Radionuclide plethysmography for noninvasive evaluation of peripheral arterial blood flow

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Submitted 6 October 2004; accepted in final form 23 February 2005

Harel, François, Jocelyn Dupuis, Ahmed Benelfassi, Nathalie Ruel, and Jean Grégoire Radionucle plethysmography for noninvasive evaluation of peripheral arterial blood flow. Am J Physiol Heart Circ Physiol 289: H258–H262, 2005. First published February 25, 2005; doi:10.1152/ajpheart.01021.2004.—We validated a noninvasive radionuclide plethysmography technique to evaluate peripheral arterial blood flow during reactive hyperemia. This method, based on the measurement of blood volume variations during repetitive venous occlusions, was compared with strain-gauge venous impedance plethysmography. The technique uses 99mTc-labeled autologous red blood cells scintigraphy to determine the rate of change of forearm scintigraphic counts during venous occlusion. Thirteen subjects were simultaneously evaluated with radionuclide and impedance plethysmography. Six baseline flow measurements were performed to evaluate the reproducibility of each method. Twenty-seven serial measurements were then made to evaluate flow variation during forearm reactive hyperemia. After 30 min of recovery, resting forearm blood flows were again evaluated. Impedance and radionuclide methods showed excellent reproducibility with intraclass correlation coefficients of 0.96 and 0.93, respectively. There was also good correlation of flows between both methods during reactive hyperemia (r = 0.87). Resting flows at 30 min after reactive hyperemia were slightly lower than at baseline with both methods. We conclude that radionuclide plethysmography could be used for the noninvasive evaluation of forearm blood flow and its dynamic variations during reactive hyperemia.

VENOUS PLETHYSMOGRAPHY OF the arms and legs has been extensively used to evaluate vascular function in normal and diseased states (4, 14). With this method, vascular reactivity can be tested by the local infusion of pharmacological agents or by evaluation of hyperemic responses after a brief period of limb ischemia. Although it has been well validated, the use of venous plethysmography for the study of arterial reactivity has been limited to research because specialized equipment is needed and because expertise is not available in most clinical centers.

Recently, there has been a growing interest in the clinical evaluation of peripheral vascular reactivity during reactive hyperemia because part of this physiological response is modulated by the integrity of the vascular endothelium (2, 8). Endothelial dysfunction is a diffuse process that occurs early in the development of atherosclerotic vascular disease (7, 9). Evidence shows that evaluation of endothelial function may be useful in risk stratification and in the assessment of response to pharmacological treatment (5). In that context, development of a simple, noninvasive, and reproducible test of the reactive hyperemic response is desirable not only for preclinical and clinical research purposes but also for its potential clinical utility.

We have thus developed a noninvasive radionuclide plethysmography technique to evaluate peripheral arterial blood flow during reactive hyperemia. This method, based on the measurement of blood volume variations during repetitive venous occlusion, is analogous to impedance plethysmography (3, 12) and can also evaluate arterial blood flow into arms or legs during pharmacological intervention and reactive hyperemia. The technique uses 99mTc-labeled autologous red blood cell scintigraphy to determine the rate of regional changes of scintigraphic counts in response to the inflation and deflation of proximal forearm cuffs.

In this study, we aimed to evaluate the feasibility of this technique for arterial blood flow measurement during forearm reactive hyperemia and to validate the results by comparison with the simultaneously performed accepted gold standard, venous impedance plethysmography.

MATERIALS AND METHODS

Patient population. Thirteen subjects (12 men, one woman) were recruited among patients scheduled for gated equilibrium radionuclide ventriculography scan at our nuclear medicine department. Mean age was 70 ± 7.5 yr, and body mass index was 29 ± 3.4 kg/m². Subjects had the following conditions: stable congestive heart failure (n = 2), stable angina (n = 6), prior myocardial revascularization (n = 10), diabetes (n = 1), high blood pressure (n = 9), and dyslipidemia (n = 11). None of the subjects was a smoker. The protocol was approved by the internal research and ethics committees of our institution. All patients signed an informed consent form before the study was started.

Venous plethysmographic study. Patients sat in front of the camera with arms supine and comfortably laying on the surface of the detector, which was positioned above the level of the heart to preclude venous congestion and to make easier spontaneous venous return. Venous cuffs were plugged into an automatic pneumatic inflator (E-20 rapid cuff inflator, Hokanson, Bellevue, WA) set to 50 mmHg. A second cuff was placed proximally on the right arm and connected to another pneumatic cuff inflator to provide arterial occlusion. For impedance plethysmography, calibrated mercury-in-silastic strain gauges were placed around both forearms every 5 cm below the elbow’s crease and connected to the plethysmograph (model EC-4, Hokanson).

For radionuclide plethysmography, because we are interested in sensitivity rather than in spatial resolution, a custom collimator was designed to optimize detection sensitivity to vascular compartment variations. This collimator consists of a 3.18-mm-thick lead sheet with two rectangular openings (10 × 22 cm), which allowed detection of radioactivity of forearms only. This sheet was placed in a standard collimator holder, thus providing direct and simple fixation to the camera. We labeled autologous red blood cells using the usual in vivo technique along with intravenous administration of cold stannous...
pyrophosphate (Amerscan, Amersham Health, Piscataway, NJ). We injected a small amount of activity (111 MBq) of $^{99m}$Tc 20 min later. This method provides labeling that is stable for many hours and that has very good efficiency (>95%) (10). The scintillation camera was configured for dynamic image acquisition every second in a 64 × 64 pixel matrix for a total of 15 min. Both plethysmographic techniques were then performed simultaneously. We digitized data from the impedance plethysmograph using a four-channel analog-to-digital converter (DI-154 RS, DATAQ Instruments, Akron, OH). For radionuclide images, regions of interest were drawn over the forearm and summations of counts were made for each acquired image. Signals were normalized by individual baseline counts of forearm that were acquired before venous occlusion. This transformation provides compensation to variations in forearm volume and radionuclide dose injected and allowed interpatient comparisons. Arterial inflow was calculated in the usual fashion by determination of the upslope of impedance signals. In a similar manner, isotopic flow was derived from the computation of the upslope of the radionuclide signals. Both techniques therefore provide relative flow changes.

The transformation of the radionuclide data was performed in two steps. First, we corrected for interpatient differences. Differences were related to variations in forearm volume and in the injected dose of radionuclide. These two variations were corrected by normalization of each patient data set with a baseline record. Baseline records consisted of an evaluation of the amount of radioactivity in the opening window in the collimator. This activity was directly related to the volume of the forearm and the dose of radiotracer injected. To perform this correction, we allowed static acquisition of forearm volume before starting the venous occlusion. This stable period allows the computation of baseline forearm radioactivity. The data set was then divided by the baseline evaluation, allowing interpatient comparisons.

Second, we performed a calibration to convert radionuclide signals into flow units. We computed the mean of all posthyperemic baseline-corrected data, providing data with the same ratio of the two means. This calibrating factor was then applied to the posthyperemic baseline-corrected data, providing data with the same units (ml·100 ml$^{-1}$·min$^{-1}$) as the impedance method. This conversion also provides easier comparisons between both methods and allows us to perform Bland-Altman computations.

Measurement protocols. Baseline flow measurements were performed with successive cycles of 10-s occlusion and 10-s deflation of the venous cuff. Six resting basal flow measurements were thus made, allowing calculation of repeatability with both methods. After 3 min of rest, rapid arterial occlusion of the right arm was provoked by inflation of the arterial cuff for 5 min at 50 mmHg above the resting systolic pressure. The arterial cuff was then suddenly deflated, and 27 cycles of venous compressions were performed. To increase the number of flow measurements in the posts ischemic period, we decreased the time of venous compression and deflation to cycles of 5 s for the first 2 min (12 cycles) followed by cycles of 10 s for the last 5 min of acquisition (15 cycles). After 30 min of recuperation, resting flow was again measured.

Statistical analyses. Statistical analysis was performed using statistical package version 10.0 from SPSS (Chicago, IL). Reproducibility of baseline flow measured with both methods was assessed by intraclass correlation coefficient (ICC). Correlation of the posthyperemia arterial blood flows derived from the two methods was calculated using Spearman’s correlation coefficient (r). Comparisons of initial and 30-min postischemic hyperemia mean baseline arterial flow were conducted by Wilcoxon’s signed-rank test. Areas under the curves (AUC) were expressed as means ± SE. Statistical significance was accepted when the two-tailed P value was <0.05.

RESULTS

During the baseline period, venous cuff occlusions generated variations of the arm blood pool compartment that could be easily detected on scintigraphic images (Fig. 1A). The deflation period allowed enough time to reach the equilibrium of blood pool activity between the cycles. The simultaneously obtained impedance plethysmography signal is shown in Fig. 1B.

Reproducibility. To determine the reliability of both plethysmographic methods, six repeated measurements of resting arterial blood flow data were acquired, and the statistical ICC of mean slopes was computed across all subjects. The impedance method showed excellent reproducibility with an ICC of 0.96 [95% confidence interval (CI): 0.92–0.99; P < 0.0001]. The radionuclide method also showed very good reproducibility, with an ICC of 0.93 (95% CI: 0.84–0.98; P < 0.0001), which was not different from the impedance approach.

Correlation of flow during reactive hyperemia. The effect of reactive hyperemia on forearm blood flow as a function of time is depicted for both the radionuclide and the impedance meth-
Some methods in Fig. 2. Successive blood flows measured with both methods during reactive hyperemia showed good concordance (Fig. 3) with a correlation coefficient \( r \) of 0.87 (95% CI: 0.85–0.90; \( P < 0.0001 \)). Bland-Altman analysis was performed and expressed graphically in Fig. 4. Radionuclide method tends to underestimate blood flow at high levels.

Further exploration between methods was obtained by calculating the AUC of the flow-time curves from deflation to 1 min of reactive hyperemia and from deflation to 5 min of reactive hyperemia. For the 1-min period, mean AUC was 11.45 ± 1.22 ml/100 ml for the radionuclide method and 17.23 ± 2.34 ml/100 ml for the impedance method. Both AUCs over 1 min correlated closely with an \( r = 0.85 \) and \( P < 0.001 \). For the 5-min period, mean AUC was 28.36 ± 3.16 for the radionuclide method and 33.73 ± 5.26 for the impedance method with \( r = 0.85 \) and \( P < 0.001 \).

DISCUSSION

This study demonstrates that the use of venous occlusion plethysmography combined with the external detection of a blood pool tracer can be used to noninvasively measure forearm blood flow and its dynamic variations during reactive hyperemia. This method provides excellent reproducibility and good correlation with simultaneously performed impedance plethysmography, a method commonly used and recognized for the evaluation of endothelial function and for vascular pharmacology studies (2, 8).

Although overall correlation between both phlethysmographic methods was good, we did observe a greater dispersion of the CI as flow values increased (Fig. 3). This difference at higher flows may be ascribed to inherent differences in these methods, which do not measure flow in the same manner, and to methodological limitations common to both methods at higher flows. Although both approaches are based on the principle that variations in forearm volume during venous occlusion is proportional to arterial inflow, the measurement of forearm volume changes is obtained differently. Impedance plethysmography uses calibrated gauges that convert forearm perimeter variations to volume, whereas radionuclide plethysmography directly measures the activity of a blood pool tracer (labeled red blood cells) and assumes that it is proportional to blood volume. Small regional changes in the hematocrit do occur with higher blood flows and could affect the results (6). Technically, measurement of the upslope of the variations in forearm volume with time is more difficult and subject to experimental error at the higher flows. Although we subjectively found this limitation to be equally valid for both methods, it may significantly contribute to the greater variability with higher flows. Because of the presence of arterial shunts inside the hands, it is customary to use a wrist cuff and exclude the hand circulation during impedance plethysmography studies (11, 13). This measure could not be used in the present experiments where we created a reactive hyperemia, and,
although its true impact is unknown, it may also contribute to some of the variability observed at high flows. Use of other stimuli such as mental or cold-pressor testing may overcome this limitation and should be evaluated in the future. We observed, however, that one technical advantage of the radioisotope method is that it is less sensitive to patient’s movements compared with the impedance gauges, which require absolute immobilization of the arms, as any small movement can lead to a recorded artifact.

Meredith et al. (8) previously studied reactive hyperemia with the use of impedance plethysmography. They also computed areas under the flow-time curves from deflation to 1 min and from deflation to 5 min and obtained measurements in good agreement with those reported in the present study. We also demonstrated that resting forearm blood flow did not return to baseline values and was in fact slightly lower 30 min after reactive hyperemia. This would suggest that a reduced resting vascular tone in response to ischemia is still present after 30 min. The impedance method showed mildly better reproducibility, probably in relation to less variability in the signal. A better collimator design could possibly improve the detection sensitivity and reduce the radionuclide variability.

Others previously used vascular radioisotopes to quantify the hyperemic response. These studies however used a different approach and evaluated the first-pass activity of vascular tracers after bolus injection. This differs substantially from our methodology because we evaluated variations of blood pool activity at equilibrium. Zicot (15) injected 131I-labeled albumin to study first-pass lower limb blood flow during reactive hyperemia to evaluate peripheral vascular disease of the legs. Compared with impedance plethysmography, Zicot found a good correlation between the time to peak of the isotopic activity curve and the width of the impedance hyperemic flow-time curve ($r = 0.74; P < 0.001$). In another study, Parkin et al. (16) studied patients who underwent brachial arteriotomy for coronary angiography. Using 99mTc-labeled albumin and a first-pass method associated during reactive hyperemia, they detected forearm ischemic complications of the brachial procedure (11). More recently, Dupuis et al. (1) used 99mTc-labeled tetrofosmin and compared the hyperemic responses in a group of patients with demonstrated coronary artery disease and a group of subjects with no known coronary artery disease and no risk factors. Dupuis et al. compared the first-pass activity of the hyperemic and nonhyperemic arms to derive parameters that were found to be predictive for the presence of coronary artery disease. These and the present study confirm the clinical potential of the use of radioisotopes for the dynamic evaluation of limb blood flow.

To our knowledge, this trial represents the first report of the use of a vascular isotope as a plethysmographic signal during reactive hyperemia. Our initial attempts, realized with a standard parallel collimator and the usual clinical amount of injected 99mTc activity (1,110 MBq), were unsuccessful. We therefore had to design a custom collimator to reach enough detection sensitivity. This custom collimator offered the best trade-off between spatial resolution and detection sensitivity since the former was not required in our study. The amount of injected activity was limited to 111 MBq to prevent any saturation of the camera detectors. This detection limit was established by evaluation of the dead time of our detectors.

The advantages of radionuclide plethysmography over the impedance method were related to the detection mode. The radionuclide method provides sensitive detection with the special collimator. The impedance method is also sensitive, but the strain-gauge signals are easily perturbed by very small movements and by muscle contractions. These perturbations inevitably occur during acquisition and create artifacts in the impedance signal. However, these contractions do not change the amount of red blood cells included in the selected region, leaving the radionuclide signal uncontaminated. Also, radionuclide plethysmography does not use special radiotracers. Technetium is available in all nuclear medicine departments and is very inexpensive. The patient irradiation is very low, and, due to the short half-life of radionuclide used, environmental load is negligible.

The custom collimator was easy to build at an affordable cost. However, a limitation of our method is that the calibration factor used to convert the units obtained from the radionuclide method to flow units was specific to our own apparatus (custom collimator) and would need to be standardized before any widespread use of this method.

We believe that significant improvements in the detector system could be realized in the future and allow better sensitivity with superior signal-to-noise ratio. This would enable higher sampling rate during image acquisition and possibly better temporal resolution to allow visualization of discrete events throughout the cardiac cycle such as cardiac pulsations. Dedicated probe systems, like those used for thyroid uptake, could provide an avenue of solution.

In conclusion, the measurement of resting forearm blood flow and its dynamic variations during reactive hyperemia is feasible with radionuclide plethysmography. This method has excellent reproducibility and good correlation with the simultaneously performed gold standard, impedance plethysmography. It has the potential of easy incorporation in any clinical center with nuclear medicine facilities and could provide a useful method for the evaluation of vascular reactivity.

REFERENCES

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