**Purpose:** To develop a 2nd dose validation software for helical TomoTherapy, study the sensitivity of the commission data variation on the final dosimetry impact, and inter-fraction setup uncertainty effect for patient quality assurance.

**Method and Materials:** A 2nd dose validation software for helical TomoTherapy, called *MU-Tomo*, has been developed to independently validates point dose upon archived patient documents, initial coordinates and planned dose of point of calculation, and common dosimetric functions. *MU-Tomo* has been validated with a hundred cancer cases (30 prostate, 26 head&neck, 18 lung, 17 pelvis, and 9 brain patients). Sensitivity studies were performed by oscillating fluctuation regions of off-axis profiles, shifting, and rotating profiles. Daily setup shifts were quantified into systematic and random shifts to evaluate dosimetric variations, separately.

**Results:** For dose validation, 98% of dose differences are within ±5% with mean 0.20%±2.06%. Sensitivity studies show linear response by oscillating OARy, 15 times larger dose variation by shifting OAR, than OARx, and less than 1.5% difference by rotating OARx in ±6° and more than 5% in ±1° by rotating OARy. Systematic variations are up to -10.02%±3.00%. Mean random variations are up to -5.65%±1.90%. ANOVA analyses show significant differences among patient random dosimetric variations and systematic dosimetric variations between head&neck-brain group and body group. Variations are not significantly correlated with treatment fraction number with the Pearson correlation analysis. The overall random dosimetric impacts to each patient are -0.0053%±1.11%.

**Conclusion:** *MU-Tomo*, has been developed for TomoTherapy dose validation. Sensitivity studies on fifty patients have been evaluated that OARy profiles are more sensitive than OARx, in dose calculation. Dosimetric consequences due to inter-fractional setup shifts on a hundred helical tomotherapy patients were assessed.

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