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EDITORIAL COMMENT

In Hot Blood

Quantifying the Arterial Input Function*

Stephen L. Bacharach, PHD,† Richard E. Carson, PHD‡
San Francisco, California; and New Haven, Connecticut

Measurement of absolute myocardial blood flow (MBF) and coronary flow reserve (CFR) is of great importance clinically. To make such measurements quantitatively, one must also measure the arterial input function (AIF). Many studies, including the one reported by Vasquez et al. (1) in this issue of *iJACC*, which focuses on rubidium-82 (^{82}Rb), have sought optimal ways to measure the AIF for a variety of tracers. Knowledge of the AIF is critical, not just to determine MBF and CFR, but also to measure other parameters of myocardial physiology (e.g., glucose and oxidative metabolic rates). It is worthwhile, therefore, to further reflect on the AIF. What is it? How is it used? How can it be measured? How might errors in its measurement affect clinical decisions?

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The AIF is simply the concentration of the radiotracer in the arterial blood measured over time. This arterial blood is the only supply of tracer to the myocardium and to most other organs in the body. The amount of tracer delivered to the myocardium is influenced by a complex set of factors: How much was injected? For a venous injection, how much makes it through the lungs? What fraction of the arterial blood flows to the myocardium compared with other organs? How much is removed from the arterial blood by other organs and is trapped or cleared from the body? How much of the tracer that reaches various tissues is then released back into the venous blood, and thus can recirculate? (We ignore here the potential problems associated with labeled metabolic products.) By measuring the AIF we can avoid answering these questions. The AIF tells us directly how much and for how long the tracer was available to the myocardium. Without some knowledge of the AIF, it is impossible to make accurate physiological measurements from radiotracer experiments.

There are many tracers that can be used to measure myocardial perfusion. A "perfect" tracer would be one that the myocardial cells could completely extract from the arterial blood. Water ($H_2{}^{15}O$) is an example of a tracer that is nearly 100% extracted. Ideally, for imaging purposes, the tracer would also be trapped once it entered the myocardium, but water washes out rapidly, proportional to MBF. Radioactive labeled microspheres, on the other hand, would be an example of a "perfect" tracer with ideal imaging properties.

To determine MBF with such a perfect, trapped tracer, one need only measure the amount of tracer taken up by the myocardial region of interest and then divide by the total amount available to the myocardium. The total amount available is simply the area under the AIF curve (AUC). This ratio of uptake divided by the AUC gives the absolute MBF (ml/min to the region or ml/min/g tissue). This division by the AUC is also the basis for the simplified computation of MBF and CFR of Vasquez et al. (1).

For the special case of a "perfect" tracer, it is easy to see how errors in the AIF would produce errors in the measurement of MBF. A certain percentage of underestimation of the AUC causes the MBF to be too high by that same percentage, and vice versa.

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From the †Center for Molecular and Functional Imaging, Department of Radiology, University of California, San Francisco, San Francisco, California; and the ‡Yale PET Center, Diagnostic Radiology, Yale School of Medicine, New Haven, Connecticut. Both authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Absolute MBF values have rarely been used clinically, although this is changing as it becomes easier to make such measurements. CFR, however, is known to be of great practical importance. Because CFR is simply the ratio of MBF during stress to MBF at rest, if a percentage error is made in the AIF at rest and the *same* percentage error is made at stress, the errors will cancel out. Therefore, any systematic underestimates or overestimates of the AUC do not matter, providing the same percentage error is made at both rest and stress. Random errors, or nonsystematic errors, however, will affect both MBF and CFR.

What about real-world, nonideal tracers like ^{82}Rb or ^{13}N-ammonia ($^{13}NH_3$)? For these tracers, the calculation of MBF is more complex and usually (but not in the case of Vasquez et al. [1]) requires not just the AIF AUC, but an accurate measurement of the shape of the AIF (i.e., how it changes with time).

Measuring the AIF. To see how systematic and nonsystematic errors can arise, consider how the AIF is measured. The most direct way to measure the AIF would seem to be by drawing blood over time from an arterial catheter and determining the blood activity. There are machines that perform these steps automatically (2). These are used in research environments and are clearly not clinically practical. In addition, this approach is not as free of error as it sounds. Unless the rate of blood drawn is very high, there will be dispersion and delays in the measured activity compared with the true arterial activity. In addition, a cross-calibration is needed to convert counts measured from the collected blood to counts measured from the scanner. The obvious noninvasive way to measure the AIF is to use the image data. Blood pool regions including the left ventricular (LV) cavity, the left atrial (LA) cavity, and the aorta (ascending and/or descending) are all within the field of view of a cardiac scan and contain arterial blood. A dynamic set of images acquired from the time of injection on can be used to produce an image-derived input function (IDIF) to estimate the AIF. Simply place a volume of interest (VOI) over the blood pool and create a time activity curve (TAC); that curve will be the AIF. The IDIF method avoids any need for cross-calibration; the activity concentrations measured by the scanner are used for both the AIF and the tissue. What then are the problems with using such image-based methods? There are 3 principal pitfalls: 1) count rate limitations of the scanner; 2) statistical fluctuations; and 3) the partial volume (PV) effect.

The effect of count rate on the AIF. Clinical ^{82}Rb or $^{13}NH_3$ images are usually taken after the blood pool activity has decreased considerably, 90 to 120 s after the end of injection for ^{82}Rb and several minutes or more for $^{13}NH_3$. By then, the activity has decayed and distributed throughout the body, greatly reducing the activity in the scanner field of view. To measure the AIF, however, the dynamic scan must begin at the start of injection because the entire dose (as much as 50 mCi for ^{82}Rb) passes through the heart. This can cause very high count rates, requiring significant corrections for accidental coincidences and dead time. In older 2-dimensional scanners (i.e., septa in), many but not all scanners can perform such large corrections accurately. In modern 3-dimensional only (septa out) scanners, the dose delivered must be significantly reduced. Thus, although 3-dimensional scanners provide high sensitivity, which is very beneficial when count rates are low, the count rate limitation requires lower activity injections for accurate measurement of the AIF.

Statistical fluctuations. The second problem with accurate AIF measurements is due to counting statistical fluctuations. For determining MBF from the "perfect" tracer mentioned earlier, all one needs is the AUC (i.e., total integrated activity under the AIF). This total area, although not free of statistical fluctuations, is less influenced by them than point-by-point TACs. For ^{82}Rb and $^{13}NH_3$, however, the shape of both the AIF and the tissue curves is usually important. If one uses a small VOI for the IDIF, then these statistical fluctuations can be large. For example, if the AIF was measured 3 times using an LV, LA, or aortic VOI, the 3 IDIFs would differ slightly in height, shape, and area due to statistical fluctuations alone, even if all other factors were equal. Fortunately, if the AIF is used in a kinetic model, the noise effects are mitigated by the integrating nature of the model equations (see the following), or by integration if the AUC is used. Statistical fluctuations can also be minimized by making the VOI larger, encompassing as much of the blood pool (LV cavity, aorta, or LA cavity) as possible. Unfortunately, this strategy produces other errors caused by the so-called PV effect.

The PV effect. To better understand the PV effect, consider a structure like the aorta, which might have a diameter of 2 to 3 cm. With a perfect scanner, a cross-sectional image would show uniform activity concentration across the aorta. Real cardiac images, however, usually have reconstructed resolutions of no better than 8 mm full width at half

maximum (FWHM), often much larger. This will cause the aorta image to blur, smearing counts from inside to outside the aorta's anatomic borders, reducing the measured peak activity. The blurring does not reduce the total activity; it simply smears it into a larger volume. A VOI drawn around the aorta will therefore measure less than the true concentration of activity. The fraction of the true activity concentration measured in a VOI is called the recovery coefficient or simply the percentage of recovery. In addition, if there were any structures containing activity near the aorta's anatomic borders (e.g., the liver in the descending aorta), counts from these structures would blur into the aorta. If there were no activity-containing structures outside the aorta, one could draw a very large VOI and capture all the counts originally in the aorta, before blurring. But because the AIF is a measure of the activity *concentration*, one would have to divide this total activity by the true aorta volume to get an accurate AIF.

This unwanted mixing of counts from inside and outside a structure is the hallmark of the PV effect. A rule of thumb to minimize the PV effect is to keep the VOI's edge, ideally, at least ~1.5 × the resolution distance from the edge of the structure. By resolution distance, we mean the actual reconstructed image resolution, measured as FWHM, *not* the resolution quoted by the manufacturer. Typical clinical cardiac scans have an FWHM reconstructed resolution on the order of 8 to 12 mm. Therefore, the LA and, to a lesser extent, the LV cavities, which are large structures, can have larger VOIs drawn on them than the aorta before incurring PV effects. VOIs drawn on the aorta, on the other hand, will have smaller percentage of recovery, unless the VOI is small and/or the reconstructed resolution is high.

Because activity mixes in all directions, the PV effect can produce insidious errors in the AIF. Shortly after injection, the activity outside the blood pool region is usually comparatively low, so the PV effect causes the activity measured with too large a VOI to be underestimated due to spill-out. The percentage of recovery can be kept high by using a smaller VOI, drawing it on multiple slices to minimize the statistical fluctuations. At late times, the situation for the LV cavity is reversed; the myocardium is hot, so an oversized LV VOI may overestimate the AIF because counts from the myocardium blur into the VOI (spill-in). For a given size VOI, the PV effect will be worse for the aorta than for the LV or LA cavities because the aorta is smaller in diameter. At late times, however, neither the aorta nor the LA is as close to the hot myocardial walls as the LV. In addition, the LV walls contract, causing additional blurring into an LV VOI. For this reason, when one uses an LV cavity VOI, one usually restricts it to the basal aspect, where contraction is limited. The left atrium has very little contraction (and low myocardial uptake) by comparison. Note that the aorta can pulsate with each heartbeat, causing a worsening of effective resolution. In theory, the aortic PV effect (apart from motion effects) can be corrected if one knows the real resolution of the images and the exact sizes of the VOI and of the object (e.g., the aorta) (3). Unfortunately, the motion component of the resolution is more difficult to measure. Nonetheless, the aorta has been successfully used to measure the AIF after PV corrections. Its location can be defined using early scan data and its size from a computed tomography scan.

One useful approach to minimizing the PV effect in the AIF is worth mentioning because it works well for ^{82}Rb and $^{13}NH_3$ scans. One acquires the dynamic part of the scan at the highest possible resolution (e.g., very little filtering). These images often look very noisy and are nearly useless for reading or defining VOIs; this does not matter because they will be used only to make TACs. To define the VOIs, one can use smoother images (e.g., summed, smoothed early data for the blood pool, and smoothed late clinical images for the myocardial VOIs). Although these VOIs might have a poor percentage of recovery for these smoothed images, they can be applied to the higher resolution, noisy data to make the AIF and the tissue TACs with a higher percentage of recovery.

Effect of AIF errors in MBF models. We spoke only of "perfect" tracers earlier, although the same PV and statistical effects occur for real-world tracers. The effects of errors in the AIF on ^{82}Rb and $^{13}NH_3$ MBF are more complicated. These tracers are not completely extracted by the myocardium, and the extracted molecules may wash out. To account for this requires a mathematical/physiological model. Typically, the AIF is fed into the model and is fit to the measured tissue TAC to calculate a value proportional to MBF. The proportionality factor is the fraction of the tracer that is extracted by the tissue; that is, the model yields flow (F) times extraction fraction (E): F × E. The extraction fraction is itself influenced by MBF, being greater at low flows (when the low flow gives the tracer more time to enter the myocardial cells) than at

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high flows. Unfortunately, it is difficult to determine E, so that one must usually rely on animal models or other ancillary information to estimate E (4). For CFR, a ratio of MBF at stress to MBF at rest is needed. One might hope that the Es would cancel out, but because E is flow dependent, this is not the case.

There are 2 tracer kinetic models commonly used to measure flow from ^{82}Rb and $^{13}NH_3$: a 1-compartment (1C) model (4) and a 2-compartment (2C) model (5) (Vasquez et al. [1], however, do not rely on these models). In general, these models behave in many of the same ways as described earlier for the "perfect" tracers. If the AIF is systematically underestimated, the flow value is overestimated and vice versa. The 2C model arguably describes the physiology most accurately. Unfortunately, in physics as in life, one rarely gets "something for nothing." The extra information gained from the 2C model comes at a price; flow values are more susceptible to noise in the AIF (and noise in the tissue TAC). For a given amount of noise in the AIF and tissue TAC, the 1C model produces less variability in flow. For this reason, the 1C model is more often used for ^{82}Rb. In addition, errors in measuring the shape of the AIF (due to time-dependent spill-out and spill-in) have a more complicated effect on MBF errors.

The tissue TAC is also affected by noise and PV effects, but in the reverse manner as the AIF; the tissue TAC is often too high at early times (due to spill in from the hot blood pool) and too low at late times. One important advantage of using mathematical models to estimate MBF is that these models can often incorporate corrections for spill-in and spill-out of the tissue TACs (4,5). For ^{82}Rb and $^{13}NH_3$, such corrections can be quite useful in minimizing errors in MBF, but they do not correct for inaccuracies in the AIF.

Previous research. These cited facts have been used by many investigators searching for an optimal way to draw VOIs to determine the AIF for various tracers. Some (6) compared using different size VOIs in the left ventricle, the left atrium, and the ascending and descending aorta, concluding that for 18F-fluorodeoxyglucose, the aortic arch gave the best agreement with the lowest variability, despite the fact that the left ventricle and left atrium gave higher peak AIF values. Others (7) compared the left atrium, left ventricle, and thoracic aorta for $H_2{}^{15}O$, and found that the left atrium seemed optimal (they did not examine the aortic arch). The effect of AIF errors on MBF caused by myocardial spill-in has also been studied for $^{13}NH_3$ at both rest and stress (8). Some papers have compared various VOI sizes and locations using the MBF or CFR computed with actual arterial sampling as their gold standard. The paper by Vasquez et al. (1) examines VOI selection for ^{82}Rb without a gold standard such as arterial sampling. Instead (using a 1-pixel VOI), they assume that the VOI having the largest AIF (as measured by its 2-min AUC) must be the "correct" value. Vasquez et al. (1) compare MBF and CFR from other VOIs with this "correct" value. The assumption that the largest AUC is the "correct" one requires that VOI size and location and image resolution be such that the percentage of recovery is 100%. In other words, this approach assumes that there is no spill-in or spill-out contamination and that the largest 1-pixel AUC is not the largest simply due to statistical fluctuations (assumed to be small because the AUC is used). They refer to a previous phantom study to assess percentage of recovery, although the current paper does not explicitly state the reconstructed resolution of their images. If the authors' assumptions are true, one could try many VOIs and select the one with the largest 2-min AUC. Of course readers would need to assure themselves that the assumptions were valid for their own imaging systems and protocols.

Conclusions. Many studies suggest that accurate measurements of AIF and MBF are quite possible in clinical situations, and commercial software has even been developed. However, there are many pitfalls. These pitfalls are slightly different for different tracers and different methods of computing flow and have different impacts on rest versus stress studies. Thus, it is important to choose an appropriate AIF method within the context of the MBF model and analysis that are used. But armed with an understanding of the underlying difficulties, it is possible to make accurate measurements of MBF and CFR with a variety of tracers. It is important, in both research *and* clinical settings, that the "consumers" of positron emission tomography MBF and CFR measures have a good appreciation of the limitations and caveats of these remarkable quantitative in vivo assays.

Reprint requests and correspondence: Dr. Stephen L. Bacharach, Center for Molecular and Functional Imaging, Department of Radiology, University of California, San Francisco, 185 Berry Street, Suite 350, San Francisco, California 94107. *E-mail: steve.bacharach@ucsf.edu.*

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