Relation of Nonperfused Myocardial Volume and Surface Area to Left Ventricular Performance in Coronary Microembolization

Nasser M. Malyar, MD; Lilach O. Lerman, MD, PhD; Mario Gössl, MD; Patricia E. Beighley; Erik L. Ritman, MD, PhD

Background—After occlusion of an epicardial artery, left ventricular (LV) dysfunction is closely related to the volume of nonperfused myocardium (NPM). The impact of coronary microembolization (ME) on LV function, however, is larger relative to the total volume of NPM. We hypothesized that the total surface area (SA), rather than the total volume, of NPM is the major determinant of ME-induced LV dysfunction.

Methods and Results—We injected microspheres of 10-, 30-, or 100-μm diameter at each of 3 doses selectively into the left anterior descending coronary artery of 48 anesthetized pigs. Electron beam computed tomography (CT) was used to measure regional myocardial perfusion and changes in LV wall thickening (ΔWT) and stroke volume (ΔSV) after ME. At postmortem, a transmural “biopsy” of 1 to 2 cm³ of embolized myocardium was imaged by micro-CT, resulting in 3D images that provided volumes and SAs of the individual nonperfused foci. Additionally, in 9 pigs, creatine phosphokinase (CK) activity in embolized myocardium was measured as an index of washout of substances from the NPM. After ME, ΔWT, ΔSV, and CK washout were correlated more closely with the total SA (r=0.95, P<0.001; r=0.68, P<0.01; and r=0.88, P=0.01, respectively) than with the total NPM volume (r=0.59, P>0.05; 0.46, P>0.05; and r=0.69, P=0.04, respectively).

Conclusion—After coronary ME, LV dysfunction is more closely related to the total SA than to the total volume of nonperfused microregions in the myocardium. (Circulation. 2004;110:1946-1952.)

Key Words: microcirculation • embolism • infarction • ventricles • tomography

Impairment of global left ventricular (LV) function due to occlusion of an epicardial artery is closely related to the size of the myocardial perfusion defect.1,2 The decrease in regional contractile function of the LV is linearly related to the decrease in transmyocardial blood flow.3,4 However, in coronary microembolization (ME), a different relation exists between regional myocardial blood flow, total infarct volume, and impairment of LV contractile function.5 The total volume of myocardium that is rendered ischemic after such ME is too small to solely explain the magnitude of LV contractile dysfunction and other adverse consequences of ME.6,7 Coronary ME, because of its frequent incidence after coronary interventions and the subsequent adverse outcomes in patients with ME, has recently been the focus of investigations.8–10 Inflammatory cytokines have been shown to depress myocardial function11; hence, it is likely that ME-induced generation of proinflammatory cytokines plays a role in myocardial contractile dysfunction after ME.12–14 It seems evident that such humoral agents can readily diffuse from the surface zone of small, ischemic volumes (ie, “microperfusion defects”) into nearby normal tissue, whereas this diffusion is increasingly likely from tissue deeper within the individual ischemic volumes. Therefore, we hypothesized that in ME, the total surface area (SA) rather than the volume of embolized, nonperfused myocardium (NPM) predominantly determines regional and global LV dysfunction.

In the past, convenient tools were lacking to quantify the entities and determinants of ME, such as the number, individual volume, and SA of microinfarcts in a certain perfusion territory. In this study, we quantified in vivo ME-induced changes in regional myocardial perfusion and its impact on regional and global myocardial contractile function in a porcine model by way of LV wall dynamics (change in wall thickening: ΔWT%) and stroke volume (ΔSV) by using an electron beam computed tomography (EBCT) scanner. At postmortem, the total number of individual volumes and SAs of embolism-induced nonperfused microterritories were measured from 3D images of the embolized myocardium with a micro-CT scanner. Reduction of creatine phosphokinase (CK) activity in the embolized myocardium was measured as...
Figure 1. Cross-sectional EBCT image of in situ porcine heart. From these images myocardial perfusion as well as LV anterior and posterior W1% were calculated. RV indicates right ventricle; CS, coronary sinus. All other abbreviations are as defined in text.

EBCT Studies

The characteristics of the EBCT scanner and the methodology used in this are detailed elsewhere. The field of view was 21 cm (pixel size, 0.58 mm²; 7-mm slice thickness, 50-ms acquisition time). Two target rings were used, resulting in 4 contiguous tomographic images (levels) of the LV, with level 1 close to the apex and level 4 at the basal part of the LV (indicated by the coronary sinus; Figure 1).

EBCT Scan Protocol and Interventions

Initially, contrast agent was injected selectively into the LAD (4 mL over 2 seconds) to highlight the LAD perfusion territory. This was followed by a series of flow studies at 20-minute intervals, as illustrated in the following schematic. Each scan was performed immediately after a bolus injection of the nonionic contrast agent iopamidol (Isovue-370, Squibb Diagnostics; 0.33 mL/kg over 2 seconds) into the superior vena cava.

Each arrow represents a 20-minute wait. In each animal, the baseline scan was followed by 5 minutes of continuous selective intracoronary infusion of adenosine (100 μg · kg⁻¹ · min⁻¹) to achieve coronary microvascular dilatation. Before the next scan (dilatation + microspheres), the coronary bed was dilated again with a 5-minute intracoronary infusion of adenosine followed by selective injection of polymer microspheres (Duke Scientific Corp) of 1 of 3 calibrated sizes (10, 30, or 100 μm), each at 1 of 3 different doses (½, ¼, or ½ the fatal dose) into the LAD. The number of injected microspheres, corresponding to a fraction of the fatal dose (Table 1), had been empirically established and used in previous studies.

EBCT Data Analysis

Regional myocardial perfusion, cardiac output, and stroke volume (SV; cardiac output/heart rate) were calculated as others and

<p>| TABLE 1. Myocardial Perfusion (Flow) in Anesthetized Pigs in Relation to Size and Dose of Injected Microspheres at Different Scan Conditions |
|---|---|---|---|---|---|
| Flow, mL/g of Myocardium per Minute at Different Scan Conditions |</p>
<table>
<thead>
<tr>
<th>Size of Microspheres, μm</th>
<th>No. of Injected Microspheres</th>
<th>Baseline</th>
<th>Adenosine</th>
<th>Adenosine + Microspheres</th>
<th>20 min After ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.25×10⁶</td>
<td>0.92±0.27</td>
<td>1.92±0.36*</td>
<td>1.95±0.26</td>
<td>1.12±0.28†</td>
</tr>
<tr>
<td>10</td>
<td>2.5×10⁶</td>
<td>1.05±0.20</td>
<td>1.73±0.55*</td>
<td>1.37±0.53*</td>
<td>1.10±0.38*</td>
</tr>
<tr>
<td>10</td>
<td>5×10⁶</td>
<td>0.77±0.21</td>
<td>1.80±0.38*</td>
<td>1.29±0.29*</td>
<td>0.86±0.23*</td>
</tr>
<tr>
<td>30</td>
<td>3.75×10⁶</td>
<td>0.85±0.22</td>
<td>2.06±0.22*</td>
<td>1.50±0.24*</td>
<td>1.06±0.12†</td>
</tr>
<tr>
<td>30</td>
<td>7.5×10⁶</td>
<td>0.83±0.19</td>
<td>1.92±0.79*</td>
<td>1.37±0.52*</td>
<td>1.04±0.31†</td>
</tr>
<tr>
<td>30</td>
<td>1.5×10⁷</td>
<td>0.96±0.27</td>
<td>2.61±1.48*</td>
<td>1.38±0.60*</td>
<td>1.23±0.62†</td>
</tr>
<tr>
<td>100</td>
<td>1.25×10⁷</td>
<td>1.09±0.35</td>
<td>2.09±0.71*</td>
<td>1.72±0.66*</td>
<td>1.11±0.32*</td>
</tr>
<tr>
<td>100</td>
<td>2.5×10⁷</td>
<td>0.97±0.39</td>
<td>2.48±0.37*</td>
<td>1.74±0.39*</td>
<td>0.97±0.21*</td>
</tr>
<tr>
<td>100</td>
<td>5×10⁷</td>
<td>0.78±0.18</td>
<td>1.89±0.42*</td>
<td>1.03±0.30*</td>
<td>0.83±0.04*</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text. Values of flow are presented as mean±SD. The number of injected microspheres for each size corresponds to ½, ¼, and ½ of the fatal dose.

*P<0.05 vs previous scan; †P<0.05 vs baseline.
we have previously shown. In 10 of the 36 animals, the EBCT scanner was also used in cine mode for geometric analysis of LV dynamics. The WT of the anterior (LAD perfusion territory) and lateral (left circumflex coronary artery [LCX] perfusion territory) walls were individually measured, and systolic wall thickening (WT%) was calculated as\(^\text{WT/WT at end-diastole} \times 100\). Measurements before and after ME were made with coronary artery branches and/or papillary muscle used as reference markers (Figure 1).

**Micro-CT Studies**

The micro-CT\(^{21}\) and specimen preparation methods\(^{18,22}\) have been described in detail elsewhere. Four hours after embolization, the animals were euthanized with a fatal intravenous dose of sodium pentobarbital, and the heart was excised and infused with a lead chromate–containing contrast material (Microfil). From the embolized myocardial region, approximately 1-cm\(^3\) transmural samples were cut out and scanned with the micro-CT scanner, resulting in 3D images (20-\(\mu\)m cubic voxels). From these images (Figure 2), the volumes of embolized myocardial perfusion territories and the corresponding SA were computed as described previously.\(^{18}\)

**CK Activity in Embolized Myocardial Tissue**

In 9 pigs (1 animal for each size and dose of microspheres), CK activity in the embolized (LAD perfusion territory) and nonembolized (LCX perfusion territory) myocardial tissue was measured as an index of washout of intracellular substances from ischemic myocardial and other cells. Approximately 1- to 2-g pieces from embolized and nonebolized myocardium were excised and homogenized.\(^{23}\) The supernatant of homogenized tissue was diluted (100\(\times\)) before CK activity measurement, performed by our Central Clinical Laboratory (Mayo Clinic).

**Statistical Analysis**

Continuous variables are presented as mean\(\pm\)SD. The 2-tailed, paired, Student’s \(t\) test was used for comparison of hemodynamic and LV contractile parameters at different scan conditions. The Mann-Whitney \(U\) test was used to compare values between the 9 different groups of animals, each group embolized with 1 of the 9 possible combinations of microspheres. Linear regression analysis was used to express the relation between variables. A probability value \(\leq 0.05\) was considered significant.

**Results**

**General Findings**

The study was successfully accomplished in 36 of 48 animals (75%). Twelve animals were either lost or excluded from the study because of refractory ventricular fibrillation during

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Adenosine</th>
<th>Adenosine + Microspheres</th>
<th>20 min After ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>75(\pm)16</td>
<td>87(\pm)17(^*)</td>
<td>91(\pm)16(^+)</td>
<td>85(\pm)17(^+)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>109(\pm)20</td>
<td>108(\pm)20</td>
<td>102(\pm)24(^$)</td>
<td>106(\pm)21(^$)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79(\pm)17</td>
<td>82(\pm)17</td>
<td>83(\pm)19</td>
<td>83(\pm)18</td>
</tr>
<tr>
<td>(\Delta\text{WT}_{\text{LAD}})%</td>
<td>46.1(\pm)5.9</td>
<td>…</td>
<td>…</td>
<td>26.5(\pm)7.9(^+)</td>
</tr>
<tr>
<td>(\Delta\text{WT}_{\text{LCX}})%</td>
<td>52.0(\pm)4.4</td>
<td>…</td>
<td>…</td>
<td>74.8(\pm)7.6(^+)</td>
</tr>
<tr>
<td>SV, mL</td>
<td>29.5(\pm)10</td>
<td>28.7(\pm)9(^+)</td>
<td>25.5(\pm)9(^+)</td>
<td>26.1(\pm)8(^$)</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>2.47(\pm)0.6</td>
<td>2.41(\pm)0.6</td>
<td>2.23(\pm)0.7(^+)</td>
<td>2.15(\pm)0.5(^+)</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. All other abbreviations are as defined in text. Data are presented as mean\(\pm\)SD.

\(^*P<0.01\) vs previous scan; \(^+P<0.01\) for adenosine + microspheres scan vs baseline scan; \(^\$P<0.01\) for 20-min post-ME scan vs baseline scan; \(^\$P<0.05\) vs previous scan.

**Figure 2.** Representative micro-CT images (left) of myocardium embolized with 10- (upper panel) and 100- (lower panel) \(\mu\)m microspheres containing radiopaque (white) polymer in microvasculature. Embolized (ie, Microfil-lacking) areas were manually outlined (middle) and computed to 3D map, where each nonperfusion focus is represented by different color (right). Abbreviations are as defined in text.
instrumentation or embolization (n=6); no washout of contrast medium from the LAD and its perfusion territory (n=4), indicating no blood flow; and pneumonia (n=1) and anaphylactic reaction to the injected contrast medium (n=1) with fatal outcome. Hemodynamic parameters at different scan conditions are presented in Table 2. In all animals, ME decreased systolic blood pressure significantly (P<0.05), whereas diastolic blood pressure was not changed. Heart rate increased significantly after ME (P<0.01).

EBCT Results

Myocardial Perfusion
At baseline, flow was similar among all groups. Adenosine increased flow to the perfusion territory from 0.90±18 to 1.9±0.39 mL·g⁻¹·min⁻¹ (P<0.001), whereas ME decreased flow (to a mean of 1.34±0.34 mL·g⁻¹·min⁻¹; P<0.001 versus baseline and adenosine scans), proportional to the dose and diameter of the injected microspheres (Table 1). At the post-ME scan, flow was significantly increased compared with baseline scan (P<0.05) in animals embolized with 10- and 30-μm microspheres.

Regional and Global LV Function
Injection of all sizes and doses of microspheres led to decreases in WT% in the embolized region (LAD perfusion territory), whereas WT% in the nonembolized control region increased proportionately to the decrease in WT% in the territory, whereas WT% in the nonembolized control region decreases in WT% in the embolized region (LAD perfusion territory). Increasing diameter and increasing dose (ie, number) of microspheres resulted in a decrease of the total number of nonperfused foci (Figure 3, top) but an increase in total NPM volume (Figure 3, middle). The total SA of the different-sized microspheres at the same fatality dose, however, was essentially constant (Figure 3, bottom).

After ME with microspheres, WT% as an index of regional myocardial contractile function decreased in proportion to the total SA of the nonperfused foci (r=0.95, P<0.001, Figure 4, left) but was correlated poorly to total NPM volume (r=0.59, P>0.05, Figure 4, right). The decrease in SV, as an index of global LV contractile function, was also significantly correlated to the total SA of the nonperfused foci (r=0.68, P<0.01, Figure 5, left) but not to the total NPM volume (r=0.46, P>0.05, Figure 5, right).

Washout of CK From Embolized Myocardial Tissue
Total CK activity in 1 cm³ of myocardial tissue was 205 900±20 150 U/L in the nonembolized and 177 545±23 230 U/L in the embolized myocardium (P<0.01). The decrease in CK activity in the embolized compared with the nonembolized myocardium showed a more significant correlation with the micro-CT–derived total SA (r=0.88, P<0.01, Figure 6, top) than with the micro-CT–derived total NPM volume (r=0.69, P=0.04, Figure 6, bottom).

Discussion
This study shows that neither myocardial perfusion nor ME myocardial volume is correlated significantly with the ME-
induced decrease in anterior WT%. Instead, the total SA of the nonperfused foci was found to be the best determinant of ME-induced regional and global LV contractile dysfunction. It is of interest that the ratio of the fatal dose of microspheres from 1 microsphere diameter to another corresponded closely to the ratio of the number of microvessels of those same diameters, as calculated by Murray’s Law (ie, diameter of mother branch cubed = sum of the cubed daughter branch diameters) and assuming symmetrical bifurcation. Interestingly, we observed that for a given fraction of fatality dose of microspheres, the micro-CT–derived total NPM volume (Figure 3, middle) differed substantially for the different sizes of microspheres, whereas the corresponding values for the total SA were essentially independent of microsphere diameter (Figure 3, bottom), suggesting a stronger link of SA with eventual outcomes.

The underlying mechanism for the decreasing number of microperfusion defects with an increasing number of injected microspheres (Figure 3A) is the previously described clustering behavior of the local distribution of microperfusion defects in the myocardium after ME. As the number of injected microspheres increases, the possibility of adjacent vessels being occluded increases, and coalescence results in a decrease of the number and an increase in mean size (Figure 2) of individual microperfusion defects. Thus, the total SA of multiple embolization foci is expected to actually decrease as the total foci volume continues to increase with increased embolization beyond a critical dose of microemboli. If the total volume of NPM tissue were the important determinant, then we would expect comparable values of NPM volume for the same fatality dose of the 3 different sizes of microsphere. The nature of the marked discrepancy between NPM volume and the microsphere dose effect and the consistency of SA values for differently sized microspheres at the same dose support our hypothesis that total SA is a more critical determinant of the impact of coronary ME than is total NPM volume.

These results are consistent with previous EBCT-based studies that showed significant differences in the decrease in intramyocardial blood volume after ME with a fatal dose of 10- and 100-μm microspheres, as well as with observations made by other investigators. Dörge et al embolized the LCX perfusion territories of dogs with 42-μm microspheres and observed a progressive decrease in posterior WT, from 19.8 ± 1.9% at baseline to 6.9 ± 4.7% 8 hours after ME and an increase in LV end-diastolic pressure, from 8 ± 3 to 17 ± 6 mm Hg. Histological evaluation of the embolized myocardium in their animals, however, revealed aggregate infarct sizes of only 6.5 ± 4.5% of the area at risk. The authors concluded that this amount of infarcted myocardium was too small to account for the progressive myocardial dysfunction after ME. This discrepancy in infarct volume and consequent contractile dysfunction is striking, especially when compared with the aforementioned studies by Pfeffer et al, wherein signs of congestive heart failure, such as elevated resting filling pressures and reduced cardiac output, were not observed until 46% of the perfusion territory was infarcted by occlusion of an epicardial artery.

Previous studies have also shown that ME induces the release of inflammatory mediators such as interleukins-6 and -8, tumor necrosis factor (TNF)-α, and free oxygen radicals from the ischemic myocardium into the surrounding nonischemic myocardium. For example, Thielmann et al showed in a dog model that intracoronary infusion of exogenous TNF-α induced contractile dysfunction even in the absence of ME. Pretreatment with TNF-α antibodies resulted in prevention of contractile dysfunction after ME, suggesting a cause/effect relation of ischemia-mediator–induced contractile dysfunction in ME. These mediators have, at least transiently owing to washout in the perfused myocardium, a deleterious effect on contiguous myocardial contractile function, whereby more effectively increasing the total volume of functionally depressed myocytes beyond the actual volume of ischemic myocytes. If true, the volume of perfused...
but functionally depressed myocardium should also be proportional to the SA of the NPM.

Because the SA represents the border zone, or contact area, between perfused myocardium and NPM, SA may be regarded as an index of release of ischemia- and infarction-induced mediators into adjacent myocardium and into the systemic circulation. This consideration was supported in our study by the decrease in CK activity in the embolized myocardium, which showed a better correlation with the total SA than with the total volume of NPM (Figure 6). These results imply that the release and removal of intracellular components from the disintegrating myocytes is, at least in the acute phase, proportional to the total SA of the embolized foci rather than to the total infarcted volume.

Limitations of the Study
It must be emphasized that direct extrapolation of our results to the clinical scenario of ME remains open to some question, in that the polymer microspheres used in this study certainly do not have the intrinsic biochemical properties of biological ME, which have a different composition and which may lead to a different reaction in the organism. Nevertheless, our results suggest that prevention of even relatively small quantities of very small ME might be as important as prevention of larger emboli because the total SA of microperfusion defects resulting from such small emboli can be large despite the small volume of myocardium rendered ischemic.

Summary and Conclusion
The main finding of our study is that in the multiple microperfusion defects scenario as the sequela of ME, the total SA of microperfusion defects rather than their aggregate volume is the major determinant of LV contractile dysfunction. The total SA of the microperfusion defects additionally supports the causal link between the inflammatory response after coronary ME and progressive myocardial dysfunction. Comprehension of the pathophysiology and identification of the determinants of the consequences of coronary ME could lead to better outcomes in patients with coronary ME by developing strategies for prevention of distal embolization and to better management of the consequences of ME.

Acknowledgments
This study was supported in part by research grants HL-43025 and EB-000305 from the National Institutes of Health, Bethesda, Md.

References
6. Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death: autopsy evidence of
Relation of Nonperfused Myocardial Volume and Surface Area to Left Ventricular Performance in Coronary Microembolization
Nasser M. Malyar, Lilach O. Lerman, Mario Gössl, Patricia E. Beighley and Erik L. Ritman

_Circulation_. 2004;110:1946-1952; originally published online September 27, 2004; doi: 10.1161/01.CIR.0000143631.15077.0F
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/14/1946

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/