

Appendix 1

Contrast-enhanced transcranial Doppler (cTCD) examination

cTCD was performed using a Doppler Box transcranial Doppler ultrasound diagnostic instrument with a probe frequency of 2.0 MHz and a depth of 45–55 mm. The infusion access was established through a left cubital vein. The agitated microbubble (MB) normal saline contrast agent was prepared as follows: 8 mL of normal saline was aspirated into one syringe and 1 mL of air was aspirated into the other syringe, and the two syringes were connected through a three-way stopcock. Next, 1 mL of autologous blood was collected and the liquid was mixed more than 20 times rapidly with the syringe to ensure it was completely mixed and the mixture was thick and foamy. The patient was placed in the supine position, and the agitated saline with blood was aspirated into one syringe, and the patient was asked to perform Valsalva exercises before exhaling, the Valsalva movement should be initiated at 5 s after injection of the contrast agent and maintained for 10 s, and the Doppler spectrum of the MCA was monitored for 1 minute (25). If the patient was not cooperating well, to better complete the stimulation of the movement, auxiliary abdominal pressure was applied, or the patient was asked to cough. At least two ultrasound examinations were performed with 5-minute intervals.

Contrast transthoracic echocardiography (cTTE) examination

cTTE was performed with a Philips Epiq 7C or CVx color Doppler ultrasound diagnostic instrument using an S5-1 probe with a frequency of 1–5 MHz. The infusion channel was established through the left cubital vein, and MB normal saline contrast agent was injected as in cTCD. An electrocardiograph was connected to show the level of the apical four chambers, and contrast was promptly infused into the left calf vein while the patient performed Valsalva exercises, stopping immediately when the right atrium

was fully displayed. The MBs appearing in the left atrium were observed over three to five cardiac cycles. At least two ultrasound examinations were performed with 5-minute intervals.

Contrast transesophageal echocardiography (cTEE) examination

cTEE was performed with a Philips Epiq 7C or CVx color Doppler ultrasound diagnostic instrument using X7-2t or X8-2t probe with a frequency of 2–7 MHz. The patient strictly fasted for 6–8 hours before the examination, and underwent local pharyngeal anesthesia by the multiple oral administration of low-dose dyclonine hydrochloride mucilage. After connecting the electrocardiogram, the patient was placed in the right lateral position, and the ultrasound probe was inserted through the trachea to the middle esophageal double atrium view, and the septal ostium was scanned continuously from all sides. The images were optimized to display the presence or absence of echogenic separation at the foramen ovale, and low-speed color Doppler was used to observe the presence or absence of shunt signals. The contrast agent was prepared as described above, and then promptly injected into the left cubital vein at the best middle esophageal double atrium view, and the Valsalva movement was stopped as soon as the right atrium was completely revealed. The presence or absence of MBs from the right atrium across the fissure between the septum and the ventricular septum into the left heart and the number of MBs were assessed. At least two ultrasound examinations were performed with 5-minute intervals. The inner diameter of the PFO and the length of tunnel were measured after the Valsalva movements ceased. All the examinations were performed by two senior ultrasonographers and one nurse. The general clinical data of the patient was collected, including gender and age, as well as a history of cryptogenic stroke, migraine, dizziness, migraine, transient ischemic attack, coronary heart disease, and arrhythmias.