

## **Title page**

### **Title:**

Vulnerable carotid arterial plaque causing repeated ischemic stroke can be detected with B-mode ultrasonography as a mobile component: Jellyfish sign

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## **Abstract**

Mobile plaque is associated with increased risk of ischemic stroke, but definitions have remained unclear. We have previously reported that carotid ultrasonography can detect the mobile component of the carotid plaque surface, which rises and falls in a manner inconsistent with arterial pulsatile wall motion (Jellyfish sign). However, clinical and pathological features of Jellyfish sign remain unclear. Subjects comprised 165 patients with carotid plaque and degree of area stenosis  $\geq 50\%$  on ultrasonography. Using magnetic resonance imaging, we quantified intraplaque hemorrhage (IPH) and defined ischemic stroke in each patient. Fifteen surgical specimens were obtained by carotid endarterectomy, and pathological features (area of fibrous cap and intraplaque atheromatous lesion) were compared with ultrasonographic plaque surface movement rate. Carotid plaques with IPH were seen in 78 cases, with Jellyfish sign in 31 cases. Jellyfish sign was not detected in patients without IPH. In these 15 patients, the fibrous cap covered the atheromatous lesion, and cap thickness correlated negatively with Jellyfish-positive plaque surface movement rate. Kaplan-Meier and Cox multiple regression analysis demonstrated that the most important predictor of ischemic stroke during follow-up is Jellyfish sign, not IPH. Stroke events in patients with Jellyfish sign repeated within a short interval after diagnosis. Jellyfish sign on ultrasonography is a

sign of high-risk plaque vulnerability, suggesting rupture of the fibrous cap associated with release of thrombogenic factors into the arterial lumen, and resulting in repeated ischemic stroke during a short interval after diagnosis.

**Key words:** Jellyfish sign, mobile plaque, plaque rupture, stroke, ultrasonography

## **Introduction**

Ischemic stroke is frequently caused by cerebral embolism from a vulnerable atherosclerotic plaque, and is thought to be related to the intrinsic composition such as size of the lipid core, thinning or rupture of the fibrous cap and the presence of intraplaque hemorrhage (IPH) [1, 16-18, 21-23]. Although the degree of stenosis offers a valid marker of stroke risk [14, 20, 21], these vulnerable plaques have a high predictive value for stroke, so identification of high-risk plaque may improve risk assessment and improve prognosis for stroke patients.

Mobile or floating plaque is easily detected by routine carotid ultrasonography and is associated with increased risk of ischemic stroke [11, 15, 25]. Mobile plaque is thus a form of vulnerable plaque. To date, the definition of mobile plaque has been unclear and confused [11, 15, 25]. We have previously examined mobile plaques from the perspective of the type of motion [12]. In some cases, a section of carotid plaque surface rose and fell in a manner inconsistent with arterial pulsatile wall motion [12]. This motion did not represent floating plaque and resembled the motion of a jellyfish, so we termed this ultrasound finding as the Jellyfish sign, to be distinguished from floating plaque. Patients with Jellyfish sign frequently experienced ischemic stroke within a short interval from diagnosis, so this sign was considered to represent a strong risk

factor for stroke. However, the previous study was a case report, and further investigations were needed to examine whether Jellyfish sign could be used as a predictor of recurrent ischemic strokes.

The aim of this study was to prospectively examine whether patients with carotid plaque showing Jellyfish sign under B-mode ultrasonography are more likely to develop ischemic stroke than those without Jellyfish sign. This study represents the first attempt to examine mobile plaque showing Jellyfish sign from the perspective of clinical, radiological and pathological findings.

## **Materials and methods**

### **Subjects**

This study was performed at Hibino Hospital and Chugoku Rousai Hospital, and approval for this prospective study was obtained from the institutional ethics committees of both hospitals. Informed consent was obtained from all patients prior to enrolment. Subjects included in this study were selected from a consecutive series of 695 patients referred for carotid ultrasonographic examination at Hibino Hospital between January 2003 and December 2007. Entry criteria included: 1) carotid area stenosis  $\geq 50\%$  using established Duplex scanning criteria [9]; 2) identification of the

existence of IPH using magnetic resonance imaging (MRI); 3) serial follow-up using MRI until surgery or >6 months without surgery. Exclusion criteria included: 1) degree of carotid stenosis <50% (n=422); 2) inability to perform MRI (n=28); 3) inability to perform ultrasonography due to the existence of acoustic shadow (calcification) or multiple reflections (n=64); and 4) hemorrhagic stroke events (n=7). Patients with bilateral hemispheric symptoms or known cardiac mural thrombus (as verified on echocardiography) were also excluded because of suspected cardioembolic origin (n=9). The remaining 165 patients were included in the study. Among these, 32 patients underwent carotid endarterectomy (CEA) and 4 cases received carotid artery stenting (CAS) after providing informed consent during follow-up. As shown in Table 1, a total of 18 patients (10.9%) suffered from ischemic stroke events and 34 patients (20.6%) experienced TIA.

### **Risk Factors**

We assessed vascular risk factors based on the following. Hypertension was defined as a history of using antihypertensive agents, systolic blood pressure >140 mmHg, or diastolic pressure >90 mmHg after the first ischemic stroke event or at the first clinic attendance. Diabetes mellitus was defined as the use of oral hypoglycemic

agents or insulin, fasting glucose level  $>126$  mg/dL or glycosylated hemoglobin level  $>6.4\%$ . Hyperlipidemia was defined as the use of antihyperlipidemic agents or serum cholesterol level  $>220$  mg/dL. Current smoking status was obtained, along with history of atrial fibrillation. Coronary artery disease was defined as a history of angina pectoris or myocardial infarction.

### **Ultrasound Examination**

We used a LOGIQ 7 system (GE Yokogawa Medical Systems, Tokyo, Japan) with a 3-10 MHz broadband linear array transducer. Area of stenosis was determined at the most advanced lesion by routine Doppler criteria [9].

Jellyfish sign of the carotid arterial plaque was defined as the presence of mobile components that rose and fell in a manner inconsistent with arterial pulsatile wall motion, and was evaluated under high-resolution B-mode ultrasonography (Supplementary Movie 1, 2). Examinations were performed by an experienced ultrasonographer and recorded as a digital movie. Data were reviewed by a vascular surgeon and a neurosurgeon, both of whom were blinded to the clinical data.

The rate of surface movement of Jellyfish-positive plaque (Jellyfish motion rate) was estimated using B-mode ultrasonography (Fig. 1). Distances from the adventitia to the

top (a) and from the top to the bottom (b) of the mobile part, appearing as a rising and falling motion, were examined and applied as follows: Jellyfish motion rate =  $b/a \times 100$  (%).

### **MRI Protocol**

We performed carotid plaque imaging using a 1.5-T MRI scanner (Signa EXICTE XI, version 11.0; GE Healthcare) equipped with an 8-cm-diameter surface coil. All participants were assessed using MRI studies including a diffusion-weighted sequence, fluid-attenuated inversion recovery, and magnetic resonance angiography (MRA) at the first clinic attendance. MRI using this protocol during follow-up confirmed all strokes to be ischemic.

IPH status of the carotid arterial plaque was assessed by MRI using a 2-dimensional spin echo sequence (echo time, 14 ms; repetition time, 400 ms; bandwidth, 15.63 kHz; field of view, 14 cm; slice thickness, 2 mm; matrix, 256×192; fat suppression) and saturation pulse to eliminate signal from inflow blood (Superior and Inferior). To clarify the border between lumen and plaque surface, we checked electrocardiogram-gated black-blood T2-weighted imaging. IPH was diagnosed using T1-weighted imaging if signal intensity of the plaque exceeded that of adjacent skeletal muscle. Presence of IPH



was determined by consensus between two experienced researchers blinded to patient information.

### **Histopathology**

Excised plaques were fixed in formalin immediately after removal. Specimens were cut into 3-mm intervals along the length of the plaque for embedding in paraffin wax. Formalin-fixed, paraffin-embedded tissue blocks were serially sectioned at 3  $\mu\text{m}$  onto slides and stained with hematoxylin and eosin (HE), and Masson trichrome for cellular components, neutral lipid, calcification, fibrous tissue and thrombus.

The proportion of fibrous cap and intraplaque atheromatous elements (including hemorrhage, lipid, cell components and thrombus) in Jellyfish-positive patients was evaluated pathologically as a percentage of the entire plaque body. HE and Masson trichrome staining of plaque was recorded using digital photographs, tracing the outlines of each element, and measuring the area (in pixels) of each traced area using Adobe Photoshop CS3 extended (Adobe Systems, San Jose, CA). The proportion of each element was described as:

(1) Proportion of fibrous cap (fibrous cap coverage rate) = area of fibrous cap (pixels) / area of whole plaque (pixels).

(2) Proportion of intraplaque atheromatous lesion (atheromatous lesion rate) = area of atheromatous lesion (pixels) / area of whole plaque (pixels).

Fibrous cap thickness was examined at 9 points at equal intervals from shoulder to shoulder of the mobile part and the adjacent non-mobile part in Jellyfish-positive plaque. Average thickness was calculated, and correlations between average cap thicknesses of the mobile and non-mobile parts were examined.

### **End Points**

Neurological symptoms that occurred during observation were noted at each visit every 1-6 months. Neurological symptoms were subsequently further evaluated by MRI, and ischemic stroke was diagnosed.

The end point of the study was ischemic stroke that developed ipsilateral to the relevant carotid stenosis.

### **Statistical Analysis**

Numerical values are reported as mean  $\pm$  standard deviation. Baseline characteristics, vascular risk factors and ultrasound and MRI findings were compared among groups (IPH-negative vs. -positive or Jellyfish-negative vs. -positive). Statistical analyses were

performed as nonparametric analysis using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. For correlative analysis, the Spearman rank correlation coefficient ( $\rho$ ) was calculated. Interobserver agreement regarding presence of the Jellyfish sign was assessed using the  $\kappa$  coefficient for two observers (neurosurgeon S.H. and vascular surgeon S.W.).

The effect of IPH and/or Jellyfish sign on rate of recurrent cerebral ischemic stroke was examined using Kaplan-Meier and Cox multiple regression analysis. The follow-up period was the time from vascular assessment to recurrent ischemia. Patients were censored if they had not suffered from ischemic stroke as of last follow-up or if they had undergone CEA/CAS before suffering recurrent ischemic stroke. Jellyfish sign was not identified among patients without IPH, so Kaplan-Meier analysis was performed after categorizing 3 groups: IPH-negative (IPH-); IPH-positive and Jellyfish-negative (IPH+JF-); and IPH-positive and Jellyfish-positive (IPH+JF+).

Clinical features of recurrent ischemic stroke were compared among groups (IPH-, IPH+/JF-, IPH+/JF+) using the Kruskal-Wallis test for nonparametric analysis.

All analyses were performed using the Statistical Package for Social Sciences SPSS for Windows version 16.0 software (Chicago, Illinois).

## Results

### Baseline Structures in all Patients

Table 1 shows baseline data for all patients and groups. MRI demonstrated IPH in 78 cases. Jellyfish sign was detected in 31 IPH-positive cases, but was not detected at all among IPH-negative cases. Jellyfish sign might thus be solely associated with IPH.

Patients were divided into 2 groups according to the presence of IPH, with 87 patients in the IPH-negative group and 78 patients in the IPH-positive group. The 2 groups were matched for hypertension, hyperlipemia, diabetes mellitus, arterial fibrillation, history of ischemic heart disease and ischemic stroke and use of antihypertensive agents, antihyperlipidemic agents and aspirin. However, patients with IPH were older and showed greater stenosis of the carotid artery, and rates of smoking, male sex, TIA presentation, operation and statin use were much higher than in those without IPH.

Jellyfish sign was not seen in the IPH-negative group, so the IPH-positive group was divided into 2 subgroups according to presence of the Jellyfish sign, with 31 patients in the Jellyfish-positive group and 47 patients in the Jellyfish-negative group. These 2 groups were matched for age, outcome, and risk factors other than history of stroke ( $p=0.019$ ). Jellyfish-positive patients were older, with a less frequent history of stroke or operation than in patients without Jellyfish sign. These risk factors were thus thought to

have no marked influence on outcome statistics between Jellyfish-positive and -negative patient groups.

### **Ultrasonographic and MRI Findings**

MRI and angiographic findings of Patient 1 with Jellyfish sign are shown in Figure 2. Diffusion-weighted imaging of MRI revealed multiple small lesions in the territory of the left middle cerebral artery (Fig. 2A). MRA (Fig. 2B) demonstrated stenosis. MRI plaque imaging (T1-weighted imaging) showed that signal intensity of the plaque exceeded that of adjacent skeletal muscle, suggesting IPH (Fig. 2C). Carotid ultrasonography demonstrated stenosis with the carotid plaque, which included the mobile component (Fig. 2D, arrow). This component rose and fell in a manner inconsistent with arterial pulsatile wall motion (Fig. 2E, illustration; Fig. 2F, montage view; Supplementary Movie 1), showing Jellyfish sign. The  $\kappa$  coefficient for interobserver agreement on Jellyfish sign positivity was acceptable, at 0.80.

### **Pathological Findings**

Figure 3 shows the pathological findings for Patient 1 with Jellyfish sign. The Jellyfish sign-positive part (Fig. 3B, C) showed a thin and ruptured fibrous cap overlying the

cavity, which included free blood cells and fragments of atheroma (Fig. 3E). However, the adjacent non-mobile element (not showing Jellyfish sign; Fig. 3D) was lined by a thickened fibrous cap covering atheromatous lesion including hemorrhage, lipid and cell components. Case 1 also showed that the intraplaque atheromatous lesion communicated with the lumen through an endothelialized fissure (Fig. 3F), suggesting that this fissure was not induced by any mechanical artifact of surgery, but instead caused over time by rupture of the fibrous cap.

Another case with Jellyfish sign (Fig. 4, Supplementary Movie 2) also showed the same trend: the Jellyfish-positive part lined with thinning and disrupted fibrous cap, and the Jellyfish-negative part lined with thickened fibrous cap. The atheromatous lesion was underlying both Jellyfish-positive and -negative parts.

These findings therefore suggest that the Jellyfish sign is associated with fibrous cap thinning and rupture.

### **Correlation between ultrasonographic plaque surface motion and pathological features**

From case presentations, the vulnerability of plaque including intraplaque hemorrhage and rupture was thought to be correlated with Jellyfish sign. We thus calculated the

degree of plaque surface motion of the Jellyfish-positive part, and examined the correlation between ultrasonographic surface motion and pathological features. The shapes of fibrous caps in this pathological study were irregular and partially thinning or ruptured, and thickness was difficult to measure correctly. We therefore examined fibrous cap thinning by measuring the area of fibrous cap and calculating the fibrous cap coverage rate.

Eighteen Jellyfish-positive patients (9 cases after onset of ischemic stroke and 9 cases before onset of ischemic stroke during study follow-up) underwent operation. Among these 18 surgical specimens of Jellyfish-positive plaque, area of the plaque structures could not be calculated in 3 specimens due to blow out during surgery and formalin fixation. The remaining 15 specimens were thus used for examination and estimation.

Spearman coefficient analysis showed a significant negative correlation between plaque surface motion and fibrous cap coverage rate (Fig. 5). However, intraplaque atheromatous lesion rate did not correlate with plaque motion rate. The smaller the fibrous cap coverage rate, the larger the surface motion rates of the Jellyfish sign.

Examination of fibrous cap coverage rate is not particularly common, and fibrous cap thickness is usually measured instead. We therefore also examined fibrous cap thickness of the mobile part and adjacent non-mobile part of the Jellyfish-positive plaque. The

results resembled those for fibrous cap coverage rate, with the mobile part showing greater thinning than the non-mobile part (Mann-Whitney,  $p < 0.0001$ ; Fig. 6). The Jellyfish sign thus appears closely correlated with thinning of the fibrous cap.

### **Kaplan-Meier Analysis of Recurrent Ischemic Stroke with or without IPH and Jellyfish Sign**

To determine whether IPH or Jellyfish sign offers a better predictor of ischemic stroke, we first performed Kaplan-Meier analysis for the incidence of ipsilateral ischemic stroke events. Fig. 7 demonstrates that event-free survival was much higher in Jellyfish-negative groups (IPH- and IPH+JF-) than in the Jellyfish-positive group (IPH+JF+) (log rank,  $p < 0.001$ ). Interestingly, if the Jellyfish sign was negative, the incidence of ischemic stroke for the IPH-positive group was almost equal to that of the IPH-negative group ( $p = 0.437$ ). This suggests that the more important predictive factor for ischemic stroke is not IPH, but the Jellyfish sign.

### **Cox Multiple Regression Analysis**

Cox multiple regression analysis was used to clarify which variables, including area stenosis, IPH, Jellyfish sign, smoking, hypertension, diabetes, hyperlipemia, arterial



fibrillation, history of ischemic heart disease and ischemic stroke and medications, contribute significantly to prediction of ipsilateral ischemic stroke (Table 2).

A highly significant difference was obtained from Jellyfish sign ( $p < 0.0001$ ). Hypertension ( $p = 0.068$ ) and use of antihypertensive agents ( $p = 0.081$ ) also tended to predict ischemic stroke, whereas IPH showed no such tendency ( $p = 0.631$ ).

### **Clinical Features of Recurrent Ischemic Stroke with or without Jellyfish Sign**

We estimated the difference for recurrent features of ischemic stroke with or without IPH or Jellyfish sign (Table 3). The ischemic stroke events with Jellyfish sign during follow-up was much higher than that without Jellyfish sign. The interval between diagnosis and onset of first ischemic stroke event in patients with Jellyfish sign was shorter (frequently within 1 week after diagnosis) than in patients without Jellyfish sign. Of note was the fact that repeated ischemic stroke events occurred much more frequently in patients with Jellyfish sign than in patients without Jellyfish sign. Carotid arterial plaque with Jellyfish sign thus appears to have a high risk for repeated recurrence of ischemic stroke within a short interval after diagnosis.

## Discussion

Our study appears to be the first to describe an ultrasonographic and clinicopathological series of patients with >50% carotid area stenosis demonstrating that ultrasonographic Jellyfish sign (a kind of mobile plaque, not representing a floating plaque) associated with IPH of carotid plaque is one of the major determinants of ischemic stroke in patients affected by carotid atherosclerotic disease. Randomized controlled trials have demonstrated the benefit of performing CEA in patients with symptomatic high-grade carotid stenosis [2, 8]. Previous studies have also demonstrated that ischemic stroke is frequently caused by thromboembolism from an atherosclerotic plaque to cerebral vessels [14, 16-18, 21-23]. Although the degree of carotid arterial stenosis is a valid marker of stroke risk, identification of high-risk plaque features other than stenosis may improve risk assessment and allow for targeted intervention.

Eliasziw et al. [7] analyzed pooled data from the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and reported that patients who experienced hemispheric TIA related to internal carotid artery disease showed a high risk of stroke in the first few days after TIA. They also showed that risk of early stroke was unaffected by the degree of internal carotid artery stenosis. That result might be consistent with our present data that the Jellyfish-positive carotid plaque led to considerably increased

frequency of repeated ipsilateral multiple ischemic strokes during a short interval (within 1 weeks, 31.3% of patients suffered ischemic stroke during follow-up) after last cerebrovascular event. Jellyfish sign might be an important sign for plaque vulnerability. Patients with the Jellyfish sign should thus be considered at the earliest possible opportunity for surgery. At this point, the question must be asked, what is the Jellyfish sign?

The pathophysiological significance of altered plaque surface motion in symptomatic carotid artery plaques remains yet to be elucidated. Meairs et al. [15] examined plaque surface motion using 4-dimensional ultrasonography and suggested that maximal discrepant surface velocity (MDSV) was significantly higher in symptomatic plaques than in asymptomatic plaques ( $P < 0.001$ ). This disparity in focal movement on the plaque surface among symptomatic patients was thought to be similar to the Jellyfish sign. They speculated that such motion patterns are related to dynamic interactions between plaque geometry, plaque composition and focal hemodynamic alterations, analogous to experimental studies on plaque rupture in which pressure loading is used to identify asymmetrical plaque movement before fissuring [19]. The present data also suggest that the thinnest region of fibrous cap induced plaque surface motion, as the Jellyfish sign. Given these observations, motion changes in plaque surface, such as

Jellyfish sign, may be localized to vulnerable areas of the plaque, particularly the area of thinnest and/or disrupted fibrous cap on the carotid plaque.

A thin or ruptured fibrous cap is well known as an important morphological component of vulnerable atherosclerotic plaque [17, 18, 21, 22, 26]. Many previous studies demonstrated that thrombosis associated with plaque rupture is one of the major determinants of ischemic stroke in patients affected by carotid atherosclerotic disease [17, 18, 21, 22, 26]. Carr et al. [3] demonstrated that IPH is highly associated with plaque rupture, suggesting that IPH may play a role in thinning, weakening and rupture of the fibrous cap. Other studies have also reported that IPH and a large lipid core are strongly associated with both a thin cap and cap rupture [16, 17, 23].

Constantinides [4] originally suggested that hemorrhage into a plaque occurs from cracks or fissures originating from the luminal surface. Davies [5] later defined plaque fissure as an eccentric, intraplaque hemorrhage with fibrin deposition within the necrotic core from “an entry into the plaque from the lumen”. Fissuring of the fibrous cap occurs at the thinnest portion, typically at the shoulder region [13], thereby allowing the entry of blood into the necrotic core. The fibrous cap covers the necrotic core and separates this collection of cellular debris and cholesterol clefts from the arterial lumen [24]. Disruption of this fibrous cap (plaque rupture) thus leads to the release of

thrombogenic debris from the necrotic core into the lumen [21, 22, 24]. In this study, every Jellyfish sign was associated with IPH. Pathologically, the Jellyfish-positive part of the plaque was covered with thinning and disrupted fibrous cap, overlying the atheromatous lesion, including lipid, hematoma, cell component and thrombosis. Rupture of the fibrous cap allows dissection of blood from the lumen into the lipid pool of the plaque, resulting in the formation of intraplaque hemorrhage. This blood inflow into the plaque pushes contents of the necrotic core into the arterial lumen through the rupture site of the fibrous cap, and this released debris may thus become thrombogenic, resulting in multiple, repeated ipsilateral ischemic strokes. The Jellyfish sign was thus thought to demonstrate plaque rupture accompanied by repeated and multiple ischemic strokes.

In this study, the rate of recurrent ischemic stroke events showed no correlation with the Jellyfish motion rate (data not shown), so the actual presence of the Jellyfish sign, rather than the degree of motion seen with the Jellyfish sign, represented the important indicator of high risk for ischemic stroke events. Moreover, the Jellyfish-positive part of the carotid plaque was thought to be highly vulnerable and to easily exhaust the thrombogenic factors. The possibility remains that the stimulation provided by prophylactic surgery (CEA or CAS) may easily lead to ischemic stroke events.

Therefore, if the Jellyfish sign is detected in a carotid arterial plaque, these surgeries must only be performed with the greatest of possible care in order to prevent ischemic stroke attack.

### **Limitations of this Study**

We were unable to completely exclude ischemic strokes of origins other than carotid plaque. However, ischemic strokes that appeared to be of cardioembolic origin and hemorrhagic strokes were excluded. Second, ultrasound B-mode imaging suffers from limitations like acoustic shadowing, resulting from calcifications in the plaque, speckle diffraction and angle dependence. MRI techniques do not suffer from acoustic shadows, and could thus reduce such ultrasonographic problems. Moreover, recent studies have also shown that fibrous cap thinning and rupture can be visualized in vivo using high-resolution MRI [10, 26]. To clarify the possibility that Jellyfish sign indicates plaque rupture, we should examine signs of plaque rupture on MRI. However, MRI methods remain very expensive. Moreover, the ability to detect plaque rupture on MRI is currently limited mainly to research. Conversely, the ultrasound procedure is fast and relatively inexpensive and plaque examination is easily performed in the standard clinical visit. Moreover, for two cases in the present study, Jellyfish sign showed at the

area of mild stenosis (30 and 40% stenosis) adjacent to the part of the carotid artery severely stenosed by plaque. The Jellyfish sign could thus prove valuable in evaluating lesions that would otherwise be considered low risk. However, the number of subjects in the present study was small and further studies (particularly including patients with <50% stenosis by plaque) are needed to clarify this issue. Lastly, detecting Jellyfish sign is not difficult for an experienced ultrasonographer, although we must always bear in mind that ultrasound examination is observer-dependent. We have therefore been trying to develop a detection method using ultrasonography with which anyone can detect the Jellyfish sign.

## **Conclusion**

In conclusion, this study demonstrated that the existence of a mobile component of carotid arterial plaque (ultrasonographic Jellyfish sign) suggests thinning and rupture of the fibrous cap over the plaque, resulting in repeated ischemic stroke during a short interval after diagnosis. An important predictive factor for ischemic stroke is thus Jellyfish sign, not IPH. The vulnerable status of the Jellyfish-positive plaque, if not removed, may trigger continuous release of embolic material, which in turn may be related to subsequent cerebrovascular events. Surgical treatment of carotid

plaque with Jellyfish sign, as detected by carotid ultrasonography, should thus be kept in mind as a treatment option. In particular, surgical intervention may be justified in cases with repeated ischemic stroke events during a short interval, as in our cases.

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## Figure legends

**Figure 1:** Schema for measurement of ultrasonographic plaque surface movement (A) and pathological area of plaque elements (B). **A)** Distance from the adventitia to the top (a) and from the top to the bottom (b) of the mobile part. Jellyfish motion rate =  $b/a \times 100$  (%). **B)** Schema for HE and Masson staining of the Jellyfish-positive plaque, showing fibrous cap (c), atheromatous lesion (d), lumen (e) and media (f). Traced areas of fibrous cap (upper), atheromatous lesion (middle) and whole plaque (lower) are shown.

**Figure 2:** MRI and ultrasonographic findings in Case 1.

A representative case showing Jellyfish sign of fibrous cap rupture and hemorrhage into a carotid atherosclerotic plaque on baseline MRI and ultrasonography. The subject experienced ipsilateral stroke and subsequently underwent CEA 2 weeks after stroke onset. **A)** DWI shows multiple, small high-intensity spots, suggesting fresh ischemic infarctions, in the left middle cerebral arterial territory. MR angiography **(B)** shows mild wall stenosis in the left cervical internal carotid artery. **C)** Signal intensity patterns of hemorrhage are hyperintense on T1-weighted images, suggesting intraplaque hemorrhage. **D)** A longitudinal B-mode ultrasonographic image of the cervical internal

cerebral artery shows a mobile lesion (arrow) in the carotid plaque; the mobile component of the carotid plaque rose and fell in a manner inconsistent with arterial pulsatile wall motion (**E**: schema of mobile plaque). **F**) Movement by montage images.

**Figure 3:** Plaque histological features in CEA specimens in Case 1. Panel A indicates a longitudinal B-mode ultrasonographic image of Jellyfish-positive carotid plaque in order to show the mobile component (B and C, white allow) and the adjacent non-mobile component (D). Mobile component (Jellyfish-positive element) of the carotid plaque overlying the atheromatous lesion (B-1, B-2 and E). The thinning fibrous cap was disconnected by fissure (C-1 and C-2) from the adjacent lesion of this atheromatous lesion. This fissure became endothelialized and connected between the lumen and intraplaque atheromatous lesion (F). The non-mobile component (adjacent to the mobile component) is lining with thickened fibrous cap, overlying the atheromatous lesion (D). HE staining: B-1, C-1, D-1, E and F, and Masson trichrome staining: B-2, C-2 and D-2. Scale bars: 1 mm in B-1, B-2, C-1, C-2, D-1 and D-2; 500  $\mu\text{m}$  in E and F.

**Figure 4:** Representative ultrasonographic B-mode imaging and micrographs of plaque of patient (case 2) presenting with (D) or without (E) Jellyfish sign. Panel A shows a

longitudinal B-mode ultrasonographic image of the cervical internal cerebral artery with or without Jellyfish sign. Square outline (D) shows the plaque area showing Jellyfish sign (white arrow), with movements also shown in illustration (B) and montage view (C). Pathological findings (Masson trichrome staining) demonstrate that thinning and ruptured fibrous caps overlying an atheromatous lesion (D). However, the area without Jellyfish sign shows a thickened fibrous cap overlying the atheromatous lesion (E).

Scale bars: 1 mm in D and E.

**Figure 5:** Correlation between Jellyfish motion rate and both atheromatous lesion rate (A) and fibrous cap coverage rate (B) (Spearman rank tests). Each circle represents a patient.

**Figure 6:** Box plots showing differences in cap thickness ( $\mu\text{m}$ ) between non-mobile and mobile parts of carotid arterial plaque showing a positive Jellyfish sign. Box plots shows median, quartiles, and 10<sup>th</sup> and 90<sup>th</sup> percentiles.

**Figure 7:** Kaplan-Meier survival estimates of the proportion of patients remaining free of ipsilateral ischemic stroke event for subjects of the 3 groups: IPH(-); IPH(+)/JF(-);



and IPH(+)JF(+). IPH, intraplaque hemorrhage; JF, Jellyfish sign.

**Supplementary Movie 1:** Digital video of plaque surface motion of Jellyfish sign in

Case 1. Arrow shows the Jellyfish sign-positive part. CCA: Common carotid artery.

ICA: Internal carotid artery.

**Supplementary Movie 2:** Digital video of plaque surface motion of Jellyfish sign in

Case 2. Arrow shows the Jellyfish sign-positive part. CCA: Common carotid artery.

ICA: Internal carotid artery.

**Statement justifying the involvement of each author in the production of this manuscript:**

Shinji Kume conceived and designed the research and analyzed and interpreted the clinical data.

Seiji Hama conceived and designed the research and performed statistical analysis.

Kanji Yamane analyzed and interpreted the clinical data (findings of surgical procedure), and handled funding and supervision.

Seishi Wada made critical revision of the manuscript for important intellectual content.

Toshihiro Nishida analyzed and interpreted the pathological findings.

Kaoru Kurisu handled funding and supervision, and draft the manuscript.

**Article summary:** Ultrasonographic rising and falling motion of the plaque surface in the carotid artery (Jellyfish sign) is a sign of high-risk plaque vulnerability suggesting rupture of the fibrous cap associated with release of thromobogenic factors into the lumen, resulting in repeated ischemic stroke during a short interval after diagnosis.

Table 1. Characteristics of 165 cases with &gt;50% carotid stenosis with or without IPH and Jellyfish sign

	ALL (n=165)	IPH-negative (n=87)	IPH-positive (n=78)	p	IPH-positive (n=78)		p
					Jellyfish-negative (n=47)	Jellyfish-positive (n=31)	
Age, median years (interquartile range)	70.9±8.6 (41-94)	69.9±8.7 (46-94)	71.9±8.4 (41-88)	<0.001	70.2±9.2 (41-85)	74.5±6.6 (59-88)	0.031
Sex: Male, n (%)	134 (81.2)	64 (73.6)	70 (89.7)	0.009	41 (87.2)	29 (93.5)	0.467
Ultrasound parameters							
Area stenosis (%)	75.3±13.1	69.1±12.2	82.2±10.5	<0.001	81.0±11.0	83.9±9.6	0.226
Presence of Jellyfish sign, n (%)	31 (18.86)	0 (0)	31 (100)	<0.001	47 (100)	0 (0)	
Risk factors							
Hypertension, n (%)	111 (67.3)	53 (60.9)	58 (74.4)	0.070	35 (74.5)	23 (74.2)	1.000
Hyperlipemia, n (%)	71 (43.0)	39 (44.8)	32 (41.0)	0.640	21 (44.7)	11 (35.5)	0.485
Diabetes, n (%)	76 (46.0)	35 (40.2)	41 (52.6)	0.121	26 (55.3)	15 (48.4)	0.645
Arterial fibrillation, n (%)	8 (4.9)	5 (5.8)	3 (3.8)	0.723	1 (2.1)	2 (6.5)	0.560
Previous ischemic heart disease, n (%)	49 (29.7)	26 (30.0)	23 (29.5)	1.000	13 (27.7)	10 (32.3)	0.800
Previous ischemic stroke, n (%)	93 (56.4)	49 (56.3)	44 (56.4)	1.000	32 (68.1)	12 (38.7)	0.019
Smoking, n (%)	56 (34)	21 (24.1)	35 (44.9)	0.005	20 (42.6)	15 (48.4)	0.648
Type of symptom on presentation							
Stroke, n (%)	18 (10.9)	10 (11.5)	8 (10.3)	0.001	5 (10.6)	3 (9.7)	0.971
TIA, n (%)	34 (20.6)	8 (9.2)	26 (33.3)		16 (34.0)	10 (32.3)	
Medication							
Antihypertensive agents, n (%)	101 (61.2)	50 (57.5)	51 (65.4)	0.339	31 (66.0)	20 (64.5)	1.000
Hypoglycemic agents, n (%)	57 (34.6)	26 (29.9)	31 (39.7)	0.194	19 (40.4)	12 (38.7)	1.000
Antiplatelet agents, n (%)	94 (57.0)	40 (46.0)	54 (69.2)	0.003	33 (70.2)	21 (67.7)	1.000
Aspirin, n (%)	68 (41.2)	35 (40.2)	33 (42.3)	0.874	20 (42.6)	13 (41.9)	1.000
Statins, n (%)	24 (14.6)	8 (9.3)	16 (20.5)	0.048	12 (25.5)	4 (12.9)	0.254
Outcomes							
Recurrence of ischemic stroke, n (%)	29 (17.6)	7 (8.1)	22 (28.2)	<0.001	5 (10.6)	17 (54.8)	<0.001
Operation, n (%)	36 (21.8)	9 (10.3)	27 (34.6)		18 (38.3)	9 (29.0)	

To test correlations between the two groups (IPH-positive or -negative, and Jellyfish sign-negative or -positive), Fisher's exact test was used to compare categorical variables and the Mann-Whitney test was used to compare continuous variables. IPH, intraplaque hemorrhage detected using MRI; TIA, transient ischemic attack.

Table 2. Relationship between risk factors and ipsilateral cerebrovascular events during follow-up, determined by Cox Regression

	Hazard ratio	95% confidence interval	p
Age (years)	1.004	0.933-1.081	0.907
Sex (male)	1.476	0.368-5.912	0.583
Stenosis rate (%)	0.986	0.945-1.029	0.521
Presence of IPH	1.386	0.366-5.250	0.631
Presence of Jellyfish sign	13.201	3.923-44.418	<0.001
Smoking	0.701	0.276-1.779	0.455
History of hypertension	4.149	0.901-19.093	0.068
History of diabetes	0.838	0.302-2.323	0.734
History of arterial fibrillation	0.262	0.024-2.891	0.274
History of hyperlipemia	1.389	0.521-3.702	0.511
History of ischemic heart disease	1.441	0.602-3.450	0.413
History of stroke	1.385	0.492-3.902	0.538
Use of antihypertention agent	0.319	0.088-1.149	0.081
Use of antilipemia agent	0.618	0.238-1.605	0.323
Use of statin	1.420	0.427-4.720	0.567
Use of antiplatelet agent	2.241	0.810-6.198	0.120
Use of aspirin	1.419	0.585-3.442	0.439

IPH, intraplaque hemorrhage

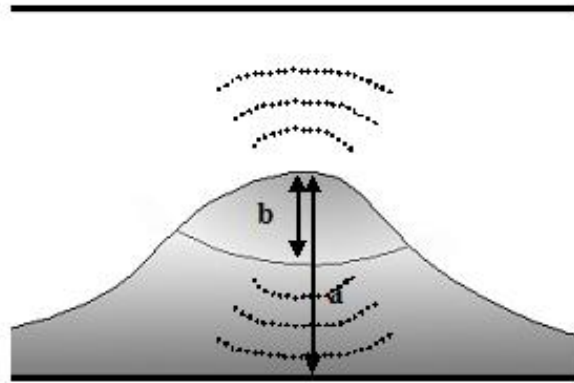
Table 3. Features of recurrent ischemic stroke with or without IPH or Jellyfish sign

	IPH-negative (n=87)	IPH-positive (n=78)	
		Jellyfish-negative (n=47)	Jellyfish-positive (n=31)
Ischemic stroke events during follow up, n (%)	7 (8.0)	5 (10.6)	17 (54.8)
Interval between diagnosis and onset of first ischemic stroke event, days	607.6±346.5	998.8±535.4	366.9±448.0
Ischemic stroke events within 1 week after diagnosis, n	0	0	4
Cases with repeated ischemic stroke events during follow-up, n	1	1	7

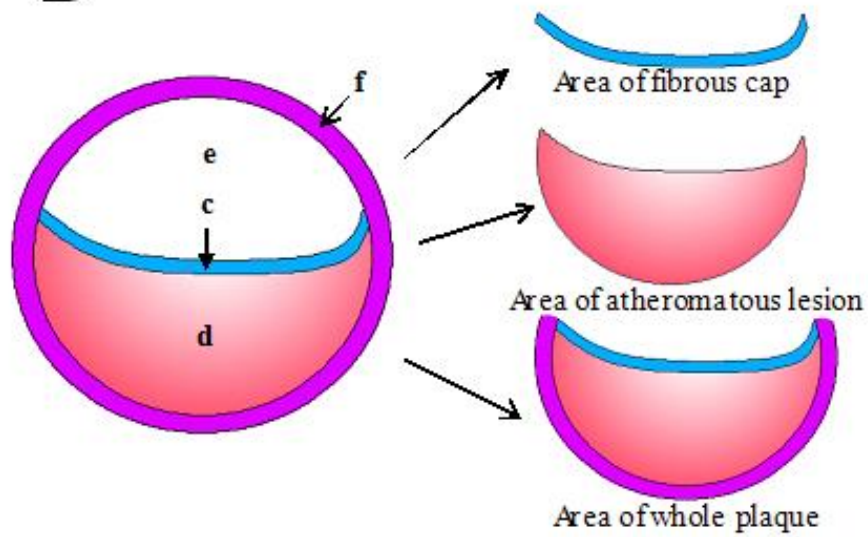
IPH, intraplaque hemorrhage

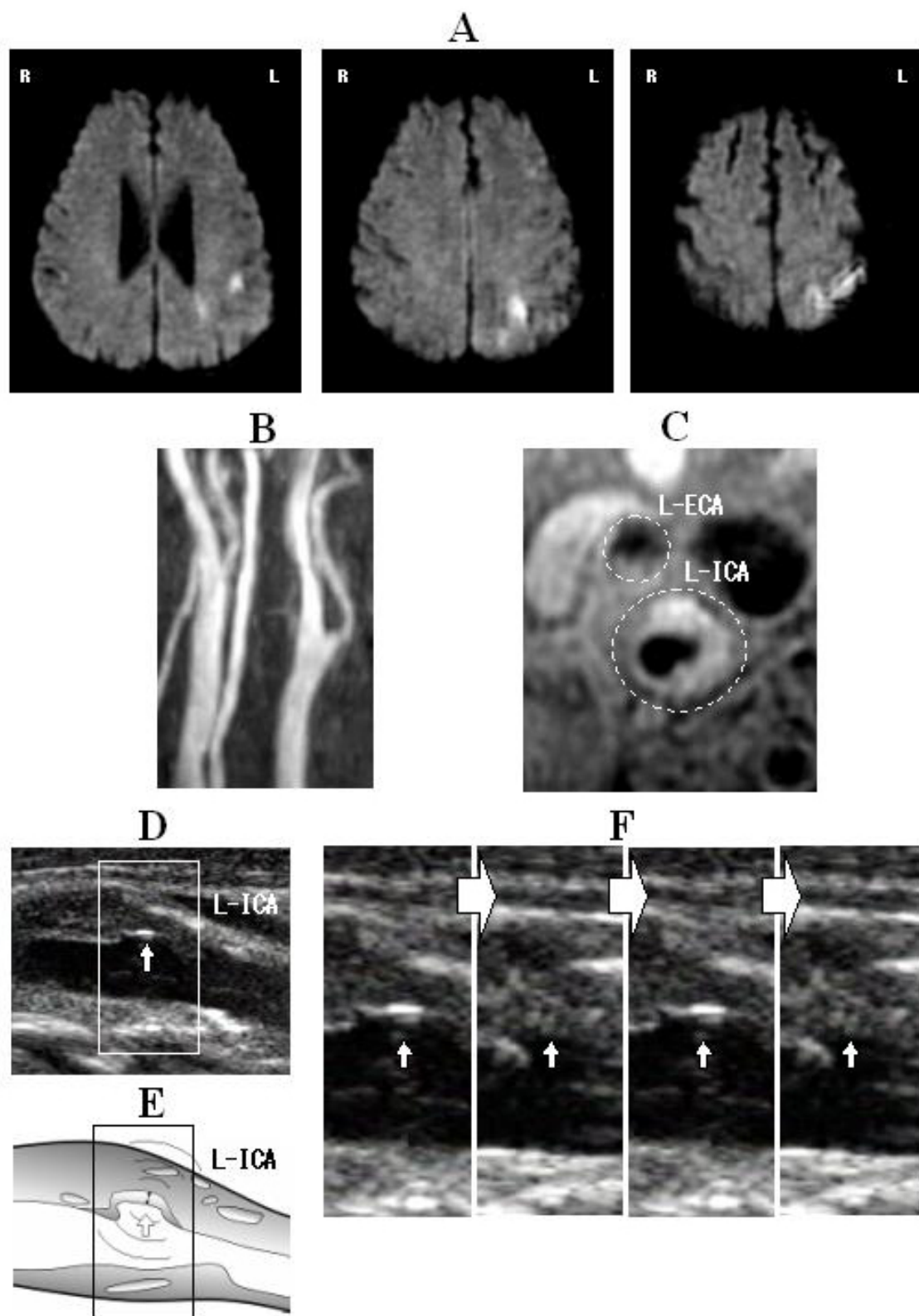
Repeated ischemic stroke event indicates more than 2 events occurred during follow-up.

Figure 1

**A**

$$\text{Jellyfish motion rate (\%)} = b/a \times 100$$

**B**

**Figure 2**



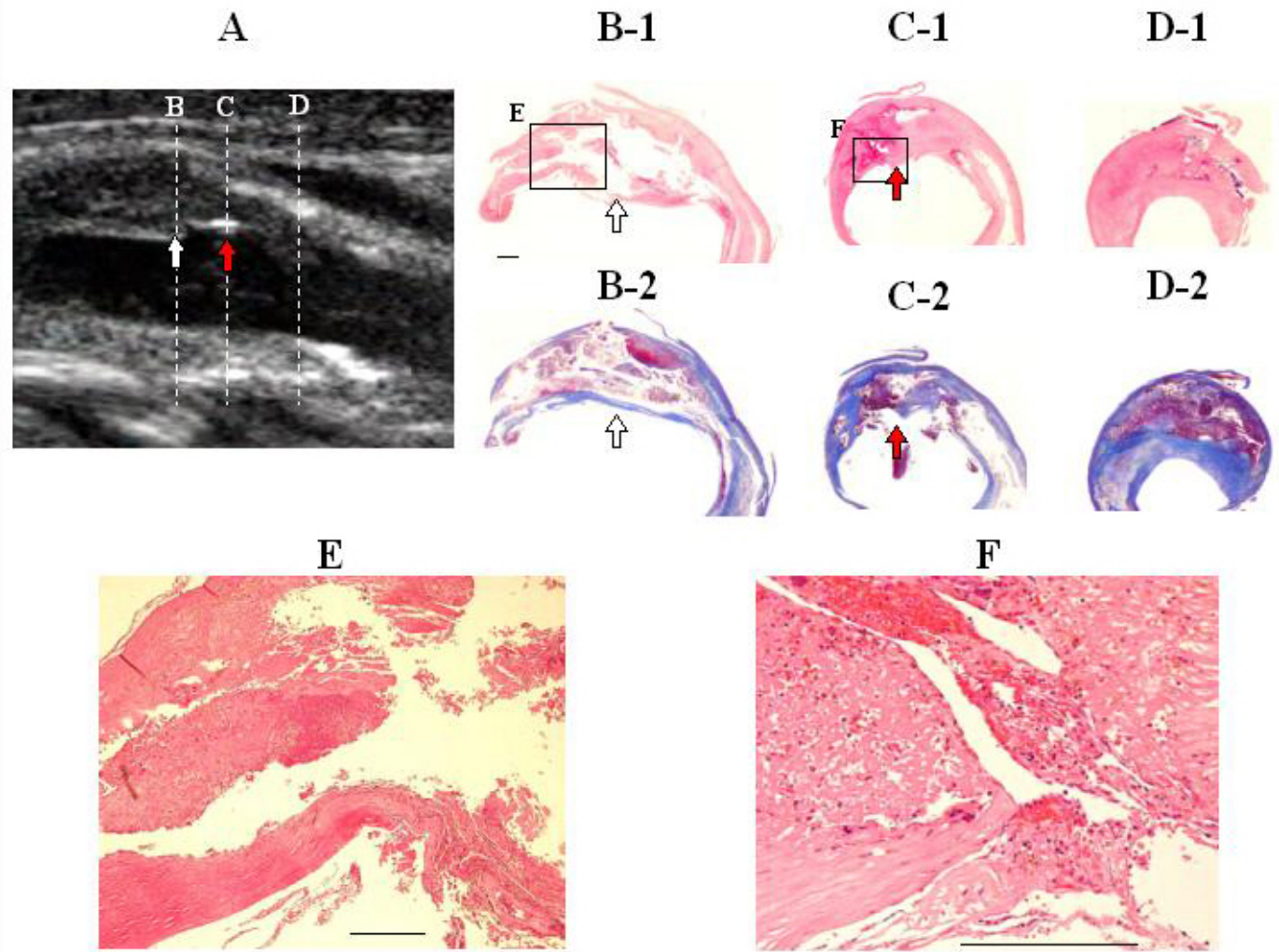
**Figure 3**

Figure 4

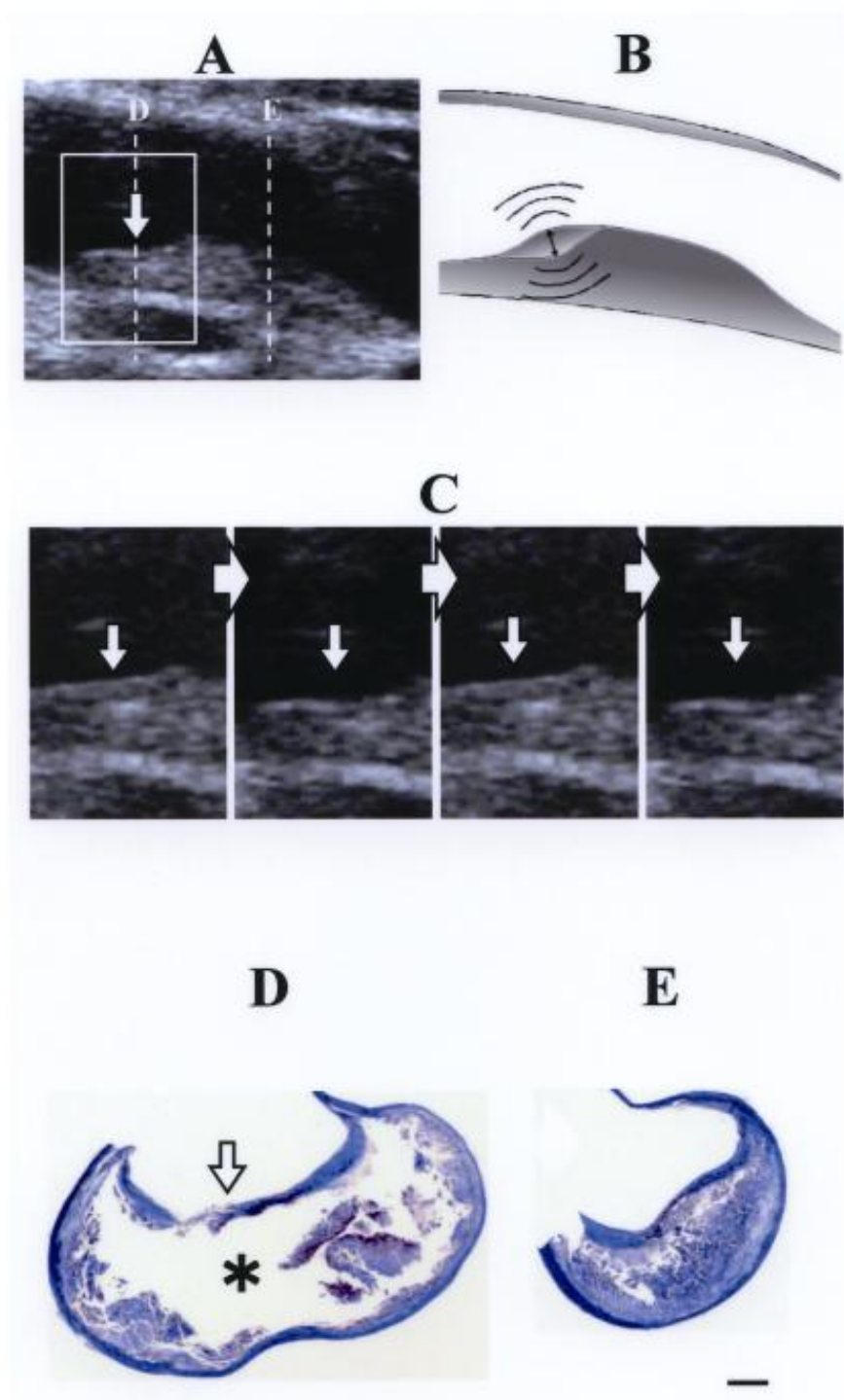


Figure 5

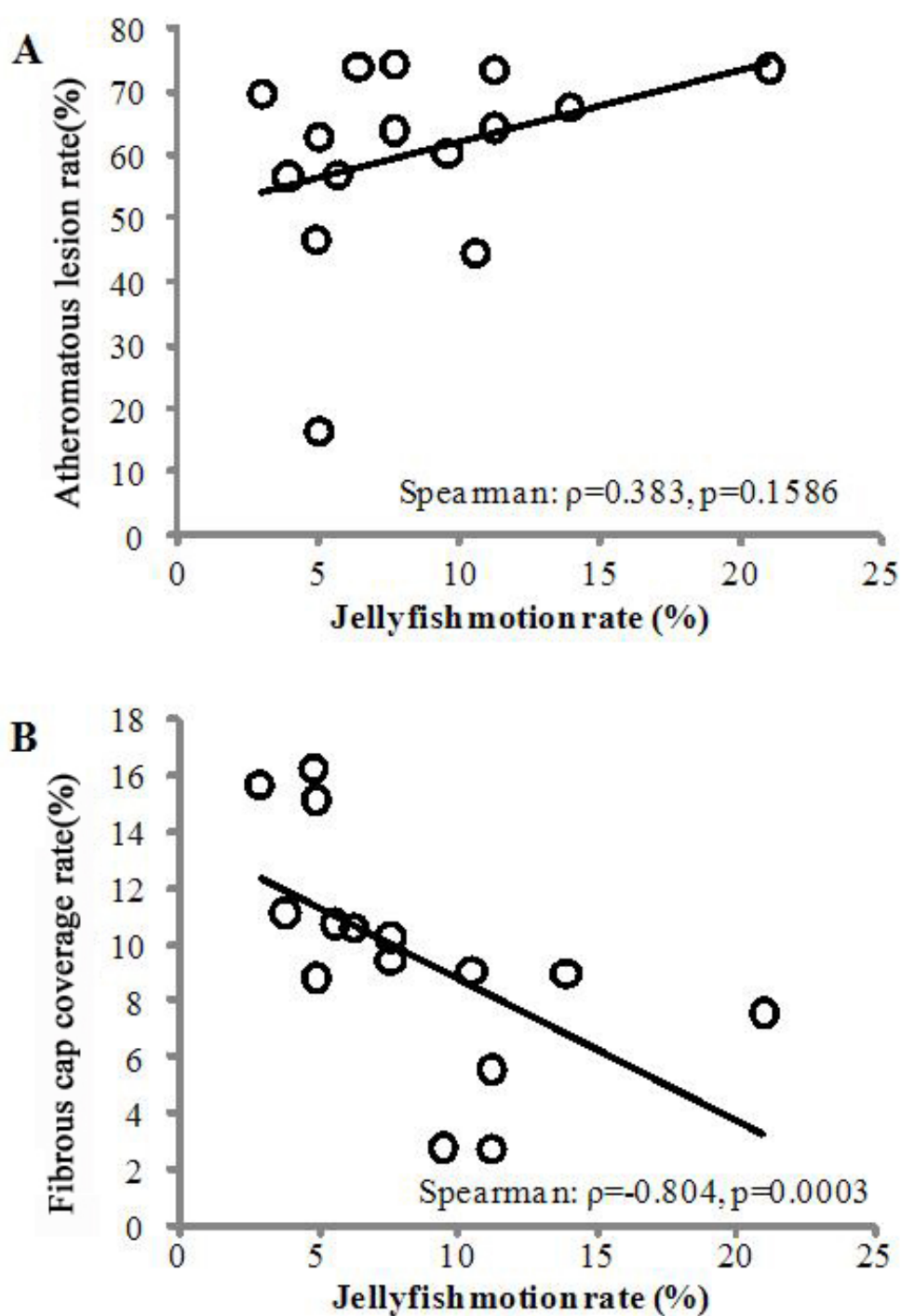


Figure 6

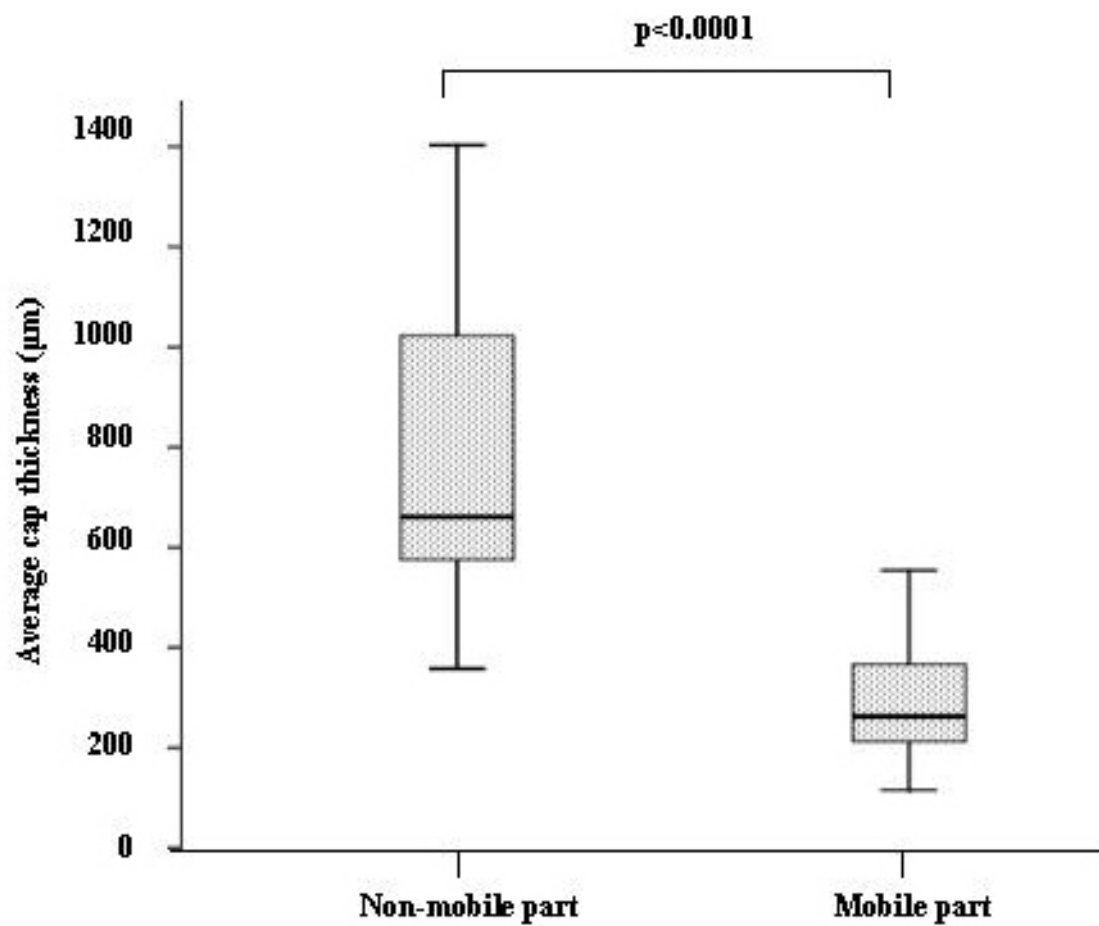


Figure 7

