

# Comparison of Agitated Saline Mixed with Blood to Agitated Saline Alone in Detecting Right-to-Left Shunt during Contrast-Transcranial Doppler Sonography Examination

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## Abstract-

**Purpose:** To evaluate a technique for contrast agent preparation as mixing the patients' blood with agitated saline and to compare it with agitated saline alone in diagnosis of cardiac right-to-left shunt in regard to their sensitivity, time window, and distribution of artificially induced microembolic signals.

**Methods:** Fifty-two patients with stroke who had Transesophageal echocardiography proven right-to-left shunt underwent contrast-transcranial Doppler sonography with injection of agitated (i) 9 ml saline with 1 ml air with Valsalva maneuver, (ii) 9 ml saline with 1 ml air without Valsalva maneuver, (iii) 8 ml saline, 1 ml of the patient's fresh blood and 1 ml air with Valsalva maneuver, and (iv) 8 ml saline, 1 ml of the patient's fresh blood and 1 ml air without Valsalva maneuver.

**Results:** The sensitivity of the bilateral middle cerebral artery monitoring in diagnosis of right-to-left shunt was 94.2%, 71.2%, 96.2% and 76.9% for agitated saline with Valsalva maneuver, agitated saline without Valsalva maneuver, agitated saline and blood with Valsalva maneuver, and agitated saline and blood without Valsalva maneuver methods, respectively. Severe right-to-left shunt was detected in 100% of patients when agitated saline and blood with Valsalva maneuver was used. Application of Valsalva maneuver resulted in detection of more right-to-left shunt ( $P = 0.002$ ).

**Conclusion:** Agitated saline mixed with blood with Valsalva maneuver is a sensitive method to detect right-to-left shunt, especially in the case of severe shunt. Mixing agitated saline with blood may increase the sensitivity of the test.

**Key Words:** transcranial Doppler ultrasonography, patent foramen ovale, stroke, contrast media, blood

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## INTRODUCTION

Patent Foramen Ovale (PFO) is haemodynamically insignificant interatrial right-to-left communication

found in 24.3% of randomly sampled subjects during Transesophageal echocardiography<sup>(1)</sup>. Patent foramen ovale presents with higher prevalence among individuals with cryptogenic stroke<sup>(2)</sup>. However, the presence of

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PFO as an independent cerebral stroke risk factor in the general population is still under debate<sup>(3)</sup>.

Right-to-left shunt (RLS) across PFO can be detected with contrast materials with two different modalities: contrast-transesophageal echocardiography (TEE) and contrast-transcranial Doppler sonography (c-TCD). Currently, TEE is accepted as a gold standard for detection of PFO<sup>(3)</sup>. However, c-TCD has shown high sensitivity and valuable specificity in detecting PFO as compared to TEE<sup>(4,5)</sup>. In addition, c-TCD has some advantages compared to TEE. It is less invasive, and the results are more quantifiable.

Previous studies focused more on commercial contrast agents as the contrast agent of choice with the highest sensitivity for RLS detection<sup>(6,7)</sup>, but these commercial agents are expensive and not broadly available. It is usually recommended to perform Valsalva maneuver (VM) during TEE and c-TCD. The performance of VM needs the patient's cooperation and cognition, which is impaired in some post-stroke patients. Therefore, this study was conducted to find a simple method with higher sensitivity which does not depend on the patient's cooperation.

The aim of this study is to evaluate a technique for contrast agent preparation as mixing blood with agitated saline (AS) and to compare it with AS alone to detect RLS in regard to their sensitivity, time window, and distribution of artificially-induced microembolic signals (MES). In addition, we compared the sensitivity of these techniques in detecting severe and minimal RLS.

## METHODS

In this cross-sectional study, we enrolled 52 consecutive patients with a recent ischemic stroke or transient ischemic attack who had a PFO, between January 2008 and December 2009. The patients were admitted to the stroke center in Shiraz, South Iran. The patients with non-severe stroke (modified Rankin disability Scaling score not more than 3) or transient ischemic attack within 30 days of enrollment, age more than 18 years, and presence of PFO during TEE examination were recruited. Exclusion criteria included refusal at the time of eli-

gibility, intracranial hemorrhage, congenital heart disease, a known case of pulmonary arterio-venous fistula, poor compliance during c-TCD and TEE, cardio-respiratory instability, and absence of proper temporal window for c-TCD.

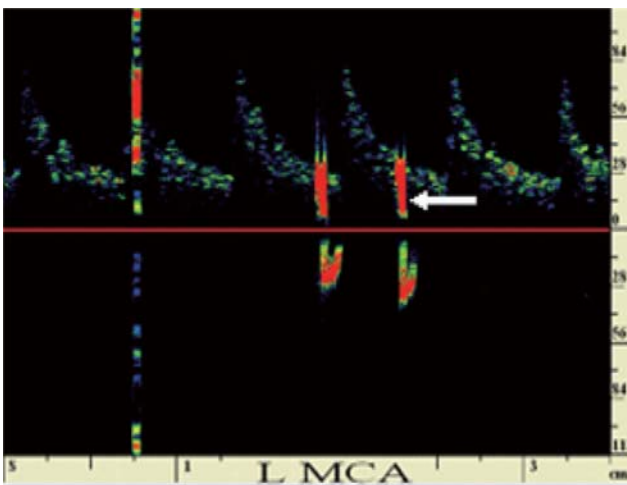
Age and sex of the patients and presence of any vascular risk factors including hypertension, diabetes mellitus, hyperlipidemia, ischemic heart disease, previous cerebral ischemic event, and cigarette smoking in the previous 10 years were recorded. Brain computed tomography scan or magnetic resonance imaging, routine laboratory tests, electrocardiograms, color duplex sonography of extracranial arteries, and TEE were conducted for all of the patients. Contrast-transcranial Doppler sonography examination was performed within a week after ischemic event for the patients who had a PFO. The informed consent was obtained from the patients or their first degree relatives. This study was approved by the local ethics committee.

Transesophageal echocardiography was performed under local pharyngeal anesthesia with a topical lidocaine spray. A Vivid 3 Echo machine with a 5-MHz TEE bi-plane probe was used (GE medical systems, Norway). Contrast (agitated saline) was injected in the mid esophageal four chamber view during VM, for the detection of any RLS across the PFO. Agitated saline as the contrast material was generated by agitating a mixture of 9 ml of normal saline and 1 ml of air in two 10-ml syringes connected by a 3-way stopcock. Once the contrast's color got milky, 10 ml of the contrast agent was immediately injected as a bolus into a cubital vein that had previously been cannulated with a large gauge indwelling intravenous catheter. Valsalva maneuver was induced by asking the patient to inhale deeply and straining against closed glottis, 5 seconds after the beginning of the injection of contrast material and then expiration 10 seconds later.

Diagnosis of RLS was based on the appearance of any bubbles in the left atrium during five cardiac cycles after the opacity of the right atrium with contrast bubbles. The number of bubbles in the left atrium was counted and graded as follows: minimal if  $< 50$ , and severe if  $\geq 50$  bubbles, visualized in the left atrium. The

frequency of bubbles was counted on-line.

For c-TCD, a Sunray transcranial Doppler ultrasound device machine version FD-T98II (Guangzhou Doppler Electronic Technologies, China) was used for all the cases. While the patients were lying supine, both middle cerebral arteries (MCA) were insonated simultaneously. Two 2-MHz transducers were fixed on the temporal windows using a helmet. The device was set to a small sample volume of 10 mm in length and the minimum possible gain to provide an optimal setting for air MES discrimination from the background spectrum. The definition for MES used was a typical visible and audible (click, chirp, and whistle) of short duration and a high-intensity signal within the Doppler flow spectrum (Figure 1). The same protocol as TEE for VM was used. The effectiveness of VM was monitored by observing a decline in the Doppler spectrum. The following methods for contrast preparation and injection was used: injection of agitated (i) 9 ml saline with 1 ml air with VM, (ii) 9 ml saline with 1 ml air without VM, (iii) 8 ml saline, 1 ml of the patient's fresh blood and 1 ml air with VM, and (iv) 8 ml saline, 1 ml of the patient's fresh blood and 1 ml air without VM. We proposed that mixing the contrast agent with a minimum amount of blood would emulsify the bubbles and prevent them to solvate in sys-



**Figure 1.** A spectral transcranial Doppler recording of the middle cerebral artery flow of a 65 year old man with patent foramen ovale shows a high-intensity transient (white arrow) after injection of agitated saline mixed with blood.

temic circulation. Adding more blood to the agent may result in increasing the viscosity of the contrast agent that may further diminish the rate of injection. All of the tests were performed consecutively, which each test required about 2 minutes to perform and with at least 1 minute interval between tests from the last observed MES. The number of MES reported was obtained by counting the MES on the spectrum of both arteries for 1 minute after injection of contrast or 40 seconds after the last MES observed. The defined threshold to diagnose RLS was appearance of  $\geq 1$  air MES in the Doppler spectrum. The frequency of MES was counted on-line and off-line. Two ultrasound experts analyzed the results and counted MES by listening to each device-recorded sound and watching each signal on the screen.

All the statistical analyses were performed in SPSS version 15.0 software. The results are expressed as absolute frequencies and percentages where appropriate. Descriptive results are presented as the mean value  $\pm$  standard deviation. Comparison of the findings between subgroups of patients was performed using t-test, Mc Nemar test and Kappa Coefficient. Inter-observer variability was measured using Pearson correlation. A P value  $< 0.05$  was considered as significant.

## RESULTS

From the 440 patients with ischemic stroke or TIA who were admitted to our stroke center over the 2 years of the study, 85 were diagnosed with PFO during TEE examination. Considering inclusion and exclusion criterion, 65 patients had undergone c-TCD examination. Of excluded patients, six patients did not have a bilateral proper temporal window for c-TCD examination. Thirteen patients did not receive four contrast injections; therefore, they were excluded as well. The mean age of the patients was  $61.3 \pm 13.5$  years. Thirty-two patients (61.5%) were male and 20 (38.5%) female. Severe cardiac RLS during TEE was presented in 36 (69.2%) patients and minimal RLS in 16 patients (30.8%). Inter-observer variability for AS with VM ( $r = 0.911$ ,  $P = 0.001$ ), AS without VM ( $r = 0.957$ ,  $P = 0.001$ ), AS and blood with VM ( $r = 0.891$ ,  $P = 0.001$ ), and AS and blood

without VM ( $r = 0.869$ ,  $P = 0.001$ ) was excellent. No major side effects were observed during contrast agent injections.

The sensitivity of bilateral MCA monitoring in diagnosis of RLS was 94.2%, 71.2%, 96.2% and 76.9% for AS with VM, AS without VM, AS and blood with VM and AS and blood without VM methods, respectively. In combination of the results of AS with VM and AS and blood with VM, 98.1% of the TEE proven PFO had been detected. The application of VM significantly resulted in detection of more RLS with AS ( $P = 0.002$ ), and with AS and blood ( $P = 0.002$ ) compared to AS, and AS and blood without VM. Using blood as a contrast material resulted in detection of more RLS compared to AS alone, with VM ( $P = 0.897$ ) and without VM ( $P = 0.453$ ); however, this was not statistically significant. Figure 2 shows the percentage (sensitivity) of the detected PFOs after injection of different contrasts in time.

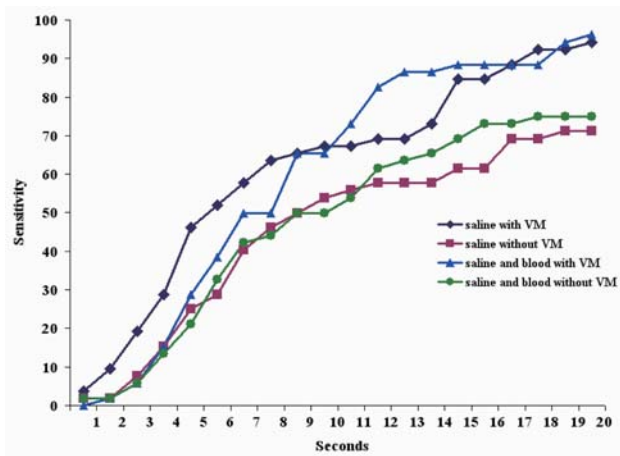


Figure 2. The percentage (sensitivity) of the detected patent foramen ovales after injection of different contrasts in time

The difference of single artery monitoring versus bilateral monitoring is shown in Table 1.

Severe RLS was detected in 35/36 (97.2%) patients with AS with VM, 29/36 (80.6%) with AS without VM, 36/36 (100%) with AS and blood with VM, and 30/36 (83.3%) with AS and blood without VM. Minimal RLS was detected in 14/16 (87.5%) patients with AS with VM, 8/16 (50%) with AS without VM, 14/16 (87.5%) with AS and blood with VM, and 10/16 (62.5%) with AS and blood without VM. This is shown in Figure 3.

The mean interval between contrast injection and MES appearance on TCD spectrum was  $7.95 \pm 5.54$ ,  $8.78 \pm 6.07$ ,  $8.58 \pm 4.44$  and  $9.12 \pm 6.51$  seconds for AS with VM, AS without VM, AS and blood with VM, and AS and blood without VM methods, respectively. The mean interval was significantly shorter in AS with VM compared to AS without VM ( $P = 0.024$ ) and AS and blood without VM ( $P = 0.015$ ). There were no sig-

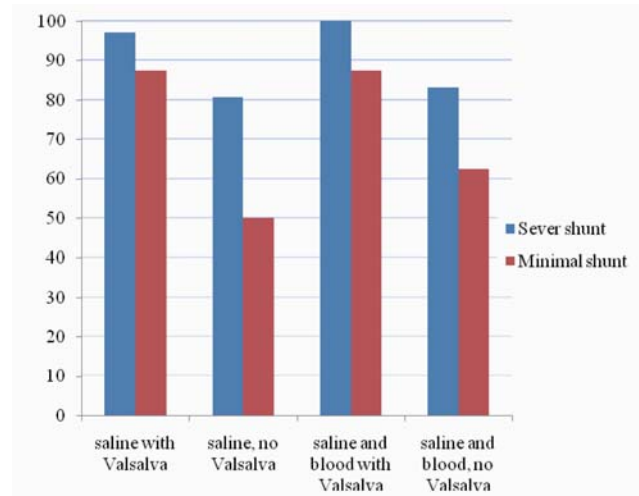


Figure 3. The percentage (sensitivity) of the detected patent foramen ovales after injection of different contrasts in time

Table 1. Number of detected patent foramen ovale with monitoring of different arteries (total = 52)

	Bilateral MCA	Right MCA	Left MCA	$\kappa$ for agreement (P value)
AS with VM	49 (94.2%)	47 (91.4%)	47 (91.4%)	0.557 (0.001)
ASB with VM	50 (96.2%)	49 (94.2%)	50 (96.2%)	0.790 (0.001)
AS without VM	37 (71.2%)	34 (65.4%)	35 (67.3%)	0.871 (0.001)
ASB without VM	40 (76.9%)	36 (69.2%)	40 (76.9%)	0.764 (0.001)

MCA, middle cerebral arteries;  $\kappa$ , kappa coefficient; AS, agitated saline; VM, Valsalva maneuver; ASB, agitated saline and blood

nificant differences in the time of MES appearance between AS and blood with VM and other methods ( $P > 0.05$ ). All of the first MES appeared in the first 20 seconds in different methods.

## DISCUSSION

The results showed that c-TCD, detected TEE proven PFOs with a sensitivity of 96.2% when AS and blood with VM was used and when both MCA had been monitored for at least 20 seconds. The best results were achieved when VM was administered; this is in agreement with what previously reported<sup>(5,8)</sup>. This was somehow more than what reported by Zanette et al (79%)<sup>(9)</sup>. If a patient is unable to perform VM, agitating the contrast material with the patient's blood may improve the sensitivity of the test. A clinician must agitate and inject the mixture before a blood clot could be formed. In our observation, this method was safe and showed no side effects. More importantly, it is always available and it is not dependent on the patients' compliance.

Droste et al have found that c-TCD detects TEE proven RLS with a sensitivity of 100% when the results of galactose-based agent (Echovist-300, Schering AG) with VM are combined with those of Echovist with coughing. Single test with Echovist with VM had a sensitivity of 90%. In addition, the sensitivity of the test with Echovist without VM in a diagnostic time window of 25 seconds was 60% less than what we achieved with AS mixed with fresh blood<sup>(4)</sup>. In another study, the same author found that the sensitivity of c-TCD to find TEE proven PFO was 94% when the combined results of Echovist with and without VM were used<sup>(10)</sup>. In comparison of our results with what was achieved previously by Echovist revealed that if VM is applied, the sensitivity of Echovist and AS seems to be almost similar; and without VM, the sensitivity of AS is more than Echovist alone. We may improve the sensitivity by mixing AS with blood. In addition, Echovist is expensive and not always available in contrast to AS and patients' fresh blood which are simply preparable and always available.

According to the current results, no artery was preferred for single artery monitoring (Table 1). In a study

by Horner et al, similar results were found and side preponderance of MES could not be demonstrated<sup>(8)</sup>. The accuracy of the test improved with bilateral monitoring of the arteries. Monitoring of both MCAs is especially warranted if we look for minimal RLS.

In our observation, AS and blood with VM had a sensitivity of 100% in detecting severe RLS. However, not all the minimal RLSs had been detected by different c-TCD methods. The main limitation of c-TCD, with or without blood as a contrast agent, was diagnosis of minimal RLS which was concluded by other authors as well<sup>(11)</sup>. There are reports indicating that with increasing the size of PFO, the risk of further stroke would increase<sup>(12)</sup>. We propose that c-TCD would not miss larger PFOs with more potential for paradoxical emboli.

When VM was used, first MES appeared in the first 20 seconds in all patients. This helps us to differentiate the cardiac RLS from other shunts such as lung arterial-venous shunts which allows the bubbles to bypass the pulmonary capillaries. Theoretically, MES needs more time to pass through the pulmonary shunts than through PFO and many authors believe that the MES from shunts other than PFO tend to appear later in the cerebral vasculature<sup>(13)</sup>; however, others found that transient time through these RLSs may overlap<sup>(4,8)</sup>. If a monitoring for MES is performed for a longer duration, the specificity of the test will decline<sup>(4)</sup>. In our observation, all the first MESs appeared in the first 20 seconds. We may propose that appearance of MES later in the Doppler spectrum may result from RLSs other than PFO.

In our experience, injection of mixture of agitated blood and saline had no reverse side effects. Recently, Romero et al presented 5 cases of cerebral ischemic events following contrast injection to detect RLS<sup>(14)</sup>. They did not mention the type of contrast which used. The true prevalence of cerebral ischemic event during c-TCD or contrast-TEE examinations is unknown, which may need further study with larger sample size.

Our study had some limitations. Thirteen patients were excluded from the study, primarily due to incompliance to receive four injections of contrast agents and denial at the time of c-TCD examination. The lack of proper temporal window to perform c-TCD in six



patients might influence the results. TEE was performed by one echocardiographer and lack of inter-observer variability for TEE findings is another shortcoming of the current study. However, the value of the work is that introduce a new method for c-TCD study with higher sensitivity, and even safety profile.

We conclude that mixing fresh blood with AS probably improves the detection of more RLS; however, this was not statistically significant. This technique is especially useful when a patient is unable to perform VM and when we look for severe RLS. Contrast-transcranial Doppler sonography had a high sensitivity compatible to Echovist in diagnosing of RLS if the results of AS with VM were combined with AS and blood with VM and when both MCA had been monitored for at least 20 seconds.

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