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Summary & discussion
Chapter 11. Summary

11.1 Summary

The studies in this thesis describe and validate methods for obtaining quantitative parametric images, showing fully quantitative data at the voxel level, for three different PET tracers used in nuclear cardiology: $^{15}\text{O} \text{H}_2\text{O}$, a tracer of myocardial blood flow (MBF), $^{11}\text{C}\text{HED}$, a tracer of myocardial sympathetic innervation, and $^{11}\text{C}\text{acetate}$, a tracer of myocardial oxygen consumption. Using quantitative parametric data rather than qualitative uptake data may yield additional information in several myocardial diseases, as these images display the specific signal without confounding (background) signals that are present in uptake images. Although this has always been possible at a region of interest level (usually myocardial segments) for several tracers, parametric images allow for analysis at the level of the intrinsic resolution of the scanner. This is of particular interest for abnormalities that are present in only a part of a segment that could be missed (‘diluted’) in a segmental analysis.

Chapters 2, 3, 4, 5 and 6 focus on generation and application of parametric images of absolute myocardial blood flow and myocardial viability as measured using $^{15}\text{O} \text{H}_2\text{O}$. The aim of this first part was to validate quantitative accuracy of these parametric images.

Chapter 2 provides a theoretical comparison between $^{15}\text{O} \text{H}_2\text{O}$ and other MBF radiotracers. It also provides an overview of the compartment model used for quantifying MBF using $^{15}\text{O} \text{H}_2\text{O}$ and the associated equations used for calculating parametric images of both MBF and PTI.

In Chapter 3, automatic generation of parametric MBF images was validated for $^{15}\text{O} \text{H}_2\text{O}$. These images were generated using a basis function approach. Arterial image derived input function (IDIF) was extracted automatically using four different segmentation algorithms and, as a ‘gold standard’, was obtained using manual delineation of the aorta. Parametric MBF values correlated highly with MBF values obtained from segmental analyzes (intraclass correlation coefficient, ICC = 0.984). In addition, the highest accuracy (ICC = 0.977) of MBF values obtained with automatically extracted IDIF was obtained using cluster analysis. However, a consistent bias of > 10% was observed for all segmentation algorithms, which was lowest for cluster analysis. Finally, reproducibility was high (coefficient of variation, CoV < 5%) and segmentation was fast. Using basis function methods and cluster analysis for automatic generation of parametric MBF images was found to be feasible for routine clinical use.

In Chapter 4, quantitative accuracy of MBF was evaluated for $^{15}\text{O} \text{H}_2\text{O}$ scans without attenuation correction. Since, in case of $^{15}\text{O} \text{H}_2\text{O}$, MBF is estimated from the washout phase, absolute scaling of PET data is not essential and therefore attenuation correction could, in principle, be omitted. To validate this approach, MBF values were calculated for reconstructions with (CTAC) and without (NAC) attenuation correction. High correlation and agreement were found between CTAC and NAC MBF values ($r^2$ of 0.99 and 0.97 for rest and stress, respectively). NAC MBF was 4% and 8% lower than CTAC MBF for rest and stress, respectively, and this difference was smallest for basal segments.
and largest for apical segments. Similarly, high correlation and agreement were found for NAC and CTAC coronary flow reserve (CFR, the ratio of stress and rest MBF), especially when only the clinically relevant range of CFR < 2 was considered ($r^2$ of 0.95 and 0.97, slope of the linear fit of 0.92 and 1.01 for all CFR and CFR < 2 values, respectively). These results indicate that omitting attenuation correction does not significantly affect derived MBF values. This makes MBF as measured with $[^{15}\text{O}]\text{H}_2\text{O}$ insensitive to mismatch between PET and CT.

In Chapter 5, a method for calculating parametric perfusable tissue index (PTI) images using PET/CT data was developed and validated. Instead of using a $[^{15}\text{O}]\text{CO}$ scan to estimate blood volume, parametric blood volume ($V_B$) images were derived from the $[^{15}\text{O}]\text{H}_2\text{O}$ scan itself. These images were combined with attenuation data and with parametric PTF images to calculate PTI images. Good correlation was observed between PTI values obtained using $[^{15}\text{O}]\text{CO}$ and parametric $V_B$ images ($r^2$ of 0.73, slope of 0.90). Quantitative accuracy of parametric PTI images was high ($r^2$ of 0.91, slope of 0.98) as compared PTI derived from regional analyzes. Finally, simulations showed that noise sensitivity of PTI was low (CoV < 10% at typical voxel noise levels). These results show that parametric PTI images can be calculated from a $[^{15}\text{O}]\text{H}_2\text{O}$ PET/CT scan without an additional $[^{15}\text{O}]\text{CO}$ scan. This enables simultaneous assessment of MBF and viability from a single $[^{15}\text{O}]\text{H}_2\text{O}$ scan.

In Chapter 6, two clinically validated analysis software packages, Cardiac VUer (Amsterdam, the Netherlands) and Carimas (Turku, Finland), were compared for data acquired at two different sites. For MBF quantification to become widely accepted, calculated MBF should be similar, irrespective of the data analysis package used. A novice observer analyzed data from 50 patients scanned in Amsterdam and 50 patients scanned in Turku using both software packages. An excellent correlation was found between MBF values obtained with either package for both stress and rest MBF and for both global and regional MBF ($r > 0.94$ for all). Results obtained with Cardiac VUer were slightly, but significantly, higher for RCA and LCx in rest and lower for LAD in rest and stress as compared with Carimas. When the most distal apex segment, which is prone to motion and spillover artifacts, was excluded from analysis, differences in LAD disappeared. For both packages, excellent reproducibility was obtained (ICC > 0.90). These results show that both packages provide similar MBF values and can be used for routine clinical quantification of MBF using $[^{15}\text{O}]\text{H}_2\text{O}$.

Chapters 7, 8, 9 and 10 focus on quantification of kinetics of the sympathetic innervation tracer $[^{11}\text{C}]$-meta-hydroxyephedrine. The ultimate aim was to define the optimal parameter to describe HED kinetics at the voxel level, and to obtain this parameter non-invasively.

In Chapter 7, the optimal kinetic model and several simplifications for quantification of HED kinetics were assessed, using the gold standard arterial plasma input function obtained with an on-line blood sampler in combination with a number of discrete manual samples. Although the reversible two tissue model yielded the best fits in > 70% of all clinical data, fitted parameters were unstable for clinical data and especially at realistic voxel noise levels. Re-
sults from the reversible one tissue model were more stable (CoV of 22.5% and 82.3% for reversible one tissue and reversible two tissue models, respectively) and correlated highly with reversible two tissue results ($r^2 = 0.94$). In addition, for clinical data, stable results were obtained more often for reversible one tissue model (94.6% of the segments) as compared to reversible two tissue model (77.1% of the segments). Therefore, the reversible one tissue model was preferred. Population averaged metabolite data yielded a bias in volume of distribution data of 30% in some patients, as compared with results obtained with individual metabolite data. Finally, standardized uptake values and retention index showed a non-linear relation with volume of distribution data and their use can not be recommended. Therefore, absolute quantification with individual metabolite corrections using the reversible one tissue model was recommended as optimal method for quantification of HED kinetics.

In Chapter 8, validity of using IDIF and venous rather than arterial blood samples was assessed. Arterial blood sampling requires arterial cannulation, which is a significant burden to the patient. Since HED has high tissue and low blood concentrations at late time-points, small errors in scatter correction may have large effects. Therefore, IDIF was evaluated using three different reconstructions algorithms: time-of-flight (TF) reconstructions as found on modern PET/CT scanners and non-TF reconstructions algorithms with both standard and Monte Carlo scatter corrections. It was shown that only non-TF reconstructions yielded accurate results for the tail of the blood curve and all reconstructions yielded different estimates in the peak of the blood curve, as compared to blood sampler data. This, in turn, resulted in an accurate estimation of $V_T$ and a small bias in $K_1$ with best results obtained using Monte Carlo scatter corrections ($r^2 = 0.91$ and slope = 0.93 for $K_1$, $r^2 = 0.90$ and slope = 0.99 for $V_T$ for non-TF with normal scatter corrections; $r^2 = 0.91$ and slope = 0.93 for $K_1$, $r^2 = 0.98$ and slope = 1.01 for $V_T$ for non-TF with Monte Carlo scatter corrections). When venous instead of arterial blood data was used, a large bias (on average 18%) was introduced in $V_T$, but not in $K_1$. Venous data could, however, be converted into 'arterial' data using a pragmatic approach and, for converted data, bias in $V_T$ was reduced to < 5%. This work shows that noninvasive quantification of HED kinetics is feasible, provided that converted venous blood data and the appropriate reconstruction methods are being used.

In Chapter 9, parametric images of both $[^{11}C]HED$ and $[^{11}C]acetate$ were developed. Parametric images were calculated for the reversible one tissue model for $[^{11}C]acetate$ and for the reversible one and two tissue models for $[^{11}C]HED$ using a basis function approach. Quantitative parameters derived using parametric images were compared with those derived using non-linear least squares regression, the gold standard for curve fitting. In addition, new analysis software incorporating these images was compared with existing analysis software by letting 3 observers with varying experience analyze 10 $[^{11}C]acetate$ scans twice in an analysis software package incorporating parametric images. In addition, obtained regional values were compared with those obtained with an existing analysis software package, CAPP, for all observers. Average segmental values of obtained parametric images correlated highly with fitted segmental values using
normal, non-linear least squares techniques (ICC > 0.90 for all parameters). A small bias (-2 to 6%) was observed in some parameters, which could be addressed to heterogeneity within a myocardial segment. Simulations showed that at voxel noise levels, accuracy and precision of parametric images were similar or improved as compared with the non-linear fitting techniques. Reproducibility of new software was excellent (ICC > 0.91) for all observers and results correlated well with those obtained in CAPP. This shows that parametric images can be calculated and analyzed accurately for $^{11}$C|HED and $^{11}$C|acetate.

In Chapter 10, the unidirectional rate of uptake $K_1$ of $^{11}$C|HED, derived using the reversible one tissue model, was compared with MBF as measured using $^{15}$O|H$_2$O. The purpose of this study was to assess whether mismatches between innervation and MBF defects could be assessed from a single $^{11}$C|HED scan. $K_1$ is the product of first pass extraction fraction and MBF, and if extraction remains constant, it could be used as an index of MBF. Since MBF represents MBF in perfusible tissue only, MBF$^T$, defined as the product of MBF and perfusible tissue fraction, was used in this study. A good correlation between $K_1$ and MBF$^T$ was observed, although the slope of the linear fit was significantly lower than 1 ($r^2 = 0.65$, slope = 0.40). MBF$^T$ and $K_1$ defect sizes correlated highly ($r^2=0.90$) and there was no significant difference between defect sizes ($p = 0.80$). Finally, Dice’s similarity coefficient showed good agreement of defect location (DSC = 0.88±0.05, range 0.77-0.98). This study shows that $K_1$ of $^{11}$C|HED can be used rather than a separate MBF scan, when assessing mismatch between innervation and MBF$^T$.

## 11.2 General discussion

### 11.2.1 Clinical application of $^{15}$O|H$_2$O

Historically, widespread clinical application of $^{15}$O|H$_2$O has been challenging. The need for on-site tracer production, elaborate post-processing and the lack of clinically useful images have been major obstacles. Small, dedicated $^{15}$O cyclotrons are being developed although these are not yet widely used and clinical application of $^{15}$O|H$_2$O remains limited.

The elaborate post-processing required for analysis and the lack of clinically useful images have been overcome using methods described in chapters 2, 3, 4 and 5 of this thesis. As shown, parametric images of absolute MBF could be obtained reliably, with little user intervention and images were of good quality. This enables a visual but fully quantitative assessment of MBF and viability (using PTI) using $^{15}$O|H$_2$O at the highest possible resolution. The methods used for MBF are insensitive to mismatch between PET and corresponding low-dose CT scan, to a point where the CT scan could be omitted completely. This is in contrast with studies performed with $^{82}$Rb (181) where mismatch between PET and CT had a significant effect on MBF. Of course, when viability is of interest, low-dose CT scans are still required as CT data are required for calculation of ATF and PTI. Whilst this shows that patient motion
between PET and CT scans is not an issue, patient motion during a dynamic scan occurs with high frequency, especially during stress (220), and can result in artifacts in parametric images and inaccurate MBF values (180), especially when motion occurs during the early phase of the scan. Due to the lack of contrast and limited signal-to-noise ratio in raw $[^{15}\text{O}]\text{H}_2\text{O}$ images, motion correction techniques cannot be applied directly to $[^{15}\text{O}]\text{H}_2\text{O}$. To overcome this issue, alternative motion correction techniques such as improved image registration algorithms or external markers placed on the patient could be explored. Introduction of fully hybrid PET/MR systems, capable of simultaneous PET and MR measurements, may enable motion correction using MR images taken at different time points during the PET scan (221). Alternatively, scan duration can be reduced significantly (222), which reduces the risk of patient motion during the scan. Finally, the use of more tolerable stress agents than adenosine, such as regadenoson, could reduce the risk of patient motion during stress scans and should be further evaluated.

Adding ECG gating to $[^{15}\text{O}]\text{H}_2\text{O}$ may lead to important additional information that is currently available for qualitative analysis of uptake MBF tracers (223–226) or FDG (227), but not for $[^{15}\text{O}]\text{H}_2\text{O}$. The low signal-to-noise ratio of $[^{15}\text{O}]\text{H}_2\text{O}$ has essentially ruled out ECG gating for $[^{15}\text{O}]\text{H}_2\text{O}$, and to some extent for dynamic scanning in general. Improvements in signal-to-noise ratio on modern PET/CT scanners using LSO or LYSO detectors may be sufficient for ECG-gated dynamic $[^{15}\text{O}]\text{H}_2\text{O}$ scans. Future work should explore the feasibility of ECG-gated dynamic $[^{15}\text{O}]\text{H}_2\text{O}$ scans, both with and without application of denoising techniques such as HYPR-LR, which has recently been validated for $[^{15}\text{O}]\text{H}_2\text{O}$ (228), or motion-freezing of dynamic PET data (143). Parametric images of blood volume are relatively insensitive to noise and may be calculated separately for each phase of the cardiac cycle. This shows contraction of the heart and could be used to calculate left ventricular ejection fraction. In addition, three to four out of eight phases of the cardiac cycle can be considered end-diastolic and could be combined into a ‘cardiac-motion frozen’ dynamic scan. This results in a dynamic scan which still contains up to half of the original data, which is likely to be sufficient for accurate MBF quantification, without blurring or partial volume effects due to cardiac motion. In addition, using ECG gated data enables differentiating between absolute endocardial and epicardial blood flow, which may be clinically highly interesting (229,230). Further research is needed to assess quantitative accuracy and feasibility of ECG gated dynamic $[^{15}\text{O}]\text{H}_2\text{O}$ scans.

The work presented in Chapter 6 shows that absolute quantification of MBF using $[^{15}\text{O}]\text{H}_2\text{O}$ is robust and independent on the analysis software package used. Interest in quantification of MBF and coronary flow reserve for clinical purposes has increased recently (8, 231) as excellent clinical results have been obtained with quantitative MBF measurements (21,22,232) and the benefit of quantitative over qualitative analyzes has been shown (26). Whilst some radiotracers are inherently different than $[^{15}\text{O}]\text{H}_2\text{O}$, effects of different analysis software packages, observer variation and variations between scanner types should be minimal for routine quantification of MBF to become clinical reality, even if differences
between radiotracers cannot be overcome. Chapter 6 is a first step towards this goal and further studies, including more software packages, tracers and multiple centers, are warranted.

11.2.2 Clinical application of $^{11}$C\textsubscript{HED}\textsuperscript{+}

The work in this thesis presents the simplest method for quantification of $^{11}$C\textsubscript{HED} kinetics that still provides accurate results. Correlation between absolute quantitative parameters and semi-quantitative uptake parameters is moderate and non-linear and therefore, absolute quantification is recommended.

Using a single tissue compartment model over a more accurate, but less stable two tissue compartment model was the simplest method that still provided accurate results. However, this method still requires metabolite corrected arterial plasma input data. Complete omission of metabolite analysis, through the use of population-averaged data, was not feasible as it introduced a large bias in some patients. On the other hand, use of venous blood samples was shown to be feasible. While this does not overcome the need for metabolite analysis facilities, it does result in a significant reduction in patient burden, as samples can be taken from the infusion line, rather than from an arterial line which requires arterial cannulation.

Use of an IDIF was found to be a feasible approach for $^{11}$C\textsubscript{HED} and this, combined with the feasibility of venous blood samples, enabled complete omission of arterial cannulation. $^{11}$C\textsubscript{HED} proved to be a very challenging tracer for defining an accurate IDIF due to the very high tissue to blood activity ratios (> 10) at late time-points which led to overestimates in IDIF at these time-points. In addition, total activity in the first minute post injection was significantly higher for IDIF as compared to BSIF. These problems were encountered in other groups as well (201), where a combination of IDIF data at early time-points and BSIF data at late time-points was used. This method still requires arterial cannulation and was therefore considered not an improvement over BSIF only. Using appropriate reconstruction and scatter correction algorithms, blood concentrations could be estimated accurately, although some differences in peak activity remained. At present, it is not clear what causes these differences. The scanner response at the high count rates observed during peak activity, was still in the linear range, indicating that dead-time correction was not an issue. On the BSIF side, small errors in delay and dispersion corrections could play a role in the measured shape of the peak, although the total activity in a peak is unaffected by these corrections. Interestingly, the newest time of flight reconstructions were shown to be inaccurate in estimating blood activity during the late phase of the scan. For a small number of scans, additional reconstructions were performed with longer frame durations to eliminate potential effects of bias due to a low number of noise equivalent count rates, but no improvement in quantitative accuracy was observed. In addition, overestimation of late blood activity was not related to liver uptake or the fraction of the liver within the field of view (data not shown). More studies are required, evaluating quantitative accuracy of dynamic time of flight reconstructions, especially...
when blood concentrations are much lower than surrounding tissue concentrations as is the case for $^{11}$C-HED. It has to be noted that feasibility of IDIF has to be evaluated for every new tracer. However, since IDIF was feasible for the challenging tracer $^{11}$C-HED, it is expected that for other tracers, which typically have smaller differences in tissue and blood concentrations, IDIF is feasible as well.

Quantification of $^{11}$C-HED kinetics was performed using a model that only includes corrections for spill-over from the left and right ventricular cavity, while omitting a correction for arterial blood volume. It is not possible to distinguish between left ventricular spill-over and arterial blood volume in the myocardium, since the time-activity curves in the left ventricular cavity (spill-over) and in the coronary arteries (arterial blood volume) are similar. Therefore, a single correction had to be defined. Assuming all arterial activity to be due to arterial blood volume in the myocardium would result in a factor $1-V_A$ added to the model (as in eq. 7.6), which is omitted when all arterial activity is assumed to be left ventricular spill-over (as in eq. 7.5).

In contrast to most other tissues, the myocardium is adjacent to the blood pool and therefore, large spill-over fractions (in the order of 30-50%) are typically observed. Assuming all of this arterial activity to be blood volume would yield blood volume fractions of 30-50%, which is unrealistic even when the myocardium is moving during the cardiac cycle. In addition, arterial blood volume in the myocardium is relatively constant (165) and therefore, bias in the spill-over model is expected to be more consistent. This has been shown before for $^{15}$O-H$_2$O (14). Finally, simulations in Chapter 7 clearly showed that bias due to different arterial blood volume and spill-over fractions were smaller for the spill-over model, and therefore the spill-over model was selected for further use.

Since its first introduction (184), $^{11}$C-HED has been used in many clinical studies, regarding heart transplantation (233–235), diabetes (53, 199), dilated (59) and ischemic cardiomyopathy (197, 200, 236) and other heart conditions (201, 237). Only a limited number of studies performed actual quantification (201, 202, 237) rather than using semi-quantitative uptake images, all of which used the single tissue compartment model without a systematic assessment of the optimal kinetic model. In addition, these studies used a combination of blood sampler and image-derived input functions, without validation of these combined input functions. The work presented in the second section of this thesis first systematically identified the optimal quantitative model, and then determined the optimal trade-off between accuracy, stability and simplicity.

It is important to note, however, that the findings in this thesis are based on the assessment of actual quantification. It is possible that clinical differences are sufficiently large, i.e. due to near-complete denervation, and in that case, the non-linearity of the relation between quantitative and uptake data is irrelevant and a further degree of simplification is justified. Indeed, simplified methods, such as scaled uptake images (retention index, RI), have been used extensively and successfully (53,184,197,199–201,233–237) and the added value of the methods proposed in this thesis should be evaluated in a clinical validation study. It is likely that more information can be gained from quantitative volume of
distribution (VT) data, as the relationship between RI and VT was nonlinear. This may be especially relevant during early phases of disease or for treatment response monitoring, where smaller differences are expected. However, since no data are available on test-retest variability of both RI and VT, it is possible that the gain in sensitivity of VT is counteracted by poorer test-retest variability. In addition, when a CT scan for attenuation is performed at the end of a dynamic scan (for RI) or if a short, static scan is performed (for SUV), simplified uptake methods are less sensitive to motion artifacts or a mismatch between PET and CT data. Finally, absolute quantification requires dynamic scans with long scanning durations and this increases risk of motion within a scan.

It should be noted that for [11C]HED and [11C]acetate, different methods were used to derive IDIF than the ones described in Chapter 3. All methods described in Chapter 3 result in a cluster containing both left atrial and left ventricular cavities, together with all large arteries within the field of view. As both [11C]HED and [11C]acetate show high uptake in the myocardium and rapid clearance from blood, blood concentrations in the left ventricular cavity are overestimated at late time-points. Consequently, when using cluster analysis, the resulting accuracy of an IDIF is insufficient. In case of [15O]H2O, Iida et al. (117) have proposed a method to correct for spill-in from the myocardium into the blood. However, this method is only valid for radiotracers that are not metabolized, it requires an accurately derived recovery coefficient and is only accurate when tissue uptake in the left ventricle is homogeneous. This method could therefore not be applied to automatically derive IDIFs for [11C]HED and [11C]acetate, and IDIFs had to be obtained by manually drawing circular ROIs on the dynamic images. Furthermore, for the [15O]H2O scans of Chapter 10, the same manual method for defining IDIF was used rather than the methods described in Chapter 3, in order to preserve consistency within this study.

The work of Chapter 10 gives rise to an interesting hypothesis. Since innervation can be quantified using VT of [11C]HED and blood flow distribution in the myocardium can be approximated using K1 of [11C]HED, mismatch between blood flow and innervation could, in theory, be represented using k2 of [11C]HED. VT is defined as the ratio of K1 and k2 and therefore, mismatch, presented as a decrease in VT (denervation) but no decrease in K1 (blood flow), can only be due to an increase in k2. This opens up possibilities for simplified methods, where a patient is scanned shortly after injection and at a late time-point, or the use of k2 as a quantitative parameter of mismatch or as a global parameter of disrupted innervation. This should be a topic for further research.

11.3 Conclusion

In conclusion, parametric images of the myocardial PET radiotracers [15O]H2O, [11C]HED and [11C]acetate can be obtained rapidly, non-invasively and with high quantitative accuracy. This enables a simultaneous visual but fully quantitative assessment of myocardial blood flow, innervation and oxidative metabolism at the highest PET resolution possible.