Single-scan rest/stress imaging ^{18}F -labeled flow tracers

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Purpose: The authors report a novel measurement strategy to obtain both rest and stress blood flow during a single, relatively short, scan session.

Methods: Measurement of rest-stress myocardial blood flow with long-lived tracers usually requires separate scan sessions to remove the confounding effects of residual radioactivity concentration in the blood and tissue. The innovation of this method is to treat the rest-stress scan as a single entity in which the flow parameters change due to pharmacological challenge. With this approach the fate of a tracer molecule is naturally accounted for, no matter if it was introduced during the rest or stress phase of the study. Two new dual-injection kinetic models are considered that represent the response to pharmacological stress as a transitional or transient increase of myocardial blood flow. The authors present the theory of the method followed by the specific application of the theory to ^{18}F -Flurpiridaz, a new myocardial flow-imaging agent.

Results: Myocardial blood flow was accurately and precisely estimated from a single-scan rest/stress study for the long half-lived tracer ^{18}F -Flurpiridaz. By accounting for the time-dependence of the kinetic parameters, the proposed models achieved good accuracy and precision (5%) under different vasodilators and different ischemic states.

Conclusions: Detailed simulations predict that accurate and precise rest-stress blood flow measurements can be obtained in 20–30 min. © 2012 American Association of Physicists in Medicine. [<http://dx.doi.org/10.1118/1.4754585>]

Key words: kinetic modeling, rest/stress, cardiac PET, myocardial blood flow

I. INTRODUCTION

Cardiac rest-stress studies have long been used to diagnose and guide treatment of coronary artery disease (CAD) with SPECT (Refs. 1–3) and PET.^{4–6} Most studies have been qualitative in that the interpretation relies on visual comparison of single images obtained during rest and stress. A long-standing problem is how to temporally separate the spatial distributions of radionuclide laid down during rest and stress. Typically, each rest/stress study includes two tracer injections, one for the baseline condition and one during the stress test. To avoid significant commingling of activity from rest and stress studies, one must delay the start of the stress study for 3–5 half-lives, a delay that adds logistical complexity and cost while inconveniencing the patient. Short-lived positron emitting radiopharmaceuticals offer one solution to this problem but require either an on-site cyclotron for production of ^{15}O -, ^{13}N -, ^{11}C -compounds or, alternatively, an ^{82}Rb generator. The goal of our work is to develop the theoretical basis and a practical measurement strategy that allows for fully quantitative measurements of myocardial blood flow (MBF) to be obtained during a single 20–30 min rest-stress imaging session using tracers with long physical half-lives.

This goal is timely because ^{18}F -based tracers are currently under commercial development for cardiac imaging applications. Their long physical half-life (109 min) allows these new

tracers to be distributed for clinical studies without the necessity for an on-site cyclotron. In addition, contemporary PET cameras have advanced to the point where quantitative measurements of myocardial radioactivity concentration can be made with high temporal sampling and good statistical precision. Because the heart and great vessels can be included in the field of view, it is possible to measure simultaneously the concentration history of activity in the myocardium and ventricular cavities, opening the way for absolute quantitation of local myocardial blood flow during rest and pharmacologically induced stress. However, as explained above, the long half-life of ^{18}F also creates a technical challenge for performing both a rest and stress study within a single image session.

In this paper, we report a novel measurement strategy to obtain both rest and stress blood flow during a single, ~20 min scan session, using ^{18}F -labeled tracers and PET. The work also extends prior methods to more rigorously include nonsteady state measurements. We consider the prior art in addition to a study of two new dual-injection kinetic models that represent the response to pharmacological stress as a transitional or transient increase of myocardial blood flow. We present the theory of the method and evaluate the feasibility of clinical measurement by realistic simulation of one such ^{18}F flow tracer, namely ^{18}F -Flurpiridaz, a new myocardial imaging agent now in Phase III trials.

II. METHODS

II.A. Overview

We analyze and compare three procedures for measuring rest/stress myocardial blood flow. These are: (1) Rest and stress measurements, separated in time so that residual radioactivity from the rest studies decays to negligible levels. We refer to this as the Standard method. (2) An adaptation of the method developed by Rust *et al.*⁷ to analyze MBF measured with ¹³N-Ammonia, with short decay period between rest and stress. We refer to this as the Rust method. (3) Our new protocol, which we refer to as the MGH method. These procedures feature different experimental protocols, kinetic assumptions, and analytic methods.

The basic methodological approach is to compare the three methods by Monte Carlo simulation. These simulations are based on a digital phantom with anthropomorphic cardio-pulmonary geometry. The simulations produce ensembles of dynamic measurements that realistically predict both the four-dimensional evolution of ¹⁸F-concentrations and the statistical noise propagation for a variety of cardiac abnormalities in different myocardial segments and with different flow responses. The simulations are designed to compare the noise-to-signal ratio of flow measurements for the Standard method versus the MGH method of combined rest-stress. The effect of nonsteady state response to vasodilation is included in the simulations, to investigate the effects on estimation bias. The ensembles of simulated studies are analyzed according to the Standard and MGH methods described above, to determine the mean and standard deviation of MBF.

Prior to describing the results of Monte Carlo simulations we look at the basic optimization of the MGH method with ¹⁸F-Flurpiridaz. The first question addressed is the effect of shortening the observation time: Short observation time ensures that the curves depend primarily on flow, but with the trade off of higher noise levels; whereas protocols with longer measurement times may yield better signal-to-noise ratio but must account for the reaction of Flurpiridaz with the mitochondrial complex I. Next, we study the effect of imperfect knowledge of k_3 on the estimation of K_1 . And, finally, we assess the noise propagation and bias in k_2 when the measurement period is short compared to $\ln(2)/k_2$.

II.B. Combined rest-stress measurement protocol

Figure 1(a) shows a conceptual diagram of the Standard method in which the rest study and stress study are done on completely different occasions, so there is no residual activity from the rest study appearing during the stress measurement. The first panel in Fig. 1(a) shows the time-line of the separated rest and stress studies, with arrows indicating the injection times. There are at least ten half-lives between the two studies to allow for tracer decay. The second panel shows that K_1 takes different constant values in rest ($K_{1,r}$) and stress ($K_{1,s}$). The third panel sketches the expected concentration histories for the input function and the tissue curve along with their relation to important fiducial markers, such as start of infusion

and the moment of peak stress. For the purpose of our studies, estimation of MBF from the Standard method is assumed to be unbiased. The Standard protocol is also expected to yield the lowest noise estimates of MBF at any fixed radiation dose. As discussed above, the price is logistical complexity, higher cost per rest-stress exam, difference in bed positions and the inconvenience of two separate scan sessions. The Standard method does not account for nonsteady state flow during the stress measurement; it may yield a biased estimate of flow during stress imaging.

We include in Fig. 1(b) the conceptual design for the rest-stress imaging protocol developed for rest-stress imaging by Rust *et al.*⁷ The first panel shows the time-line of a single-scan rest-stress protocol, with the arrows indicating time of injection. The second panel shows the assumed time dependence of K_1 . During rest and stress, Rust *et al.* assume steady state, with different constant values for rest and stress MBF. The third panel sketches the expected shape of the input functions during rest and stress. The fourth panel sketches the compartment model used to describe the tissue activity. Their approach allows the stress study to begin before the activity from the rest study decays away. As shown in Fig. 1(b), they assume that the activity from the first injection evolves throughout the entire experimental period unaffected by the pharmacological perturbation. The sketch also shows that, during the stress measurement period, it is the sum of the residual activity from the first injection and the new activity from the second injection, which is measured by the PET camera. The input function from the first injection, $C_{p,1}$, must be known throughout the entire rest-stress measurement period but only the sum of the two injections is measured during the stress period. Extrapolation and subtraction are used to estimate separate input functions. The PET curve is modeled as the sum of rest and stress, using two separate two-tissue models, as detailed in Sec. II.C.

Figure 1(c) shows the conceptual design for the MGH protocol reported here. The same injection protocol as in the Rust method is used: The rest phase, with the first tracer injection followed about 7 to 10 min later by a vasodilator infusion and then the second tracer injection and stress imaging period. The first panel in Fig. 1(c) shows that during the rest state K_1 is constant, but as vasodilator is infused at time T_s blood flow starts to increase, reaches a maximum at peak stress (time T_p), and the peak hyperemia ends at time T_E . The flow is expected to return to the baseline at T_B . In the MGH protocol list mode acquisition starts with the first injection and continues, without interruption, throughout the stress period. At peak stress, the second tracer injection is made. As the whole study duration is expected to be less than 30 min, the residual activity from the first tracer injection will be mixed with the activity from the second tracer injection in the period of the stress scan. As shown in the second panel of Fig. 1(c), the residual tracer concentration is affected by the infusion of vasodilator even before the second tracer injection as a result of the pharmacological action of the vasodilator. The effect of the vasodilator continues to affect the residual activity throughout the rest of the study. Due to the vasodilator, the subject is now in a nonsteady state with respect to blood flow; that is blood

FIG. 1. The experimental diagrams of the (a) Standard, (b) Rust, and (c) MGH methods, including the injection time-line, time-dependence of MBF, blood and tissue time-activity curves and the compartment models. Note that the MGH method shares the same experimental design with the Rust model, but the model configuration is different.

flow may be changing during the measurement. A basic assumption underlying conventional kinetic modeling, constant blood-to-tissue transport rates and fluxes, is no longer valid. Therefore, we extend compartment modeling, allowing flow-related parameters to vary with time in response to the action of vasodilators.

II.C. Kinetic models

As shown in Fig. 1, all three protocols are analyzed with similar kinetic models, but there are some key defining differences in the detailed assumptions. The fourth panel of Fig. 1(a) depicts the kinetic model underlying the Standard

model; it is designed to analyze rest and stress imaging as independent events. The fluxes and rate constants are considered to be fixed at constant values. The compartment diagram for rest imaging is the same for the Rust and MGH models; they differ only in how stress imaging is analyzed. The fourth panel in Fig. 1(b) shows the compartment diagram for stress imaging in the Rust model. The Rust model predicts the PET curve will be the sum of activity from the rest and stress injections. Rust and colleagues assume that the two tracer injections are independent and superimpose a rest and a stress study, each with constant flow, to predict/simulate the observed composite PET tissue curve. The Rust model does not explicitly model the effect of pharmacological challenge on the first injection. As a result, the Rust model assumes that residual activity from the rest phase of the study is unaffected by the changes wrought by vasodilator challenge. The state equations for the Rust model can be expressed as

$$\begin{aligned}\frac{dC_{F,1}}{dt} &= K_{1,r}C_{P,1} - (k_{2,r} + k_3)C_{F,1} + k_4C_{B,1}, \\ \frac{dC_{B,1}}{dt} &= k_3C_{F,1} - k_4C_{B,1}, \\ \frac{dC_{F,2}}{dt} &= K_{1,s}C_{P,2} - (k_{2,s} + k_3)C_{F,2} + k_4C_{B,2}, \\ \frac{dC_{B,2}}{dt} &= k_3C_{F,2} - k_4C_{B,2},\end{aligned}\quad (1)$$

where the subscript “1” and “2” denote the activity concentration from the first and second injection, respectively.

In the case of the MGH method, we first consider how a hypothetical ^{18}F -tracer gains access to an elemental tissue volume. We assume that the tracer is not metabolized in plasma and is free or in instantaneous equilibrium with plasma proteins and possibly other cellular and subcellular constituents. We denote the molar concentration of the tracer in plasma as $C_p(t)$, no matter whether there are one or more tracer injections. The inward flux is $K_1(t) \cdot C_p(t)$, where $K_1(t) = F(t) \cdot E$ is the product of the local myocardial blood flow and the unidirectional extraction fraction, E . For simplicity, we assume that E is a constant, independent of time. The time-varying clearance rate of tracer from tissue to blood is described by $k_2(t)$. The tissue space is conceptualized as two compartments, one representing free tracer, $C_F(t)$, the other compartment representing reacted tracer molecules, $C_B(t)$. Only the free tracer molecules in blood and tissue can respond to changes in blood flow; increases in flow will affect $K_1(t)$ and $k_2(t)$, resulting in observable changes in the kinetic curves measured by the PET camera. The reacted molecules are considered to be unaffected by flow changes and they cause the long tracer residence time in tissue. For generality, the reaction is left unspecified. But, for example, it may represent a receptor binding reaction or the committed step in a metabolic process. For short measurement times, we can assume that the amount of tracer returning from the reacted pool is negligible.

Analysis of the MGH protocol uses the two tissue-compartment kinetic model, to allow for tracers with high first pass extraction and long tissue residence time. The two-tissue compartment model is extended to include important charac-

teristics of a rest-stress study, as shown in the bottom panel of Fig. 1(c): First, the two injections of tracers are treated as a single entity—the input function $C_p(t)$. The ventricular concentration history is measured during the entire study. During the transition from rest to stress there is a change in physiological state. Residual tracer molecules from the rest study cannot be distinguished from those injected later. Residual activity from the rest study is observed in combination with the radioactivity injected during pharmacologic stress. Second, stress imaging is reconsidered to account for the changes due to the use of vasodilating drugs. It has been assumed, either implicitly or explicitly, that blood flow changes abruptly from one constant value in the rest condition to a different constant value in the stress condition. However, previous reports have shown that the myocardial blood flow is altered by slow intravenous infusion of vasodilating drugs in a progressive way over 2 to 7 min, depending on the drug.^{8–11} One way to deal with this problem is to assume that K_1 and k_2 can be represented by smooth functions of time that can be described by a small number of extra parameters. In the simplest case, K_1 and k_2 could merely undergo a step change but in a more realistic approximation, K_1 and k_2 are constant during the rest phase and then have a unimodal shape as a result of stress. We discuss these points in more detail in the theoretical section that follows.

Second, we extend the compartment representation for rest-stress applications. As in conventional compartment modeling, we retain the concept that the concentration in a compartment is spatially homogeneous and temporal changes occur simultaneously throughout the compartment. The innovation in our development is that we allow the flow-related transport rates to change as a function of time. We further assume that the probability per unit time for transport (k_3) into the reacted compartment is unaffected by vasodilating drugs. Accordingly, the basic differential equations are formulated as

$$\begin{aligned}\frac{dC_F(t)}{dt} &= K_1(t)C_p(t) - [k_2(t) + k_3]C_F(t), \\ \frac{dC_B(t)}{dt} &= k_3C_F(t),\end{aligned}\quad (2)$$

where $K_1(t)$ and $k_2(t)$ are functions of time dependent on the pharmaceutical properties of vasodilators. During the rest phase of the study we assume they are constant. Following the infusion of vasodilator, $K_1(t)$ and $k_2(t)$ are represented by time-varying functions, specified with a few parameters that will be estimated from the PET data. Equation (2) has a closed-form solution given by

$$\begin{aligned}C_F(t) + C_B(t) &= e^{-\int_0^t (k_2(z) + k_3) dz} \bullet \int_0^t \left[e^{\int_0^u (k_2(z) + k_3) dz} K_1(u) C_p(u) \right] du \\ &+ k_3 \int_0^t \left\{ e^{-\int_0^s (k_2(z) + k_3) dz} \bullet \int_0^s \left[e^{\int_0^u (k_2(z) + k_3) dz} K_1(u) C_p(u) \right] du \right\} ds.\end{aligned}\quad (3)$$

We considered two functional forms, namely, MGH1 and MGH2, for parameters $K_1(t)$ and $k_2(t)$. For the MGH1 model,

K_1 and k_2 are altered as a smooth function of vasodilator kinetics, infusion rate, and duration. Two drugs, adenosine and dipyridamole, have been commonly used in clinical studies. It has been reported that during the intravenous infusion of adenosine and dipyridamole, there is a transitional phase of flow increase until peak hyperemia is reached for both vasodilators^{10,11} but the functional form of the transition is unclear. For simplicity it is approximated as a linear function in this work, as shown in the first panel of Fig. 1(c). Similarly, once the blood flow begins to decline from peak hyperemia, the transitional change is assumed to be a linear function of time until the baseline flow is reached. $K_1(t)$ is expressed as

$$K_1(t) = K_{1,r}u(T_S - t) + \frac{K_{1,r}(T_P - T_S) + (K_{1,s} - K_{1,r})(t - T_S)}{(T_P - T_S)} \\ \times u(T_P - t)u(t - T_S) + K_{1,s}u(t - T_P)u(T_E - t) \\ + \frac{K_{1,r}(T_E - T_B) + (K_{1,s} - K_{1,r})(t - T_B)}{(T_E - T_B)} \\ \times u(t - T_E)u(T_B - t) + K_{1,r}u(t - T_B), \quad (4)$$

where $u(t)$ is the unit step function. In Eq. (4), four time points are used to describe the transitions: (1) beginning of infusion (T_S), (2) peak hyperemia (T_P), (3) end of peak hyperemia (T_E), and (4) baseline flow (T_B). Note that the peak hyperemia may not end right after the end of vasodilator infusion, especially for dipyridamole. The baseline and peak MBF is defined as $K_{1,r}$ and $K_{1,s}$. The same ramp function is also used to describe the time course of k_2 with $k_{2,r}$ and $k_{2,s}$ as k_2 at the baseline condition and peak hyperemia,

$$k_2(t) = k_{2,r}u(T_S - t) + \frac{k_{2,r}(T_P - T_S) + (k_{2,s} - k_{2,r})(t - T_S)}{(T_P - T_S)} \\ \times u(T_P - t)u(t - T_S) + k_{2,s}u(t - T_P)u(T_E - t) \\ + \frac{k_{2,r}(T_E - T_B) + (k_{2,s} - k_{2,r})(t - T_B)}{(T_E - T_B)} \\ \times u(t - T_E)u(T_B - t) + k_{2,r}u(t - T_B). \quad (5)$$

Although Eq. (5) is a realistic in terms of the physiology, using it to estimate the MBF and CFR (cardiac flow reserve) require prior knowledge or the estimation of T_P , T_E , and T_B (T_S is fixed and known). In practice, such timing information might be acquired with external monitoring of the blood pressure or by analyzing the ECG signals. To simplify the model and eliminate the need to measure these three time points, a simplified model MGH2 is also proposed by removing the transition of the parameters and just assuming the parameters to change their values by an immediate switch at the time of vasodilator infusion, as the time-dependence of K_1 and k_2 described by

$$K_1(t) = \begin{cases} K_{1,r}, & t < T_S \\ K_{1,s}, & t \geq T_S \end{cases} \\ k_2(t) = \begin{cases} k_{2,r}, & t < T_S \\ k_{2,s}, & t \geq T_S \end{cases} \quad (6)$$

II.D. Modeling the observed measurements

Regardless of the kinetic model chosen or the detailed physiologic assumptions, it is necessary to account for the transiently high blood radioactivity concentrations and finite resolution effects that are included in the PET measurements. Some myocardial radioactivity recorded by the PET camera is not due to radioactivity in myocardial tissue. We model this effect and estimate its magnitude as part of the fitting process, according to Eq. (3):

$$\text{PET}(t) \approx f_{lv} \cdot C_{lv}(t) + f_{rv} C_{rv}(t) + e^{-\int_0^t (k_2(z) + k_3) dz} \\ \bullet \int_0^t e^{\int_0^u (k_2(z) + k_3) dz} K_1(u) C_p(u) du \\ + k_3 \int_0^t e^{-\int_0^u (k_2(z) + k_3) dz} \\ \times \int_0^\mu K_1(u) C_p(u) e^{\int_0^\mu (k_2(z) + k_3) dz} du \cdot d\mu, \quad (7)$$

where f_{lv} and f_{rv} represent the spillover fractions for left and right ventricles, respectively.

II.E. ¹⁸F-Flurpiridaz

To evaluate and compare the methods proposed in this work, we used simulation studies based on the kinetic parameters derived from human studies conducted with ¹⁸F-Flurpiridaz, formerly known as ¹⁸F-BMS747158-02 (Lantheus Medical Imaging, North Billerica, MA).^{12,13} As a pyridazinone analog, ¹⁸F-Flurpiridaz binds to the mitochondrial complex I with high affinity. The combination of high first pass extraction and complex I binding ensure a high uptake in the myocardium with long residence time, due to the high mitochondrial density in the myocardial wall.¹³ Data from a rat model¹² have shown that ¹⁸F-Flurpiridaz has an extraction fraction of greater than 90% over the physiological MBF range, which makes it an attractive perfusion tracer. Validation of MBF measured with ¹⁸F-Flurpiridaz has been performed by Nekolla *et al.* in pigs by comparison to microsphere and ¹³N-Ammonia¹⁴ flow measurements. Good agreement between the MBF from ¹⁸F-Flurpiridaz and microspheres has been demonstrated to exist for a wide range of blood flow values.

II.F. Kinetic analysis based on ¹⁸F-Flurpiridaz clinical data

To determine the local myocardial kinetics used in our simulations, we used ¹⁸F-Flurpiridaz data furnished to us under research agreement by Lantheus Medical Imaging Inc. These data included three subjects who underwent rest and stress imaging. In these studies, rest and stress imaging sessions were separated by at least 2 h. First, the rest and stress studies for each of the subjects were analyzed separately. We fit these data to the two-compartment tissue model, using methods similar to the work of Nekolla *et al.*¹⁴ For each subject, the acquired stress images were first aligned to the images of the rest study using the nonrigid image registration tool from SPM8.¹⁵ The LV and RV input functions for each

TABLE I. Kinetic parameters of K_1 and k_2 estimated from the clinical ^{18}F -Flurpiridaz studies and used for Monte Carlo simulations.

Subject	Myocardium	Rest		Stress	
		K_1	k_2	K_1	k_2
1	Normal	0.68	0.08	1.96	0.07
	LAD	0.56	0.07	1.04	0.05
	RCA	0.74	0.08	1.26	0.08
	LCX	0.58	0.08	0.99	0.05
2	Normal	0.74	0.11	2.29	0.12
	LAD	0.78	0.11	1.60	0.07
	RCA	0.68	0.08	1.43	0.11
	LCX	0.94	0.13	2.10	0.10
3	Normal	0.60	0.05	2.09	0.23
	LAD	0.64	0.04	1.58	0.18
	RCA	0.58	0.03	0.68	0.17
	LCX	0.69	0.05	1.61	0.24

subject and each study were extracted with generalized factor analysis on dynamic series (GFADS).^{16,17} The LV input function was used to approximate the plasma input function. No metabolite correction was performed. Because there is a 2-h gap between the rest and stress studies, the residual myocardial activity from the first tracer is mostly tracer bound to MC 1 and is nearly unaffected by the stressor. The rest and stress image volumes of the heart were segmented into 17 standard segments.¹⁸ Time-activity curves were calculated for each segment. The residual activity from the rest study was estimated and subtracted from the stress time-activity curve. We then estimated the kinetic parameters for each segment under the resting state with its 25-min time-activity curve. k_3 was fixed as 0.026 (1/min) during the curve fitting. For the time-activity curve of the stress study, only data within the first 7.5 min were used in the curve fitting. After the parameters were estimated for all 17 segments, the cardiac flow reserve ($\text{CFR} = K_{1,s}/K_{1,r}$) of each segment was calculated. For the left anterior descending (LAD) territory, the segments with the two lowest CFR values were selected and the mean of each kinetic parameter was calculated from the estimated parameters of those two segments. The same calculation was repeated for right coronary artery (RCA) and left circumflex (LCX) as well. After two segments were picked for each territory, the kinetic parameters of the other 11 segments were averaged to compute the average kinetic parameters of the normal myocardium. The computation of the kinetic parameters of the normal myocardium, LAD, LCX, and RCA territories was performed separately for each subject. The estimated parameters are summarized in Table I.

Due to the relatively short measurements obtained in human studies, we made longer duration ^{18}F -Flurpiridaz measurements in a healthy 4.1-kg cynomolgus monkey. The animal was imaged under resting state and general anesthesia with 1%–2% isoflurane mixed with 100% oxygen. A 100-min dynamic ^{18}F -Flurpiridaz PET study, with 1.25 mCi injected dose, was acquired with a microPET P4 system from Concorde Microsystems, Inc. Images were reconstructed with filtered back-projection (FBP) into 24 5-s, 6 30-s, 5 60-s,

and 18 300-s frames. The LV and RV input functions were extracted with GFADS. A region of interest was drawn over the myocardium and used to calculate the time-activity curve which was then fitted to the standard four parameter, two-compartment model, with corrections for blood-to-tissue spillover in Eq. (3). We used compartment model kinetic analysis tool (COMKAT) (Refs. 19 and 20) under MATLAB R2009a (Mathworks, Natick MA) to perform the nonlinear least squares for parameter estimation with the frame duration used as the statistical weight. After the kinetic parameters were estimated from the 100-min time-activity curve, we refit the data while progressively reducing the observation period. First, K_1 , k_2 , f_1 , and f_{RV} were estimated as a function of study duration of the time-activity curves, ranging from 1 to 10 min. Due to the short measurement periods used, k_3 and k_4 were fixed to the values estimated by the 100-min data. The parameter variance of each parameter was approximated by the covariance matrix from the nonlinear least squares fitting. We then fixed the duration of the time-activity curve to 10 min and repeated estimation of K_1 , k_2 , f_1 , and f_{RV} with k_3 fixed at values ranging from 0 to 0.1, incremented by 0.005. k_4 was fixed as the value derived from fitting the 100-min data. Again, variance of each estimated parameter was approximated by the covariance matrix.

II.G. Monte Carlo simulation studies

To provide an initial evaluation of the proposed new single-session rest/stress measurement strategy and to compare with the conventional model for estimating the MBF estimation, we used Monte Carlo techniques to simulate realistic dynamic PET studies based on kinetic parameters obtained from human studies with ^{18}F -Flurpiridaz. The kinetic parameters were estimated as described in Sec. II.F. We also considered whether the proposed models will work for different vasodilator kinetics. The generation of simulated data was performed as follows: First, we used the NCAT torso phantom²¹ to generate static activity maps of the LV, RV, lung, liver, and the soft tissue. The heart activity maps were also generated with NCAT, with transmural defects in the LCX, RCA, and LAD territory and the rest of the myocardium is treated as the normal tissue. With those activity maps, we simulated high counts for each region with SIMSET,²² using a validated procedure for simulating the Philips Gemini System.²³ The simulated list-mode data were Fourier-rebinned into two-dimensional sinograms as 256×256 matrices over 87 slices. The simulated noise-free sinogram of each tissue type were then combined into a dynamic study by first scaling each sinogram with the corresponding time-activity curve and then summing those to form a simulated, noise-free sinogram for a dynamic PET study. The simulated study duration is 20 min, with the framing scheme of 6 5-s, 3 30-s, 5 60-s, 3 120-s, 6 5-s, 1 30-s, 2 60-s, 2 120-s frames. Then, Poisson deviates were added to the noise-free sinogram to form sinogram data with noise levels similar to those observed in clinical studies.

We simulated dynamic PET studies with two different ^{18}F -Flurpiridaz injection doses and two different vasodilators:

TABLE II. The initial guess and bounds for parameter estimation.

	$K_{1,r}$	$k_{2,r}$	$K_{1,s}$	$k_{2,s}$	f_1	f_{RV}	Dipyridamole		Adenosine		
							$T_{P,S}$	$T_{E,S}$	$T_{P,S}$	$T_{E,S}$	$T_{B,S}$
Initial guess	0.5	0.1	1	0.1	0.05	0.05	11.5	23.5	12	17.6	19
Lower bound	0	0	0	0	0	0	8.5	21.5	11.5	17.5	18.5
Upper bound	5	0.3	10	0.3	0.25	0.25	14.5	26.5	13.5	18.5	21

^aNote: T_B is not estimated for dipyridamole because T_E is greater than 20 min, which is the total study duration.

adenosine and dipyridamole. 2 mCi was used for the ^{18}F -Flurpiridaz dose for the first injection at time zero. The second injected dose was either 2 mCi (single-dose) or 4 mCi (double-dose) at 13 min after the first injection. $K_1(t)$ and $k_2(t)$ are both modeled as piecewise linear functions as Eqs. (4)–(6), respectively. For dipyridamole, the following timing information was used to simulate the data: $T_S = 6.5$, $T_P = 13$, $T_E = 23$, and $T_B = 29$ (minutes). The choice of T_P and T_E was based on the dipyridamole kinetics reported previously.¹⁰ For adenosine, the following timing information was used to simulate the data: $T_S = 11.5$, $T_P = 13$, $T_E = 17.5$, and $T_B = 20$. T_P and T_B were chosen based on the kinetics reported¹¹ under a 6-min adenosine infusion ($T_E = T_S + 6$).

For each combination of vasodilator and stress tracer dose, we simulated twenty noise realizations for each of the three simulated subjects. When simulating data for each subject, the kinetic parameters used to simulate the time-activity curve of a specific myocardial region were taken from the corresponding region of this specific subject. For example, the time-activity curve simulated the normal myocardium for the second subject is calculated with the parameters $K_{1,r} = 0.738$, $k_{2,r} = 0.108$, $K_{1,s} = 2.292$, and $k_{2,s} = 0.118$. In addition, for each noise realization, we simulated both the sinogram of a stress-only study and a single-scan rest/stress study. The stress-only study will be later used as the reference for comparison.

After the sinograms were simulated with the previous steps, images were reconstructed with FBP (Hamming, cut-off = 0.7) for 50 slices that cover the whole heart. In the normal myocardium and the three territories, a ~ 2 mL ROI was used to calculate the time-activity curves. For each type of tissue and each combination of subject/vasodilator/stress dose, there will be two time-activity curves per noise realization: the stress-only curve and rest/stress curve.

II.H. Parameter estimation

After the time-activity curves were extracted from the reconstructed images, they were fitted to the kinetic models previously described. First, the stress-only time-activity curve was used to estimate $K_{1,s}$ and $k_{2,s}$. The reference peak MBF is set to be equal to $K_{1,s}$ estimated from the stress-only time-activity curves. For the time-activity curves of the rest/stress study, it is fitted to the MGH1 model in two steps: First, the portion of the time-activity curve that is before the vasodilator infusion is fitted to MGH1 for estimating $K_{1,r}$, $k_{2,r}$, f_1 , and f_{RV} . After that, $K_{1,r}$, $k_{2,r}$, f_1 , and f_{RV} were fixed to values es-

timated from the previous step while $K_{1,s}$, $k_{2,s}$, T_P , T_E , and T_B were estimated with Eqs. (4) and (5). The initial guess and bounds of these parameters are summarized in Table II. For the MGH2 model, the same procedure was used for fitting the time-activity curves. The parameters $K_{1,r}$, $k_{2,r}$, f_1 , and f_{RV} were first estimated from the time-activity curve before vasodilator infusion and then fixed when estimating $K_{1,s}$, $k_{2,s}$. All the parameter estimation procedures were performed with COMKAT using the nonlinear weighted least squares with weights equal to the frame durations.

II.I. Statistical analysis

For each combination of region/subject/vasodilator/stress dose, we computed the sample mean and standard deviation of the estimated $K_{1,s}$ over the twenty noise realizations. The $K_{1,s}$ estimated from the stress-only study was used as the reference. The mean and SD of $K_{1,s}$ from different models are plotted as bar graphs to compare the estimated results between those from the Standard method and those estimated from MGH1 and MGH2. For each vasodilator/stress dose combination, the average bias percentage is calculated by averaging the bias percentage either over the subjects or over the tissue types (four tissue types, three subjects). To evaluate the precision of MBF estimation for each model, we compute the SD percentage as follows: First, the SD is first calculated from the twenty noise realizations for each tissue type/subject/dose, and then it is divided by the mean of estimated MBF to form the relative SD percentage. Following that, the average SD percentage for each method is calculated by averaging over regions or the subjects. The average bias and SD percentages are plotted as bar graphs.

III. RESULTS

The first result deals with estimating blood flow at rest. We show, as expected on theoretical grounds, that the part of the PET curve most highly dependent on blood flow is the initial period after IV injection of ^{18}F -tracers, such as ^{18}F -Flurpiridaz. Figure 2(a) shows a graph of K_1 values versus measurement time for the rest state. The measurement duration is varied in 1 min increments, from 1 to 10 min. For short measurements, less than 3 min duration, there is a positive bias. Increasing the measurement period, reduces the statistical error in estimating K_1 , though gains in signal-to-noise ratio diminish, becoming minimal beyond a 5 min measurement period. The SD of estimated K_1 was 2% with 7 min of

FIG. 2. The sensitivity analysis of K_1 as a function of study duration and k_3 . The error bars represent the SD estimated from the covariance matrix. (a) K_1 as a function of study duration. (b) K_1 as a function of k_3 .

data and 1.8% with 10 min of data. Figure 2(b) shows the variation in estimated K_1 as a function of the nominal value of k_3 . The result clearly shows that K_1 is nearly the same (ranged from 0.498 to 0.507), for k_3 values between 0 and 0.1 min⁻¹.

The clearance rate for tracer from tissue to blood is represented by k_2 . Simulations (data not shown) demonstrated that a longer measurement period is needed to achieve stable k_2 values and lower noise levels in the estimates. With 7 to 10 min of data, the SD for k_2 was 10.4% and 6.2%. We also found a strong positive correlation between k_3 and k_2 ; fixing k_3 at higher values increases the least squares estimate of k_2 .

Bar graphs are presented in Fig. 3 summarizing the range of simulated abnormal responses to dipyridamole stress. The

FIG. 3. Bar graphs for the estimated MBF when dipyridamole is used as the stressor. The second tracer injection dose is equal to the first tracer dose. On the x-axis, the number 1–3 denote the simulation performed based on different representative subjects.

FIG. 4. Plots of the average bias calculated from the MBF estimated with MGH1 and MGH2 models using the MBF from the stress-only as reference. The mean bias is computed by pooling the same region over the subjects.

injected doses for Fig. 3 for the two tracer injections were identical. The different stress models/protocols include: (1) simulations of stress only as the Standard method, (2) simulations of MGH1, the piecewise linear ramp, and (3) simulations of MGH2, with step-change in flow between rest and stress. Estimates from the MGH1 were found to be close to the estimates from the Standard method in all combinations. MGH2, on the other hand, shows a greater bias in some simulations, usually modest underestimation of MBF.

The comparison of bias and variance of MBF estimation from each model is presented in Figs. 4 and 5. Figure 4 shows the bias averaged over the subjects for each region. MGH1 is found to be nearly unbiased, with bias between -1% and 3%. There is no significant pattern of bias dependency on the myocardial sector. In general, a higher tracer dose for the stress study reduces the bias for MGH1 method. On the other hand, MGH2 has a higher bias than the MGH1 method. For dipyridamole, an underestimation of MBF is found in all regions with bias ranging between -1% and -10%. Higher bias is observed in the single-dose stress studies. When adenosine is used as the vasodilator, the bias of MGH2 is fairly low, ranging between -2% and 2%. The average SD of the estimated MBF is shown in Fig. 5. The stress-only protocol, as the Standard method, has the lowest noise estimate, less than 5% in

FIG. 5. Plots of the average SD calculated from the MBF estimated with MGH1 and MGH2 models using the MBF from the stress-only as reference. The mean SD is computed by pooling the same region over the subjects.

FIG. 6. Representative rest/stress ^{18}F -Flurpiridaz PET study. (a) The 10-min frame reconstructed image corresponding to 15–25 min of the rest and stress studies as a fused image over the three axes (short axis, vertical and horizontal long axes). The rest study is in gray color scale and stress is in color. (b) The polar map of the estimated coronary/cardiac flow reserve (CFR) for this subject.

SD, for all the segments and conditions simulated. MBF estimates from MGH1 and MGH2 are of higher variance, ranging between 2% and 9%. Variance of the MGH1 and MGH2 estimates is reduced to less than 6% when double tracer dose is used for the stress study. In general, MGH2 has a lower SD than the MGH1 method.

Finally, Fig. 6 shows a representative myocardial rest and stress study with the short axis, vertical and horizontal long axes with rest in gray scale and stress in color along with the corresponding coronary flow reserve (ratio of absolute myocardial blood flow at rest and during peak stress).

IV. DISCUSSION

Presently, there is great enthusiasm for ^{18}F -based perfusion tracers, such as ^{18}F -Flurpiridaz and ^{18}F -BFPET,²⁴ due to their long half-life which enables commercial distribution and does not require on-site production.^{25,26} The validity of measuring MBF using ^{18}F -Flurpiridaz with a two-compartment model has been shown by comparison with microsphere reference measurements.¹⁴ Phase III clinical trials of ^{18}F -Flurpiridaz are now ongoing. However, the long half-life of ^{18}F also creates a challenge for a single-scan rest-stress study, as pointed out by Camici and Rimoldi in their review paper of ^{18}F -Flurpiridaz.²⁶ In this work, we examined the feasibility of absolute quantification of the MBF within a short, single imaging session by kinetic modeling approaches and simulation.

We began this research by reconsidering what we call the Standard method. As seen from the foregoing discussion, most contemporary quantitative methods assume that measurement of resting MBF can be described as a steady state condition and analyzed by compartment models with constant transport rates and rate constants. This is similar to the approach taken by Nekolla *et al.* who validated ^{18}F -Flurpiridaz in animals by comparison with microsphere measurements.¹⁴ Our analysis deviates from the conventional Standard method in that we allow blood flow and flow-related rate constants to be time dependent in response to pharmacological stress. Based on prior research,^{8–11} we may expect different time-variation, depending on the detailed properties of the vasodilator. Previous work has not considered this effect and it should be taken into account, as not doing so may add to the bias and variability of measurements. Our solution is to parameterize a mathematically convenient functional form for $F(t)$ so that estimating these parameters provides a summary description of the temporal flow variation. We recognize that, as a practical matter, adopting a more realistic mathematical function may lead to unidentifiable estimates of MBF. So, a carefully thought out trade off is required.

Another point to consider is the validation of flow measurements made during stress imaging. Microsphere flow estimates are considered to be the gold standard but the derivation of the microsphere flow equations also assumes constant flow. The assumption of constant flow enters the

microsphere method through the Fick equation $\int_0^T F(t)C_a(t)dt \equiv F \int_0^T C_a(t)dt$, where F is the myocardial blood flow and $C_a(t)$ is the arterial concentration history of microspheres. This means that the accuracy of the microsphere method during stress imaging depends on the magnitude of the systematic error made by factoring the Fick equation.

There are a number of approaches to combined rest-stress imaging that have been considered. Among those are what can be called semiquantitative or *ad hoc* methods that use larger doses of radionuclide for stress imaging and neglect the effect of prior rest imaging entirely. "Subtraction" of rest images from stress images can improve contrast but it is not based on rigorous physiological and physical principles.⁷ More quantitative methods have been proposed in various reports.^{7,27–29} Kim *et al.*³⁰ proposed and Iida *et al.*³¹ implemented a related model and measurement protocol for measuring the change in cerebral blood flow elicited with pharmacologic challenge. The Rust model is most relevant to imaging with ¹⁸F-labeled MBF tracers. Their kinetic model predicts that the activity measured during the stress exam is the sum of the residual rest activity plus the new activity injected during pharmacological stress. Using the kinetic parameters estimated from fitting the rest images they predict the residual contributions to the stress images. Rust *et al.* assume that MBF is constant during stress imaging but they also mention in their report⁷ that "kinetic parameters would not be constant due to the infusion of adenosine during the scan" as a limitation of their model.

In our work, we re-examined the feasibility of absolute quantification of rest/stress MBF within a single, short imaging session. Unlike previous methods, we assume that free tracer molecules will "feel" the effects of stress as a change in state whether they be part of residual activity from a previous injection, or "newer" molecules injected during infusion of a pharmacological stressor. In effect, we assume that the system has no memory of the previous state, once pharmacologic stress is started. This key insight allows us to treat the entire rest-stress measurement as a single study with a single input function that includes the contributions from both the rest and stress tracer injections. Transport and rate constants describing the inflow and egress of unreacted tracer molecules become time dependent and the equations no longer describe a classical compartment system. Such assumption of time-dependent kinetic parameters has been previously used for other applications in brain studies.^{27,32} Since we do not know, *a priori*, the functional form of the time dependence, it is necessary to assume a convenient functional form for the fluxes and rate constants. Both a piecewise linear (MGH1 method) and a simpler two state formulation (MGH2 method) are described and evaluated by the simulation. Despite the more general and realistic assumptions the new method is not significantly more complicated than more conventional analyses. Future work will investigate the feasibility of forming high quality parametric images of MBF with the MGH method using principal component clustering^{33,34} and our previously developed 3D orthogonal grouping of voxels with similar time course.¹⁶ Finally, double gating/dynamic imaging is an interesting research direction that is not presently performed rou-

tinely. It would be more realistic to model the change in heart rate at rest and during peak hyperemia and generate the corresponding gated studies and then average these gates for each time frame. This would require a substantial increase in simulation time (16× if we model 16 cardiac gates) and may not yield a major change in our findings because of the averaging of cardiac gates for each dynamic frame.

Working with a long rest study obtained with ¹⁸F-Flurpiridaz, we show that the first few minutes of the dynamic sequence is insensitive to the mechanisms that account for the long residence time of Flurpiridaz. In terms of compartment modeling, Figure 2 shows that estimate of MBF reaches a high precision, requiring only a few minutes of data, while being very insensitive to any physiologically plausible value of k_3 . This means that binding of Flurpiridaz can be treated as a nominal effect which does not vary from subject to subject. From Fig. 2, it can be concluded that estimates of MBF with low bias and good statistical precision can be obtained in less than 10 min. Therefore, there is little reason to prolong the rest study and pharmacological stress infusion may begin promptly, allowing for a shorter study design.

While the finding that only a few minutes are needed to obtain quantitative measurements of resting flow is of basic importance, there are other important questions to answer about combined rest/stress: (1) Under plausible physiologic assumptions, is it possible to obtain accurate and precise MBF with a rapid single-scan rest/stress protocols? (2) What is the trade off between accuracy and signal-to-noise ratio due to overlapping rest and stress studies? Considering the Standard method with completely separate rest and stress measurement to be method of lowest radiation dose, how much more activity need to be used to get similar signal-to-noise ratio with the MGH protocols? These questions are answered by realistic Monte Carlo simulations based on a digital torso phantom with an anthropomorphic cardiac geometry. By following millions of photon histories we built up an ensemble of simulated dynamic studies with both normal and abnormal myocardial segments. We simulated ensembles of data emulating the Standard method and the MGH1 method. The myocardial time-activity curves from simulated data were fitted to the Standard method, the MGH1 and MGH2 methods. We found the following properties of these three methods by evaluating the bias and precision performance. First, the Standard method produced the most precise estimates for the peak MBF during stress. This is because, in the Standard method, rest and stress studies are performed separately, so there is no mixture of the activity from the two tracer injections. This results in a simple and straightforward parameter estimation strategy, which provides the best precision. Second, by combining a rest and stress study, the price to pay is the inevitable increase in the parameter variance. For both MGH1 and MGH2, the activity after the onset of vasodilator infusion is affected by the time-dependence of K_1 and k_2 . Furthermore, the model becomes more complicated than the Standard method and since the measured activity comes from two injections, the parameter variance will be increased for both MGH1 and MGH2. Therefore, choosing between the Standard method and the MGH methods become a trade-off

between the study complexity, injected dose and the parameter precision. Considering all these factors, we believe that the convenience of a single scan session will outweigh the minor increase in the variance of MBF.

Based on the current simulations, we believe that the MGH method is promising for rest/stress studies. By examining the performance of the MGH1 method, we found that the MBF bias is very small ($<3\%$). As previously mentioned, MGH1 method has a higher variance than the Standard method, but this increase of variance is modest as shown in Fig. 5. The SD of MBF is less than 5% for double-dose and less than 9% for single-dose stress. The Standard method requires that the subject be repositioned for the rest study; whereas the MGH method offers a short, single-scan session protocol.

There are two variants of the MGH method. The MGH1 method is more accurate due to the more detailed model of the effects of vasodilators. The MGH2 method, as a simplified version of MGH1, also produced reasonable performance. By modeling the transition of MBF as a step change, fewer parameters need to be estimated for MGH2. As a result, MGH2 is a more precise than MGH1. However, since the approximation of the step change does not fully describe the kinetic behavior of MBF, MGH2 is more biased than the MGH1 method. This is particularly obvious in the case as dipyridamole, as it takes longer (6.5 min) to reach the peak hyperemia than does adenosine (1.5 min). In other words, the MBF change in the MGH2 model better approximates the fast transition in adenosine than dipyridamole. As a result, the MGH2 method is more appropriate for fast-acting vasodilators as its accuracy is more dependent on the functional form of the time-dependent kinetics.

Compared to the Rust method, the MGH method is different in several important features: First, residual tracer molecules in plasma and myocardium are transported according to flow conditions which prevail during stress. Second, we model flow and clearance as a continuous function during stress imaging to account for the effect of the vasodilator. The modeling is the same whether the tracer molecule was introduced at rest or during vasodilation. Because the residual activity of the rest study is more properly modeled we expect a lower bias. Third, the input function of the tracer is treated as a single entity in the MGH method while the Rust method requires the input functions from the two injections to be separated and extrapolated. In the current work, we did not make a judgment on the performance of the Rust method because the simulated data are based on the K_1 and k_2 kinetics described by the MGH1 model. Since the MGH1 model is also an approximation and requires further validation with animal or human data, it would not be fair to compare the estimates of the Rust method using data simulated in this work. The bias/precision performance of the Rust and MGH methods both require further validation with real experimental data against reference MBF measured with microspheres.

Although this work is aimed toward application of ^{18}F -Flurpiridaz, the modeling approach is general and may be applied to other tracers with relatively long half-lives such as ^{13}N -Ammonia. The model may also be applied for vasodila-

tors other than the adenosine and dipyridamole or even exercise stress studies that can be performed with patient under the fixed bed position within the scanner.

V. CONCLUSION

We have shown with simulation studies that the MBF can be accurately and precisely estimated from a single-scan rest/stress study for long half-lived tracer ^{18}F -Flurpiridaz. Our rest/stress model requires a single input function for rest and stress, eliminating the need for extrapolation or subtraction. Accounting for the time-dependence of the kinetic parameters, the proposed models (MGH1 and MGH2) both achieve good accuracy and precision for different vasodilators and a range of different MBF states.

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