Appendix E1

CT Perfusion Acquisition

Patients underwent CT perfusion scanning in the supine position. The CT perfusion scans were preceded by localization scans without contrast material enhancement to identify the CT coordinates of the target lesion. For localization for phase 1, an inspiratory breath-hold helical scan was performed with the following settings: tube voltage, 120 kV; tube current, 60 mA; section thickness, 5 mm; section interval, 5 mm; pitch factor, 0.984:1; speed, 39.37; rotation speed, 0.8 second; field of view, 32–40 cm; and matrix, 512 × 512.

After the localization image, phase 1 cine scans were performed by using a single level 4 cm thick (0.5-cm contiguous section thickness for eight sections, 8i mode) at the midpoint of the target lesion. CT data were collected at that single location by using the cine mode, with the following settings: tube voltage, 120 kV; tube current, 90 mA; field of view, 32–40 cm; matrix, 512 × 512. Images were obtained during a 30-second breath hold in inspiration. Data acquisition started 5 seconds after intravenous injection of 50 mL of a nonionic contrast agent (ioversol [Optiray], 320 mg of iodine per milliliter; Mallinckrodt, St. Louis, Mo) by using an automatic injector (MCT/MCT Plus; Medrad, Pittsburgh, Pa) and an injection rate of 7 mL/second. Images were reconstructed every half second and to a thickness of 5 mm.

Phase 2 delayed scans were eight intermittent short inspiratory breath-hold helical scans, each of 3.6 seconds duration. Scan parameters were similar to those for localization: tube voltage, 120 kV; tube current, 90 mA; section thickness, 5 mm; section interval, 5 mm; pitch factor, 0.984:1; speed, 39.37; rotation speed, 0.8 second. The first phase 2 helical scan started 20 seconds after the end of the phase 1 acquisition; the subsequent seven helical scans were obtained at increasing intervals as illustrated in Figure 1. The final helical acquisition started 590 seconds after the start of Phase 1 acquisition. Images were reconstructed to 5 mm thickness, as they were for the phase 1 scans. The estimated effective dose for this CT perfusion protocol was 28 mSv.

When required for clinical purposes, routine staging CT scans of the chest, abdomen, and/or pelvis were performed after the CT perfusion study by using further intravenous administration of 100 mL of contrast medium.

Appendix E2

Statistical Analysis

Acquisition Duration

Deconvolution modeling requires acquisition durations of sufficient length to provide accurate quantification of a patient's CT perfusion parameter values. Before reaching relative steady states, these models are characterized by dynamic periods of noisy fluctuation. Ensuring stable quantification requires the identification of acquisition durations that correspond to time-invariant mappings. Nonparameteric regression was used to evaluate CT perfusion parameters for evidence of time invariance as functions of acquisition duration during a period of 12–590 seconds. This approach obviates predetermination of the underlying functional relationships,

which are unknown, while facilitating direct evaluation of the expected rate of fluctuation in CT perfusion parameter quantification as a function of acquisition time.

If $t > 0$ is acquisition duration and $f(t) > 0$ is the mapping of a particular CT perfusion parameter as a function of t, mapping $f(t)$ is considered to be δ-stable at acquisition time point t_0 if

$$\left| f\left(t_0\right) - f\left(t^*\right) \right| < \delta, \text{ for all } t^* > t_0, \text{ where } \delta > 0, \quad (1)$$

where $f'(t)$ is the derivative of the CT perfusion parameter function at acquisition time t, $f'(t)$ characterizes the infinitesimal rate of change in $f(t)$ with respect to the change in acquisition time t. The stability condition in Equation (1) is satisfied for all $\delta > 0$ if $f(t)$ is time invariant beyond t_0: $f'(t^*) = 0$, for all $t^* > t_0$. Therefore, we evaluated CT perfusion parameter acquisition durations for time invariance by fitting smooth curves to the observed data and conducting inference on the corresponding derivatives to assess their relative proximity to zero as a function of time.

If y denotes the random response variable associated with a single CT perfusion parameter, in general, the model assumes that log (y) varies symmetrically around $f(t)$ with random error ε,

$$log\left(y\right) = f\left(t\right) + \varepsilon, \text{ where } \varepsilon \sim \mathrm{N}\left(0, \sigma_\varepsilon^2\right). \quad (2)$$

Observed CT perfusion parameter values were transformed to the natural logarithm scale for the purpose of adjusting for conditionally asymmetric residual error at a given acquisition time and to mitigate heteroskedasticity as a function of acquisition time. We modeled $f(t)$ with a truncated piece-wise linear spline basis consisting of 16 knots placed at evenly spaced quantiles of the observed acquisition time points. The model approximates the derivative with a step function over the time axis partition. We incorporated random effects to account for dependencies in the nested structure of the data, which involves in-ROI and in-patient repeated measurements, inducing compound symmetric covariance structure. The model facilitates direct evaluation of the quantity of interest, the expected derivative of the ROI subject-specific curves. A mixed model framework was used to facilitate penalized estimation of the spline basis coefficients with restricted maximum likelihood estimation (32).

If $L_\alpha(t)$ and $U_\alpha(t)$ are the lower and upper bounds of the 100 $(1 - \alpha)$% confidence interval for $f'(t)$ at acquisition time t, we considered $f(t)$ stable at acquisition duration t_0 if the confidence intervals obtained at all subsequent acquisition durations were contained within a small neighborhood of zero $(-\lambda, \lambda)$:

$$-\lambda < L_\alpha\left(t^*\right) \text{ and } U_\alpha\left(t^*\right) < \lambda, \text{ for all } t^* > t_0. \quad (3)$$

The approach is analogous to testing null hypotheses of nonequivalence at zero with equivalence region $(-\lambda, \lambda)$. For each CT perfusion parameter, we defined λ to be a scaled multiple of the estimated residual error standard deviation, $\lambda = \hat{\sigma}_\epsilon / k$. This facilitates standardized comparisons among the five CT perfusion parameters and between tumor and normal tissues. However, the choice of scaling constant, k, is subjective and largely determined by the sample size. We fixed $k = 3$ in the inference. Regression models were fit to the observed data by using the statistical software R (R Development Core Team, *http://www.r-project.org*) version 2.12.2 with the package "amer."

Tumor versus Normal Liver Tissue

Linear mixed model was used to compare CT perfusion parameter values acquired at 360 seconds between tumor and normal tissue. The model incorporates random effects to account for correlation among CT perfusion values obtained from multiple ROIs in a given patient, inducing compound symmetric covariance structures. In addition, the model accounts for heteroskedasticity between tumor and normal liver tissue. The statistical software R (R Development Core Team) version 2.12.2 with the package "nlme" was used for statistical analysis.

32. Ruppert D, Wand MP, Carroll RJ. Semiparametric regression during 2003-2007. Electron J Stat 2009;3:1193–1256.