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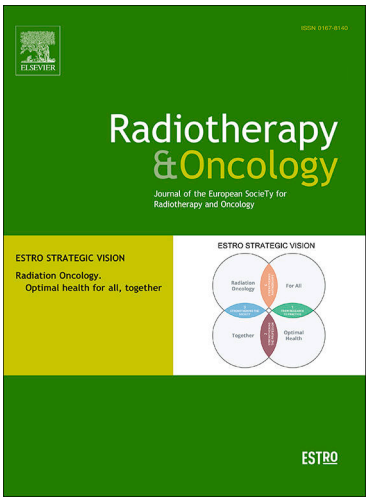

Original Article

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Short title

Coronary artery calcium score and survival in LA-NSCLC patients.

Does Coronary artery calcium score have an impact on overall survival for locally advanced non-small cell lung cancer treated with definitive radiotherapy

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Introduction

The treatment for locally advanced non-small cell lung cancer (LA-NSCLC) is definitive radiotherapy with or without concomitant chemotherapy, potentially followed by immunotherapy [1-3]. Despite treatment with curative intent, the five-year survival is poor. However, overall survival is improving because of advanced radiotherapy [4, 5] and immunotherapy [3]. The heart is an important organ at risk when irradiating thoracic malignancies [6, 7]. Emerging evidence suggests that dose to the heart may be associated with worse overall survival in patients with LA-NSCLC [7-10].

Coronary artery calcium score (CACs) measures calcified plaques in the coronary arteries and indicates coronary atherosclerosis; it can be quantified using the Agatston method in non-contrast CT scans [11, 12]. CACs is an excellent prognostic marker for overall survival and development of cardiovascular disease in non-cancer patients [13]. CACs is mostly absent in men until age 55 and 65 in women, hereafter increasing with age [14]. Traditionally, CACs is categorized into four groups: 0, 1-99, 100-399, and ≥400. The risk of developing cardiovascular disease is low in patients with CACs of zero while increasing in patients with CACs 1-99, 100-399, and six- to tenfold in patients with CACs ≥400 [13, 15, 16].

Traditional risk factors for cardiac diseases, such as hypertension, diabetes, and dyslipidemia [17], are rarely systematically collected in patients with LA-NSCLC, so there is a need for a prognostic marker that can identify LA-NSCLC patients at an increased risk of cardiac disease, in need of cardiac risk factor optimization before radiotherapy treatment. The role of CACs as a prognostic marker for overall survival in LA-NSCLC patients has previously only been tested in a few small studies suggesting an impact of CACs on overall survival [18, 19].

Our aim was to evaluate how CACs impact the mortality of LA-NSCLC patients treated with definitive radiotherapy. The main objective is to assess overall survival in patients with CACs of 0, 1-99, 100-399, and ≥400 while controlling for known risk factors such as age, sex, Eastern

Cooperative Oncology Group - performance status (PS), tobacco consumption, treatment dose in 2 Gy equivalent doses (EQD2), Body Mass Index (BMI), tumor stage and gross tumor volume (GTV).

Methods

Patient population and study design

This is a national, multicenter observational cohort study of patients with LA-NSCLC treated with definitive radiotherapy. We included patients treated throughout 2014-15 in six radiotherapy centers in Denmark with a prescription dose of 50-66 Gy in 24-33 fractions. The patients were identified at each center using local radiotherapy treatment codes. The Danish Patient Safety Authority (3-3013-2847/1) and National Committee on Health Research Ethics (2102012) approved the study. In addition, the Region of Southern Denmark approved data processing (20/21547).

Treatment

Patients with a good PS (0-1), normal kidney function, and no severe comorbidities (e.g., heart failure) were treated with concurrent chemotherapy. If chemotherapy was administered, it was a platinum doublet with oral vinorelbine. Platinum was administered intravenously every third week in three cycles, and vinorelbine tablets were administered orally. Radiotherapy was delivered in 2 to 2.75 Gy fractions with five weekly fractions. The prescribed dose for most patients was 60-66 Gy in 30-33 fractions. For patients treated with 50 Gy in 24 fractions in the HERAN trial (NCT03742687), the dose was escalated to 66 Gy in the Gross Tumor Volume (GTV). The mean dose to GTV for all patients was calculated in 2 Gy equivalent using an alpha/beta ratio of 10 Gy (EQD2).

Clinical data

Patients were identified through all radiotherapy centers in Denmark. Clinical data such as date of diagnosis, disease stage, PS, sex, weight, smoking status, BMI, and date of death were obtained from the Danish Lung Cancer Registry (DLCR). However, treatment data as chemotherapy and radiotherapy treatment are not complete in the DLCR. The date of death derived from the Danish Civil Registration System transferred to the DLCR and was updated at data retrieval in May 2022. The inclusion rate of lung cancer patients in DLCR is estimated to be above 95%, with very high completeness of data (larger than 90%) and a high data validity [20]. Data from DLCR were received in June 2022. Data on stage were dichotomized into a ≤IIB category and ≥ IIIA and recurrence of previously treated lung cancer.

Radiotherapy Data and CT scans

The patient's radiotherapy planning CT scans, CT scans without intravenous contrast agent, radiotherapy doses, target, normal tissue delineations, and treatment plans were transferred in DICOM-RT format from the participating centers to the Danish DICOM Collaboration system [21, 22]. A MATLAB algorithm automatically analyzed DICOM files for GTV volume; radiotherapy mean dose to the GTV. For the analysis, the volume of the GTV was logarithmically transformed to avoid outliers affecting the analysis.

Coronary calcium scoring

CACs were measured on non-ECG-gated non-contrast CT scans. Most of the scans used to evaluate CACs were treatment-planning CT scans, but if intravenous contracts had been used during treatment-planning, a CT scan related to a diagnostic scan prior to radiotherapy was utilized. All CT scans used for CACs were performed within three months before the initiation of radiotherapy. CACs were analyzed manually by the primary investigator (AO) in a clinical Siemens Healthineers syngo.via system. CACs were measured within the left main, the left anterior descending, the circumflex, and the right coronary artery by quantifying the area of calcification with Hounsfield Units above 130 utilizing the Agatston method [11], thus yielding total CACs. The total score was categorized into four groups: CACs 0, CACs 1-99, CACs 100-399, and CACs ≥ 400 [15]. As a quality control of CACs measurement, a senior cardiologist AD blindly assessed 30 randomly picked patients with no patients changing CACs group (Supplementary S1, Bland-Altmann plot)

Data management and statistics

As recommended by the Equator network, a Statistical Analysis Plan (SAP) based on the "Liverpool Clinical Trial Centre-LCTC" [23] was made before having access to the survival data (Supplementary S2, SAP).

Based on an initial power calculation, it was estimated that a single-sided test on a population of 500 patients would enable the detection of a hazard ratio (HR) exceeding 1.2 with a statistical significance of 5% (α) and a power of 80% (1-β). CAC-related HRs less than 1.2 were not considered of clinical interest. Statistical analysis was performed in STATA statistical software (Version BE 17.0, StataCorp, College Staton, Texas, USA) and R-statistical software version 4.2.

Continuous variables were summarized by median and interquartile ranges (IQR) and compared by the Kruskal Wallis test. Categorical variables were summarized by numbers and percentages and compared using Fisher's exact test. A p-value below 5% was considered statistically significant. Reported confidence intervals were made as two-sided 95% confidence intervals. For the CACs-related coefficient in the multivariable analysis, a single-sided 95% confidence interval (CIs) was chosen since it is unlikely that an enhanced CACs level can affect survival positively. Survival time was defined as the time from the planning CT scan until death. Patients are right-censored if still alive. Follow-up time was estimated by the reverse Kaplan-Meier method.

The selection of variables for the multivariable model was performed using cross-validation and bootstrap. The possible parameters for the multivariable model were age, sex, BMI, PS, dichotomized stage, log GTV, pack years, and CACs group. Chemotherapy is administered according to PS; only PS is included in the model. Chemotherapy was not included in the model since the data on chemotherapy was not complete. Before each bootstrap, the missing data were imputed (Multivariate Imputation by Chained Equations – the MICE package in R) [24], thereby using multiple imputations. For each bootstrap, Cox proportional hazard models were made for all combinations of variables during the model selection (best subset selection). For each model, the cross-validated likelihood was calculated utilizing the patient data not included in the bootstrap (out-of-boot patients, on average, approximately 1/3 of the patients). The selected model was the model that includes CACs and performs best within the cross-validation (thus, CACs is forced into the model). The model selection process was based on 50 bootstraps, while calculating the 95% CI of the regression constants of the final model utilized 2000 bootstraps.

To match a recent study [18], the analysis was repeated with CACs divided into two groups: CACs < 100 and ≥100. The entire analysis was also performed in two additional unplanned analyses: 1) without forcing the CAC into the model and 2) excluding patients with mean tumor dose below 60 Gy.

The multivariable model was validated by dividing the patients into three risk groups based on the predicted hazard. The cut-points for the risk groups will be the 25 and 75 percentile values of the entire cohort's predicted hazard values. The Kaplan-Meier estimator and the model prediction were calculated for each risk group. As for the confidence intervals mentioned above, the estimators for the risk groups were based on 2000 bootstraps to utilize the multiple imputation method. The average value of the bootstraps will be shown as the final performance within each risk group. Finally, the overall survival for the entire cohort will be visualized using the Kaplan-Meier estimator.

Results

Initially, 731 eligible patients were identified, from all Danish radiotherapy centers, as having received definitive radiotherapy for LA-NSCLC throughout 2014-15. Excluded patients due to non-NSCLC histology or included in the ongoing national NARLAL 2 dose escalation study (NCT02354274) are seen in figure 1. Patients (n=42) were also excluded if they did not have non-contrast or CT scans of too low quality to perform CACs measurement. Lastly, we excluded 18 patients for whom we did not receive data from DLCR (Figure 1). Patients were followed until death or to the data retrieval time in May 2022, yielding a median follow-up time of 7 years with no patients lost to follow-up.

Patient characteristics are listed in Table 1. The median age was 68 years. Age increased according to the CACs group, with the patients in the highest CACs group being the oldest. Male patients were predominant in the highest CACs group. The median prescribed EQD2 was 66.6 Gy (IQR 66.1-67.2), with no significant differences according to the CACs group. No significant differences were observed in the distribution of patients in the different CACs groups between the participating centers. Most patients were treated with 30-33 fractions (99 %), and nine were treated with 24 fractions (Table 1).

The median survival time for all patients was 26 months (95% CI 24-29). 1-year and 2-year survival rates were respectively 73% (95% CI 70-76) and 52% (95% CI 48-56). 5-year overall survival was 25% (95% CI 22-29) (Figure 2). Median survival differed between the CACs groups (univariable p-value 0.49); in CACs 0, median survival was 28 months (95% CI 23-36), while in CACs 1-99, 32 months (26-40), and decreasing median survival in CACs 100-399 and CACs ≥400, with a median survival of respectively 24 months (95% CI 17-29) and 20 months (95% CI 16-26). Kaplan-Meier survival estimates for the overall survival grouped by CACs are shown in Figure 2.

Univariable analysis was performed to evaluate the separate associations of each variable with survival (Figure 3); statistically significant associations with survival were observed for age, PS ≥2, logarithmic GTV, treatment dose (EQD2) to GTV, and CACs above 100. We did not find a statistically significant association on the univariable analysis for sex, BMI, stage, and pack years.

The best-performing cross-validated multivariable model, forcing CACs, included age, PS, EQD2, and logarithmic GTV (Figure 4). When adjusting for these variables, CACs lost its statistical significance. An unplanned supplementary analysis that did not force CACs into the model was performed and did not select CACs to be included in the model but selected PS, EQD2, and log GTV (Supplementary-S3), underlining the likely modest direct impact of CACs on survival in the current patient cohort. In this dataset, we also included patients treated with a mean dose to GTV below 60 Gy; in a supplementary analysis, we excluded these patients and found almost identical results to the primary analysis (Supplementary –S4 and S5). In supplementary 6, dichotomizing patients in CACs <100 and ≥100 and forcing CACs into the model also resulted in almost the same result as the primary analysis (Supplementary –S6).

As for all generalized linear models, the multivariable model depends on the sum of the regression constant (β_i) times the observed patient value (x_i), also referred to as the linear predictor (LP= $\sum_i \beta_i x_i$). The validity of the multivariable model was tested by dividing the patients into three groups based on their LP values (low, middle, and high-risk groups). The LP division coefficient between the groups was -0.15 and 0.33, reflecting the 25th and 75th percentile of the LP values within the cohort.

Kaplan-Meier estimates for each of the three groups and the related model predictions are shown in figure 5. The similarity of the Kaplan-Meier estimates and model predictions show the model's predictive power for both low and high values of the linear predictor. The median survival of the three risk groups was 46 months (95% CI 36-58), 26 months (95% CI 20-30), and 16 months (95% CI 13-20) for the low, middle, and high-risk groups, respectively.

Discussion

This study is one of the first to assess the impact of CACs divided into four groups on overall survival in a large national cohort of patients with LA-NSCLC treated with definitive radiotherapy. While a univariable analysis hinted towards a decreasing overall survival with increasing CACs, the association did not remain statistically significant when tested in a cross-validated multivariable model, including age, PS, EQD2, and Log GTV. This model showed excellent correspondence between prediction and Kaplan Meier estimator within the defined risk groups.

Over recent years, several lung cancer-screening trials have been published [25, 26]. Several of them included secondary analyses of CACs to assess the predictive value of CACs for overall survival and the risk of developing cardiac disease. In the Danish Lung Cancer Screening Trial, Rasmussen et al. evaluated the prognostic value of CACs measured in a non-ECG-gated CT scan, utilizing the Agatston method in 1,945 individuals. They divided the patients into three CACs groups 0, 1-400, and above 400 [27], and found that CACs 1-400 for all-cause mortality had a significant HR of 1.7 and 2.1 for patients with CACs >400. In a meta-analysis by Gendarme et al. assessing the impact of CACs on cardiovascular and all-cause mortality, including 20,175 subjects participating in lung cancer screening trials, dividing them into CACs 0, >400, and >1000. They found an increased relative risk (RR) of 2.29 for all-cause mortality and RR of 2.02 for cardiovascular mortality [28]. For both lung cancer screening trials, high-risk subjects with an extensive smoking rate are included, but with differences from our population, including younger

age and the vast majority without lung cancer diagnosis (none of the studies reported the prevalence of lung cancer in their cohorts).

Few studies have investigated the prognostic value of CACs in NSCLC patients. Osawa et al. analyzed a novel method of measuring coronary calcium and included 309 early-stage lung cancer patients after resection and concluded that coronary calcium was associated with all-cause death HR 1.18 [29]. However, they only adjusted for chemotherapy, cardiac risk factors, and CACs; furthermore, the study only included early-stage and resected lung cancer patients with better median survival than our study.

A study published by Atkins et al. measured coronary calcium in 428 LA-NSCLC patients treated with definitive radiotherapy. The ungated planning CT scans underwent a deep learning-based coronary calcium measurement and divided the patients into coronary artery calcium of 0 and ≥ 1, revealing a higher all-cause mortality risk in the CAC ≥ 1 group HR 1.51 (95% CI 1.01-2.26) [19]. Patients in the Atkins et al. publication are similar to our population regarding tobacco use, PS, sex, stage, and age. In contrast to our study, Atkins et al. found an impact of CACs on overall survival. However, Atkins et al. differ from our study regarding the division of CACs in no CACs or CACs ≥ 1; they did not adjust for tumor size and radiotherapy dose, which may have predictive value for the population in this study. Finally, they had a high rate of patient exclusion due to planning CT with contrast, which could introduce some bias.

A recent small study by Koutroumpakis et al. utilizing the same CACs method as in our study and dichotomizing patients into CACs <100 and ≥100 found an overall survival of HR of 0.61 (95 % CI 0.41-0.90) with the group with a high CACs serving as reference [18]. However, we found no effect on overall survival in a supplementary analysis (supplementary S-6) dichotomizing CACs <100 and ≥100. Besides the study by Koutroumpakis being smaller, the patients in the study were slightly younger and assumable in a better PS (despite different methods of reporting Karnofsky vs. ECOG PS) as the patients primarily were enrolled in a prospective study randomizing between photon and proton treatment, which could affect the results.

In our study, tumor volume was the strongest predictor of survival in lung cancer patients; this is probably the most evident reason to why CACs in this study shows no impact on survival when compared to other similar studies [19, 30]. In addition, the results might be more valid since the patients in our study represent real-life patients, with only few LA-NSCLC patients excluded. Recent evidence suggests that radiation dose to the heart impacts overall survival [9, 10]. There is an increasing need to identify patients at risk of developing cardiac disease and worse overall survival due to heart irradiation. CACs are an excellent prognostic marker in non-cancer patients, patients participating in lung cancer screening [27, 28], and early-stage lung cancer patients [29, 31], this is not the case for patients with LA-NSCLC despite an increased CACs in 73 % of patients suggesting a high risk of cardiac comorbidities at baseline. CACs is also a good prognostic marker for the development of cardiac disease. Therefore, it is essential to perform studies including information on cardiac disease before and after radiotherapy and irradiation dose to the heart.

The main strengths of our study are that it represents a national cohort including all LA-NSCLC patients from all Danish radiotherapy centers and that no patients were lost to follow-up during a seven years potential follow-up. Furthermore, we applied a validated method of CACs measurement. Potential limitations of this study could be that CACs were measured in a non-gated

CT. However, several studies have shown that this does not pose problems and that CACs can be measured in non-gated CT [32-34].

Furthermore, we quality assured the CACs measurement in 30 patients randomly selected by an experienced cardiologist with a specialty in heart CT. Clinical data originate from patient registries, but the data are collected prospectively. The Danish registries have a high level of validity [20, 35, 36], however with lack of quality on chemotherapy treatment. This is a potential limitation, as chemotherapy can induce heart disease [37], but PS is included in the model. However, this does not impact the assessment of CACs on the primary outcome of overall survival.

In this study, we could not confirm that CACs are prognostic markers of survival in LA-NSCLC patients. Our analyses showed that factors such as age, performance status, and tumor volume substantially impacted survival, suggesting that the greatest attention should be on treating lung cancer, despite of a high risk of cardiac comorbidity at baseline. However, several questions remain to be addressed about the implication of CACs in the field of LA-NSCLC patients treated with definitive radiotherapy, one of them being CACs and radiotherapy dose to the heart and substructures, which could identify patients at risk after irradiation.

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Figure 1: *Flowchart of patient inclusion and exclusion in the attempt to perform manual coronary artery calcium score according to the Agatston method. CT, computed tomography, LA-NSCLC, Locally advanced non-small cell lung cancer. NARLAL 2, a prospective Danish dose-escalation trial.*

Figure 2: *Overall survival grouped by Coronary artery calcium score (CACs).*

Figure 3: *Forest plot of univariable Cox regression analysis for overall survival. HR, Hazard Ratio. CI, Confidence intervals. EQD2, treatment dose in 2 Gy equivalent doses. Log GTV, Logarithmic Gross Tumor Volume. Coronary artery calcium score with single-sided confidence Intervals, while the rest are two-sided.*

Figure 4: *Forest plot of multivariable Cox regression analysis for overall survival. HR, Hazard Ratio. CI, Confidence intervals. EQD2, treatment dose in 2 Gy equivalent doses. Log GTV, Logarithmic Gross Tumor Volume. Coronary artery calcium score with single-sided confidence Intervals, while the rest are two-sided.*

Figure 5: *Survival of the validated model, based on the multivariate analysis and bootstrapping; thus, there is no well-defined number of patients at risk at specific time points. Full line Kaplan-Meier estimate and 95 % CI for the Kaplan-Meier estimate, dotted lines Cox estimates and 95 % CI.*

Figure 1

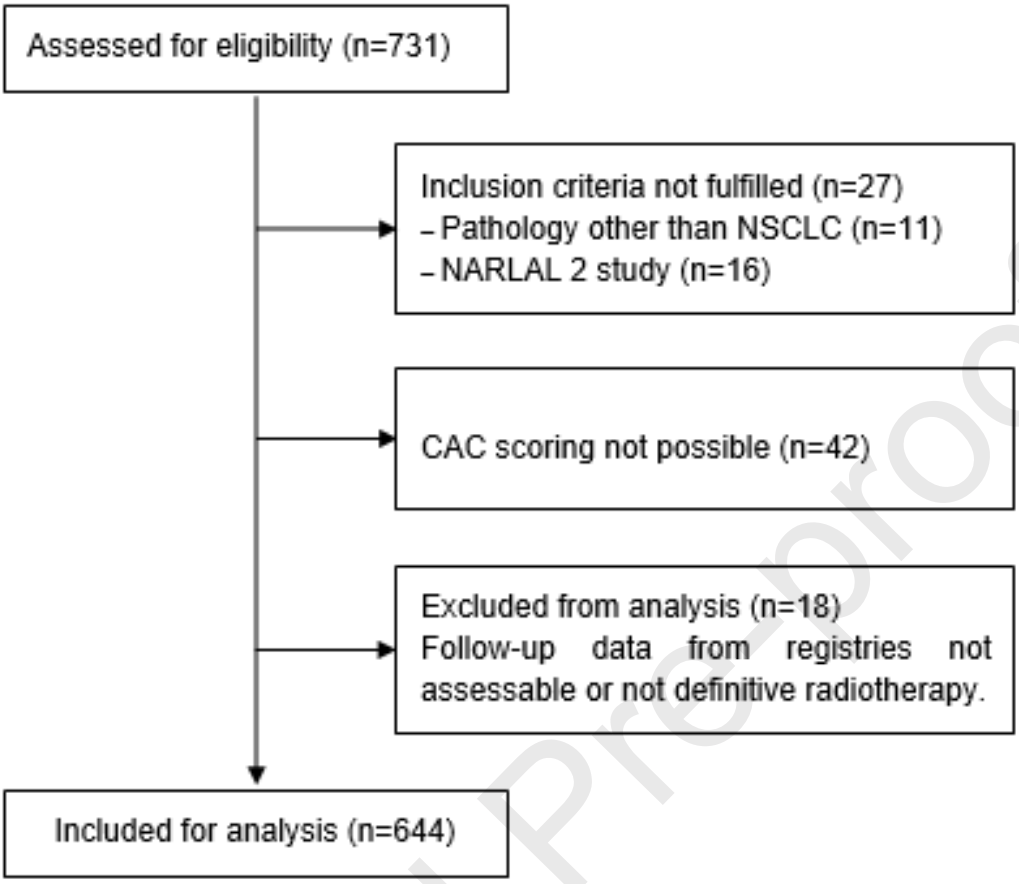


Figure 2

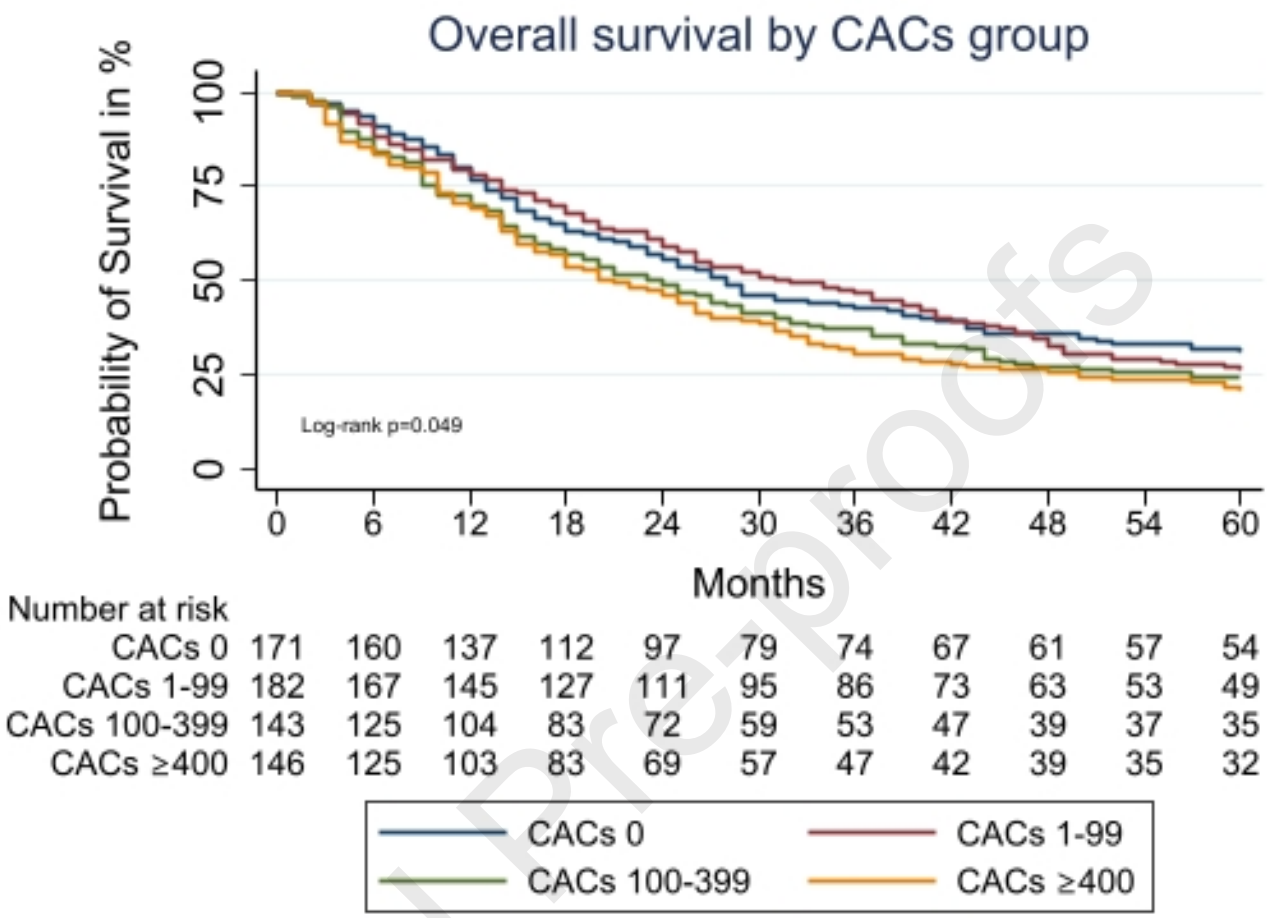


Figure 3

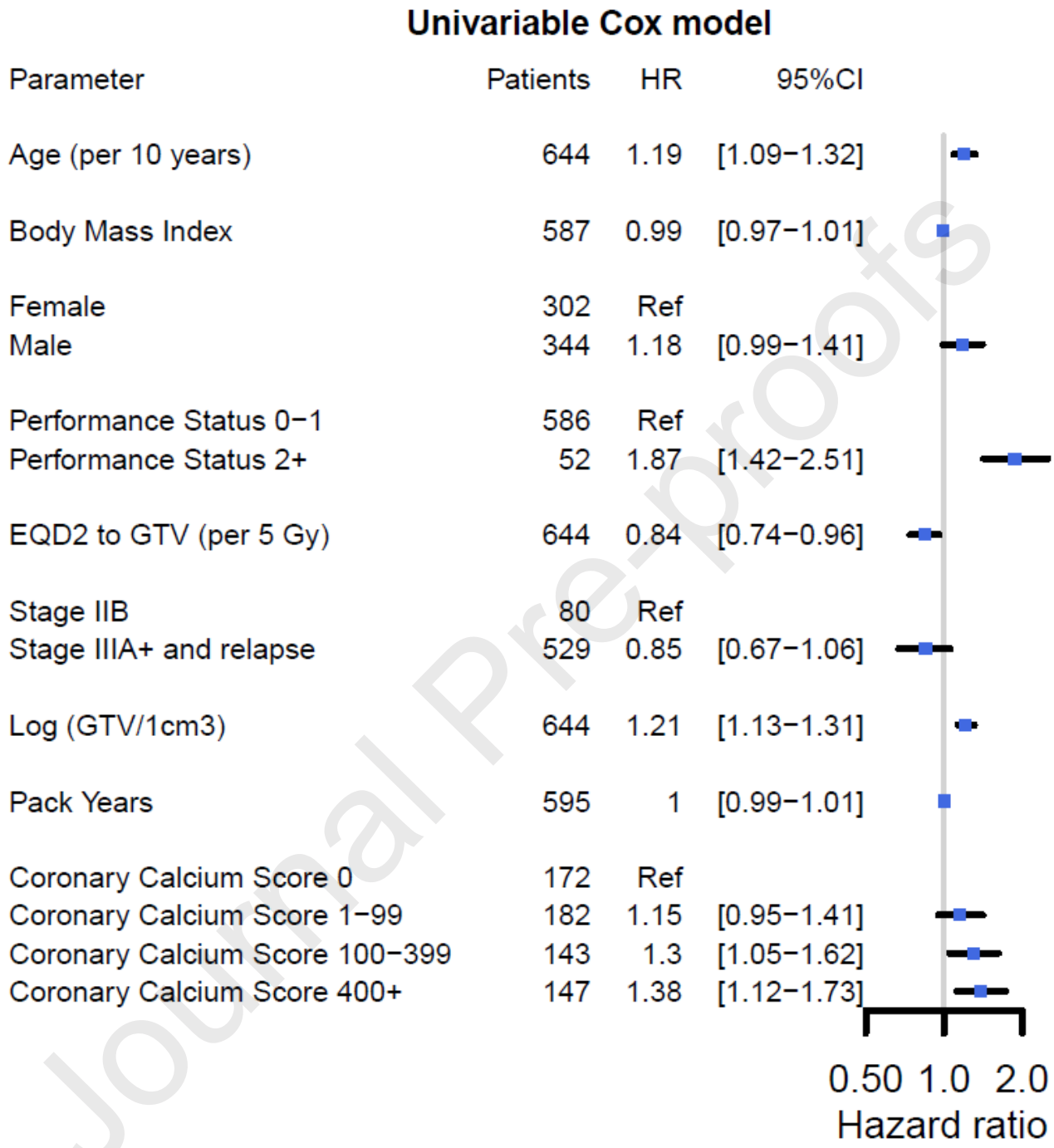


Figure 4

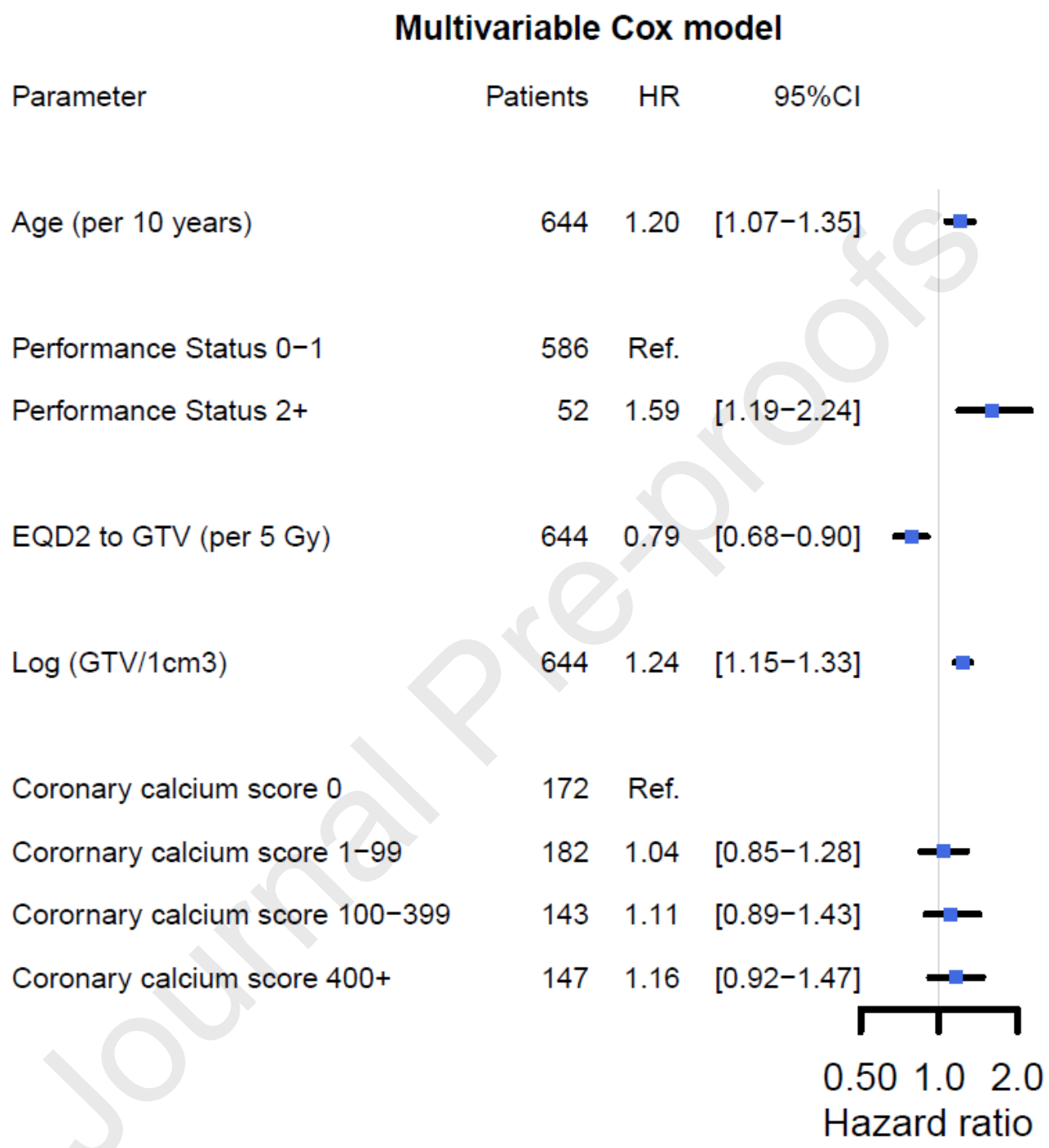
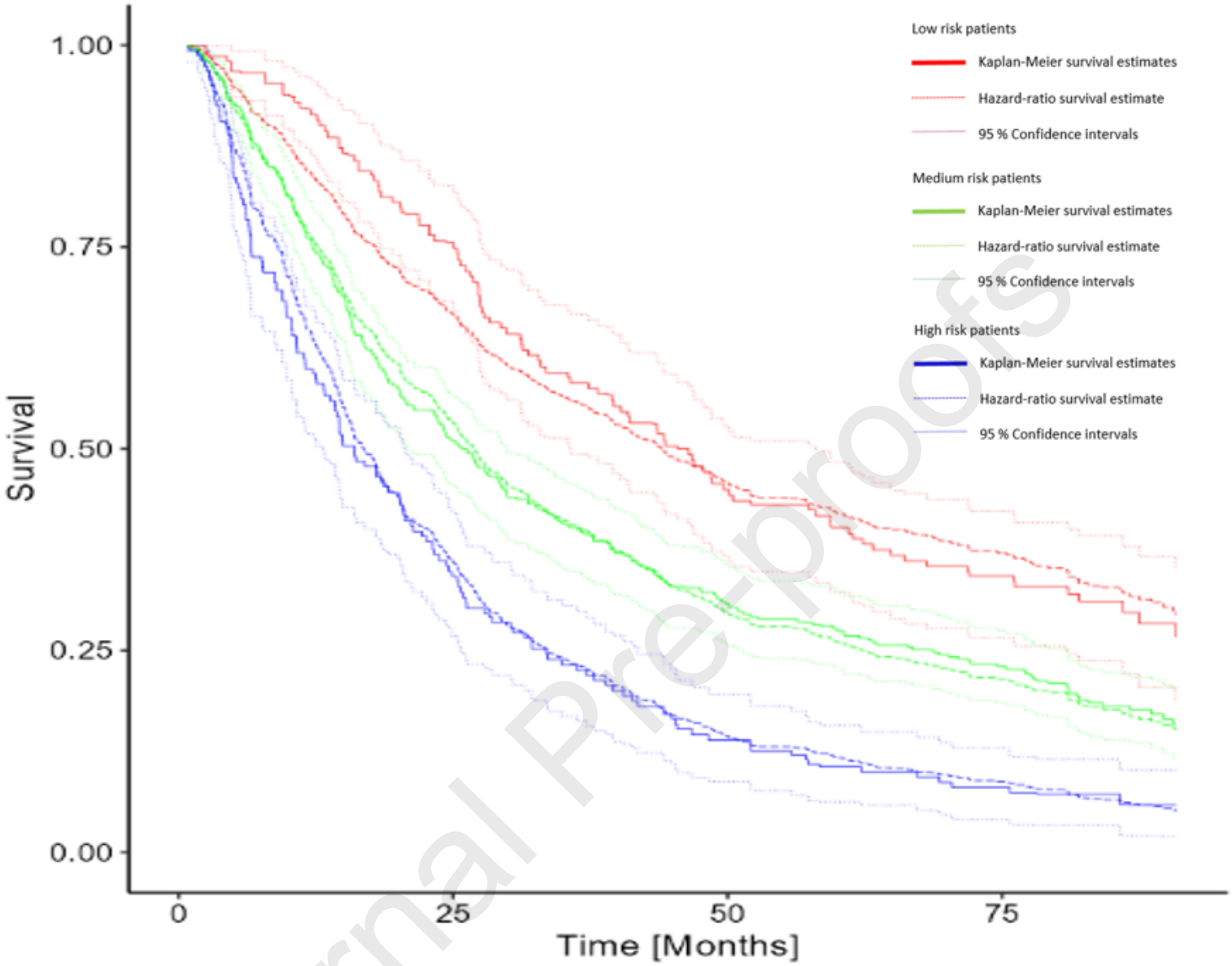


Figure 5



Panel Figure 5

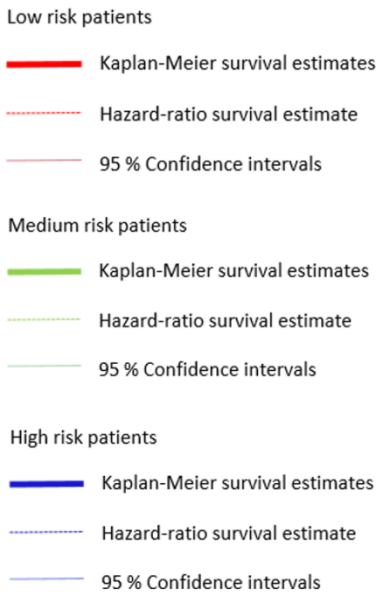


Table 1: Table 1 Patient and treatment characteristics, provided as overall and divided by CACs (Coronary artery calcium score). Categorical variables were compared using Fisher's exact test; continuous variables were compared using Kruskal-Wallis Test. GTV (Gross Tumor Volume). BMI (Body Mass Index). RT (Radiotherapy). Missing values in continuous variables: Weight (55 missing), BMI (57 missing), and Pack Years (49 missing). No missing values for age, EQD2 (treatment dose in 2 Gy equivalent doses), GTV and RT technique. IQR (Interquartile range).

	Total	**CACs = 0**	**CACs = 1-99**	**CACs = 100-399**	**CACS ≥400**	**p-value**
	N=644	**N=172**	**N=182**	**N=143**	**N=147**	
Age in Years, median(IQR)	68 (62-73)	62 (54-68)	68 (62-73)	70 (65-74)	71 (67-76)	<0.001
Weight in kg, median(IQR)	72 (63-83)	72 (62-83)	72 (62-86)	72 (65-82)	74 (63-83)	0.92

BMI, median (IQR)	24.5 (21-27)	23.9 (21-27)	24.5 (22-28)	24.6 (21-27)	24.8 (21-27)	0.77
Performance Status						0.33
0-1	586 (91%)	161 (94%)	164 (90%)	131 (92%)	130 (88%)	
≥2	52 (8%)	9 (5%)	16 (9%)	10 (7%)	17 (12%)	
Missing	6 (10%)	2 (1%)	2 (1%)	2 (1%)	0 (0%)	
Sex						<0.001
Female	302 (47%)	108 (63%)	93 (51%)	64 (45%)	37 (25%)	
Male	342 (53%)	64 (37%)	89 (49%)	79 (55%)	110 (75%)	
EQD2 in Gy, median(IQR)	66.6 (66-67)	66.5 (66-67)	66.7 (66-67)	66.5 (66-67)	66 (66-67)	0.17
Tumor stage						0.001
≤IIB	80 (12%)	13 (8%)	17 (9%)	20 (14%)	30 (20%)	
≥IIIA and recurrence	529 (82%)	155 (90%)	150 (83%)	117 (82%)	107 (73%)	
Missing	35 (6%)	4 (2.3%)	15 (8%)	6 (4%)	10 (7%)	
Smoking history						<0.001
Never	30 (5%)	16 (9%)	10 (5%)	3 (2%)	1 (1%)	

Active/ previous	565 (88%)	148 (86%)	149 (82%)	131 (92%)	137 (93%)	
Missing	49 (7%)	8 (5%)	23 (12.6%)	9 (6%)	9 (6%)	
Pack Years, median(IQR)	40 (25-50)	35 (20-45)	39 (25-50)	40 (25-50)	43 (30-50)	<0.001
GTV in cm3, median(IQR)	64 (29-133)	66 (31-118)	61 (27-124)	66 (30-163)	64 (29-136)	0.68
Institution						<0.001
1	127 (20%)	35 (20%)	35 (19%)	25 (17%)	32 (22%)	
2		35 (20%)	36 (20%)	41 (29%)	26 (18%)	
3	138 (21%)	34 (20%)	46 (25%)	25 (17%)	21 (14%)	
4	126 (20%)	12 (7%)	35 (19%)	23 (16%)	36 (24%)	
5		41 (24%)	19 (11%)	14 (10%)	16 (11%)	
6	106 (16%)	15 (9%)	11 (6%)	15 (11%)	16 (11%)	
	90 (14%)					
	57 (9%)					
RT technique						0.11
3D-CRT	57 (9%)	15 (9%)	11 (6%)	15 (11%)	16 (11%)	
IMRT	264 (41%)	69 (40%)	82 (45%)	66 (46%)	47 (32%)	
VMAT	323 (50%)	88 (51%)	89 (49%)	62 (43%)	84 (57%)	

Conflict of interest

Nothing to declare.