Right and left ventricular adaptation to hypoxia: a tissue Doppler imaging study

Sandrine Huez,1 Kathleen Retailleau,2 Philippe Unger,2 Adriana Pavelescu,2 Jean-Luc Vachiéry,2 Geneviève Derumeaux,3 and Robert Naeije1

1Department of Physiology, Faculty of Medicine of the Free University of Brussels, Belgium; 2Department of Cardiology, Erasme University Hospital, Brussels, Belgium; 3EMI 0226, Lyon University Hospital, Lyon, France

Submitted 4 April 2005; accepted in final form 2 May 2005

EXPOSURE TO HYPOXIA in humans is associated with a preserved cardiac output at rest but limited cardiac output during exercise (26). The mechanisms accounting for this limitation of maximal cardiac output remain unclear. Echocardiographic studies in hypoxic healthy volunteers have shown a preservation of left ventricular (LV) contractility but reported altered mitral flow patterns, suggestive of LV diastolic dysfunction (2, 6, 19). How hypoxia and hypoxic pulmonary vasoconstriction-related increase in afterload affect RV function is not exactly known.

Standard Doppler echocardiography to evaluate RV function is limited by its complex geometry and nonconcentric contraction patterns (3, 4, 16). We wondered whether tissue Doppler imaging (TDI) could provide a more exact assessment of RV function. This recently introduced approach allows the noninvasive determination of myocardial velocity in the human heart and has the potential of shape-independent assessment of ventricular contractile function (31). It has been applied with high recovery rate of good quality signals, with demonstration of aged dependency in healthy subjects (1), and has been reported to be sensitive to early function changes in experimental and clinical cardiomyopathy (18, 23).

We therefore investigated RV and LV adaptation to hypoxia in healthy volunteers using standard Doppler-echocardiography, pulsed TDI, and longitudinal systolic strain and strain-rate imaging. To sort out intrinsic effects of hypoxia from those of reflex sympathetic nervous system activation, we compared hypoxia-induced changes with those of dobutamine administered at a dose devoid of intrinsic flow-independent effects on pulmonary vascular tone (25) and titrated to reproduce the same increase in heart rate as in hypoxia.

MATERIALS AND METHODS

Study Population

Twenty-five healthy volunteers aged 32 (8) years [mean (SD)], 14 men and 11 women, were included in the present study, which was approved and registered by the Institutional Review Board of the Erasme University Hospital of Brussels and is compliant to the Belgian law of May 2004. None of the subjects had a previous history of cardiac or pulmonary disease. Physical examination, 12-lead electrocardiogram, pulmonary function tests, and standard Doppler echocardiography were normal.

Study Design

Hypoxia test. A complete standard Doppler echocardiography with TDI acquisition was performed when the subjects were in a stable state as assessed by continuously monitored systemic blood pressure (BP), heart rate (HR), and arterial O2 saturation (SaO2), first at baseline in normoxia and then after 90 min of hypoxic breathing with an inspired fraction of O2 of 0.12. This severity of hypoxia corresponds to an altitude of 4,500 m and has been shown to be well tolerated with minimal changes in arterial Pco2 (14). The 90-min duration was chosen because it has been shown to produce a maximal hypoxic pressure response in healthy humans (14).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Dobutamine infusion. One week later, echocardiography with TDI acquisition was repeated in 19 subjects during baseline conditions, assessed by a return to the initial values of BP, HR, and $\text{SaO}_2$, recorded the week before, and repeated during a dobutamine infusion titrated to reproduce the same increase in HR as elicited during the hypoxia test. This target was achieved in all the subjects at a dose 9.6 (1.1) μg·kg\(^{-1}\)·min\(^{-1}\). This dose of dobutamine has no intrinsic effect on the pulmonary circulation in intact experimental animal preparations (25).

Experimental Protocol

Hypoxia test. The low inspired fraction of $\text{O}_2$ was administrated by using a tightly collar-fitted helmet (Castar, Brussels, Belgium). Inhaled and expired $\text{O}_2$ and $\text{CO}_2$ were checked with analyzers (Datex, Aartselaar, Belgium). $\text{SaO}_2$, was continuously monitored by a pulse oximeter (Nellcor, Tyco Healthcare, Mechelen, Belgium). BP was determined by an automated blood pressure cuff, and HR was continuously monitored by three electrocardiographic leads (Siemens SC 6000, Drager Medical, Wemmel, Belgium).

Dobutamine infusion. Dobutamine was administrated into a brachial vein at the initial dose of 5 μg·kg\(^{-1}\)·min\(^{-1}\) and progressively increased by 2 μg·kg\(^{-1}\)·min\(^{-1}\) steps every 5 min while HR was monitored.

Echocardiography

Data acquisition. Echocardiography was performed with a Vivid 7 ultrasound system (GE Ultrasound) equipped with a 3-MHz transducer and TDI technology. Pulsed and two-dimensional color TD images were acquired from an apical four-chamber view during a short end-expiration pause. Pulsed TDI volume samples were recorded at the lateral side of the mitral annulus and at the free-wall side of the tricuspid annulus. Two-dimensional color TDI cineloops were obtained with high frame rate (>150 frames/s) by reducing sector angle to each wall separately. Pulse repetition frequency was adjusted visually to avoid aliasing.

Data analysis and measurements. A single observer performed off-line analysis using a workstation (EchoPac software). All the measurements were done in triplicate and averaged.

End-systolic and end-diastolic LV areas and volumes were estimated from an apical four-chamber view, and LV ejection fraction (LVEF) were calculated from these data. End-systolic and end-diastolic RV areas were also measured, and the RV area shortening fraction (RV area SF) was calculated (17). Cardiac output was calculated as the product of the velocity-time integral of the pulsed-Doppler tracing in the LV outflow tract, the cross-sectional area of the LV outflow tract, and the HR. Mitral and tricuspid inflow patterns were obtained from an apical four-chamber view to measure early (E) and late (A) diastolic wave maximal velocities. Tricuspid annular plane systolic excursion (TAPSE) was recorded with M-mode as previously described (17). Pulmonary artery pressure was estimated from the maximal of the tricuspid regurgitant jet to calculate a systolic RV pressure gradient (SRVG) (22) and pulmonary artery prejection time (PET) and acceleration time (PET) divided by ejection time (ET) of pulmonary artery blood flow (15, 22).

Mitral and tricuspid annuli peak velocities during isovolumic contraction (ICV), along with systolic ejection (S), early diastole (E) and late diastole (A) were measured. Acceleration of the ICV wave (ICA) was calculated as the difference between baseline and peak velocity divided by their time interval (31). Isovolumic relaxation time (IRT) was measured as the time between the onset of the QRS complex and the onset of E wave (time to E), was also calculated. To minimize any influence of tachycardia on these indexes, these times were divided by the RR intervals between two QRS complexes.

For TDI velocity measurement, three operator-selected regions of interest were positioned (basal, mid, and apical segment) for the interventricular septum and the LV lateral wall (18) and two for the RV (basal and apical segment). Systolic strain rate and strain were determined as previously described (18). Therefore, peak systolic tissue velocity (S), peak systolic strain rate (SR), and systolic strain (ε) were calculated from exactly the same sample volume.

Statistical Analysis

Results are expressed as means (SD). Intraindividual variability was assessed by calculation of agreement between baseline conditions and expressed by the repeatability coefficient, calculated as 1.96$\sqrt{\text{SD}^2}$ of observations during the two baselines (5). Comparisons consisted of a repeated measures two-factors analysis of variance (time: baseline, intervention; and period: hypoxia, dobutamine). When the F ratio of the analysis of variance reached a $P < 0.05$ critical value, paired t-tests were used to compare specific situations (33).

RESULTS

Agreement Between Baseline Measurements

There were no significant differences between the mean of the two baseline measurements. Repeatability coefficients between the two baseline conditions were 2.8 cm/s for pulsed-TDI S wave peak velocity, 3% for IRT/RR, 6.3% for systolic $ε$, and 0.59/s for systolic SR. Therefore, only the first of the two baseline measurements are shown in Tables 1 and 2.

Effects of Hypoxic Breathing

Hemodynamics and conventional echocardiography. During hypoxic breathing ($n = 25$ subjects), continuously monitored $\text{PCO}_2$ did not change or showed a minimal decrease, and $\text{SaO}_2$, decreased to below 80%, as previously described (14) (Table 1).

Hypoxia increased HR, LVEF, cardiac output, SRVG, and pulmonary PET/ET. It decreased mitral and tricuspid inflow E/A ratio, pulmonary AT/ET but did not change mean BP, TAPSE, end-diastolic RV area, and RV area shortening fraction.

Pulsed TDI indexes. At the tricuspid annulus, hypoxia did not change ICV, ICA, S, and ICT when related to the RR interval (ICT/RR), increased A, IRT, time to E, and also these

<table>
<thead>
<tr>
<th>Table 1. Conventional echocardiographic measurements at baseline and during hypoxic breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
</tr>
<tr>
<td>$\text{SaO}_2$, %</td>
</tr>
<tr>
<td>Mean BP, mmHg</td>
</tr>
<tr>
<td>CO, l/min</td>
</tr>
<tr>
<td>LVEF, %</td>
</tr>
<tr>
<td>SRVG, mmHg</td>
</tr>
<tr>
<td>Mitral inflow E/A</td>
</tr>
<tr>
<td>Tricuspid inflow E/A</td>
</tr>
<tr>
<td>EDRV area, cm$^2$</td>
</tr>
<tr>
<td>RV area SF, %</td>
</tr>
<tr>
<td>TAPSE, cm</td>
</tr>
<tr>
<td>Pulmonary AT/ET</td>
</tr>
<tr>
<td>Pulmonary PET/ET</td>
</tr>
</tbody>
</table>

Values are means (SD); $n = 25$ subjects. HR, heart rate; $\text{SaO}_2$, arterial saturation in oxygen; mean BP, mean blood pressure; CO, cardiac output; LVEF, left ventricular ejection fraction; SRVG, systolic RV pressure gradient; EDRV area, end-diastolic right ventricular area; RV area SF, right ventricular area shortening fraction; TAPSE, tricuspid annular plane systolic excursion; AT, acceleration time; PET, prejection time; ET, ejection time; E and A, early and late diastolic waves peak velocities.
two indexes when related to RR interval (IRT/RR and time to E/RR), and decreased RR and E/A (Table 2).

At the mitral annulus, hypoxia increased ICA, S, A, IRT/RR, and time to E/RR. It did not change ICV, E, ICT/RR, and IRT, and decreased RR, E/A ratio, and time to E.

Comparison Between Hypoxia and Dobutamine Infusion

Hemodynamics and conventional echocardiography. Hypoxia and dobutamine increased HR, LVEF, cardiac output, and SRVG and did not change EDRV area (n = 19) (Table 3). Mean BP, RV area SF, and TAPSE were unchanged by hypoxia and increased by dobutamine. Tricuspid and mitral inflow E/A were decreased by hypoxia, whereas dobutamine increased mitral inflow E/A and did not change tricuspid inflow E/A. Hypoxia decreased pulmonary AT/ET and increased PET/ET, whereas dobutamine did not change AT/ET and decreased PET/ET.

Pulsed TDI indexes. At the tricuspid annulus, hypoxia and dobutamine decreased the RR interval and E/A, increased A, and did not change ICT/RR. Hypoxia did not change ICV, ICA, S, and E, whereas dobutamine increased all of these variables (Table 4, Figs. 1 and 2). IRT/RR, IRT, time to E/RR, and time to E were increased by hypoxia and decreased by dobutamine.

At the mitral annulus, hypoxia and dobutamine decreased RR and time to E; increased ICV, E, ICT/RR, and IRT, and did not change ICT/RR and time to E/RR. E was unchanged by hypoxia and increased by dobutamine, and E/A was decreased by hypoxia but unchanged during dobutamine. IRT was unchanged by hypoxia and decreased by dobutamine. When related to RR, IRT/RR was increased by hypoxia and unchanged by dobutamine.

Regional deformation by strain and strain rate analysis. Regional deformation was able to be analyzed in 13 subjects with the highest two-dimensional quality (Fig. 3). At the RV free wall, S, ε, and SR at the basal and apical segments were unchanged by hypoxia and increased by dobutamine. At the interventricular septum, S, ε, and SR in the three segments were increased by dobutamine, whereas the only change associated with hypoxia was an increased ε in apical segment. At the LV lateral wall, both hypoxia and dobutamine increased S, ε, and SR in all segments.

DISCUSSION

This study shows for the first time the effects of hypoxic breathing on concomitant RV and LV function in humans, as assessed by Doppler echocardiography, pulsed TDI, and regional indexes of myocardial strain and strain rate imaging. The results show that functional changes induced by hypoxia predominate in the RV during diastole and in the LV during systole.

Hypoxia decreases exercise capacity and maximum cardiac output (9). However, whether RV or LV function changes contribute to the limitation of maximum cardiac output and exercise capacity in hypoxia remains unclear. Right heart catheterization studies in healthy volunteers progressively decompressed in a hypobaric chamber during 40 days to a level of hypoxia equal to that at the summit of Mount Everest showed a maintained cardiac output at rest but a decreased stroke volume associated with tachycardia and decreased RV and LV filling pressures (26). Echocardiographic studies in the same subjects, focused on the LV, showed decreased stroke volume and end-diastolic and end-systolic volumes.
with unaltered LVEF and ratio of peak systolic pressure to end-systolic volume suggestive of well-preserved contractility (29). Recent hypobaric chamber experiments confirmed the preservation of LV systolic function but showed an alteration of mitral inflow pattern with a decrease of the E/A ratio, suggestive of an alteration of diastolic function (6). This observation was confirmed in high-altitude newcomers (2, 19), with mitral annulus pulsed TDI measurements in one of these studies (2).

Hypoxia should exert predominant effects on the RV because of increased loading due to hypoxia-induced pulmonary vasoconstriction (21). Accordingly, an inhibition of hypoxic

### Table 4. Comparison between tricuspid and mitral annuli pulsed-tissue Doppler measurements during hypoxia and dobutamine infusion in the same volunteers

<table>
<thead>
<tr>
<th></th>
<th>Tricuspid Annulus Pulsed-TDI</th>
<th>Mitral annulus pulsed-TDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>RR</td>
<td>889 (142)</td>
<td>769 (146)*</td>
</tr>
<tr>
<td>ICV, cm/s</td>
<td>9.6 (2.1)</td>
<td>9.5 (3.2)</td>
</tr>
<tr>
<td>ICA, cm/s²</td>
<td>0.34 (0.09)</td>
<td>0.39 (0.15)</td>
</tr>
<tr>
<td>S, cm/s</td>
<td>15.3 (2.6)</td>
<td>16.4 (2.6)</td>
</tr>
<tr>
<td>E, cm/s</td>
<td>13.1 (3.6)</td>
<td>12.6 (3.3)</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>9.9 (2.6)</td>
<td>13.1 (4.1)*</td>
</tr>
<tr>
<td>E/A</td>
<td>1.4 (0.5)</td>
<td>1.1 (0.5)*</td>
</tr>
<tr>
<td>ICT/RR, %</td>
<td>6.4 (1.3)</td>
<td>6.3 (1.7)</td>
</tr>
<tr>
<td>IRT/RR, %</td>
<td>3.2 (2.2)</td>
<td>10.8 (4.4)*</td>
</tr>
<tr>
<td>IRT, ms</td>
<td>28 (19)</td>
<td>84 (39)*</td>
</tr>
<tr>
<td>Time to E/RR, %</td>
<td>46 (6)</td>
<td>57 (8)*</td>
</tr>
<tr>
<td>Time to E, ms</td>
<td>401 (36)</td>
<td>432 (48)*</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 19 subjects. *P < 0.05 intervention vs. baseline; †P < 0.05 dobutamine vs. hypoxia.
pulmonary vasoconstriction together with an increased exercise capacity was recently reported to occur in normal volunteers exposed to hypobaric hypoxia at a base camp of Mount Everest (10). These authors reasoned that the sildenafil-induced improvement in hypoxic exercise capacity could be related to unloading of the RV, allowing for an increased cardiac output and convensional O₂ transport to the exercising muscles (10). However, this concept remains unproven in the absence of functional studies on the adaptation of the RV to hypoxia (27). The RV has a complex anatomy and contraction pattern (3), making functional studies in this chamber difficult. We reasoned that this could be improved with TDI because of its capacity to directly visualize myocardial motion, thereby providing shape-independent assessment of ventricular contractile function (12, 13, 31, 32). The TDI also allows for longitudinal motion recording as the main determinant of RV performance (4).

In the present study, hypoxic breathing was associated with an increased exercise capacity was recently reported to occur in normal volunteers exposed to hypobaric hypoxia at a base camp of Mount Everest (10). These authors reasoned that the sildenafil-induced improvement in hypoxic exercise capacity could be related to unloading of the RV, allowing for an increased cardiac output and convensional O₂ transport to the exercising muscles (10). However, this concept remains unproven in the absence of functional studies on the adaptation of the RV to hypoxia (27). The RV has a complex anatomy and contraction pattern (3), making functional studies in this chamber difficult. We reasoned that this could be improved with TDI because of its capacity to directly visualize myocardial motion, thereby providing shape-independent assessment of ventricular contractile function (12, 13, 31, 32). The TDI also allows for longitudinal motion recording as the main determinant of RV performance (4).

In the present study, hypoxic breathing was associated with a decrease in SaO₂, an increase in HR, mean BP and cardiac output, and an increase in SVR and decreased pulmonary flow acceleration time indicating hypoxic pulmonary vasoconstriction. These changes are in keeping with previous studies on normal subjects in similar experimental conditions (14, 30).

LVEF and ICV, ICA, and S at the mitral annulus all increased during both hypoxic breathing and dobutamine, indicating an enhanced contractility. This is in keeping with previous hemodynamic and echocardiographic measurements, indicating an enhanced LV systolic function in hypoxic healthy volunteers (2, 6, 19, 24, 29). In contrast, hypoxia did not affect RV area SF, TAPSE, nor any of the pulsed TDI indexes of systolic RV function, all of which were increased by dobutamine. This was particularly true for ICA recorded at the tricuspid annulus, which has been recently shown to be correlated to LV maximal pressure change and to maximal systolic elastance (31), generally considered to be the reference load-independent measure of contractility (20). The present observations are in keeping with a previous report of preserved RV isotropic ejection fraction in hypoxic normal subjects (24). However, studies on the effects of similar severity of inspiratory hypoxia on the coupling of RV function to the pulmonary circulation in experimental animals point to a moderate increase in maximal systolic elastance allowing for adaptation to increased hydraulic load (7). This apparent discrepancy could be related to the fact that TDI velocities are affected by heart translation and motion of adjacent myocardial segments. Systolic ε (relative deformation) and SR (rate of deformation) have been shown more appropriate to assess both segmental and global LV contractility (12, 13, 32). Longitudinal ultrasonic ε and SR at the RV free wall have recently been closely correlated to deformation indexes recorded by sonomicrometry (16). In our study, hypoxia increased deformation indexes in the LV wall but not in the interventricular septum or RV free wall. The same indexes increased on each myocardial wall during dobutamine infusion, thus confirming the absence of increased

![Fig. 2. Left: mean pulsed TDI curves at the mitral annulus (n = 19), at baseline and during hypoxia (top) and at baseline and during dobutamine infusion (bottom). Hypoxia increased ICV, S, and A and did not change E and IRT. Dobutamine increased ICV, S, E, and A and decreased IRT. Right: example of the pulsed TDI curves at mitral annulus in same volunteer. *P < 0.05 compared with baseline.](http://ajpheart.physiology.org/10.220.32.246)

AJP-Heart Circ Physiol • VOL 289 • OCTOBER 2005 • www.ajpheart.org
RV systolic function in response to increased pulmonary artery pressures. Why RV contractility did not appear to increase in the present study is unclear. It may be that TDI indexes of systolic function remain less sensitive than invasive measurements of maximal systolic elastance. On the other hand, negative inotropic effects of hypoxia (28), insufficiently counterbalanced by positive inotropic effects of sympathetic nervous system activation (8) or systolic ventricular interaction, could have limited RV systolic function adaptation to afterload.

Hypoxia was associated with an increase in IRT/RR and time to E/RR, indicating an alteration of early diastolic function. This result is in keeping with previous studies that used simultaneous phonocardiography and Doppler echocardiography to establish an inverse relationship between RV isovolumic relaxation time and systolic pulmonary artery pressure, as such this measurement has been proposed as a noninvasive method to evaluate pulmonary hypertension (15). Dobutamine decreased IRT/RR and time to E/RR, indicating an improved diastolic function. There are two possible explanations for hypoxia-induced alteration RV early diastolic function. The first would be that hypoxia could have altered active cellular mechanisms of calcium reuptake and thereby prolonged muscle relaxation. However, this was described with more severe hypoxia than in the present study (28). The second explanation relates to the concept of contraction-relaxation coupling, leading to prolonged relaxation phase in relation to magnitude and timing of systolic loading, as previously described for the LV (11). Pulsed TDI indexes of early diastolic function were only slightly increased at the mitral annulus, suggesting a minor role of cellular hypoxia compared with increased RV afterload.

Hypoxic breathing was also associated with decreased tricuspid and mitral inflow and annuli E/A ratio, resulting from an increase in atrial contraction (A wave). These results are in

---

**Fig. 3. Effects of hypoxia and dobutamine on S (top), ε (middle), and peak systolic strain rate (SR) (bottom) along right ventricle (RV), interventricular septum (IVS), and left ventricle (LV) lateral walls (n = 13).** Hypoxia increased deformation indexes along LV lateral wall but not along IVS and RV free wall, whereas the same indexes increased on each wall during dobutamine infusion. *P < 0.05 compared with baseline.
keeping with previous reports of hypoxic exposure-induced LV diastolic dysfunction on the basis of transmitial flow (2, 6, 19) and recently of mitral annulus pulsed TDI increased A wave in high-altitude newcomers (2). The authors of this last study suggested that increased atrial contraction may prevent the appearance of LV diastolic dysfunction in healthy subjects. The present results indicate that at least part of hypoxia-induced changes in mitral and tricuspid inflow and pulsed TDI E/A might be explained by a sympathetic nervous system activation-mediated increase in the A wave, because dobutamine was associated with variable increases in the A wave and decreases in E/A ratios. Hypoxic exposure has been shown to be associated with an activation of the sympathetic nervous system (8), accounting for an increase in HR, which in turn, can affect ventricular diastolic function (34). In the present study, there was no increase in RV end-diastolic dimensions, and therefore it is unlikely that diastolic ventricular interaction due to ventricular competition for space within a nondistensible pericardium could have influenced valvular inflow or TDI indexes of LV or RV diastolic function.

The Doppler echocardiography evaluation of pulmonary hypertension usually relies on the measurement of the maximal velocity of tricuspid valve regurgitant jet and the calculation of a systolic transtricuspid pressure gradient based on the simplified form of the Bernoulli equation (14, 15, 22, 30). The resulting systolic pulmonary artery pressure estimate is generally correlated to invasively measured pressure. However, pulmonary artery pressure may increase because of increases in pulmonary vascular resistance, flow, or atrial pressure. Accordingly, the SRVG determined in the present study increased either as a consequence of pulmonary artery vasoconstriction (hypoxia) or increased pulmonary blood flow (dobutamine). Mean pulmonary artery pressure can also be estimated from the morphology of pulmonary artery blood flow, with a decreased AT/ET and increased PET/ET in proportion to increased mean pulmonary artery pressure (19, 22, 30). These measurements may be more sensitive to pulmonary vasoconstriction-related increase in RV afterload (15, 22), as also shown in the present study: AT/ET decreased during hypoxia, whereas remaining unchanged during dobutamine infusion, and PET/ET increased during hypoxia, whereas decreasing during dobutamine infusion.

In conclusion, short-term hypoxic exposure in normal human subjects is associated with changes in echocardiographic, pulsed-TDI, and regional ventricular deformation indexes of function that are predominantly early diastolic for RV and systolic for LV. These differences may be explained by increased RV afterload due to hypoxic pulmonary vasoconstriction and by the effects of an activation of the sympathetic nervous system on both ventricles.

GRANTS
The study was supported by Grant 3.4516.02 from the Fonds de la Recherche Scientifique Médicale and by the Foundation for Cardiac Surgery. Sandrine Huez is fellow of the Fonds National de la Recherche Scientifique, Belgium.

DISCLOSURE
There is no conflict of interested related to this study.

REFERENCES


