Validation of myocardial blood flow estimation with nitrogen-13 ammonia PET by the argon inert gas technique in humans

Jörg Kotzerke[1], Gerhard Glatting[1], Jörg van den Hoff[3], Martin Höher[2], Bernd Neumaier[1], Jochen Wöhrle[2], Sven N. Reske[1]

[1]Department of Nuclear Medicine, University of Ulm, Robert-Koch-Strasse 8, 89081 Ulm, Germany
[2]Department of Cardiology, University of Ulm, Germany
[3]Department of Nuclear Medicine, Hannover Medical School, Germany

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Abstract. We simultaneously determined global myocardial blood flow (MBF) by the argon inert gas technique and by nitrogen-13 ammonia positron emission tomography (PET) to validate PET-derived MBF values in humans. A total of 19 patients were investigated at rest (n=19) and during adenosine-induced hyperaemia (n=16). Regional coronary artery stenoses were ruled out by angiography. The argon inert gas method uses the difference of arterial and coronary sinus argon concentrations during inhalation of a mixture of 75% argon and 25% oxygen to estimate global MBF. It can be considered as valid as the microspheres technique, which, however, cannot be applied in humans. Dynamic PET was performed after injection of 0.8±0.2 GBq ^{13}N-ammonia and MBF was calculated applying a two-tissue compartment model. MBF values derived from the argon method at rest and during the hyperaemic state were 1.03±0.24 ml min^{-1} g^{-1} and 2.64±1.02 ml min^{-1} g^{-1}, respectively. MBF values derived from ammonia PET at rest and during hyperaemia were 0.95±0.23 ml min^{-1} g^{-1} and 2.44±0.81 ml min^{-1} g^{-1}, respectively. The correlation between the two methods was close (y=0.92x+0.14, r=0.96; P<0.0001). No indication was found for limited extraction of ammonia in the myocardium. The high concordance of global MBF values derived with argon and ammonia indicates that the implicit correction of spillover and recovery effects, incorporated in the model by including an effective blood volume parameter, works correctly quantitatively. Our data provide the previously missing human validation of MBF measurements from ^{13}N-ammonia PET.

Keywords: Argon – Nitrogen-13 ammonia – Positron emission tomography – Myocardial blood flow

Jörg Kotzerke (✉)
Department of Nuclear Medicine, University of Ulm,
Robert-Koch-Strasse 8, 89081 Ulm, Germany
e-mail: joerg.kotzerke@medizin.uni-ulm.de
Tel.: +49-731-5024507, Fax: +49-731-5024512

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Introduction

Regional myocardial blood flow can be estimated qualitatively by means of flow tracers (e.g. methoxyisobutylisonitrile, tetrofosmin) and single-photon emission tomography (SPET) to detect regional ischaemia. Global and regional myocardial blood flow (MBF) can be measured quantitatively and non-invasively by means of various tracers (e.g. rubidium-81, rubidium-82, nitrogen-13 ammonia, oxygen-15 water) and positron emission tomography (PET) [1, 2, 3]. Various techniques have been proposed to calculate the MBF, mainly based on compartment models. However, results depend on the detailed model assumptions and analysed time intervals as well as the treatment of limited extraction and/or retention fractions. Evaluation of regional perfusion based on differences in tracer retention is error-prone for most tracers: retention fraction is usually reduced in high-flow areas, which implies that the regional differences in tracer retention are smaller than the underlying flow differences. Adequate modelling of the tracer exchange between blood and tissue, therefore, is necessary. For ammonia, the compartment analysis proposed by Hutchins et al. [4, 5] separates tracer delivery (MBF) from metabolic retention (myocardial uptake). However, the technique was validated only in animal experiments using the microspheres technique as gold standard [6, 7]. In humans, the microspheres technique is not applicable and, therefore, blood flow estimations using ^{13}N-ammonia were compared sequentially only with the freely diffusible tracer ^{15}O-water and PET [8]. Values for MBF under resting conditions were reasonable and changes during hyperaemia were as high as expected. However, validation in humans against a true reference is still lacking.

The argon inert gas method allows repeated measurements of the global MBF by means of arterial and coronary venous argon concentrations during inhalation of the inert gas argon for several minutes. The method was introduced by Bretschneider, Rau and Tauchert [9, 10, 11, 12, 13, 14] and is considered a valid reference for the measurement of global MBF in humans [1, 3, 15, 16]. In this study, we estimated global MBF under resting conditions and during pharmacological vasodilation by applying dynamic ^{13}N-ammonia PET and the argon method simultaneously in patients with suspected microvascular disease.

Materials and methods

The study was approved by the ethics committee of the University of Ulm. Written informed consent was given by all study participants after the investigative aim of the study, its risks and its merits had been explained.

Study population. Nineteen patients (mean age 59.0±7.7 years; range 46–72 years; nine women, ten men) were studied at rest and during hyperaemia. Patient selection was done after coronary angiography. Clinical indications for angiography were angina pectoris in 16 patients and cardiac examination prior to an electrophysiological study due to syncope in three patients. None of the patients had a history of myocardial infarction. Of the 16 patients with angina, nine had angina on exertion or during tachycardia, five had atypical angina and two had angina at rest. None of the patients had significant coronary artery stenosis (>50%), but 11 had indirect signs of microvascular disease (e.g. decreased peripheral run-off of the contrast medium, increased end-diastolic left ventricular pressure). Of the remaining eight patients, three had hypertensive cardiac disease and five had normal findings. Coronary risk factors were hypertension (n=6), diabetes mellitus (n=1), smoking (n=5) and hyperlipidaemia (n=8).

The patients were carefully instructed to refrain from intake of caffeine-containing beverages during the 12 h before the study.

Adenosine-induced hyperaemia. Adenosine (Adenoscan Sanofi Winthrop GmbH, München, Germany, 0.14 mg kg^{-1} min^{-1}) was infused over 9–12 min. After reaching stable conditions and well-being of the patient (3–5 min), the argon–oxygen mixture (75% argon and 25% oxygen) was supplied by a mouth pipe, prohibiting further conversation, and ^{13}N-ammonia was injected. The hyperaemic state was maintained until the final blood sample for the argon concentration in coronary venous sinus had been withdrawn (additional 6–7 min). Measurements were repeated in a subset of patients after complete desaturation of argon from blood (Fig. 1).

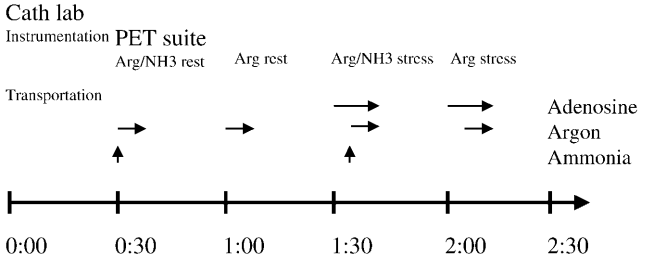

Fig. 1. Time schedule (h:min) of the blood flow investigations. *Arg*, argon; *NH3*, ^{13}N-ammonia

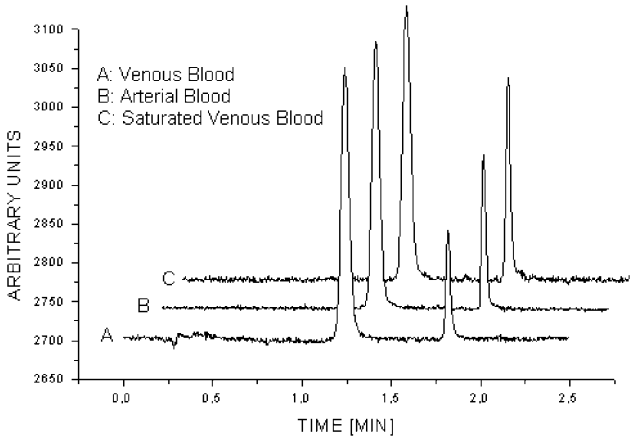

Fig. 2. Typical gas chromatographic profiles from coronary sinus (**A**), arterial blood (**B**) and argon-saturated venous blood (**C**) after 5 min of argon inhalation. The x-axis represents retention time. The first peak (*left*) represents nitrogen, and the second one (*right*) represents argon. The different area of argon peak in arterial and saturated venous blood is mainly caused by dilution of the arterial argon concentration from argon-free dead space in the catheter

Argon inert gas technique. Placement of a venous coronary sinus catheter was performed in the cath lab under angiographic control. Correct placement before and after transportation to the PET suite was checked by an oxygen saturation <40%. An arterial line for blood sampling (radial artery) and a venous line for injection of the tracer and infusion of the pharmacological stress agent (adenosine, 0.14 mg kg^{-1} min^{-1}) were added. Neutral blood samples were collected to correct for argon concentration in room air. Continuous arterial and coronary venous sampling was performed by means of a pump (2 ml min^{-1}) for 5 min during inhalation of the argon–oxygen gas mixture. Dead space of catheter material (2.3 ml) was compensated by adequate prolongation of the sampling interval. Subsequent sampling of coronary venous blood was performed to estimate the final argon saturation in blood. Argon dissolved in blood was extracted in an extraction chamber and relative argon concentrations of all samples were measured by gas chromatography within 72 h [17]. Typical profiles are displayed in Fig. 2. Loss of argon from locked glass syringes was estimated to be below 3% within 1 week when stored at 7°C.

Determination of MBF derived from argon data. MBF was calculated according to:

$$F = \frac{p \cdot c_v(T)}{\int_0^T (c_a(t) - c_v(t))dt}$$

where $c_v(T)$ is the saturated venous argon concentration, $c_a(t)$ and $c_v(t)$ are the arterial and coronary sinus argon concentrations and p=1.1 is the partition coefficient for argon [14]. This relationship follows a simple one-compartment model. The procedure was repeated during resting (15 patients) and under hyperaemic conditions (three patients) to estimate the reproducibility of the argon method.

PET image acquisition and reconstruction. Images were acquired with a Siemens/CTI model 931/08-12 or HR+ tomograph. Photon attenuation was corrected using a 20-min transmission image. A dynamic study of 14.5 min duration (12×10 s, 4×30 s, 3×90 s, 1×360 s) was started after injection of 0.8±0.2 GBq ^{13}N-ammonia at rest and following 0.9±0.2 GBq ^{13}N-ammonia during hyperaemia. One hour was allowed between the two studies for decay of the tracer and exhalation of any argon. ECGs were monitored continuously throughout all studies and blood pressure was measured every minute during argon inhalation.

Determination of MBF derived from PET data. Quantification of the ammonia studies was performed after transformation of the transaxially acquired image data into dynamic polar maps. The transformation was achieved by using an automatic rendering of the centre of the myocardial wall in the original image volume [18]. The arterial input function was derived from the cavum of the left ventricle by shrinking the detected surface to 30% of its original size. Quantification was performed on a pixel by pixel basis in the dynamic polar maps using the model developed by Hutchins [4, 19]. We also adopted Hutchins' approach [19] for correction of spillover and recovery by including an effective blood volume as a free parameter in the model equations. Data fitting was extended over the whole time course of the acquisition. An averaged metabolite correction was performed according to the results given in [20]. Average myocardial perfusion was determined by averaging over the respective parametric polar maps. Care was taken to ensure correct regional weighting when calculating the averages.

Statistical analysis. Statistical analysis was performed with a commercially available personal computer software program (Microcal Origin, Microcal Software, Inc., Northampton, Mass., USA). Values are expressed as mean±1 standard deviation. A probability of the α-error $P<0.05$ was considered statistically significant. For each pair of MBF data, the difference between the measurements was expressed as a percentage of the mean value of the two measurements (Bland-Altman plot) [21].

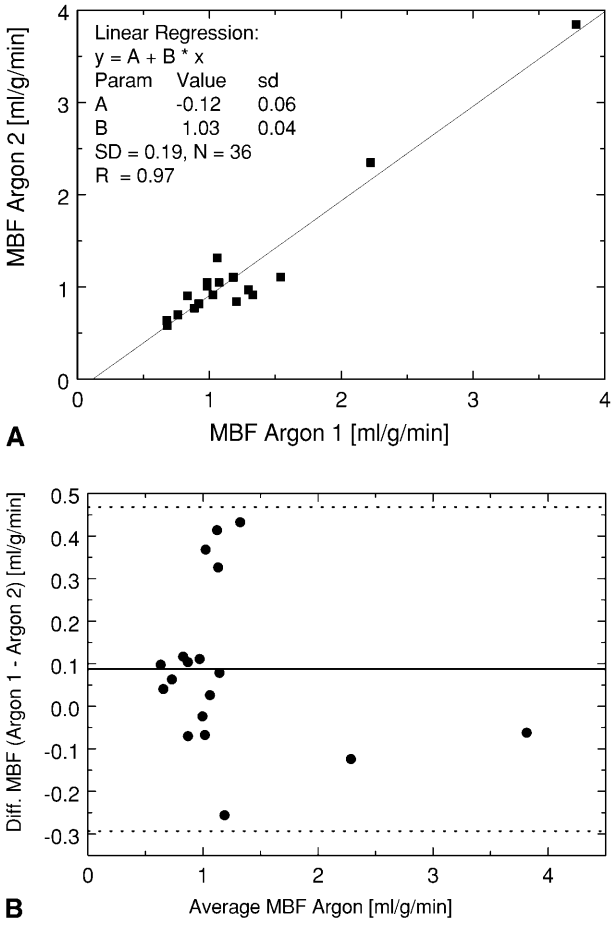

Fig. 3. Reproducibility of myocardial blood flow (*MBF*) estimations by the argon inert gas method under resting and hyperaemic conditions (**A**). Investigations were repeated within 1 h. The relative differences are also displayed in relation to the average MBF values of both investigations (Bland-Altman analysis of agreement) (**B**)

Results

Haemodynamic findings

Heart rate in beats per minute (bpm) at rest and during hyperaemia was 65±11 bpm and 81±13 bpm, systolic blood pressure (mmHg) at rest and during hyperaemia was 147±24 mmHg and 143±24 mmHg, respectively. Rate-pressure product (bpm×mmHg) at rest and during hyperaemia was 9,488±1,900 and 11,487±2,021, respectively.

Estimates of global MBF by the argon inert gas technique

Oxygen saturation estimated in coronary sinus blood during rest was 28%±6%, confirming the correct placement of the catheter. Myocardial blood flow averaged 1.03±0.24 ml min^{-1} g^{-1} (range 0.68–1.54) at rest and increased to 2.64±1.02 ml min^{-1} g^{-1} (range 1.06–4.33) in the hyperaemic state. Repeated measurements in a subset of patients did not differ significantly (Fig. 3).

Estimates of global MBF by ^{13}N-ammonia PET

Polar map analysis suggested that all participants were free of significant coronary artery disease, as they had homogeneous tracer distribution throughout the entire left ventricular myocardium at baseline and in the hyperaemic state. Myocardial blood flow averaged 0.95±0.23 ml min^{-1} g^{-1} (range 0.58–1.41) at rest and increased to 2.44±0.81 ml min^{-1} g^{-1} (range 1.28–3.82) in the hyperaemic state. A detailed regional analysis is provided in Table 1.

Comparison of global MBF values

Overall, all blood flow measurements under resting and hyperaemic flow conditions were closely correlated, with a slope approaching unity ($y=0.92x+0.14$, $r=0.96$; $P<0.0001$) (Fig. 4). Flow reserve was impaired at least

Table 1. Global and regional myocardial blood flow under resting and hyperaemic conditions

	MBF rest (ml min^{-1} g^{-1})	CV (%)	MBF stress (ml min^{-1} g^{-1})	CV (%)
Global	0.95±0.23	24±8	2.44±0.81	24±6
Anterior	0.94±0.25	24±8	2.15±0.96	24±7
Lateral	0.94±0.30	24±9	2.53±1.13	25±8
Inferior	0.99±0.30	25±9	2.41±1.20	25±9
Septal	0.93±0.32	24±9	2.67±1.42	24±9

MBF, Myocardial blood flow; CV, coefficient of variation

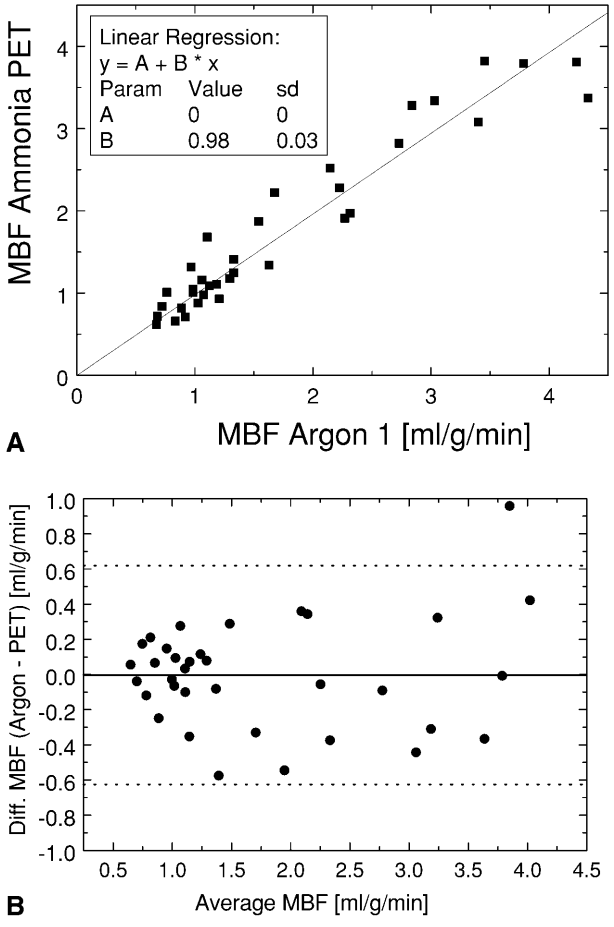

Fig. 4. Comparison of myocardial blood flow (*MBF*) derived from the argon inert gas technique and ^{13}N-ammonia PET (**A**). Values from simultaneous measurements (*MBF Argon 1*) are used. The relative differences are also displayed in relation to the average MBF values of both investigations (Bland-Altman analysis of agreement) (**B**)

partially, as was to be expected in this study group: coronary flow reserve derived from the argon and PET technique was 2.6±1.0 and 2.7±1.1, respectively. Eight patients had a coronary flow reserve <2.5, consistent with small-vessel disease.

Discussion

The "ideal" blood flow tracer is completely extracted by the myocardium during a single capillary transit. Radiolabelled microspheres fulfil these criteria with nearly 100% first-pass extraction at all flow levels [22]. ^{13}N-ammonia and ^{82}Rb have been employed as "chemical microspheres" for the measurement of myocardial blood flow; however, the myocardial retention of these tracers (due to metabolic trapping) is incomplete, resulting in a markedly non-linear relation between net tissue retention of the tracer and blood flow [23]. Several quantitative approaches have been proposed for the measurement of MBF, mostly based on compartment models. The approaches differ in interpretation of the observed tracer kinetics and in the handling of tracer washout from tissue. Although the methods have yielded comparable results and have been validated in animal models, none has been validated with an independent gold standard for humans.

Although the argon inert gas technique is not non-invasive (owing to placement of a coronary sinus catheter), it has been considered a valid method for quantitative assessment of MBF for 30 years [9, 11, 14]. Because the isolation of dissolved argon from blood is time consuming and expensive, the argon method was abolished in favor of simplified techniques, including PET. However, the argon method is a proven valid reference method applicable in animal experiments and in humans [9, 12, 13]. An additional important advantage of use of the argon method was that MBF estimation by PET and the argon method could be performed simultaneously.

Reproducibility of MBF estimations by the argon inert gas technique within 1 h was satisfactory, with a mean difference of 11%±17% which includes technical and physiological variations (Fig. 3). Repeated MBF estimation by ammonia PET in 30 healthy volunteers yielded values under resting and hyperaemic conditions of 16%±16% and 11%±9%, respectively [24]. Similar values were obtained by Sawada et al. in normal volunteers as well as in patients with stable coronary artery disease using a semi-automatic sampling routine [25]. In that study the most important factor influencing MBF values was cardiac work, and the authors showed that normalization for rate-pressure product minimized the differences.

Regional analysis of the PET data demonstrated variation coefficients of about 25%, with the highest MBF values obtained inferiorly and laterally under resting and hyperaemic conditions, respectively. Only limited data are available on the extent to which spatial heterogeneity of MBF exists in normal humans. Hutchins et al. [4] did not detect any regional difference while Czernin et al. [26] and Gewirtz et al. [27] observed variation coefficients of 12% and 22%, respectively. However, analysis of the spatial MBF heterogeneity in vivo has always been limited by the resolution of imaging modalities.

Comparisons of MBF estimations by ^{13}N-ammonia and ^{15}O-water with the microspheres technique in animal models were performed by Bol et al. and Muzik et al. [7, 28], the results demonstrating similarly high correlations of both positron emitters with the reference method. However, MBF estimations by ^{13}N-ammonia and compartment analysis in humans were compared only with ^{15}O-water PET, without any further external reference [8]. It is worth pointing out that the sequential application of both tracers cannot rule out physiological changes in MBF within the time interval employed. To date ammonia PET has been applied without further validation because MBF values at rest and during hyperaemia have been in the expected range.

It is well recognized that even in the normal heart there is regional spatial and temporal heterogeneity in MBF and contractile function. However, this variability is generally small, being in the order of 10%–15%, and its detection in vivo has been limited by the spatial and temporal resolution of routinely available imaging modalities [29, 30, 31]. While ammonia PET allows estimation of regional as well as global blood flow values, the argon inert gas technique estimates only global values. Short-lasting changes in MBF can be recognized neither from PET data nor using the argon inert gas technique because both methods analyse time-indicator curves of about 5 min or longer and need a further 30 min for decay of the tracer and re-breathing of the argon from blood to room air. However, investigation of resting and hyperaemic states can be completed within 90 min by both techniques. Moreover, the sampling time for both techniques is very similar, excluding differences in MBF calculations due to sampling time spread.

Another important factor is the type of model used to derive MBF values from PET data. In a recent study, Choi et al. reported that differences in absolute flow values amount to about 25% at most when using different algorithms. However, despite these differences in absolute MBF values, estimation of coronary flow reserve was always accurate [32]. As noted by the authors, remaining differences can easily be explained by details regarding region of interest positioning and implementation, so that all compared algorithms can be used for quantification of myocardial perfusion. In the present study we decided to use the model of Hutchins [4, 5], which explicitly takes into account tracer back-flow from tissue, thus obviating the need for the empirical extraction corrections that are necessary with the other approaches.

The data presented in Fig. 4 provide no clear evidence for incomplete first-pass extraction of ammonia. Fitting a straight line through the origin yields a slope of 0.98±0.03 (interpreting all deviations between data and fit as being caused by statistical fluctuations). This result is compatible with the assumption of complete first-pass extraction of ammonia at all physiological flow levels, justifying the use of the tracer for perfusion quantification of myocardial perfusion.

Contrary to the results reported by Choi et al. [32], we found no overestimates of perfusion when using the model of Hutchins [4, 5]. Moreover, the high concordance of global MBF values derived with argon and ammonia indicates that the implicit correction of spillover and recovery effects incorporated in the model by including an effective blood volume parameter is quantitatively correct.

The parameter K_1 of the approach proposed by Hutchins [4] is related to the permeability-surface area PS [33, 34], extraction and flow F, according to $K_1=F\,E$, with E=1-exp(-PS/F). A fit to our data results in PS=(10.6±1.9) ml min^{-1} g^{-1}. For normal flow, i.e. F=0.8 ml min^{-1} g^{-1}, the extraction calculated with PS=10.6 ml min^{-1} g^{-1} yields E=1–exp(–PS/F)=1.00, whereas for F=3.2 ml min^{-1} g^{-1} we obtain E=0.96. Thus, for a perfusion reserve of 4 the error when assuming a constant extraction fraction of E=1 is only about 4%. Therefore, when considering the magnitude of errors due to other sources, the possible small deviation of extraction from unity at high flow values can be neglected.

In conclusion, MBF values under resting and hyperaemic conditions derived with a two-tissue compartment model (Hutchins' approach) in the flow range between 0.7 and 3.8 ml min^{-1} g^{-1} demonstrated a close correlation with MBF values derived by the gold standard argon inert gas method. No indication was found for over- or underestimates of perfusion under low- and high-flow conditions when using the model of Hutchins. The assumption of complete initial extraction of ammonia as implied in this model can therefore be considered valid in humans. Thus, extraction correction is unnecessary when using this model for perfusion quantification.

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