Uncertainty in Measurements of ^{18}F Blood Concentration and Its Effect on Simplified Dynamic PET Analysis

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The purpose of this study was to assess the accuracy and practicality of well counter– and thyroid probe–based methods, commonly available in nuclear medicine facilities, for measuring the concentration of ^{18}F-FDG in blood samples. The degree to which the accuracy of such methods influences quantitative analysis of dynamic PET scans was also assessed. **Methods:** Thirty-five patients with cancer of the head and neck underwent dynamic PET imaging as part of a study intended to evaluate the utility of quantitative, image-based metrics for assessment of early treatment response. The activity in blood samples from the patients, necessary to provide an estimate of the input function for quantitative analysis, was measured both using a thyroid probe and using a well counter. Three calibration techniques were compared: single-point calibration using a standard solution for the thyroid probe (ProbePoint technique), single-point calibration using a standard solution for the well counter (WellPoint technique), and multiple-point calibration over the full range of expected blood activities for the well counter (WellCurve technique). The WellCurve method was assumed to provide the most accurate estimate of blood activity. The precision of measuring blood volume using a micropipette was also evaluated by obtaining multiple blood samples. Simplified-kinetic-analysis multiple-time-point (SKA-M) uptake rates for the primary tumor were calculated for all 35 patients using PET images and each of the 3 methods for assessing blood concentration. **Results:** Errors in blood activity measurements ranging from −9.5% to 7.6% were found using the ProbePoint method, whereas the error range was much less (from −1.3% to 0.9%) for the WellPoint method. The precision in blood volume measurements ranged from −6% to 12% in the 10 patients assessed. The errors in blood activity and volume measurements were reflected in the SKA-M measurements in the same range. **Conclusion:** The WellPoint method provides a compromise between accuracy and clinical practicality. Random errors in both blood activity and volume measurements accumulate and may compromise parameters—such as the SKA-M estimate of tumor uptake rate—that depend not only on images but also on blood concentration data.

Key Words: blood activity; dynamic PET; micro-pipette; thyroid probe; well counter

J Nucl Med Technol 2014; 42:21–27
DOI: 10.2967/jnmt.113.131789

Nuclear medicine technologists are sometimes asked to measure the concentration of activity in blood samples. Such data may be required, for example, for pharmacokinetic studies of new radiopharmaceuticals (*1*), to aid in calculating expected radiation dose to the bone marrow per unit administered activity in radionuclide therapy (*2*), and as a method of deriving an input function for kinetic analysis exploiting data from dynamic PET. In the current case, blood sample concentration measurement was required because of a current clinical trial at our center that sought to assess the ability of ^{18}F-FDG PET scanning to predict treatment response for head and neck cancer patients. As part of this trial, dynamic PET scans were acquired and used to generate measures of activity versus time for volumes of interest thought to contain tumor.

Kinetic analysis of ^{18}F-FDG PET studies normally requires measurement of absolute blood ^{18}F activity concentration (i.e., activity per unit volume) (*3*). For full kinetic analysis, the patient's arterial blood activity concentration versus time, starting at the point of tracer administration and extending to about 1 h after the injection of ^{18}F-FDG, is the input function needed for kinetic analysis. Because taking arterial blood samples continuously for 1 h is usually not clinically practical, dynamic PET studies commonly rely on a smaller number of blood samples (*4*). The technique chosen in the trial in question, the simplified-kinetic-analysis multiple-time-point (SKA-M) method, relies on a single venous blood sample (*5*). This single blood activity concentration is used to scale a population-averaged blood activity curve and thus obtain the input function. Because the SKA-M method relies on

Received Sep. 5, 2013; revision accepted Nov. 11, 2013.
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Published online Jan. 30, 2014.

a single blood sample from the patient, it is reasonable to postulate that the accuracy of any quantitative metric derived using the SKA-M technique strongly depends on the accuracy with which the blood activity concentration is measured. To obtain an accurate blood activity concentration, accurate measurements of both blood activity and blood volume are essential.

In many nuclear medicine facilities, the available detector systems for measuring blood activity are either a thyroid probe or a well counter. Direct measurement of blood activity in the dose calibrator is not practical because of the low activities involved. A cross calibration against a dose calibrator is necessary to convert the thyroid probe or well counter readings to activity (Fig. 1). A single-point calibration factor using a standard solution (*6*) can be applied to either the thyroid probe or the well counter. The former technique is referred to as "ProbePoint" and the latter as "WellPoint" in this article. Alternately, a calibration curve can be obtained for the well counter in a full range of expected blood activity. This is referred to as the "WellCurve" technique in this article. The WellCurve method is more labor-intensive but would be expected to provide the most accurate results.

Blood volume is typically measured using micropipettes. Laboratory micropipettes are designed for precise measurement of the volume of liquids such as water. Because blood is more viscous than water, the precision of blood volume measurements may be compromised. Slightly different techniques are recommended by manufacturers for pipetting blood (*7*). Because the need to pipette any liquid may be rare in some nuclear medicine departments, it is unlikely that specialized techniques for pipetting blood will be familiar to all nuclear medicine technologists. For example, neither the nuclear medicine technologists who performed the initial pipetting work for the clinical study nor the researchers requesting the pipetting work were aware of the need to use such methods.

For the purposes of the clinical trial, blood activity was originally measured using the ProbePoint method. The well counter was not used initially in the trial, because cross calibration of the well counter was considered less convenient. A standard solution of sufficient activity to be accurately measured in the dose calibrator contains too much activity to be used without decay with a standard well counter, because of detector dead-time effects. However, as the trial progressed, the data suggested there might be significant variation in measurements obtained using the ProbePoint technique. In response, potentially more accurate techniques such as WellPoint and WellCurve were investigated.

The objective of this study was to quantitatively assess the accuracy of absolute blood activity measurements using the ProbePoint, WellPoint, and WellCurve techniques. Moreover, the precision of blood volume measurements using a typical laboratory micropipette was also investigated. Finally, the effects of the accuracy of the blood activity concentration measurements on the accuracy of a SKA-M metric were evaluated.

MATERIALS AND METHODS

Thirty-five patients with advanced head and neck cancer underwent dynamic PET scans using a Gemini PET/CT scanner (Philips). Patients were injected with 5 MBq of ^{18}F-FDG per kilogram of body weight. Patients heavier than 75 kg were injected with a fixed dose of 370 MBq (10 mCi) of ^{18}F-FDG. The PET acquisition consisted of 12 frames of 2.5 min each for a total of 30 min, all acquired at a single bed position. The PET voxels were isotropic and measured 2 mm on each side. The reconstructed field of view was 576 mm. The patients underwent a low-dose CT scan for PET attenuation correction purposes before undergoing the PET scan. After the PET scan, they underwent diagnostic, contrast-enhanced CT for radiation treatment planning. The treatment was 7 wk of radiotherapy. If a patient could tolerate it, chemotherapy was also administered.

Blood samples approximately 3 mL in volume were obtained immediately after the PET/CT scans. The average time at which blood was drawn was 77.8 ± 12.4 min after injection. From each

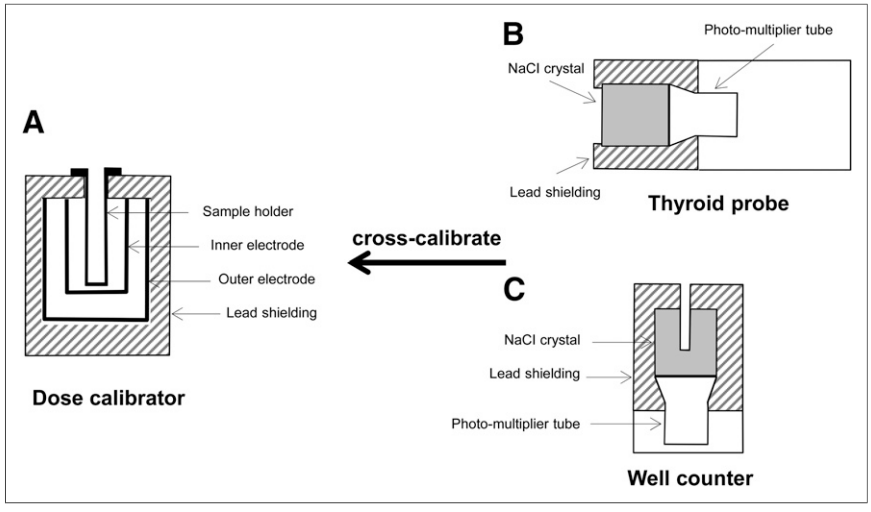

FIGURE 1. Cross calibration against dose calibrator (A) is needed to convert count readings from thyroid probe (B) and well counter (C) to units of activity (kBq or μCi) for blood samples.

gross blood sample, a testing sample of 0.9 mL was extracted using a calibrated Lambda micropipette (Corning Life Sciences). The pipette was an air displacement model with a continuously adjustable volume setting. The absolute activity of each testing blood sample was measured with 3 different techniques: ProbePoint, WellPoint, and WellCurve.

ProbePoint

A thyroid probe was cross calibrated against a dose calibrator to convert counts to activities using a single-point calibration factor. The thyroid probe used was an Atomlab 950, and the dose calibrator was an Atomlab 500 (both from Biodex Medical Systems). Cross calibration was performed on the day of the PET scan immediately after the blood sample had been obtained. A standard solution for cross calibration was obtained by diluting ^{18}F-FDG with saline such that about 370 kBq (10 μCi) in a 0.9-mL volume could be read using the dose calibrator. The test tube and the volume of the standard solution (0.9 mL) were the same as those of the blood sample to avoid geometric effects. Three 1-min count measurements of blood sample and standard solution were made using the thyroid probe, and the averages of the 3 counts were used in the calculations. Two 1-min count measurements of background were also acquired. The geometric orientation of the probe for all count measurements (i.e., standard solution, blood sample, and background) was kept the same by placing the test tubes in a holder at a fixed distance from the probe. Blood samples were corrected first for background counts and then for ^{18}F decay. With the known activity of the standard solution from the dose calibrator and the counts of the standard solution in the probe, a single-point calibration factor was obtained. This calibration factor and the blood volume (0.9 mL) were used to obtain the activity concentrations (kBq/mL) of the blood samples. The ProbePoint technique required about 20 min to measure the activity of each blood sample.

WellPoint and WellCurve

A well counter was cross calibrated against a dose calibrator to convert counts to activities using a standard ^{18}F solution. The well counter that was used was an optional accessory to the Atomlab 950 thyroid probe, and the dose calibrator was again the Atomlab 500. On the day of the patient's PET scan, the same blood sample as used in the ProbePoint method was counted in the well counter 3 times for 1 min each. The background was also counted twice. Moreover, on the same day as the patient scan, a 0.9-mL standard solution of ^{18}F was prepared in the same kind of test tube as the blood sample. The activity of this standard solution was chosen to be approximately 130 MBq (3.5 mCi) so that, after decay, its activity during the following 8-h working day would be within 1–20 kBq (0.03–0.5 μCi). This range was chosen to cover the whole expected range of activities of all blood samples. On the day after the PET scan, the counts of the standard solution were measured in the well counter 8 times 1 h apart. From these 8 measurements, a calibration curve was obtained for the well counter by calculating the known activity at each of the 8 measurement times after accounting for decay. The calibration curve was a linear fit to all 8 points. This calibration curve and the blood volume (0.9 mL) were used to calculate the activity concentration of the blood sample in kBq/mL. The WellCurve technique requires a full day of measurements to obtain the calibration curve and apply it to a blood sample. The WellPoint method used a single point randomly selected from the WellCurve calibration curve. Similar to the ProbePoint method, only 20 min were required for the WellPoint method, with the provision that the ^{18}F calibration source used in the WellPoint method needed to be decayed for about 24 h. The WellCurve technique was assumed to represent the ground truth for all measurements in this study.

Precision of Micropipette Blood Volume Measurements

For 10 of the 35 patients, multiple blood samples (3–6 samples) were obtained and the precision of blood volume measurements was evaluated. The same calibrated Corning Lambda micropipette as described above was used to draw 0.9 mL for each blood sample. The activities of all blood samples were measured using the WellCurve technique. The precision of the blood volume measurements was evaluated by calculating the percentage difference in activity of each blood sample relative to each patient's sample mean blood activity.

Accuracy and Precision of Micropipette Water Volume Measurements

A quality assurance test was performed to evaluate the accuracy and precision of water volume measurements using the micropipette, and the resulting values were compared with those stated by the manufacturer. Five nuclear medicine technologists participated in the quality assurance test. Using the micropipette, each technologist drew 4 samples of 0.9 mL of water into test tubes. The test tubes were the same as those used for the blood samples. The test tubes were weighed with and without the water using a microbalance, and the volume of the water for each test tube was calculated assuming that the density of water is 1.0 g/mL. The microbalance used was a Sartorius CPA2P (Precision Weighing Balances). The accuracy and precision (SD) of the microbalance as stated in the manual were both 1 μg.

Calculation of SKA-M Using the Different Blood Sample Measurement Techniques

For ^{18}F-FDG PET imaging, SKA-M may be considered an estimate of the better-known Patlak constant, K_i, which is linearly related to the glucose metabolic rate. The principles of SKA-M have been described by Sundaram et al. (*5*). A single venous blood sample and a series of dynamic PET images are required. The ProbePoint, WellPoint, and WellCurve activity concentration measurements of the same blood sample were used to calculate 3 values of SKA-M. Each blood activity concentration was used to scale a population-averaged blood activity curve obtained from previously published data (*5*). The scaled blood activity curve was used to estimate a patient-specific blood activity curve, $c_p(t)$. An activity-concentration-versus-time curve for every PET voxel, $A(t)$, was extracted from the 12 frames of the PET images. To suppress noise in $A(t)$, the average voxel value in a $3 \times 3 \times 3$ window centered on each PET voxel was used as that voxel's value for subsequent calculations. For each voxel, 12 points were obtained by plotting $\int c_p(t)/c_p(t)$ on the abscissa versus $A(t)/c_p(t)$ on the ordinate for each PET frame as shown in Figure 2. A line was fitted to these 12 points, and the slope of this line was taken as the SKA-M uptake rate for that voxel. This procedure was repeated for all PET voxels, and an SKA-M image was obtained for every PET study and for every blood activity measurement technique. From these SKA-M images, mean SKA-M was calculated for the primary tumor of all 35 patients for all the voxels within a physician's manually contoured gross tumor volume. All the SKA-M

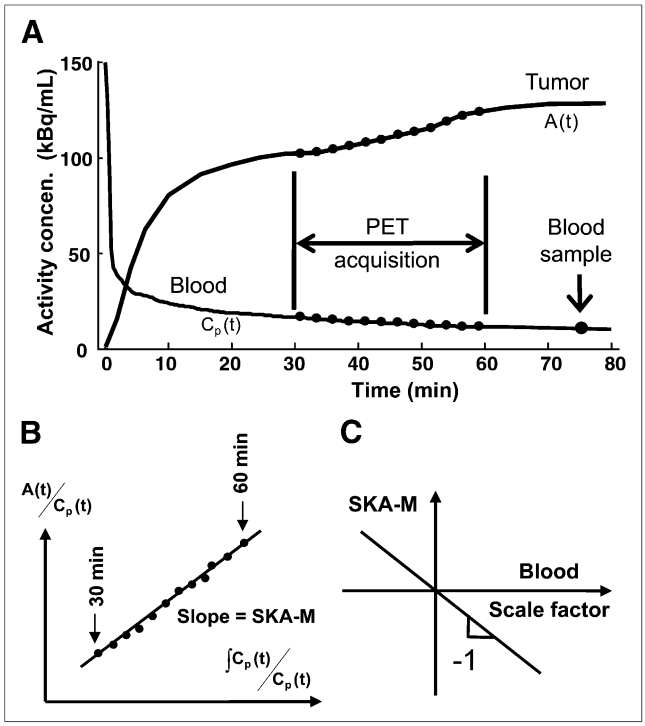

FIGURE 2. Steps for calculating SKA-M. (A) Single patient's blood sample is used to scale population average blood activity curve and estimate patient's blood activity curve, Cp(t). Tumor uptake curve, A(t), is measured during PET acquisition between 30 and 60 min after injection in 12 frames. Two time curves, Cp(t) and A(t), are used to generate plot shown in B with data points corresponding to each PET frame. Slope of line fitted to these 12 points is SKA-M parameter. (C) Relationship between blood scale factor and SKA-M parameter.

calculations were performed in Interactive Data Language, version 8.1 (Research Systems Inc.).

RESULTS

The average blood sample counts (corrected for both background and decay) ranged from 110 to 1,516 for the thyroid probe technique and from 18,595 to 177,927 for the well counter technique. Based on Poisson statistics alone, the percentage uncertainty in the thyroid probe counts thus ranged from 2.6% to 9.5% for the thyroid probe technique and from 0.24% to 0.73% for the well counter technique. A sample calibration curve that converts sample counts in the well counter to sample activities is shown in Figure 3. All 8 data points are close to the fit line, as reflected in the high R^2 value (0.9998). Similar calibration curves were obtained for all blood activity measurements acquired using the well counter. All calibration curves had good linear fits, with R^2 being at least 0.9998. However, there was some drift in well counter response over the period during which measurements were acquired. Over a period of 1 y, the slope of the WellCurve calibration changed by approximately 3.8%. Figures 4A and 4B show the accuracy of the blood activity measurements using the ProbePoint and WellPoint techniques, respectively, relative to the WellCurve technique. Sorting of the sample numbers (on the abscissa) is based on the date of blood sampling, and no trend over time (i.e., 1 y) was observed. The ordinate is labeled as the percentage error in blood activity measured using the ProbePoint or WellPoint technique relative to the WellCurve technique, since the WellCurve technique was assumed to represent the true blood activity. The average value of the percentage difference between the ProbePoint and WellCurve techniques was −0.5%, and the SD was 6.2%. A paired *t* test with 95% confidence interval showed no statistically significant differences in measured average sample activities between the ProbePoint and WellCurve techniques. Thus, there was no systematic error in blood activity measurements using the ProbePoint technique, but only random errors. Errors in ProbePoint technique ranged from about −9.5% to 7.6% as shown in Figure 4A. Errors in the WellPoint technique were significantly smaller, ranging from −1.3% to 0.9%.

Figures 4C and 4D show the accuracy of SKA-M calculated using the ProbePoint and WellPoint techniques, respectively, relative to the WellCurve technique. Since the WellCurve technique was assumed to provide the true blood activities, the SKA-M values calculated using WellCurve-based measurements were taken as the true SKA-M values. Similar to the errors of blood activity data in Figure 4A, the errors of SKA-M in Figure 4C were also found to be random and in the same range. The errors in SKA-M in Figure 4C are reciprocal to the errors in blood activities in Figure 4A. The same reciprocal relationship applies in the WellPoint techniques between Figures 4B and 4D.

Figure 5 shows micropipette volume precision with multiple blood sample volumes from the same patient. Figure 5A is the absolute blood activity concentration measurements in 10 patients labeled A to J. Each patient had multiple blood samples, which are indicated by numbers following the letters A to J. Figure 5B was obtained by calculating the percentage difference between each blood sample and the mean of each patient and thus shows

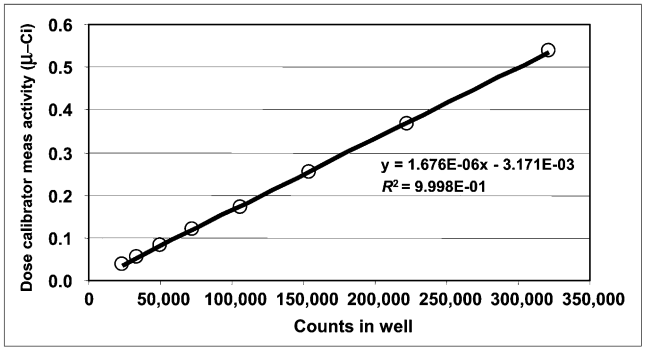

FIGURE 3. Sample calibration curve obtained for well counter in range of typical blood activities for SKA-M.

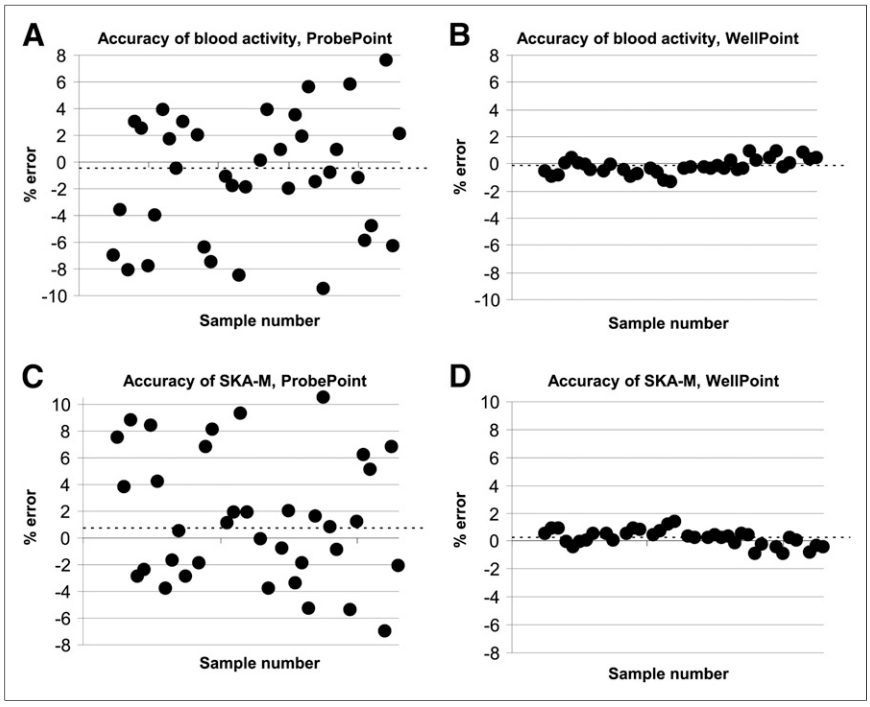

FIGURE 4. Errors in blood activity measurements using ProbePoint (A) or WellPoint techniques (B) in 35 patients. Errors are relative to WellCurve technique, which is assumed to be ground truth. Accuracy of blood sampling techniques in A and B is reflected by accuracy of SKA-M in C and D, respectively. Red lines are average values for each plot.

the precision of the micropipette in measuring blood volume. As seen in Figure 5B, estimates of blood sample volume varied widely, with differences from the mean value ranging from −6% to 12%.

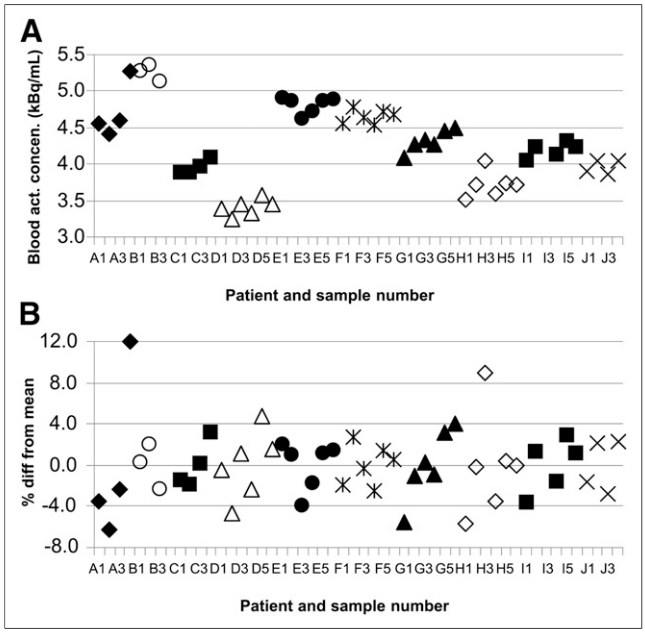

FIGURE 5. Pipette volume precision estimated for 10 patients, with each patient represented by a different symbol. (B) Absolute blood activity in 10 patients having 3–6 blood samples. Letters A to J correspond to the 10 patients. Numbers after each letter are sample numbers for each patient. (B) Percentage difference of each blood sample from mean for patient.

Figure 6 shows the results of the quality assurance test to confirm the accuracy and precision of micropipette volume measurements for water. The range of accuracy and precision (SD) for all technologists were −1% to 0.7% and 0.2% to 0.4%, respectively. These values agreed reasonably well with the corresponding values in the micropipette manual, that is, ±0.6% and ±0.2% for accuracy and precision, respectively.

DISCUSSION

Blood Activity Measurements

In this study, the WellCurve method was taken as the best available estimate of the truth since it incorporated both the more sensitive detector and multiple calibration points. The well counter has a fixed geometry to reduce setup errors,

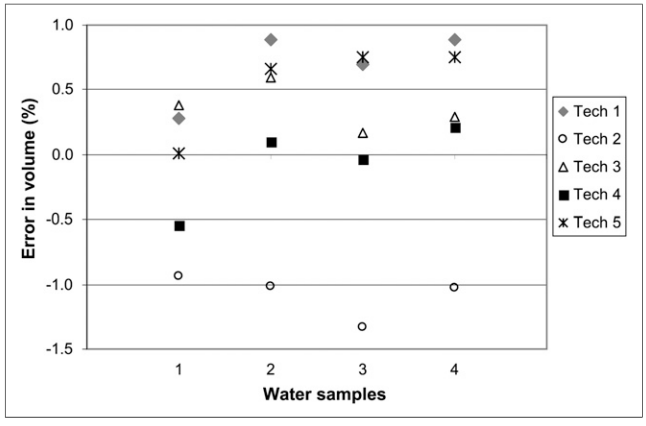

FIGURE 6. Micropipette accuracy and precision of 5 nuclear medicine technologists in measuring 0.9 mL of water.

and its geometric efficiency is higher than that of the thyroid probe because of its nearly 4-π geometry. Thus, the well counter is less likely to be affected by transient variation in background—a factor that is particularly important for measurements of low blood activity after biologic and physical decay. Unlike the ProbePoint or WellPoint techniques, which rely on a single-point calibration factor, the WellCurve technique used 8 data points to obtain calibration over the full range of expected blood activities, thus reducing the overall uncertainties. The paired *t* test showed that there are only random errors, not systematic errors, in the ProbePoint technique. This finding can be explained by the fact that thyroid probes are more susceptible to variation in background levels and setup errors, both of which have a stochastic nature. In addition, it is clear from the low number of counts acquired using the thyroid probe technique that longer counting times could have been used to reduce the percentage uncertainties in the results. However, long counting times are not convenient in a busy nuclear medicine department and can present the problem of a potential for variation in background levels as injected patients move about the department.

The disadvantage of the WellCurve technique is that it requires a full working day of measurements, whereas the ProbePoint technique needs only 20 min. The ProbePoint and WellPoint techniques also automatically account for any drift in response over time. However, in the WellCurve technique, regular calibration of the well counter (e.g., every 2 wk) is required if a drift is observed (*6*). Thus, the WellPoint technique provides a compromise between clinical practicality and accuracy (within 1.3%).

Blood Volume Measurements

The results in Figure 5B show that errors of up to 12% may be encountered if one relies on a single blood sample from the patient. Large errors such as these in reproducibility of volume measurements with lab micropipettes were not expected. The most common variety of micropipette, the air-cushion or air-displacement type, is designed for precise measurement of liquids such as water. Whole blood, however, has physical properties differing from water. Blood has both higher viscosity and higher density than water, contains proteins that can interact with the micropipette tip and cause foaming, and is not just a simple fluid but a suspension of blood cells. Part of the observed error could be due to these differences between blood and water. This error can be minimized by using pipetting techniques more suitable for blood, such as reverse pipetting with prewetting of the pipette tip (*8*). Alternatively, the use of a different type of pipette, a positive displacement pipette, may be considered (*9*). In addition, it is reasonable to have multiple blood samples and average the results to reduce uncertainties.

Effects of Blood Activity Concentration Measurements on SKA-M

Inaccuracies in blood activity or volume measurement will directly affect the accuracy of SKA-M measurement. The relationship between the blood scale factor and SKA-M is mathematically reciprocal. For example, if the measured blood activity concentration is 0.9 of its true value (i.e., 10% underestimation), then the calculated SKA-M is 1/0.9 = 1.11 of its true value (i.e., 11% overestimation). The reason for the reciprocal relationship lies in the mathematic calculation of SKA-M as shown in Figure 2B. A relative error in the estimate of blood activity concentration by a constant factor, α, will not affect the abscissa of Figure 2B but will translate to an error of $1/\alpha$ in the estimate of the ordinate. Thus, the SKA-M will also be inaccurate by a factor of $1/\alpha$. This reciprocal relationship is reflected in the results in Figure 4. The range in error of blood activity in Figures 4A and 4B also translates to the same range of error as for SKA-M in Figures 4C and 4D, respectively. A similar argument applies to Figure 5B in terms of precision of blood volume and its effect on the precision of SKA-M. Therefore, errors in precision of blood volume measurements up to 12% will be translated to errors in precision of SKA-M up to approximately 12%.

Paired *t* tests for both blood activity measurements (Fig. 4A) and SKA-M (Fig. 4C) showed that the difference between the ProbePoint and WellCurve methods is not statistically significant. Therefore, the errors in SKA-M using the ProbePoint technique are random, and this fact is of special concern. To quantify PET uptake in a tumor using SKA-M, typically pretreatment (pretx) and intratreatment (intratx) PET scans are obtained and the relative change in uptake is used for tumor response assessment:

$$\Delta\text{SKA} - \text{M} = \left(\frac{\text{SKA} - \text{M}_{\text{intratx}}}{\text{SKA} - \text{M}_{\text{pretx}}} - 1\right) \times 100\%.$$

Multiplicative systematic errors in SKA-M (e.g., due to calibration) in both pretreatment and intratreatment will cancel out whereas the random errors will combine to cause a bigger error in ΔSKA-M. Theoretically, the combined error in ΔSKA-M will be on average a factor of $\sqrt{2}$ greater than the error in SKA-M. For example, errors in both pretreatment and intratreatment of 10% in both activity and volume measurements will cause an error of $\sqrt{2} \times 10\%$ in blood activity concentration estimates and approximately an error of $\sqrt{2} \times \sqrt{2} \times 10\% = 20\%$ in ΔSKA-M.

CONCLUSION

Although one might think that measuring the concentration of activity in a blood sample would be a simple task, in practice care must be taken to select an appropriate technique and to properly apply that technique. Pipetting techniques suitable for blood should be applied to minimize errors in blood volume measurements. If possible, techniques that use a thyroid probe should be avoided in favor of those that use a well counter. The WellPoint technique provided a compromise between clinical practicality and accuracy (within 1.3%). Random errors in blood activity and volume measurements may accumulate and compromise the

SKA-M estimates of tumor uptake rate. Such errors would also be of concern in other situations in which blood activity concentration must be assessed, such as the measurement of blood concentration time–activity curves for radionuclide treatment planning applications.

DISCLOSURE

This work was supported in part by the Natural Sciences and Engineering Research Council of Canada, the Ontario Graduate Scholarship, the Ontario Institute for Cancer Research, Cancer Care Ontario, and the Beatty Fellowship. No other potential conflict of interest relevant to this article was reported.

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