Validation of minimally invasive measurement of myocardial perfusion using electron beam computed tomography and application in human volunteers

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Abstract

Objectives—To measure myocardial perfusion using an estimate of intramyocardial vascular volume obtained by electron beam computed tomography (EBCT) in an animal model; to assess the feasibility and validity of measuring regional myocardial perfusion in human volunteers using the techniques developed and validated in the animal studies.

Methods—Measurements of myocardial perfusion with EBCT employing intravenous contrast injections were compared with radioactive microsphere measurements (flow 57 to 346 ml/100 g/min) in seven closed chest dogs. Fourteen human volunteers then underwent EBCT scans using intravenous contrast injections.

Results—Mean (SEM) global intramyocardial vascular volume by EBCT was 7.6 (1.1)%). The correlation between global EBCT (y) and microsphere (x) perfusion was y = 0.59x + 15.56 (r = 0.88) before, and y = 0.72x + 6.06 (r = 0.88) after correcting for intramyocardial vascular volume. Regional perfusion correlation was y = 0.75x + 23.84 (r = 0.82). Corresponding improvements in agreement between the two techniques were also seen using Bland–Altman plots. In the human subjects, mean resting global myocardial flow was 98 (6) ml/100 g/min, with homogeneous flow across all regions. In 10 of these subjects, perfusion was studied during coronary vasodilatation using intravenous adenosine. Global flow increased from 93 (5) ml/100 g/min at rest to 250 (19) ml/100 g/min during adenosine (p < 0.001), with an average perfusion reserve ratio of 2.8 (0.2). Similar changes in regional perfusion were observed and were uniform throughout all regions, with a mean regional perfusion reserve ratio of 2.8 (0.3).

Conclusions—Accounting for intramyocardial vascular volume improves the accuracy of EBCT measurements of myocardial perfusion when using intravenous contrast injections. The feasibility of providing accurate measurements of global and regional myocardial perfusion and perfusion reserve in people using this minimally invasive technique has also been demonstrated.

Keywords: electron beam computed tomography; cardiac imaging; myocardial blood flow; indicator dilution.

Among patients with coronary artery disease undergoing selective coronary angiography, the severity of individual coronary artery stenoses is generally defined by simple geometric measurements of the visualised lumen, but has well documented limitations.1–6 Measurement of the physiological significance of such stenoses, such as the measurement of absolute myocardial perfusion (that is, as ml/g/min), would greatly complement coronary angiography.

Intracoronary Doppler measurements of coronary flow velocity and flow reserve have increased the clinical awareness of the value of physiological measurements of coronary artery stenoses7–11 but requires catheterisation, has some important technical limitations, and is unable to provide absolute measurements of flow. Coronary flow reserve can be influenced by small changes in resting values such as occur with alterations in heart rate and blood pressure. Absolute determination of the maximum flow rate will potentially overcome such limitations in the interpretation of flow reserve ratios. However, methods to quantify myocardial perfusion in absolute terms are quite limited in people compared with sophisticated techniques available in animals.12

Positron emission tomography13–15 and electron beam computed tomography (EBCT)16–19 show promise for the absolute quantification of global and regional myocardial perfusion in human subjects. EBCT measurements from our laboratory17 and others,18,19 employing venous injections of iodinated contrast, have underestimated absolute myocardial perfusion (that is, flow per unit volume), particularly at higher flow rates. Several reasons for this observation have been postulated and include the use of intravenous rather than intra-aortic injections of contrast and failure to correct for intramyocardial vascular volume (that is, the volume within the myocardium that is entirely vascular), which is known to increase as myocardial blood flow increases.20 Wang et al, using intra-aortic root injections of contrast,16 showed good correlation between measurements of myocardial flow by x ray computed tomography (CT) and radioactive microspheres in dogs when accounting for intramyocardial vascular volume. However, intra-aortic
or intracoronary injections of contrast have limited practical value in routine clinical practice.

The purposes of the present study were, first, in an animal model using intravenous injections of iodinated contrast to estimate intramyocardial vascular volume by EBCT, characterise how this changes during vasodilatation, and assess whether correction for this variable improves the validity of EBCT measurements of myocardial perfusion. Additional studies were then performed to assess the feasibility and validity of measuring regional myocardial perfusion in human subjects using the techniques developed and validated in these animal studies.

Methods

ANIMAL STUDIES

Animal preparation

Approval was obtained from the Mayo Clinic institutional animal care and utilisation committee to perform the following validation study, and each experiment was conducted in accordance with institutional guidelines. Seven closed chest dogs (mean weight 22 kg; range 18 to 24 kg) were anaesthetised with 1 ml/10 kg of Innovar-Vet (droperidol-fentanyl mixture) and 13 mg/kg of sodium pentobarbitone intravenously. Each dog was intubated and ventilated with room air using a Harvard respirator. Supplemental anaesthesia was provided with sodium pentobarbitone and/or Innovar-Vet.

The left and right femoral arteries and veins and the right internal jugular vein were surgically exposed. A 5 F angiographic pigtail catheter was inserted through the right femoral vein and positioned under fluoroscopy in the inferior vena cava at the level of the diaphragm. A 6 F catheter was inserted through the right jugular vein and into the right atrium and advanced across the atrial septum into the left atrium for injection of radioactive microspheres. An 8 F Rodrigues catheter was placed in the left femoral artery and connected to a withdrawal pump. Approximately 0.5 ml of dilute iohexol-370 (iohexol to normal saline ratio of 1:3) was continuously withdrawn at 7.5 ml/min from the femoral artery into a heparinised syringe connected to a withdrawal pump. Approximately 5 ×10⁶ uniquely labelled radiolabelled microspheres (Sn⁶⁷, Co⁷⁴, Sr⁹⁰, or Se⁸⁵) were injected into the left atrium during each scan and peripheral blood withdrawn for two minutes. At the conclusion of the experiment, the dogs were killed and the heart removed and fixed in formalin for at least four days.

Data analysis

Microsphere measurements—The left ventricle was dissected free from the excised heart, and the ativoventricular valves removed and divided into consecutive 8 mm thick slices parallel to the short axis. Four of these slices were then matched to their corresponding EBCT images using internal landmarks for reference. Each slice was cut into radially oriented sectors, divided into segments weighing 0.5 g to 1.0 g, and placed into labelled glass tubes. Reference blood samples were divided into 3 ml aliquots in glass tubes. All tubes were then placed in a gamma well counter and counted for radioactivity using a sodium iodide detector. These data were analysed by the matrix inversion technique with application of an appropriate spillover correction for each tracer.

Myocardial blood flow was calculated using the following algorithm:

\[
\text{Flow (ml/100 g/min)} = \frac{(C_r \times 7.5 \times 100)}{(W_{tm} \times C_m)}
\]

where \(C_r\) = radioactivity counts in myocardium, 7.5 = reference blood flow (ml/min), \(W_{tm}\) = weight of each segment of myocardium in grams, and \(C_m\) = counts in reference blood samples. Global and regional flows were determined by averaging the flow per unit weight for each specific slice and region.

Indicator dilution curves from EBCT—Contrast density versus time curves were recorded separately from adjacent tomographic slices at the mid-left ventricular level and were obtained through the coronary tomographic slice (global flow) of the left ventricle and four equal regions (anterior, septal, lateral, and posterior). The indicator dilution curve from the left ventricular cavity was used as the arterial input function. To avoid partial volume effects, tracing of the myocardium was performed a few pixels inside the endo and epicardial borders.

Estimation of intramyocardial vascular volume—The intramyocardial vascular volume was defined as that proportion of the total tissue volume composed of the volume of vascular space per unit volume of myocardium (\(\beta\)). It is assumed that muscle and vasculature are separate volumes and that, in vivo, the CT...
“densities” of both are virtually identical. During the first pass of iodinated contrast, which a priori is assumed to remain almost entirely intravascular,
the concentration of iodine in the myocardium relative to that in a purely vascular space (for example, the left ventricular cavity) represents a “dilution” effect of the avascular component. The proportion of myocardial tissue that is purely avascular can be considered to “volume average” the myocardial tissue that is purely vascular.

To estimate $\beta$, the mean concentration of iodine (represented by the mean CT number) in the myocardium and left ventricular cavity were determined. An index of the mean concentration of contrast in a region of interest was defined as the mean of the CT densities above baseline during the first pass. This was calculated by defining the area under the gamma variate fit of the contrast–dilution curve and the time for the first pass. Assuming symmetry of the curve about the first moment ($\int t c(t)dt$), the first pass time can be estimated as twice the mean transit time; $\beta$ was then calculated from the ratio of the mean CT numbers within the region of interest (CT$_m$) and the purely vascular left ventricular cavity (CT$_{LV}$) and expressed as a percentage of total myocardial volume: CT$_m$/CT$_{LV}$.

Calculation of myocardial perfusion by EBCT—The algorithm used for determining myocardial perfusion (in ml/100 g/min) has been described previously by Rumberger et al.,$^{17}$ and assumes a myocardial specific gravity of 1.05 g/ml:

$$\frac{\text{Area}_m / \text{Area}_{LV} \times t_m}{\times 60 \text{ s/min}} \times \frac{100}{1.05 \text{ g/ml}} \ (A)$$

where $\text{Area}_m$ and $\text{Area}_{LV}$ represent the areas under the indicator dilution curves from the myocardium and left ventricular cavity, respectively, and $t_m$ is the first pass mean transit time of the contrast through the myocardium, relative to the myocardial appearance time of contrast (identical to the “full width/half maximum time”).$^{17,23}$

This flow algorithm was used with the correction for intramyocardial vascular volume which incorporates $\beta$ (see Appendix for details of derivation):

$$\frac{\text{Area}_m / \text{Area}_{LV} \times t_m}{\times 60 \text{ s/min}} \times 100 \times \frac{1}{1.05 \text{ g/ml}} + \frac{\beta}{1.05 \text{ g/ml} \times (1-\beta)} \ (B)$$

Measurements of $\beta$ and flow through each of the four tomographic slices were averaged for each scan to provide a single measurement of global flow as well as anterior, lateral, posterior, and septal regional flows. These were compared with the average flows for the corresponding regions determined by the microspheres.

HUMAN STUDIES
Fourteen healthy young male volunteers (mean age 31 years, range 18 to 43 years) without known cardiac disease were enrolled to study the feasibility of measuring myocardial perfusion with EBCT using intravenous contrast injections. All subjects were considered to be free from cardiac disease after review of their medical history, physical examination, resting...
Figure 3  Bland and Altman plots showing comparison of regional myocardial perfusion measurements of EBCT, before (A) and after (B) correction for intramyocardial vascular volume, and radiolabelled microspheres obtained in the canine experiments. The abscissa is the ordinate is the difference (EBCT minus microsphere). The dashed lines represent 1 standard deviation from the mean of the differences.

Electrocardiogram, and serum cholesterol. No subject had a history of cigarette smoking and all were normotensive. Their mean weight was 83 kg. All subjects gave written informed consent before the examination and the study was approved by the Mayo Clinic institutional review board.

Volunteer preparation
Subjects were studied in the fasting state. A 4.5 F angiographic catheter was placed percutaneously into a right brachial vein and advanced to the superior vena cava under fluoroscopic guidance. A peripheral intravenous line inserted in the left arm was available for infusion of adenosine (Medco Research Inc, Los Angeles, California, USA). Subjects were then placed supine within the gantry of the EBCT scanner.

Myocardial perfusion was determined using algorithm B. Global and regional (anterior, lateral, and septal) flows were calculated from each of the four slices and then averaged to provide measurements of global and regional perfusion, respectively. Perfusion in these normal subjects was expected to be uniform throughout the heart and so homogeneity of measurements of flow and perfusion reserve was examined. Myocardial perfusion reserve ratio was calculated by dividing the flow measured during vasodilatation (during adenosine) by the corresponding global or regional flow at rest. Total left ventricular mass was determined using a previously validated method.26

Data analysis
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STATISTICS
Mean and standard errors of the mean are used throughout to describe continuous data. Simple linear regression using the method of least squares was used to determine correlation between EBCT and microsphere data and agreement between the two datasets was illustrated using Bland and Altman plots.27 Student’s t test (paired and unpaired where appropriate) was used for comparison of continuous data in the human studies. A level of statistical significance was assumed as p < 0.05.
**Results**

ANIMAL STUDIES

Twenty five EBCT scans were performed with simultaneous microsphere injections. Technical problems with microsphere administration and improper timing of the contrast injection during some scans resulted in the loss of four pairs of microsphere and EBCT data. There were potentially 21 EBCT scans with 21 measurements of global perfusion and 84 regional measurements (21 × four quadrants). Image misregistration and reconstruction imaging artefacts resulted in uninterpretable scans accounting for the absence of seven measurements of global flow and 21 regional measurements. Data are therefore presented from 14 global and 63 regional flow measurements.

Myocardial perfusion

Global—The estimated global intramyocardial vascular volume ranged from 2.8% at rest to 17.0% during graded infusions of adenosine. These measurements correlated closely to flow rates by microspheres of 57 to 346 ml/100 g/min (r = 0.94; fig 1). Comparison between the two measurements of perfusion is shown graphically with and without correction for intramyocardial vascular volume (fig 2). The agreement between the two measurements is modest but improved with algorithm B (fig 2B). The correlation between flow algorithm (A) and microsphere data was: y = 0.59x + 15.6 (r = 0.86). Use of algorithm B, which accounted for intramyocardial vascular volume, improved the prediction of microsphere flow: y = 0.72x + 6.06 (r = 0.88). The regression models for the two algorithms were compared statistically by examining the correlation between the differences (A minus B) and the microsphere measurements. This correlation proved to be significant (r = 0.90, p < 0.0001), indicating that the regression analysis for model B was statistically different from model A.

Regional—Bland and Altman analyses of the agreement between the two measurements of regional perfusion are shown in fig 3. The wide differences in agreement between the two measurements with no correction for intramyocardial vascular volume seen in fig 3A are much less evident when the correction was used (fig 3B). The correlation between regional EBCT measurements and microspheres using algorithm A was r = 0.74 with a regression slope of 0.55. The correlation using algorithm B was r = 0.82 with a regression slope of 0.75. Correlations for each of the four different myocardial quadrants using algorithm B are shown in table 1: the closest association between EBCT and microsphere data was found in the lateral wall (regression slope = 0.98, r = 0.87) while the association was poorest in the posterior wall (regression slope = 0.63, r = 0.82).

HUMAN STUDIES

Although the measurement of myocardial perfusion was reasonably accurate in the animal studies, several problems were evident. Imaging artefacts were a problem in some scans and probably accounted for much of the variation seen in figs 2 and 3. As some of the imaging artefacts probably represented noise in the data, it was hypothesised first, that larger myocardial slices in human subjects relative to those in dogs—particularly if imaged at end systole rather than end diastole—would improve the signal to noise ratio, as would imaging in the neutral rather than the short axis; and second, that limiting the amount of contrast to 20 ml would potentially minimise image artefacts in the adjacent myocardium as the contrast passed through the left ventricle.

All 14 subjects were studied at rest but two curves were uninterpretable in the anterior region in one subject and the interventricular septum in another. In one of the 11 subjects who received adenosine, no image data were acquired because of a technical problem with the scanner. Thus data regarding myocardial perfusion reserve were available for 10 subjects. No significant complications occurred during any of the scans, although flushing or mild chest discomfort occurred in some subjects associated with the adenosine infusion, resolving spontaneously without the need for dose reduction. Transient and asymptomatic second degree heart block occurred in one subject during the adenosine infusion but did not require any action. No significant changes in heart rate or blood pressure were documented during contrast injections (table 2). Data are shown for all patients at rest and separately for the 10 patients who received adenosine. During adenosine infusion, a small but significant increase in heart rate was observed from (mean (SEM)) 58 (6) to 79 (17) beats/min, and to 77 (15) beats/min immediately after cessation of the infusion (p < 0.01 for trend).

<table>
<thead>
<tr>
<th>Region</th>
<th>Rest (n = 10)</th>
<th>Adenosine (n = 10)</th>
<th>Perfusion reserve (n = 10)</th>
<th>p Value</th>
<th>V/Values</th>
<th>SEM, ml/100 g/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>93 (5)</td>
<td>250 (19)</td>
<td>&lt;0.0001</td>
<td>2.8 (0.2)</td>
<td>2.9 (0.3)</td>
<td>2.2 (0.1)</td>
</tr>
<tr>
<td>Anterior</td>
<td>107 (9)</td>
<td>255 (19)</td>
<td>0.0003</td>
<td>2.5 (0.3)*</td>
<td>2.8 (0.3)</td>
<td>2.0 (0.1)</td>
</tr>
<tr>
<td>Lateral</td>
<td>104 (7)</td>
<td>255 (28)</td>
<td>&lt;0.0001</td>
<td>2.8 (0.3)</td>
<td>3.0 (0.3)*</td>
<td>2.1 (0.1)</td>
</tr>
<tr>
<td>Septum</td>
<td>107 (9)</td>
<td>267 (20)</td>
<td>&lt;0.0001</td>
<td>3.0 (0.3)*</td>
<td>3.2 (0.3)*</td>
<td>2.4 (0.2)</td>
</tr>
</tbody>
</table>

*Values are mean (SEM), ml/100 g/min.*

**Table 2** Haemodynamic data recorded in human subjects at rest and with intravenous adenosine

<table>
<thead>
<tr>
<th>Haemodynamic variable</th>
<th>Rest (n = 14)</th>
<th>Adenosine (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
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<tr>
<td>Mean arterial pressure</td>
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<td></td>
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<tr>
<td>Preinfusion value</td>
<td></td>
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<tr>
<td>Post-contrast</td>
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<tr>
<td>Preinfusion value</td>
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<tr>
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<td>Preinfusion value</td>
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<tr>
<td>Post-contrast</td>
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</table>

*Values are mean (SEM). p < 0.01 = preinfusion value.
but no significant change in mean arterial pressure was noted.

The mean (SEM) resting global myocardial flow measured with EBCT in the 14 subjects was 98 (6) ml/100 g/min. Average regional flow in the anterior wall was 109 (9) ml/100 g/min, in the lateral wall 111 (8) ml/100 g/min, and in the septal wall 102 (6) ml/100 g/min (NS). In the 10 subjects who had perfusion reserve assessed (table 3), global flow at rest was 93 (5) and increased to 250 (19) during adenosine (p < 0.001) with an average perfusion reserve of 2.8 (0.2). Similar and uniform changes in myocardial perfusion and perfusion reserve were observed in the anterior, lateral, and septal regions. Average regional perfusion reserve among all subjects was 2.8 (0.3) (range 1.1 to 4.9). Compared with the animal studies, there were far fewer imaging artefacts seen in the human studies. An example of left ventricular and myocardial contrast clearance curves from one subject is shown in fig 4.

Left ventricular mass measurements were available in 13 of the 14 subjects and averaged 148 (5) g (73 (2) g/m²) which is within the range for normal subjects.28

**Discussion**

This study shows that EBCT can be used to quantify global and regional myocardial perfusion accurately when an estimate of intramyocardial vascular volume is included in the measurement. The major limitation of this technique potentially lies with imaging artefacts, which accounted for uninterpretable contrast clearance curves in approximately 25–30% of all measurements in the animal studies but was less common in the human studies. Global and regional myocardial perfusion and perfusion reserve measurements were consistent with those expected in normal subjects.

Although EBCT has been found to be applicable to the measurement of myocardial perfusion in previous animal studies, high flow rates have been significantly underestimated.16 17 19 Other investigators, using intraaortic contrast injections, 18 29 have shown that this problem can be overcome if dynamic changes in intramyocardial vascular volume during coronary vasodilatation are taken into account. We derived a parameter for estimating intramyocardial vascular volume with EBCT which was consistent with historical measurements using different measurement techniques.20 30 31 Total myocardial vascular volume is approximately 7% and can increase to approximately 15–18% when perfusion pressure is increased30 or if adenosine is given.31 EBCT estimates of intramyocardial vascular volume ranged from 2.8% at rest to 17.0% during coronary vasodilatation in our study. Incorporation of this variable in the flow algorithm resulted in better agreement between EBCT measurements of myocardial perfusion and microspheres (algorithm B), reflected by the differences in the Bland–Altman analyses in figs 2 and 3. The lateral wall measurements were particularly encouraging, with a correlation coefficient of 0.87 and regression slope of 0.98. Our data support the hypothesis that accounting for intramyocardial vascular volume is necessary for the accurate prediction of myocardial perfusion by EBCT using intravenous injections.

Encouraged by the animal data, we studied measurement of perfusion in human volunteers with EBCT and intravenous contrast injections. The studies were performed without significant complications and generally required 30 minutes or less; the actual scanning duration was less than one minute. Breath holding is a requirement for optimal imaging and many sick or elderly patients would not be able to do this for the 30–40 seconds required in this study. However, future developments with EBCT may obviate the need for prolonged breath holding. Imaging of the human
heart resulted in very smooth contrast clearance curves (fig 4). Almost all scans were interpretable in human studies, in contrast with the animal experiments. As would be expected in normal subjects, measurements of perfusion were found to be relatively homogeneous across all regions of the heart.

There are significant limitations to the currently available techniques for the measurement of myocardial perfusion and coronary flow reserve in human subjects and none was considered an ideal reference standard for use in this study. However, perfusion measurements at rest and after intravenous adenosine were similar to those reported from experiments in normal volunteers using positron emission tomographic imaging.14-15 Myocardial perfusion measurements using 15N labelled ammonia and 18O labelled water have been reported to be 70 to 90 ml/100 g/min at rest,13-15 increasing to 135 ml/100 g/min with exercise15 and to 230 to 355 ml/100 g/min after dipyridamole.14-15 Flow reserve in the study of Di Carli et al among normal subjects was 2.6 (0.7) (mean (SD)),15 compared with 2.5 to 3.0 obtained in the current study. These are also consistent with those observed in normal subjects using Doppler velocity measurements after papaverine induced coronary vasodilatation (2.9 to 3.2).11

Ludman et al have also reported EBCT measurement of myocardial perfusion in people13 but regional perfusion in normal subjects in that study was substantially lower both at rest and during coronary vasodilatation than in the current study. Corresponding regional perfusion reserve ratios ranged from 0.4 to 2.2 in the current study. This is consistent with those observed in normal subjects using Doppler velocity measurements after papaverine induced coronary vasodilatation (2.9 to 3.2).11

Much of the discordance between EBCT and microsphere measurements of flow in our animal studies may have been the result of imaging artefacts. A full description of these is beyond the scope of this paper but image reconstruction artefacts are generally related to beam hardening or photon (Compton) scatter. These effects occur as the energy spectrum of x rays passing through the vertebral column and through the iodine filled, adjacent left ventricular cavity become “distorted” in myocardial regions. Such imaging artefacts are significantly reduced when intra-aortic contrast injections are employed,13-15 although this approach requires invasive arterial catheterisation.

Comparison with microspheres in the animal studies may have resulted in errors from temporal heterogeneity in flow, since these measurements were averaged over a few minutes rather than the 20 to 30 seconds required to perform the EBCT scan. Heterogeneity in regional or spatial flow exists in many animal hearts, often with significant variability,16-19 and may have important implications for our data since the matching of the perfu-
The theoretical range of values for β is from zero to one and is estimated from the EBCD data as discussed in detail in the text.