque tissue (arrow) was observed sealing the oval window, without perilymph leakage. (B) Gross appearance of the membranous tissue. (C, D) Hematoxylin and eosin staining of the membranes. The membranous tissue was covered with a simple squamous epithelium (black arrow in C) and displayed proliferating fibrosis (blue arrow in C), papillary hyperplasia (arrows in D), and partial melanin deposition (red arrows in C).

below the floor of the stapes to probe for the presence of a membranous structure during the operation of the other 20 cases. If the soft tissue was found there, it was removed from the oval window and analyzed with immunochemistry and electron microscopy.

Histology and Immunohistochemistry

The specimens were fixed with 10% formalin, dehydrated, made transparent, immersed in wax, and embedded in paraffin. Specimens were sectioned to 4 μm in thickness, stained with hematoxylin-eosin, and observed. Immunohistochemical staining was performed with the EnVision 2-step method,[20] and anti-pan-cytokeratin (pan CK; CM-0641), anti-SOX-10 (SM-0061), anti-S-100 (SM-0011), anti-D2-40 (DM-0011), anti-β-catenin (CR-0101), and anti-smooth muscle actin (SMA; AM-0041) antibodies were purchased from Beijing Zhongshan Jinqiao Biotech Corp (Beijing, China). Pan CK-positive cells were located in the cytoplasm, SOX-10-positive cells in the nucleus; S100-positive cells in the cytoplasm and nucleus, D2-40 positive cells in the cytoplasm and cell membrane, and SMA-positive cells located in the cytoplasm. Positive and negative controls were used in this study. The basic morphology of the membranous tissues was observed under a light microscope (BX53, Olympus). Brown-yellow staining was considered to be positive staining.

Immunofluorescence Staining

The dewaxing and hydration steps for the paraffin sections are the same as those described above. The tissue was treated with 1% TritonX-100 for 20 minutes and blocked with 10% goat serum at room temperature for 1 hour. The primary antibodies were LYVE-1 (1:100, Rabbit, 3107EA18; Invitrogen, Thermo Fisher Scientific), podoplanin (PDPN; 1:100, Rabbit, ab109059; Abcam), PROX1 (1:100, Rabbit, 11-002p; AngioBio). The next day, tissues were incubated with fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit secondary antibodies (1:1000; Invitrogen), and diamidino-phenyl-indole staining at was performed room temperature in darkness for 1 hour. Specimens were imaged on a Leica confocal microscope (Leica).

Transmission Electron Microscopy (TEM)

The tissues were fixed in 2.5% glutaraldehyde, dehydrated in ascending concentrations of ethanol, embedded in Epon resin, and sectioned. Ultrathin sections were used to determine the optimal position for imaging. These ultrathin sections were stained with lead citrate and

uranyl acetate and examined using a JEM-1200 EX electron microscope (JEOL Ltd.).

Scanning Electron Microscopy (SEM)

Tissue samples were immersed in 2.5% glutaraldehyde in 0.1 M of phosphate-buffered saline (PBS; pH = 7.2) overnight at 4°C. Postfixation in 1% OsO_4 in 0.1 M of PBS (pH = 7.2) was then performed for 1 hour. The samples were washed in 0.1 M of PBS (pH = 7.2), dehydrated, critical point-dried, coated with approximately 10 nm of platinum, and examined under a scanning electron microscope (Sigma 300; Zeiss).

Statistical Analysis

Data were analyzed using SPSS v. 17.0 (IBM Corporation). A t test, χ^2 test, nonparametric test, and Fisher exact test were used to analyze the potential associations between the patients' clinical features and the presence of membranous tissue. Statistical significance was set at $P < .05$.

Results

Patient Characteristics

The 21 patients with MD comprised 8 men and 13 women, aged 60.67 ± 7.92 years (range: 49-81 years); 7 were affected on the right side and 14 on the left side. Their 3-tone average threshold at 0.5, 1, and 2 kHz was 68.10 ± 8.14 dB hearing level (HL); their mean speech recognition rate was 18.10 ± 17.05%; and the mean duration of MD was 10.56 ± 16.36 years (range: 2-70 years; **Table 1**). The patients in the control group comprised 3 men and 4 women, aged 59.86 ± 10.00 years (range: 45-76 years); 2 were affected on the right side and 5 on the left side. Their 3-tone average threshold at 0.5, 1, and 2 kHz was 72.86 ± 10.75 dB HL; their mean speech recognition rate was 17.43 ± 19.67%; and the mean duration of control group was 3.00 ± 2.08 years (range: 1-7 years; Supplemental Table S1, available online). There was no significant difference between the control group and the MD group (all $P > .05$).

The 6 patients with MD in whom the novel membranous structure was detected included 1 man and 5 women, aged 61.00 ± 6.32 years (range: 49-68 years); 4 were affected on the left side and 2 on the right side. Their 3-tone average threshold at 0.5, 1, and 2 kHz was 71.67 ± 9.83 dB HL; their mean speech recognition rate was 12.00 ± 11.31%; and the mean duration of MD was 4.17 ± 2.64 years (range: 2-8 years). There were no associations between the patients' clinical features and the presence of the membranous tissue in the MD patient group (**Table 2** and Supplemental Table S2, available online). Of these 6 patients, 5 had no prior surgical treatment but 1 underwent TSCP surgery before labyrinthectomy.

Table 1. Characteristics of the Patients With Meniere Disease

Patient no.	Sex	Age, y	Duration of MD, y	Side	Tumarkin	PTA, dB	SRR, %	UW, %	cVEMP	MRI with gadolinium	MD-related surgical history
1	F	68	2	L	No	90	8	89	abN	None	TSCP
2	F	66	8	L	Yes	65	20	None	abN	Significant	None
3	M	49	3	L	No	65	16	64	abN	Significant	None
4	F	62	2	R	No	70	0	69	abN	Mild	None
5	F	61	7	R	No	65	28	45	N	Significant	None
6	F	60	3	L	No	75	0	4	N	Normal	None
7	M	53	7	R	Yes	75	4	83.7	abN	Significant	None
8	M	49	10	L	No	60	44	74	abN	Significant	Sac decompression
9	M	81	70	L	No	65	48	40	abN	Significant	None
10	M	62	3	R	No	80	0	59	abN	Significant	None
11	M	56	0.5	R	No	60	0	9	N	Normal	None
12	F	74	4	L	No	65	4	34	abN	Mild	None
13	M	56	4	L	Yes	70	24	37.5	N	Significant	None
14	F	66	1	L	No	70	20	51	abN	Significant	None
15	F	57	17	L	Yes	60	48	60	abN	Significant	None
16	F	62	20	L	No	75	4	13.6	abN	Significant	None
17	F	62	2	L	No	60	24	35.9	abN	Mild	None
18	M	61	40	L	No	75	32	None	abN	None	TSCP
19	F	64	1	R	No	70	8	44	abN	Significant	None
20	F	50	11	R	No	60	20	71	N	Significant	None
21	F	55	5	L	No	60	16	51	abN	Significant	Sac decompression

The missing items indicate a lack of a test result due the patients' inability to tolerate the test.

Abbreviations: abN, abnormal; cVEMP, cervical vestibular-evoked myogenic potential; MD, Meniere disease; MRI, magnetic resonance imaging; N, normal; PTA, pure tone average; SRR, speech recognition rate; TSCP, triple semicircular canal plugging; UW, unilateral weakness.

Table 2. Clinical Features of Patients in Whom the Membranous Tissue Was Detected

	Patient no. 1	Patient no. 2	Patient no. 3	Patient no. 4	Patient no. 5	Patient no. 6
Sex	Female	Female	Male	Female	Female	Female
Age, y	68	66	49	62	61	60
Disease duration, y	2	8	3	2	7	3
Side	L	L	L	R	R	L
Pure-tone average, dB	90	65	65	70	65	75
Speech discrimination, %	8	20	16	0	28	0
Caloric asymmetry, %	89	None	84	69	45	4
cVEMP	Abnormal	Abnormal	Abnormal	Abnormal	Normal	Normal
MRI with gadolinium	None	Significant	Significant	Mild	Significant	Normal
Concurrent disease	Hypertension, coronary heart disease	Hypertension	None	Hypertension, diabetes, hyperlipidemia	Removed right kidney because of nephronophthisis 10 years prior	None
MD-related surgical history	TSCP	None	None	None	None	None
Tumarkin	No	Yes	No	No	No	No
Hennebert sign	No	No	No	No	No	No

The missing items indicate a lack of a test result due the patients' inability to tolerate the test.

Abbreviations: cVEMP, cervical vestibular evoked myogenic potential; MD, Meniere disease; MRI, magnetic resonance imaging; TSCP, triple semicircular canal plugging.

Discovery of a Novel Membrane

The pink opaque tissue that was found to be sealing the oval window has not been previously described in studies of either normal or pathological vestibular structures (eg, acoustic neuroma, otosclerosis, cholesteatoma involving the inner ear), and it was detected in 6 of the 21 patients with MD (28.6%) who underwent labyrinthectomy in our study. In the 7 control patients with hearing loss due to vestibular schwannoma (5 cases) or glomus jugular tumor (2 cases) and the other 15 patients with MD, the perilymph flowed out immediately after the stapes was removed, as there was no membranous structure to seal the oval window.

The main pathological features of the morbid membranous structures were found to be simple squamous epithelium-lined fibrous tissue hyperplasia, papillary hyperplasia, or cystic dilation accompanied by melanin deposition, hyalinosis, and calcification (**Figure 1C** and **D**; **Table 3**).

Staining was positive for the epithelial markers pan CK and β-catenin in the morbid membranous structures, as well as for S-100, Sox-10, D2-40, and actin. The positive expression of S-100 and Sox-10 may indicated that this abnormal membrane structure was composed of myoepithelial cells involved in contraction and secretion functions. The positive staining for actin was consistent with the characteristics of myoepithelial tissue. In addition, D2-40 is a specific marker of lymphatic endothelial cells and mesenchymal cells (**Figure 2**; **Table 3**). The positive staining of D2-40 indicated the presence of lymphatic vessels in the MD vestibule.

Confirmation of the Lymphatic Vessel Structure

SEM revealed a very rare surface ultrastructure on the unique morbid membranous structures with a limited number of microvilli and tiny round holes, which was consistent with the findings of TEM (**Figure 3A**). TEM showed that the vestibular sides of the membranous structures were covered with a layer of squamous epithelial cells with a small number of microvilli on its surface. The epithelial cells on the surface of the membranous structures were typical myoepithelial cells with dense bodies and actin filaments (**Figure 3B**), which was consistent with the positive actin labeling. A layer of loose connective tissue with fibroblasts and collagen fibers was detected beneath the basilar membranes of the membranous structures (**Figure 3B** and **C**). Below this layer of loose connective tissue, a network of lymphatic vessels and capillaries was detected (Figure **3D**), which was consistent with the positive D2-40 labeling. The membranous structures facing the side of the footplate of the stapes were covered by a layer of squamous epithelial cells.

Furthermore, we focused on the lymph capillaries in the vestibule. The lymph capillaries are the smallest type of vessel and have an enlarged blind end. Lymph capillaries are composed of a single layer of endothelial cells, which cover each other to form a membrane flap structure. These slender vessels lack a typical basement membrane. No lymphoid cells or macrophages were observed in lymphatic capillaries. The structure of the lymphatic vessels was identified using 3 classic markers of lymphoendothelial cells, including LYVE-1, PDPN, and PROX1 (**Figure 4**). LYVE-1 and PDPN are expressed in lymphatic endothelial cells while PROX1 is mainly expressed in the nucleus of lymphatic endothelial cells. For ease of understanding, a diagram of the lymphatic vessel structure is provided in **Figure 5**, showing the distribution of lymphatic capillaries in membrane tissue.

Discussion

The inner ear has many unique features, and thus it is one of the few organs where in vivo specimens for pathology study are difficult to obtain, which hinders us from obtaining a more in-depth understanding of inner ear diseases. The membranous tissue described in our study is

Table 3. Findings From Hematoxylin and Eosin Staining and Immunohistochemistry

	Patient no. 1	Patient no. 2	Patient no. 3	Patient no. 4	Patient no. 5	Patient no. 6
HE						
Fibrous tissue	+	+	+	+	+	+
Simple squamous epithelium	+	+	+	+	+	+
Papillary hyperplasia	+		+	+	+	+
Cystic dilation	+	+				
Partial melanin deposition			+	+	+	+
Partial calcification			+			
Hyaline degeneration				+	+	+
IHC						
Pan CK	+	+	+	+	+	+
SOX-10	+	+	+	+	+	+
S-100	+		+	+	+	+
D2-40	+	+	+	+	+	+
β-actin				+	+	+
β-catenin	+	+	+	+	+	+

Abbreviations: HE, hematoxylin and eosin; IHC, immunohistochemistry; pan CK, pan-cytokeratin.

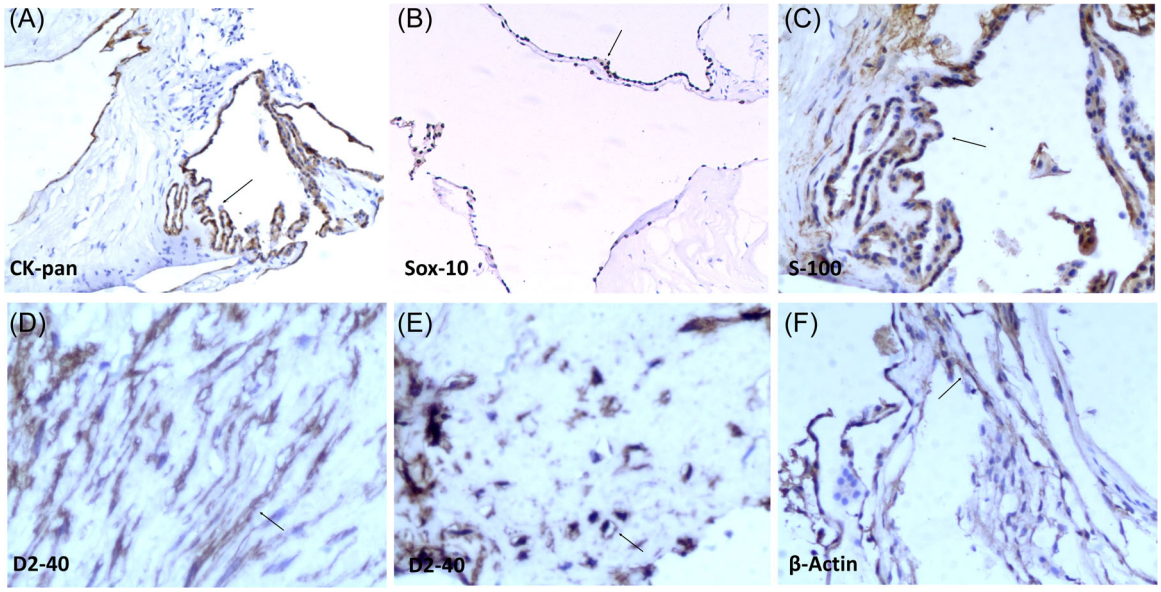

Figure 2. Immunohistochemical staining of the novel membranous tissues. Positive staining for epithelium marker pan-cytokeratin, myoepithelium marker SOX-10, and S-100 (arrows in A-C). The positive staining for actin was characteristic of myoepithelium (arrows in D). Papillary hyperplasia was also observed in A and C. The lymphatic capillary was marked with anti-D2-40 antibodies in cross- and longitudinal sections (arrows in E and F). Magnification: ×10 in A and B, ×20 in C and D, and ×40 in E and F.

a completely novel pathological finding and was observed in almost 30% of our cohort of patients with MD. To the best of our knowledge, this is the first surgical report of membranous tissue beneath the footplate of the stapes in patients with MD. In a previous report of 124 patients with MD, of whom 90 underwent transcanal labyrinthectomy as first indication or as revision surgery, there was no mention of such a finding.[21] Furthermore, our review of studies on the normal anatomy of the human inner ear did not reveal any descriptions of similar tissue in the oval window. Backous et al described a trabecular structure connecting the stapes footplate and the utricle in the temporal bone anatomy,[22] part of the stapes footplate was connected in 130 human specimens. Gacek reported visible fibrosis in the vestibular cistern in 6 of the 9 temporal bones from patients with a documented history of MD. This fibrous tissue was seen to extend from the utricular nerve to the stapes footplate.[23] Michaels and Helquist found that fibrous tissue may be present in cases of Meniere's hydrops external to the endolymphatic space in the scala vestibuli and in the vestibule deep to the footplate of the stapes. It is possible that the foci of connective tissue in these 2 situations are reactions to the irritation produced by repeated distension and subsidence of the adjacent cochlear duct and saccule, respectively.[24] Reissner's membrane can be so distorted that it adheres to the cochlear duct and the stapes footplate when hydrops is present.[10] It was also described in cases of otosclerosis where secondary hydrops attached membranes to the stapes footplate. Although these descriptions bear some resemblance to our findings, they were not followed up by further observation under high resolution, and thus the verification of the composition of this fibrous tissue and comparisons to our findings are impossible to conduct. Importantly, the specimens from these studies were all from autopsies of patients with MD, as biopsies of these patients are rarely sent for histology. Therefore, our report constitutes the first detailed description of substapedial membrane, with formation of capillary lymphatic structures, obtained from MD biopsies.

To determine whether such substapedial membrane occurs specifically in MD, we also removed the stapes in additional control patients with hearing loss due to acoustic neuroma or glomus jugular tumor. In these patients, the perilymph flowed out immediately after the stapes were removed because there was no membranous structure to seal the oval window. These findings prompted us to also review previous surgical records of patients treated at our institution with primary stapes surgery, and we did not found any reference to a similar finding. Therefore, we believe that the membranous structure is a pathological alteration specific to MD. Moreover, the population of patients in this study in whom the membrane was detected (n = 6) had substantial heterogeneity, including in age range (49-68 years) and duration of MD-related symptoms prior to surgery (2-8 years). Nonetheless, the membranous tissue histopathology was remarkably similar among these 6 patients. The relationship between membranous structure and clinical presentation remains unclear.

Histological studies and ultrastructure observations provided a fundamental understanding of the potential function of this membranous tissue. Notably, lymphatic capillaries are one of the components of the membranous

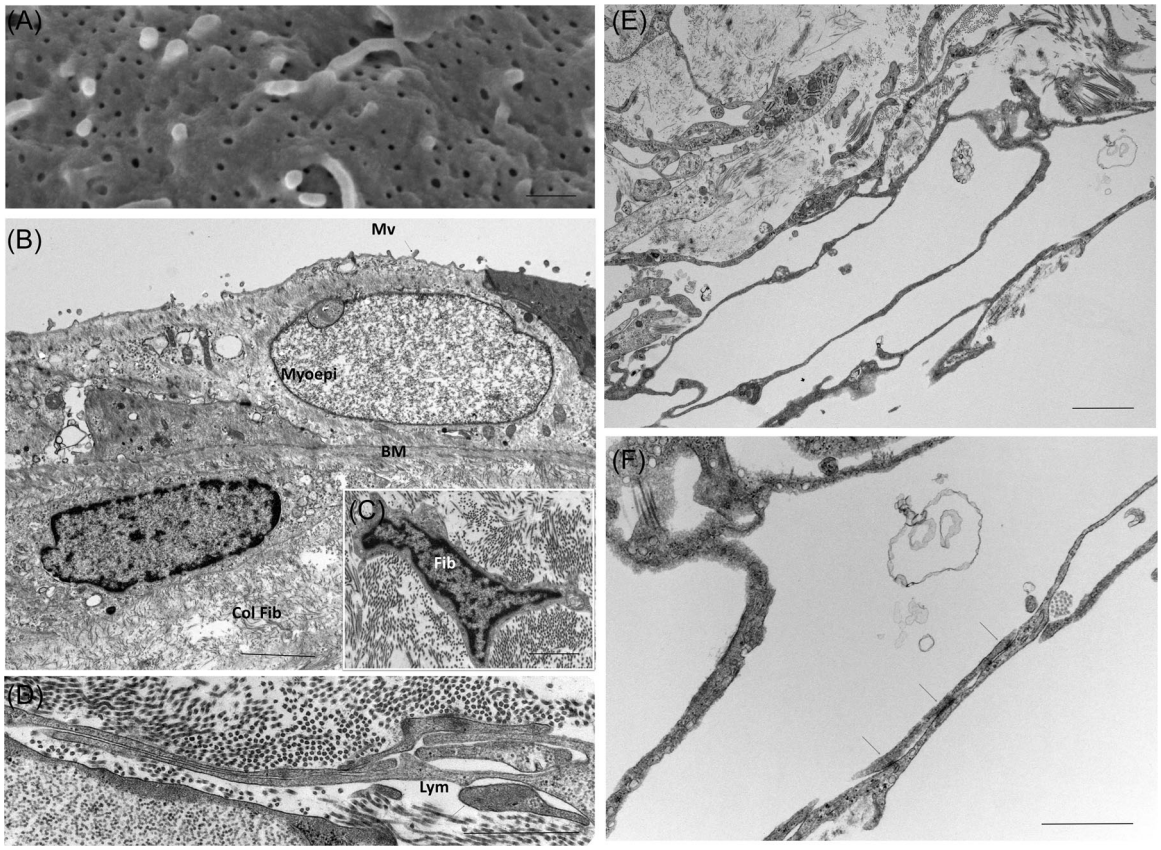

Figure 3. Ultrastructure of the membranous tissue. The membrane tissue was covered by a layer of squamous epithelial cells, approximately 15-μm thick, on the vestibular side with a limited number of microvilli on the surface. Connective structures were observed between adjacent cells. A continuous basement membrane was observed at the bottom of the cells. Some epithelial cells were typical myoepithelial cells with dense bodies and actin filaments (red arrows in B). A layer of loose connective tissue, approximately 40-μm thick, was observed under the basement membrane, with fibroblasts (C) and collagen fibers distributed throughout. A lymphatic capillary network (approximately 30-μm thick) appeared below. The lymph capillaries lacked a typical basement membrane and had a blind end (D). Lymph capillaries were composed of a single layer of endothelial cells (E), with valval connection structures between adjacent capillary epithelial cells (red arrows in F). Scale bar: 200 nm in A; 2 μm in B, C, and E; and 1 μm in D and F. BM, basement membrane; Col Fib, collagenous fibers; Fib, fibroblast cells; Lym, lymphatic capillary; Mv, microvilli; Myo, myoepithelial cell.

structure. To our knowledge, no studies on lymphatic vessels in the inner ear have been conducted. Our study confirmed the presence of lymphatic capillaries in the inner ear of patients with MD although it is not clear that the removed tissue is lymphatic in origin. Traditionally, the inner ear, as well as the brain, is considered to be an immune-privileged organ with no lymphatic vessels or lymphatic drainage. Lymphatic vessels are plastic and can form under a number of pathological conditions, such as inflammation, tissue repair, and tumor growth. Recent studies have found that lymphatic vessels exist in the dura mater, which is related to the incidence of multiple sclerosis and Alzheimer's disease, and participate in cerebrospinal fluid circulation and immune cell transport; thus, they may be a potential target for disease intervention and treatment. Given that EH is a common pathological manifestation in patients with MD, the formation of lymphatic vessels may result from compensatory hyperplasia of EH. In addition, as myoepithelial cells mediate secretion and absorption, the presence of microvilli implies anatomical characteristics that could allow for the passage of substances and thus suggest that the tissue has the capacity for absorption. Previous anatomical studies have demonstrated the presence of microvilli in the outer epithelium of the round window membrane. These microvilli participate in the secretion and/or absorption of substances traveling between the inner and middle ear.[25] These anatomical features of secretion and absorption, together with the function of lymphatic capillaries, imply that the membranous tissue may be a pathological response to EH in the inner ear.[16] On the other hand, recent studies have found that aseptic inflammation of the inner ear of MD is involved in the pathogenesis and pathological hydrops of MD.[26,27] Membrane tissue may also be a reactive product of long-term chronic inflammation in the inner ear.

Interestingly, not all patients with MD had this membrane-like structure. Of the 21 patients enrolled in this study, the tissue was observed in only 6 (28.6%).

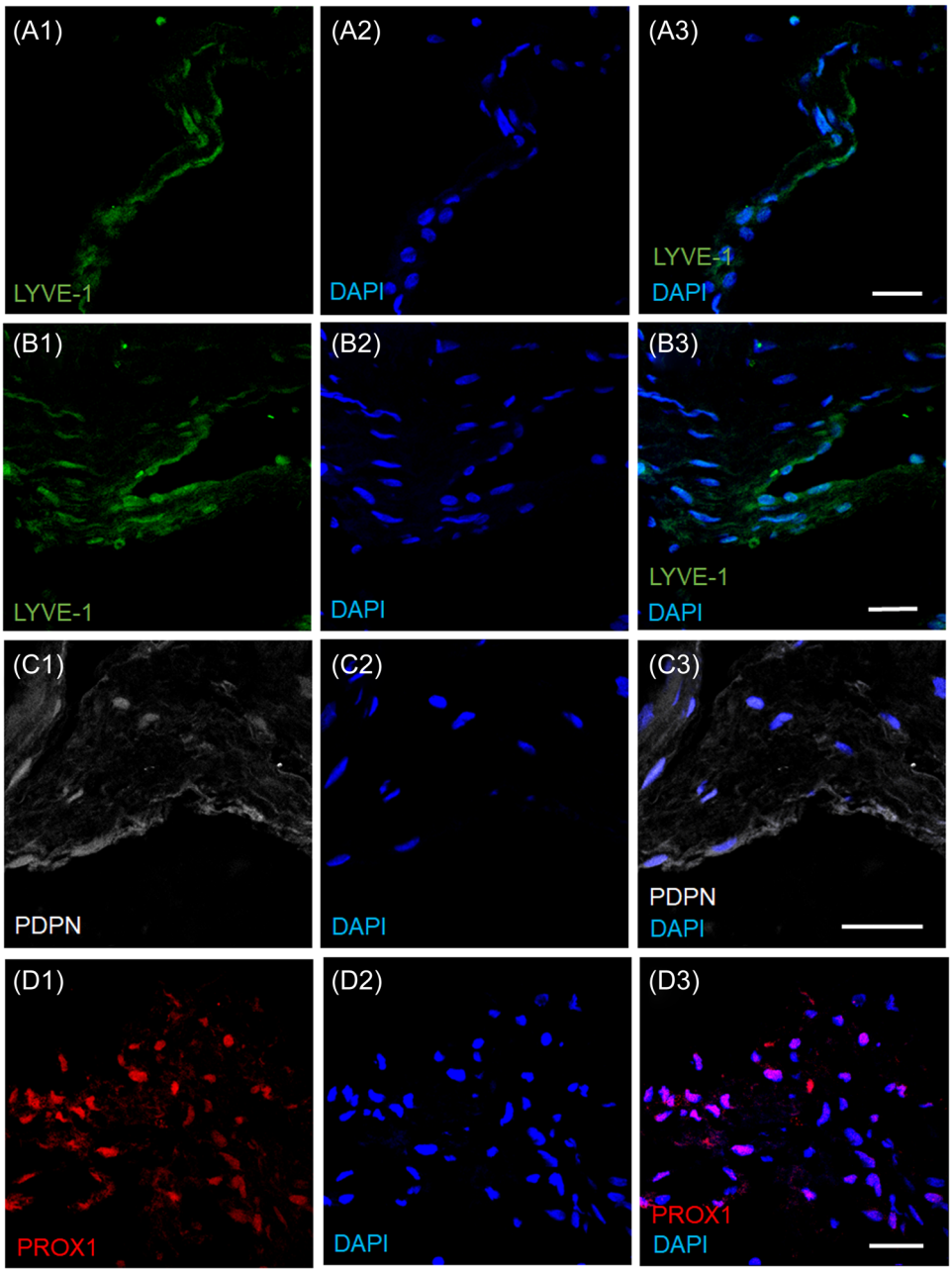

Figure 4. Identification of lymphatic vessel by immunofluorescence staining. The structure of lymphatic vessels was identified using 3 classic markers of lymphoendothelial cells, including LYVE-1 (green in A and B), podoplanin (PDPN; white in C), and PROX1 (red in D). The nuclei were stained with diamidino-phenyl-indole (DAPI) (blue). LYVE-1 and PDPN were expressed in lymphatic endothelial cells while PROX1 was mainly expressed in the lymphatic endothelial cell nucleus. Bars in B and C = 25 μm.

We also found no associations between the patients' clinical features and the presence of the membranous tissue. In addition, we found the substapedial membrane in 1 of the 6 patients that were previously treated with TSCP surgery. The prior TSCP can cause an inflammatory reaction in the labyrinth which needs to be noted. However, membrane tissue was found in the other 5 cases without any prior otologic surgery, and we speculate that substapedial membrane formation is a natural process implicated both in chronic inflammation and pressure- and flow-dependent pathological reaction, which cannot be well explained only by a reactive fibroblast, myofibroblast, or granulation tissue formation arising from intralabyrinthine intervention. The potential mechanism of development of this structure needs to be studied further and may aid in clarifying the pathogenesis of MD.

In summary, the morphological evidence was presented for the first time. The mechanism of the formation of lymphatic capillaries in the vestibule of MD is still unclear. We speculate that the formation of lymphatic

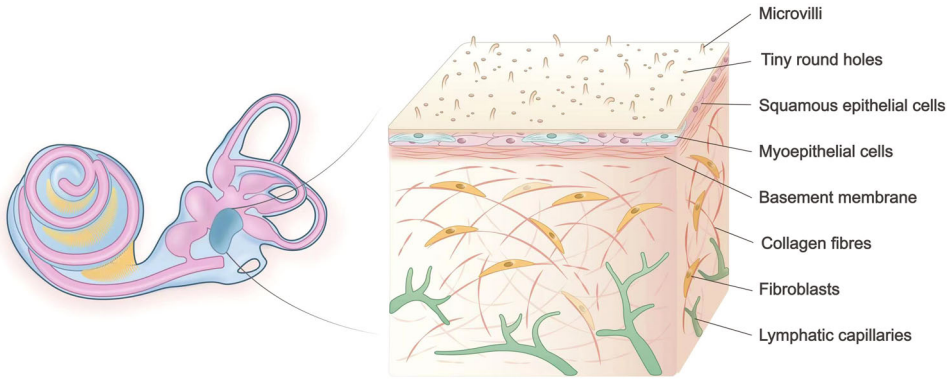

Figure 5. Schematic diagram.

capillaries in the vestibule of MD may develop as a result of an imbalance in the lymphatic circulation and a decompensation of pressure in the inner ear, as well as a chronic immunoinflammation in the labyrinth of the inner ear. This newly discovered membrane composed of lymphatic capillaries in patients with MD help improve our understanding of MD.

A limitation of this study includes a lack of large-sample data. The correlation between this pathological structure and the etiology of MD needs to be confirmed by further analysis. More cases are needed and that more tissue needs to be evaluated.

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Author Contributions

Daogong Zhang, performed the surgeries and wrote the manuscript; **Xiaofei Li**, did the immunofluorescence, created the tables, figures and wrote the manuscript; **Yafeng Lv**, managed the patients and collected the data; **Yongdong Song**, presented the TEM and SEM data; **Ligang Kong**, managed the patients and collected the data; **Boqin Li**, the TEM and SEM data; **Jinfeng Zheng**, completed the observations and evaluations of immunohistochemistry and hematoxylin and eosin staining; **Nicolas Pérez-Fernández**, revised the manuscript and interpreted the data; **Zhaomin Fan**, performed the surgeries and wrote the manuscript; **Haibo Wang**, conceived the study and drafted the manuscript.

Disclosures

Competing interests: The authors have no conflicts of interest to declare.

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Data Availability Statement

The data sets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Supplemental Material

Additional supporting information is available in the online version of the article.

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