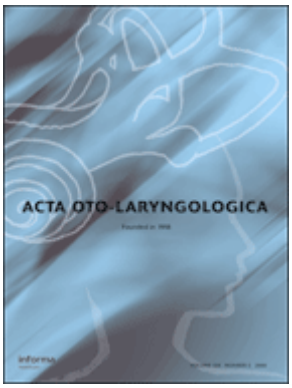


Evaluation of Blood-Perilymph Barrier in Ears with Endolymphatic Hydrops

Journal:	*Acta Oto-Laryngologica*
Manuscript ID	SOTO-2021-0322
Manuscript Type:	Regular
Classification Scheme:	inner ear
Keywords:	MRI, endolymphatic hydrops, signal intensity

Evaluation of the blood–perilymph barrier in ears with endolymphatic hydrops

Running head: Blood–perilymph barrier and endolymphatic hydrops

Address for correspondence:

Abstract

Background: Otological diseases including Meniere's disease (MD) involve endolymphatic hydrops (EH), which can be visualized by magnetic resonance imaging (MRI) with gadolinium contrast agents, but the temporal changes of contrast in the inner ear have not been evaluated.

Objectives: We investigated the permeability of the blood–perilymph barrier (BPB) in ears with EH to evaluate the severity of the inner ear disturbances.

Materials and methods: The study included 32 ears from 16 patients with EH or related diseases who underwent MRI. The permeability of the BPB was assessed by the signal–intensity ratio (SIR) at four time points: before and at 10 minutes, 4 hours, and 24 hours after administration of gadolinium for assessing EH.

Results: Cochlear EH was found in 25 of the 32 ears, and vestibular EH in 11. The rate of EH was significantly higher in symptomatic ears; however, the existence of EH was not related to SIR values. Nevertheless, SIR values in the basal turn were significantly higher 4 and 24 hours after injection of gadolinium in patients aged ≥50 years.

Conclusion and significance: Higher SIR values observed in older patients with EH indicate severe disturbances of the BPB in the cochlea, which may account for intractable inner ear disturbances in older patients.

Key words: MRI—endolymphatic hydrops—signal intensity.

Introduction

Meniere's disease (MD) is related to pathological endolymphatic hydrops (EH) of the inner ear. Gadolinium (Gd)-enhanced magnetic resonance imaging (MRI) is used to visualize EH in living humans [1,2]. Intravenously injected Gd slowly enters the

perilymphatic space but not the endolymphatic space and the difference in concentration between these two spaces is used to identify EH [3].

The mechanism responsible for the barrier function in the inner ear as provided by the blood–perilymph barrier (BPB) can be explained as follows. The maintenance of inner ear fluid homeostasis provides a barrier function between the vascular system and the inner ear fluid. Morphologically similar to cells of the blood–brain barrier (BBB), endothelial cells in the BPB lack fenestrations and their lumen is sealed with tight junctions and adherens junctions. Pericytes and perivascular resident macrophage-like melanocytes are in intimate contact with endothelial cells and add multiple basement membrane layers, which tightly regulate the exchange between the blood and interstitial fluid [4].

Recent data provide direct evidence of the impaired function of the BPB in MD. MD is associated with deteriorating BPB, as evaluated with MRI postcontrast measurements of signal intensity. Specific ultrastructural changes in capillaries constituting the BPB have been identified in utricles removed during surgery from patients with MD [4]. The increased prevalence and severity of EH with the duration of MD indicate that hydrops is a progressive degenerative phenomenon. Frequent abnormalities in the vestibule and cochlea have been noted in some histopathological investigations [5].

The mechanism of MD attacks is unknown. Foster et al. [6] reported that the unique characteristics of the attacks can be explained by the differential sensitivity of the inner ear tissues to transient ischaemia. In such attacks, the sensory tissues are vulnerable to hours-long ischaemia or reperfusion injury, and the stria vascularis is vulnerable to ischaemia because of its high metabolic rate. Foster et al. also noted an association

between EH and vascular risk factors for intracerebral ischaemia. Therefore, changes in vascular permeability of the inner ear may be closely involved in MD attacks and the development of EH. To evaluate the role in MD attacks of BPB permeability and the severity of the inner ear disturbances, we examined the signal intensity and contrast effect in multiple MRI images of the inner ear.

Methods

Patients

A total of 32 ears in 16 patients diagnosed with MD or EH-related diseases who underwent otological examination and MRI were recruited to the study between 2018 and 2020. The diagnoses of patients with MD were based on the new international consensus diagnostic criteria for MD [7].

Pure-tone audiometry

Pure-tone audiometry was performed before the MRI study using an audiometer (Model AA-78; Rion, Tokyo, Japan) in a sound-insulated chamber. If the patient did not respond to the maximum level of sound, 5 dB was added to each threshold. The average hearing level was expressed as the average score at three frequencies (500, 1,000, and 2,000 Hz).

MRI

MRI evaluation used a 3-Tesla scanner (Magnetom Skyra; Siemens, Erlangen, Germany) equipped with a receive-only, 32-channel, phased-array coil. MRI was performed before, and 10 minutes, 4 hours, and 24 hours after intravenous administration of a standard dose of Gd hydrate (gadobutrol 0.1 mmol/kg; Gadovist; Bayer AG, Leverkusen, Germany). All patients underwent heavily T_2-weighted (hT2W)

MR cisternography (MRC) for anatomical reference of the fluid space and hT2W-three-dimensional (3D) fluid-attenuated inversion recovery MRI (3D-FLAIR) for evaluation of labyrinthine fluid alterations in 7 minutes. The inversion time (T_1) was 2250 ms after MRC images using a variable flip angle 3D turbo spin–echo technique, sampling perfection with application-optimized contrasts using different flip angle evolutions (SPACE) and the repetition time was 9000 ms. The presence of EH was investigated using a hybrid of a reversed image of the positive endolymph signal and native image of the positive perilymph signal, a hybrid of the reversed image of MRC, positive perilymph signal by heavily T_2-weighted (hT_2W) 3D-FLAIR, and 3D-real inversion recovery (IR) sequences. The detailed parameters of 3D-real IR were reported previously [8].

At least two radiologists with more than 20 years of experience who were blinded to the clinical symptoms classified the degree of EH in the cochlea and vestibule into one of three groups: none, mild, or significant, according to previously described criteria [9]. Example images of EH in the cochlea are shown in Figure 1.

Evaluation of Gd contrast effects

The contrast effects on the cochlear and vestibular fluid were evaluated semi-quantitatively, as reported previously in patients with sudden deafness [10]. The signal–intensity ratio (SIR) was measured three times, and the average SIR value was calculated for each ear. The signal intensities in the basal turn of each affected and unaffected cochlea were quantified as follows. Morphological distortion of the scala tympani because of enlargement of the scala media (EH) is less than that of the scala vestibuli. Contouring of the scala tympani in the basal turn of the cochlea is easy and stable regardless of the degree of EH. Therefore, measurements of the lymph fluid

signal were performed for the scala tympani in the basal turn of the cochlea. For the scala tympani region of interest (ROI), the slice was selected at least 3 slices below the centre slice on which the cochlear modiolus appeared largest. For quantitative evaluation, the SIR between the signals of the scala tympani and cerebellum was measured on 3D-real IR images. The ROI for the cerebellum was set as a circle with a diameter of 10 mm in the ipsilateral cerebellar white matter on the MRC images. The ROI for the scala tympani was drawn manually on the MRC images. ROIs were copied and pasted onto 3D-real IR images (Figure 2). Using the signal value of the cerebellar hemisphere as a control, each SIR was calculated as the signal intensity of the ROI/signal intensity of the cerebellar hemisphere [10]. The results of simple SIR measurements have been reported to correlate well with those based on a quantitative method [8]. Gd excretion rate was calculated using (SIR 4 hours–SIR 24 hours)/SIR 4 hours.

Ethics review

The study was approved by the Ethics Review Committee of ******* (No. 2018–0218).

Statistical analysis

IBM SPSS Statistics software (version 27, IBM Corp., Armonk, NY) was used for statistical analyses. The Mann–Whitney *U* test, Fisher's exact test, and one-way analysis of variance followed by the post hoc Bonferroni test were used, as appropriate, for comparisons. The significance level was set at 5%.

Results

The study population included 6 women and 10 men (average age 50.2 years; range 26–76 years) (Table 1). The diagnoses of the 16 patients were unilateral definite MD (*n* = 3), probable MD (*n* = 1), delayed EH (*n* = 4) and other (*n* = 8). Case 7 was diagnosed as bilateral definite MD. Bilateral MD was defined as confirmed MD in one ear, with the opposite ear having inner-ear symptoms combined with documented hearing loss. The patients categorized as having the ‘‘other’’ diagnosis did not meet the new criteria because of the duration of episodes of vertigo and the level of their pure-tone thresholds. However, they were diagnosed with probable or possible MD according to the 1995 American Academy of Otolaryngology-Head and Neck Surgery guidelines [11].

EH was present in the cochlea in 30 of 32 ears (94%), and significant EH was present in 25 of 32 ears (78%). EH was present in the vestibule in 15 of 32 ears (47%), and significant EH was present in 11 of 32 ears (34%). Table 2 shows the distribution of ears with EH in the cochlea and vestibule. The degree of EH was significant in both the cochlea and vestibule in the symptomatic ear. The average duration of disease in all cases was 103.1 months (range 0–360 months). The degree of EH did not differ significantly according to age and duration of disease (Figure 3A and B).

The changes in the SIR values for the cochlear basal turn, before, immediately after, and 4 hours and 24 hours after intravenous Gd injection are shown in Figure 4A–C. Figure 4A shows the results for patients aged < 50 years versus ≥ 50 years. Figure 4B shows the data for the patients grouped according to the presence or absence of EH. Figure 4C shows the data for the patients grouped according to the average hearing level, <40 dB and ≥40 dB. The highest SIR values in all groups were obtained 4 hours

after contrast. The SIR value was significantly higher in the older group at 4 hours (mean, 29.2 vs 15.9; $p < 0.001$) and 24 hours (mean, 19.2 vs 9.0; $p < 0.001$) after contrast (Figure 4A). The excretion rate was calculated as 43.2% in patients aged <50 years and 34.4% in those aged ≥50 years, 42.7% in the absence of EH and 37.0% in the presence of EH, 36.7% in those with average hearing level <40 dB and 38.0% in those with ≥40 dB, respectively. No significant differences were found for the other comparisons.

Discussion

This is the first study to compare the inner ear findings obtained using enhanced MRI at four time points up to 24 hours after Gd administration. We found that patients aged ≥50 years with EH in the cochlea and vestibule showed greater vascular permeability of the BPB than younger patients. The excretion rate of contrast media did not differ significantly between older and younger patients. We found no relationship between SIR values and the presence of EH or hearing level.

In animal studies, inflammation produces changes in the BPB that lead to increased vascular permeability. It is thought that a compromised BPB would lead to hearing impairment through the loss of the endocochlear potential [12]. The BBB limits entry of blood-derived products, pathogens, and cells into the brain, and the BPB functions similarly in the inner ear. BBB breakdown is an early event in the aging human brain that begins in the hippocampus and may contribute to cognitive impairment [13]. BBB permeability, expressed as a ratio of infarct permeability to contralateral permeability, is greatest at 6–48 hours after the onset of acute ischaemic stroke. These findings suggest that the permeability of the BBB is elevated continuously following acute ischaemic

stroke. Moreover, the 'glymphatic system' is hypothesized to be a waste clearance system of the cerebrospinal fluid through the perivascular and interstitial spaces in the brain. MRI has also been used to evaluate glymphatic function primarily using Gd-based contrast agents as tracers [14]. Glaucoma, normal pressure hydrocephalus, and MD are consistent in their disturbance of fluid homeostasis [15]. In addition, both glaucoma and normal-pressure hydrocephalus are presumed to be dysfunctions of the glymphatic system, which is a waste excretion mechanism [14]. Therefore, MD may be caused not only by abnormalities in the BPB but also by abnormalities in the waste excretion mechanism of the inner ear. This study demonstrated that the excretion rate was delayed in the EH group and the group >50 years old, by comparing 4 and 24 hours post-contrast. Our results suggest that assessing the dysfunction of excretory function is the key to the discovery of new biomarkers for MD.

Similar to these findings in the BBB, it is also possible that vascular permeability increases with age in the BPB in the inner ear. The function of the BPB has been reported. The rate of transfer of some medicines, such as gentamicin (molecular weight, 477.6) from the blood into the perilymph is faster than from the blood into the endolymph. The rate of Gd-diethylenetriamine pentaacetic acid penetration into the BPB is slow, with a peak concentration occurring 4 hours after administration, which suggests the presence of a tight barrier between the perilymph and blood [3]. In healthy people, cochlear fluid is enhanced most intensely 4 hours after the injection [3]. The optimal timing of contrast enhancement in patients with suspected MD remains unclear, but an evaluation of EH is possible 3.5–4.5 hours after contrast administration [8].

The SIR in the cochlea and brainstem or cerebellar hemisphere has been used to evaluate the BPB. The SIR increases more in symptomatic ears than in asymptomatic

ears at 4 hours after intravenous Gd injection used in combination with hT2W-3D-FLAIR MRI [16]. Gd movement into the inner ear from the blood circulation is greater in hydropic ears than in normal ears [17]. A recent study showed that the combination of perilymphatic enhancement and EH in patients suspected of having MD increases the positive predictive value for the definite diagnosis of MD [18].

In this study, SIR values did not differ significantly between ears with and without EH. One limitation of this study is that we focused on the presence or absence of EH in ears with various types of otological diseases. Okazaki et al. [19] observed cochlear and vestibular EH in 66% and 41% of affected ears with sudden sensorineural hearing loss (SSNHL), respectively. In that study, cochlear and vestibular EH were also detected in 52% and 38% of the unaffected ears with SSNHL, respectively. These findings were deemed to indicate secondary EH. We found that the degree of EH was greater in symptomatic ears. However, EH alone is insufficient to cause symptomatic MD and vascular risk factors should be studied as possible cofactors [6]. Older people have a higher risk of vascular disease, which also increases their risk of developing inner ear diseases, including SSNHL [20].

The present study revealed a difference in vascular permeability at the BPB between older and younger patients with EH. Repeated evaluations using MRI are not applied to all patients with MD, and the number of patients enrolled in the study was small. However, such evaluation using enhanced MRI may provide important information for elucidating the role of the BPB in the pathophysiology of MD.

A limitation of this study is that we included only patients with EH. It would be more interesting to include a comparison with a control group.

In conclusion, higher SIR values were observed in older patients with EH and the excretion rate was delayed in older patients and in ears with EH. A detailed understanding of the signals that indicate a healthy BPB and the factors that promote fluctuations in BPB permeability in disease states will be key to elucidating the disease mechanisms and to identifying potential targets for diagnostics and therapeutic modulation of BPB.

Disclosure statement

The authors have no funding, financial relationships or conflicts of interest to disclose.

References

[1] Nakashima T, Pyykkö I, Arroll MA, et al. Ménière's disease. Nat Rev Dis Primers. 2016;2:16028.

[2] Nakashima T, Naganawa S, Sugiura M, et al. Visualization of endolymphatic hydrops in patients with Ménière's disease. Laryngoscope. 2007;117:415–420.

[3] Naganawa S, Komada T, Fukatsu H, et al. Observation of contrast enhancement in the cochlear fluid space of healthy subjects using a 3D-FLAIR sequence at 3 Tesla. Eur Radiol. 2006;16:733–737.

[4] Glueckert R, Chacko LJ, Rask-Andersen H, et al. Anatomical basis of drug delivery to the inner ear. Hear Res. 2018;368:1027.

[5] Fiorino F, Pizzini FB, Beltramello A, et al. Progression of endolymphatic hydrops in Ménière's disease as evaluated by magnetic resonance imaging. Otol Neurotol. 2011;32:1152–1157.

[6] Foster CA, Breeze RE. The Ménière attack: An ischemia/reperfusion disorder of inner ear sensory tissues. Med Hypotheses. 2013;81:1108–1115.

[7] Lopez-Escamez JA, Carey J, Chung W-H, et al. Diagnostic criteria for Ménière's disease. J Vestib Res. 2015;25:1–7.

[8] Naganawa S, Yamazaki M, Kawai H, et al. Visualization of endolymphatic hydrops in Ménière's disease with single-dose intravenous gadolinium-based contrast media using heavily T_2-weighted 3D-FLAIR. Magn Reson Med Sci. 2010;9:237–242.

[9] Nakashima T, Naganawa S, Pyykko I, et al. Grading of endolymphatic hydrops using magnetic resonance imaging. Acta Otolaryngol Suppl. 2009:5–8.

[10] Yang C-J, Yoshida T, Sugimoto S, et al. Lesion-specific prognosis by magnetic resonance imaging in sudden sensorineural hearing loss. Acta Otolaryngol. 2021;141:5–9.

[11] Committee on Hearing and Equilibrium guidelines for the diagnosis and evaluation of therapy in Ménière's disease. American Academy of Otolaryngology–Head and Neck Foundation, Inc. Otolaryngol Head Neck Surg. 1995;113:181–185.

[12] Hirose K, Li SZ. The role of monocytes and macrophages in the dynamic permeability of the blood–perilymph barrier. Hear Res. 2019;374:49–57.

[13] Montagne A, Barnes SR, Sweeney MD, et al. Blood–brain barrier breakdown in the aging human hippocampus. Neuron. 2015;85:296–302.

[14] Taoka T, Naganawa S. Glymphatic imaging using MRI. J Magn Reson Imaging. 2020;51:11–24.

[15] Nakashima T, Sone M, Teranishi M, et al. A perspective from magnetic resonance imaging findings of the inner ear: Relationships among cerebrospinal, ocular and inner ear fluids. Auris Nasus Larynx. 2012;39:345–355.

[16] Sano R, Teranishi M, Yamazaki M, et al. Contrast enhancement of the inner ear in magnetic resonance images taken at 10 minutes or 4 hours after intravenous gadolinium injection. Acta Otolaryngol. 2012;132:241–246.

[17] Tagaya M, Yamazaki M, Teranishi M, et al. Endolymphatic hydrops and blood–labyrinth barrier in Ménière's disease. Acta Otolaryngol. 2011;131:474–479.

[18] van Steekelenburg JM, van Weijnen A, de Pont LMH, et al. Value of endolymphatic hydrops and perilymph signal intensity in suspected Ménière disease. Am J Neuroradiol. 2020;41:529–534.

[19] Okazaki Y, Yoshida T, Sugimoto S, et al. Significance of endolymphatic hydrops in ears with unilateral sensorineural hearing loss. Otol Neurotol. 2017;38:1076–1080.

[20] Yoshida T, Sone M, Kitoh R, et al. Idiopathic sudden sensorineural hearing loss and acute low-tone sensorineural hearing loss: A comparison of the results of a nationwide epidemiological survey in Japan. Acta Otolaryngol. 2017;137:S38–S43.

Figure legends

Figure 1. Example images of endolymphatic hydrops (EH) in the cochlea. Images were obtained using a hybrid of the reversed image of the positive endolymph signal and the native image of the positive perilymph signal and acquired 4 hours after intravenous administration of single-dose gadolinium-based contrast material. The endolymphatic space is observed as the black area. Right, significant EH in the cochlea (arrows). Left, no EH in the cochlea.

Figure 2. Example images for the calculation of the region of interest (ROI) on 3D-real inversion recovery (IR) sequences. We drew the shape of each basal turn of the cochlea for the ROI on the SPACE sequence, then copied the shapes to draw the ROI on 3D-real IR sequences (dotted line). (*A*) before enhancement, (*B*) 10 minutes after, and (*C*) 4 hours and (*D*) 24 hours after enhancement using the signal value of the cerebellar hemisphere in the drawn circle as a control (*E*). The image was obtained from a patient with significant endolymphatic hydrops. SPACE, sampling perfection with application-optimized contrasts using different flip angle evolutions.

Figure 3. (*A*) Comparison of the degree of EH in the cochlea according to age. (*B*) Comparison of the degree of EH in the cochlea according to the duration of the disease.

Figure 4. Changes in mean SIR values of the cochlear basal turn before, immediately after, and 4 hours and 24 hours after intravenous gadolinium injection. SIR was measured three times, and the average SIR value was calculated for each ear. The highest SIR values in all groups were obtained 4 hours after contrast. (*A*) Data are presented according to age group <50 years and ≥50 years. (*B*) Data are presented according to the presence or absence of endolymphatic hydrops. (*C*) Data are presented according to the average hearing level <40 dB and ≥40 dB. PTA, pure-tone audiometry.

Table 1. Patients' clinical characteristics.

						EH		SIR			
Case no.	Gender	Age(years)	Side	diagnosis	Average Hearing level (dB)	Cochlea	Vestibule	4 h	24 h	Excretion rate (%)	Time from onset of symptoms(months)
1	M	27	Right	–	20.0	significant	no	6.6	3.4	48.6	–
			Left	DEH	66.7	significant	significant	10.9	4.6	57.5	168
2	M	37	Right	–	8.3	significant	no	15.0	7.4	50.9	–
			Left	Definite MD	46.7	significant	significant	26.8	8.4	68.5	156
3	M	37	Right	DEH	68.3	significant	significant	21.3	16.3	23.3	72
			Left	–	10.0	mild	no	28.2	26.0	7.9	–
4	F	76	Right	Fluctuated HL	66.7	significant	significant	25.1	12.0	52.1	36
			Left	–	10.0	no	no	32.5	12.4	61.9	–
5	M	35	Right	SNHL	105.0	significant	no	8.9	4.3	52.3	132
			Left	Fluctuated HL	61.7	significant	no	17.3	9.4	45.8	132
6	M	67	Right	Probable MD	41.7	significant	mild	26.2	18.0	31.3	204
			Left	–	8.3	no	no	24.8	20.4	17.6	–
7	M	55	Right	Definite MD	70.0	significant	significant	43.2	17.2	60.1	84
			Left	Definite MD	65.0	significant	significant	44.5	29.8	33.2	84
8	M	26	Right	Fluctuated HL	11.7	significant	no	12.8	5.5	56.8	36
			Left	Fluctuated HL	20.0	significant	no	12.7	5.2	59.1	1
9	F	63	Right	Fluctuated HL	70.0	significant	no	17.0	16.9	0.5	300
			Left	Fluctuated HL	45.0	mild	no	17.8	24.7	-38.5	1
10	M	56	Right	DEH	28.3	significant	significant	26.4	19.6	25.8	16
			Left	SNHL	86.7	significant	mild	43.8	29.8	32.0	300
11	F	32	Right	–	8.3	significant	no	11.2	5.8	48.6	–
			Left	Definite MD	30.0	significant	significant	15.3	4.9	68.0	6
12	F	72	Right	Fluctuated HL	48.3	mild	no	23.6	16.8	29.0	15
			Left	SNHL	86.7	significant	significant	39.1	21.8	44.2	96
13	F	75	Right	–	18.3	mild	no	29.2	23.7	18.8	–
			Left	Fluctuated HL	51.7	significant	no	28.2	18.8	33.3	10
14	M	44	Right	Fluctuated HL	16.7	significant	significant	17.3	13.5	22.0	24
			Left	Fluctuated HL	15.0	significant	mild	14.9	12.6	15.0	0
15	F	40	Right	SNHL	103.3	significant	no	17.6	10.1	42.9	240
			Left	Fluctuated HL	6.7	significant	mild	17.0	6.9	59.3	1
16	M	61	Right	DEH	56.7	significant	significant	24.0	11.1	53.7	360
			Left	–	21.7	mild	no	22.0	13.7	37.8	–

EH: Endolymphatic hydrops; SIR: signal intensity ratio; DEH: delayed endolymphatic hydrops; MD: Meniere's disease; HL: hearing loss; SNHL: sensorineural hearing loss

Excertion rate=(SIR 4h - 24 h)/SIR 4h

TABLE 2. Numbers and severity of EH in symptomatic and asymptomatic ears

	Cochlea			Vestibule		
Symptoms	Significant	Mild	None	Significant	Mild	None
Yes	21*	2	0	11*	3	9
No	4	3	2*	0	1	8*

EH: endolymphatic hydrops; symptoms: vestibular or cochlear symptom-related EH

*$p < 0.05$ with versus (yes) without (no) symptoms (Fisher's exact test).

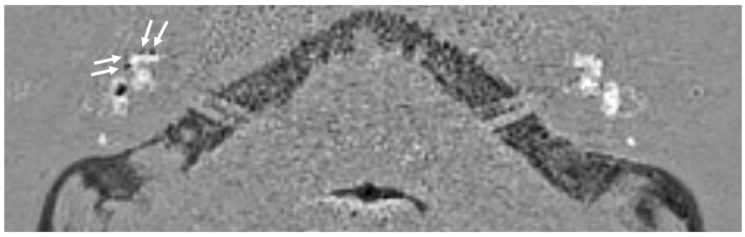


Figure 1

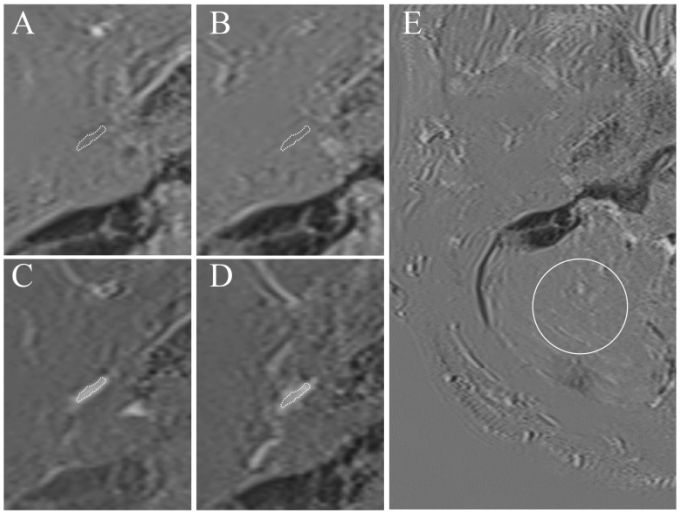
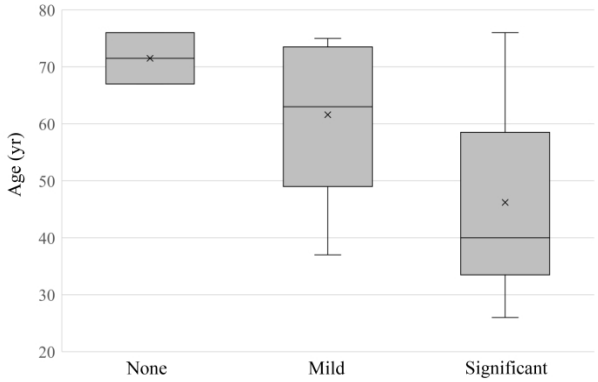
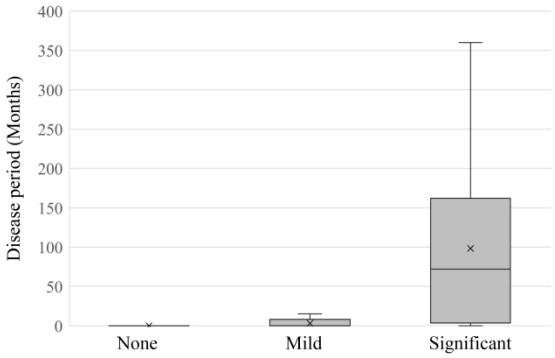


Figure 2



A

Figure 3a


Disease period (Months)
400
350
300
250
200
150
100
50
0
None
Mild
Significant

B

Figure 3b

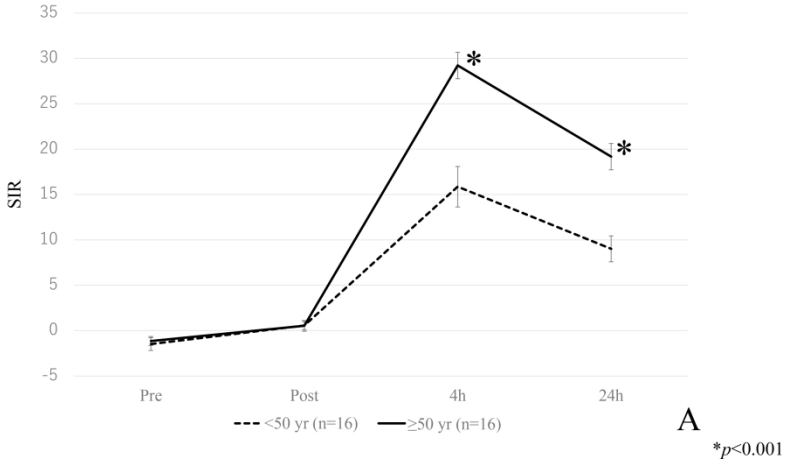

35
30
25
20
15
10
5
0
-5
SIR
Pre
Post
4h
24h
*
*
<50 yr (n=16)
≥50 yr (n=16)
A
*p<0.001

Figure 4a

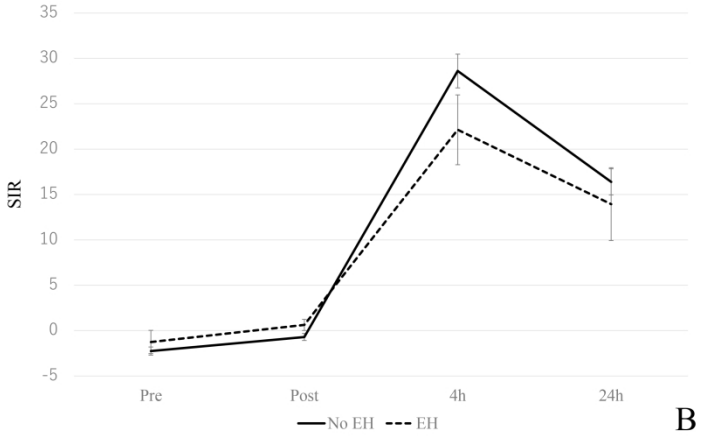


Figure 4b

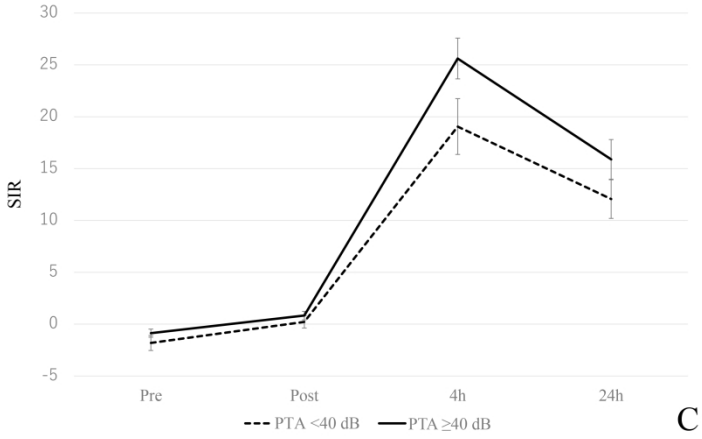


Figure 4c