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Can CT measures of tumour heterogeneity stratify risk for nodal metastasis in patients with non-small cell lung cancer?

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Abstract:	OBJECTIVES: To undertake a preliminary assessment of the potential for CT measures of tumour heterogeneity to stratify risk of nodal metastasis in patients with non-small cell lung cancer (NSCLC). METHODS: Tumour heterogeneity in CT images from PET/CT examinations in 150 consecutive patients with NSCLC was assessed using CT texture analysis. The short axis diameter of the largest mediastinal node was also measured. 42 patients without distant metastases subsequently had tumour nodal status confirmed by surgery (n=26) or Endobronchial Ultrasound (EBUS); n=16). CTTA parameters and largest nodal diameter were related to nodal status using the rank-correlation and the risk-ratio for each nodal stage (>N0, >N1, >N2) was compared between patients categorised as high and low risk by CTTA or nodal size. The most significant predictor of nodal status was related to overall survival using Kaplan-Meier analysis. RESULTS: N-stage was more significantly correlated with CTTA than nodal diameter (Rs = -0.39, p = 0.011, Rs = -0.45, p=0.0025, Rs = -0.40, p=0.0091 for normalised SD, normalised E and kurtosis respectively; Rs = -0.39, p = 0.042 for nodal diameter). The presence of 2 or more high-risk CTTA values was the greatest risk-factor for mediastinal metastasis (Risk-ratio: 11.0, 95% confidence interval 1.56 - 77.8, p=0.0014) and was associated with significantly poorer overall survival (p=0.016). CONCLUSION: CTTA in NSCLC is related to nodal status in patients without distant metastases and has the potential to inform selection of investigative strategies for the assessment of mediastinal malignancy.

CAN CT MEASURES OF TUMOUR HETEROGENEITY STRATIFY RISK FOR NODAL METASTASIS IN PATIENTS WITH NON-SMALL CELL LUNG CANCER?

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DECLARATION OF INTEREST

Kenneth Miles declares he has a financial interest in Feedback PLC who supply the texture analysis software used in this study.

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1. Guarantor of integrity of entire study – Michelle Craigie
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3. Literature research – Michelle Craigie, Kenneth Miles
4. Clinical studies – Michelle Craigie, Julia Squires, Kenneth Miles
5. Experimental studies/data analysis – Michelle Craigie, Julia Squires, Kenneth Miles
6. Statistical analysis – Kenneth Miles
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ABTRACT

OBJECTIVES: To undertake a preliminary assessment of the potential for CT measures of tumour heterogeneity to stratify risk of nodal metastasis in patients with non-small cell lung cancer (NSCLC).

METHODS: Tumour heterogeneity in CT images from PET/CT examinations in 150 consecutive patients with NSCLC was assessed using CT texture analysis. The short axis diameter of the largest mediastinal node was also measured. 42 patients without distant metastases subsequently had tumour nodal status confirmed by surgery (n=26) or Endobronchial Ultrasound (EBUS); n=16). CTTA parameters and largest nodal diameter were related to nodal status using the rank-correlation and the risk-ratio for each nodal stage (>N0, >N1, >N2) was compared between patients categorised as high and low risk by CTTA or nodal size. The most significant predictor of nodal status was related to overall survival using Kaplan-Meier analysis.

RESULTS: N-stage was more significantly correlated with CTTA than nodal diameter (Rs = -0.39, p = 0.011, Rs = -0.45, p=0.0025, Rs = -0.40, p=0.0091 for normalised SD, normalised E and kurtosis respectively; Rs = -0.39, p = 0.042 for nodal diameter). The presence of 2 or more high-risk CTTA values was the greatest risk-factor for mediastinal metastasis (Risk-ratio: 11.0, 95% confidence interval 1.56 - 77.8, p=0.0014) and was associated with significantly poorer overall survival (p=0.016).

CONCLUSION: CTTA in NSCLC is related to nodal status in patients without distant metastases and has the potential to inform selection of investigative strategies for the assessment of mediastinal malignancy.

KEY WORDS

Non-small cell Lung Cancer, Positron-emission tomography, Computed Tomography Texture Analysis, Nodal metastasis

CAN CT MEASURES OF TUMOUR HETEROGENEITY STRATIFY RISK FOR NODAL METASTASIS IN PATIENTS WITH NON-SMALL CELL LUNG CANCER?

INTRODUCTION:

Non-small cell lung cancer (NSCLC) remains one of the leading causes of cancer in the Western World with a poor prognosis. Predictive factors of disease burden are required to help with decisions regarding options for clinical management. These circumstances are illustrated by the current clinical guidance for the management of NSCLC issued by the National Institute of Clinical Health and Excellence (NICE)(1). This guidance recommends different investigative strategies for the assessment of mediastinal disease according to probability of mediastinal malignancy based on nodal size as depicted by CT. For patients with a low probability of mediastinal malignancy (15%; lymph nodes < 10 mm maximum short axis on CT), the optimum strategy was determined to be staging with PET-CT alone. PET-CT, or endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA), or endoscopic ultrasound (EUS)-guided fine needle aspiration, or non-ultrasound guided TBNA are recommended for patients with an intermediate probability of mediastinal malignancy (50%; lymph nodes between 10 and 20 mm maximum short axis on CT) whilst neck ultrasound with sampling of visible lymph nodes, or non-ultrasound guided TBNA should be offered to patients with a high probability of mediastinal malignancy (85%; lymph nodes > 20 mm maximum short axis on CT). New methods that can improve the risk stratification for mediastinal disease based on CT therefore

have the potential to improve the selection of staging procedures for patients with NSCLC.

CT texture analysis (CTTA) is emerging as a technique for derivation of prognostic biomarkers for patients with NSCLC and other tumours (2-5). CT texture analysis evaluates quantitatively the distribution of CT attenuation values within a tumour to determine its heterogeneity. Tumour heterogeneity has been shown to relate to tumour aggression and treatment response, with hypoxia, mutations in EGFR and KRAS genes, and ALK gene re-arrangements having been identified as potential biological correlates for CTTA values in NSCLC (2). Given the prognostic significance of CTTA and its associated biological characteristics, we hypothesize that CTTA can stratify risk for nodal metastatic disease in patients with NSCLC.

METHOD:

Study Design:

A prospective observational study design was adopted, using patient data that was acquired as part of routine clinical care. Our local institutional review board had waived the requirement for individual consent.

Patients:

Imaging data was collected from 150 consecutive patients undergoing PET/CT for staging of NSCLC cancer. The study cohort of 42 patients comprised all those with no distant metastasis detected on PET/CT and subsequent confirmation of nodal status either at surgery (n=26) and or by EBUS with TBNA (n=16). Overall survival was

45 determined from a median clinical follow-up period of 279 days (range: 59-437
46 days).

47 *CT Imaging protocal and data analysis:*

48 Images were acquired using a Siemens mCT PET/CT system 120kV, automated tube
49 modulation (Care dose) with reference tube current set at 80mAs, 5mm slices and
50 collimation 1.2mm(Siemens, Erhlangen, Germany). Using TexRAD software
51 (Feedback plc, Cambridge, UK), CTTA was performed on the low dose CT slice that
52 displayed the largest cross sectional area of the tumour on soft tissue windows as
53 described previously (2). Definition of the tumour boundary was assisted with
54 reference to the PET fused images and narrow CT windows (level 40HU, width
55 150HU). Automated segmentation tools were used to optimise consistency in the
56 analysis between operators were possible, for example where the tumour was
57 surrounded by aerated lung. Where the lung tumour was in contact with other
58 tissues such as the chest wall, mediastinum or consolidated lung, manual selection of
59 the region of interest (ROI) was required along that border and the automated
60 segmentation tool could be used on those areas where the tumour bordered
61 aerated lung. Segmentation tools excluded areas of tumour cavitation seen on CT
62 but were not used to exclude areas of necrosis/photopaenia seen on the PET fused
63 images.

64 Based on the filtration-histogram CTTA approach utilised by the CTTA software,
65 tumour heterogeneity at a scale of 4mm was expressed as kurtosis, standard
66 deviation (SD) and entropy (E). SD and E were both log-normalised to the tumour
67 area determined by the number of pixels in the tumour ROI.

The short-axis diameter of the largest mediastinal was also measured by a separate operator who was an accredited radiologist with more than 25 years of CT experience.

Statistics

The relationship between CTTA parameters and nodal status were determined using the rank-correlation and compared to the correlation found between N-stage and mediastinal nodal size. If a significant correlation was found, patients were categorised as high or low risk for nodal metastases using the median texture value for the study cohort. The risk for each nodal stage (>N0, >N1, >N2) was compared between high and low risk patients and expressed as the risk ratio (with 95% confidence limits), with comparison against the risk-ratios found for nodal size <10mm versus ≥ 10mm, using Fisher's exact test to assess statistical significance. The most significant predictor of nodal status was related to overall survival using Kaplan-Meier analysis.

RESULTS:

42 patients NSCLC without distant metastases went on to have nodal status confirmed either at surgery or EBUS with TBNA, the numbers of patients with N-stages 0, 1, 2 and 3 were 26, 4, 8 and 4 respectively. 5 (11.9%) patients died during follow-up.

N-stage was shown to correlate significantly with normalised SD, normalised E and kurtosis (Rs = -0.39, p = 0.011, Rs = -0.45, p=0.0025, Rs = 0.40, p=0.0091

respectively). There was a weaker but statistically significant correlation between N-stage and mediastinal nodal diameter (Rs = -0.39, p = 0.042). CTTA values were categorised as high-risk for nodal metastases if below the median for normalised SD (5.66) and normalised E (0.777), or above the median for kurtosis (-0.295). 21 (50%) patients had 2 or more high-risk CTTA values. Example CT texture results from patients with high and low risk CTTA parameters are given in Figures 1 and 2.

The greatest risk factors for node positive disease were log-normalised entropy and the presence of 2 or more high-risk CTTA values, each associated with a 4.3 greater likelihood of nodal metastases (p=0.0036, table 1). A mediastinal nodal diameter of ≥ 10mm was a significant risk-factor for mediastinal malignancy (i.e. N stage 2 or 3) but its risk-ratio of 2.82 was exceeded by that found for all CTTA parameters. The presence of 2 or more high-risk CTTA values was the greatest risk-factor for mediastinal metastases, being associated with an 11-fold risk for mediastinal malignancy (p=0.0014) and significantly poorer overall survival (p=0.016, Figure 3). The only significant risk factor for N3- disease was mediastinal nodal size ≥10mm.

DISCUSSION

In this study we investigated the potential for CT measures of tumour heterogeneity to stratify risk of nodal disease in patients with NSCLC. CTTA parameters within the primary tumour were found to be superior predictors of nodal stage >0 and nodal >1 than the measurement of mediastinal nodal diameter as currently recommended by NICE. However, mediastinal nodal diameter was the best predictor for N-stage >2. The finding that the presence of two or more high-risk CTTA values is not only associated with a greater risk of

nodal stage >1 but also reduced survival, indicates that the presence nodal disease inferred by high-risk CTTA values is of clinical importance.

CTTA has the potential to determine the optimum strategy for investigation of nodal disease more accurately than measurements of mediastinal nodal diameter as currently recommended by NICE. For example, the NICE guidance would recommend the nodal status for the patient with N2 disease illustrated in Figure 1 be determined by PET-CT alone. While PET CT is a non-invasive method in assessing for mediastinal nodal metastasis, its sensitivity and specificity of detecting mediastinal metastasis is only around 77% and 86% respectively(6). Furthermore the sensitivity of PET for detecting nodal metastasis is lower when the size of the lymph node is less than 10mm(7). A PET/CT only strategy for this patient would have resulted in underestimation of disease status whereas the presence of high-risk CTTA parameters could feasibly have indicated the need for nodal sampling prior to surgery. Similarly, due to the presence of an enlarged mediastinal node, the NICE guidance would recommend nodal sampling be considered for the patient with N0 disease illustrated in Figure 2 whereas this procedure may not have been considered necessary based on the absence of high-risk CTTA values. As the NICE strategies were optimised on the basis of cost-effectiveness, CTTA therefore also has potential health economic benefits.

The observation that tumours with increased metastatic potential exhibit greater genetic instability (8) provides a biological basis for a relationship between CT measures of tumour heterogeneity and nodal status. In the presence of genetic instability, the evolutionary dynamics of tumour development may result in the co-existence of genetically distinct sub-clones within the same tumour (9). For these sub-clones to be detected by CT the genetic status of the sub-clone must correspond to one or more phenotypic features demonstrable by imaging. Furthermore, the spatial separation between sub-clones needs to be sufficiently large relative to the spatial resolution of CT (See Figure 4). The first requirement is shown to

be met by radiogenomic studies demonstrating correlations between genomic aberrations and specific CT features in NSCLC and other tumours (5, 10-15). Spatial separation of sub-clones has also been reported and may be sufficiently large to result in sampling error during image-guided biopsy (9). Although plausible, a direct connection between genomic instability, metastatic potential and CT measures of tumour heterogeneity remains to be demonstrated empirically.

The main limitation of our study is the small size of our cohort of NSCLC patients without distant metastases for whom nodal status had been confirmed either at surgery or endobronchial biopsy. Larger multi-centre studies are required to confirm our findings. To minimise potential bias, we have adopted median textures as the thresholds for the categorisation of high and low risk for nodal metastases. Larger studies would also allow optimisation of these threshold values through use of separate training and evaluation cohorts. We have also adopted a single slice approach to assess for tumour heterogeneity which has the potential to miss regions of greater heterogeneity and hence under estimate the risk of nodal metastasis (3). The consistency of risk stratification by CTTA may also be potentially improved through automated CT ROI definition based on the PET component of PET/CT examinations(16).

The potential prognostic value of CTTA in NSCLC was first reported in 2012 (17) but as yet this finding has not been translated into a widely adopted clinical application. It has been proposed that the prognostic information afforded by CTTA could be used to identify patients with a greater risk for post-surgical recurrence who might benefit most from adjuvant chemotherapy, or to recognise those patients with advanced disease who are unlikely to get sufficient survival benefit to justify the morbidity of chemotherapy in a palliative setting (2). However, the use of CT as a prognostic biomarker represents a novel application for imaging in the management of NSCLC, and in cancer care in general; a fact

that may represent a block to the adoption of CTTA into clinical practice. On the other hand, the use of CT markers to assess risk of mediastinal malignancy is embedded within the current clinical guidelines published by NICE. The replacement of measurements of mediastinal nodal size by CTTA measurements of tumour heterogeneity would simply represent a development of an established concept and may therefore be more readily adopted into clinical practice.

In summary, CTTA in NSCLC is related to nodal status in patients without distant metastases and has the potential to inform selection of investigative strategies for the assessment of mediastinal malignancy.

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FIGURES AND TABLES

Figure 1. A: Axial CT image from a patient with a left upper lobe NSCLC categorised as high risk by CTTA using the filtration-histogram approach. B: Tumour ROI after filtration to highlight image features of radius 4mm. All three CT texture parameters derived from the histogram of values in filtered ROI (C) indicated increased risk for nodal disease: 4.74, 0.73 and -0.09 for normalised SD, normalised E and kurtosis respectively. Maximum intensity projections (D) and fused axial images (E) from the patient's PET/CT examination, which showed no nodal disease. N2 disease was confirmed at surgery.

Figure 2. A: Axial CT image from a patient with a right upper lobe NSCLC categorised as low risk by CTTA using the filtration-histogram approach. B: Tumour ROI after filtration to highlight image features of radius 4mm. All three CT texture parameters derived from the histogram of values in the filtered ROI (C) indicated a reduced risk for nodal disease: 8.61, 0.85 and -0.86 for normalised SD, normalised E and kurtosis respectively. The largest

mediastinal node measured 11mm on CT (D), indicating a high risk of mediastinal malignancy according to NICE guidance. N0 disease was confirmed at surgery.

Figure 3: The survival curve for patients with less than two high-risk CTTA values (above) showed no deaths in the follow-up period, compared to five deaths for patients with 2 or more high-risk CTTA values (below; p=0.016).

Figure 4: Diagrammatic representation of how genetic instability can lead to genetically distinct subclones within a tumour, which express different phenotypes that lead to imaging heterogeneity. This in turn can be assessed quantitatively with CT Texture analysis

Table 1. Risk of advancing N-stage for patients categorised by the median texture values with mediastinal nodal size at CT as comparator.

Figure 1

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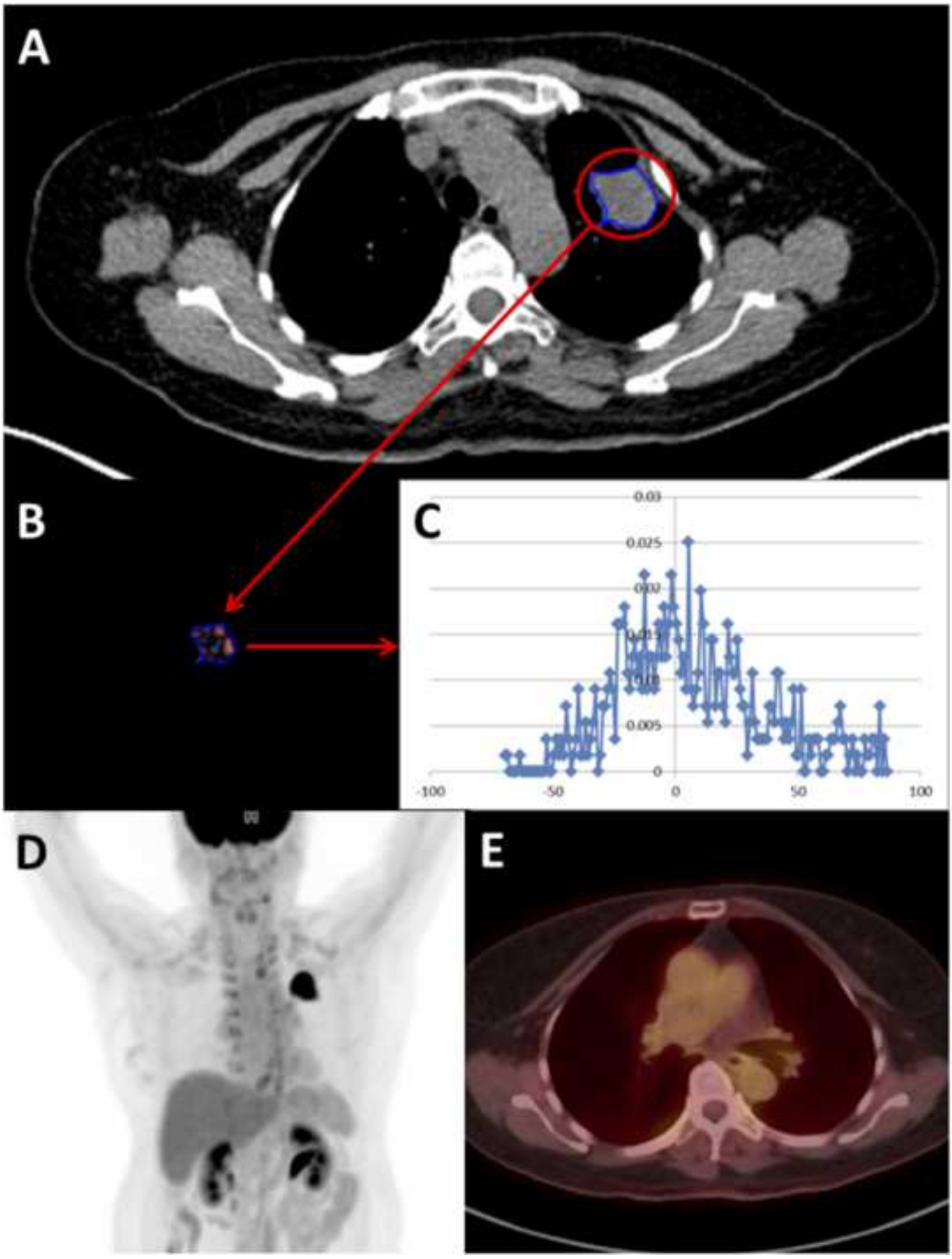

Figure 2

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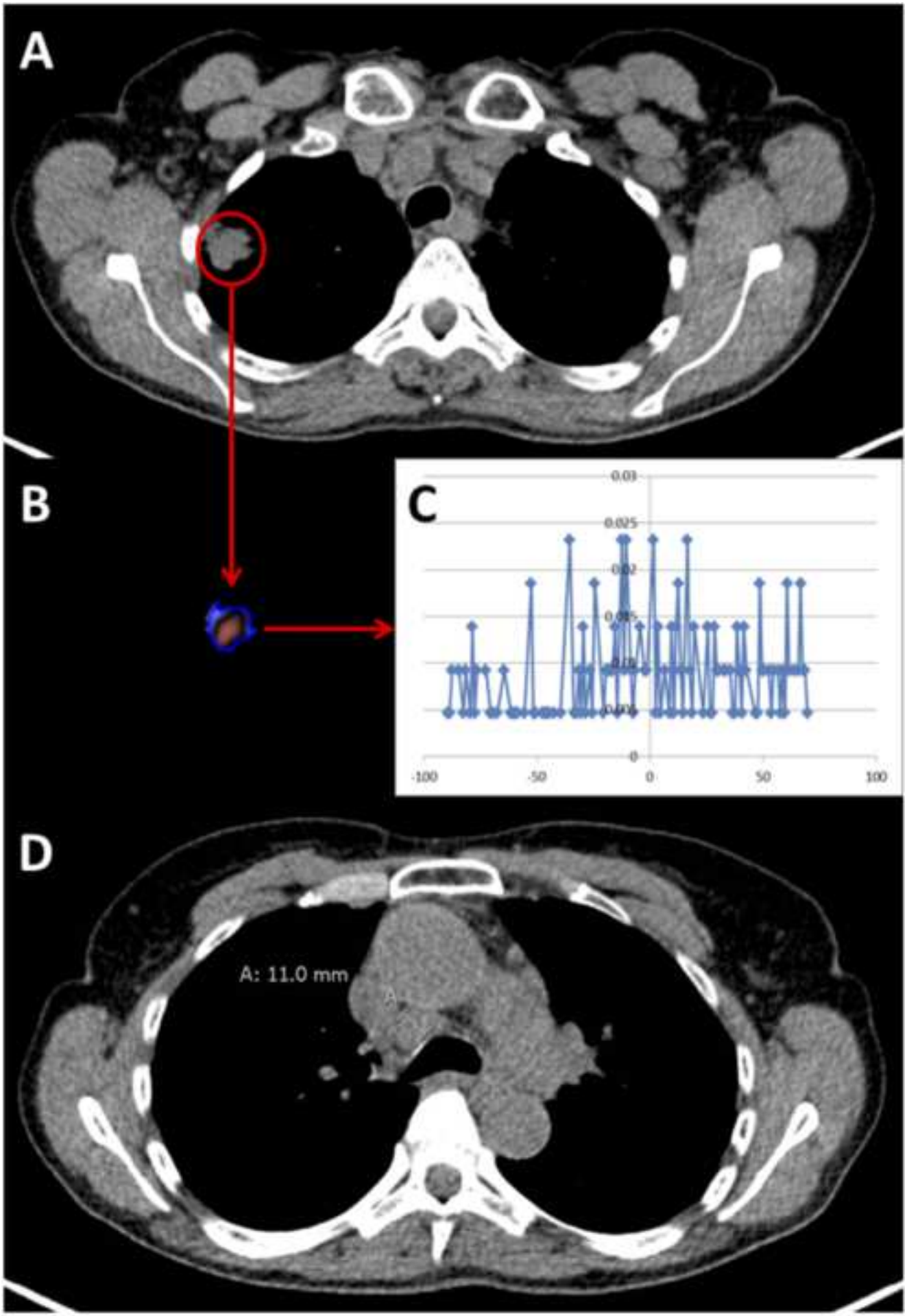

Figure 3

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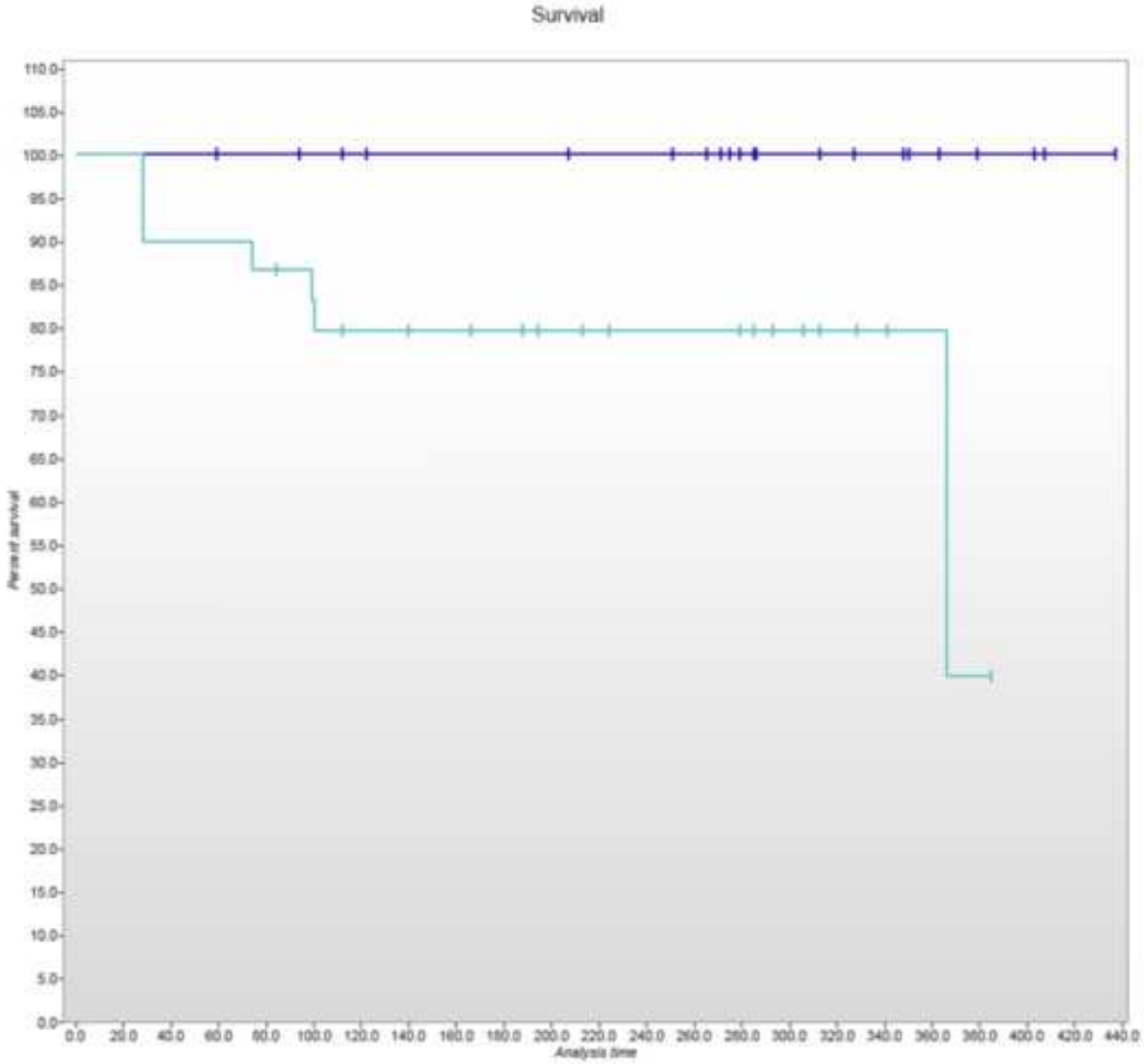

Figure 4

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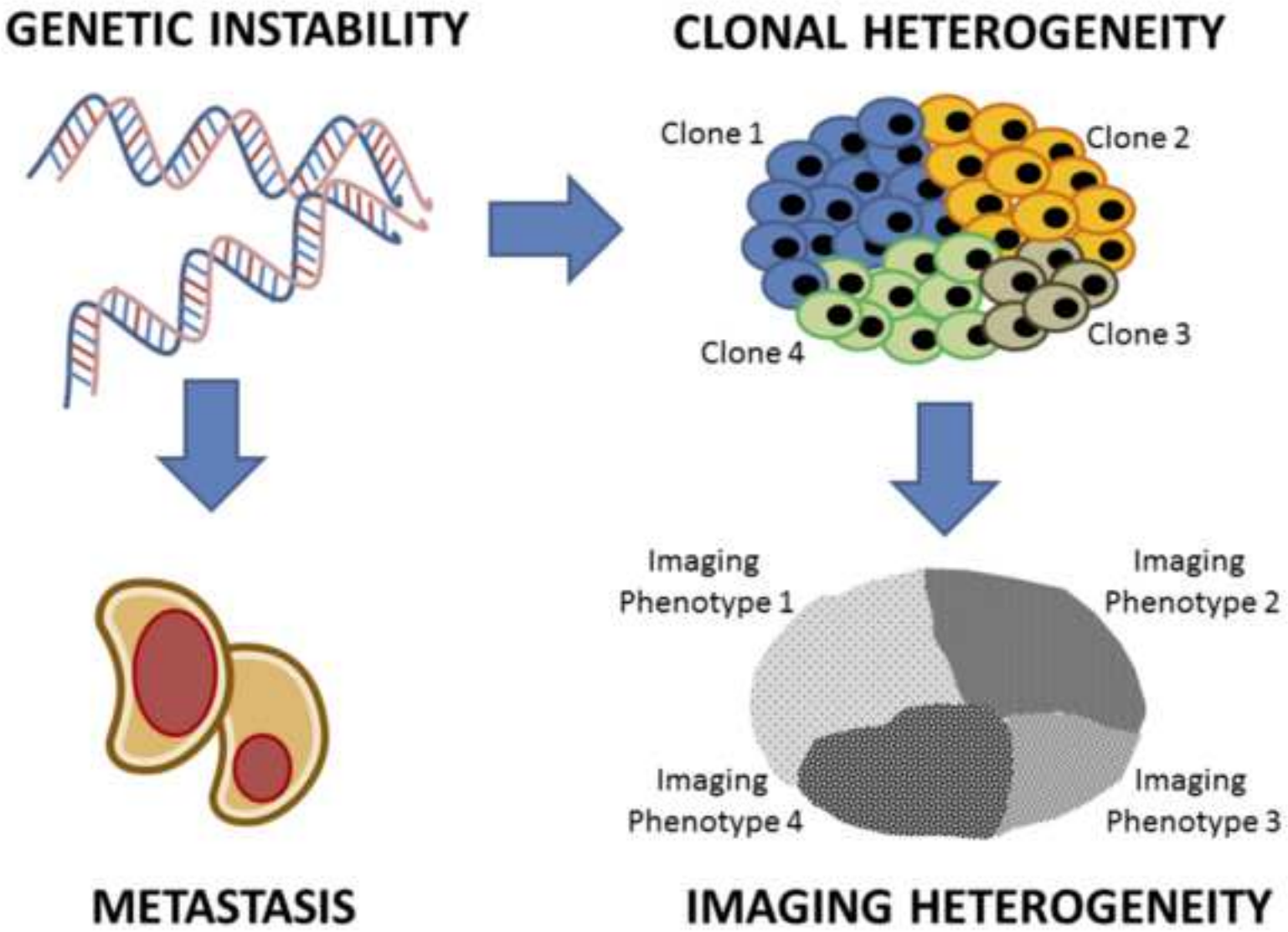

Table

Nodal Status	Parameter	High Risk	Low Risk	Risk Ratio (95% CI)	p-value (Fisher's test)
>0	Normalised SD	52.4%	23.8%	2.2 (95% CI 0.92-5.24)	0.11
	Normalised E	61.9%	14.3%	4.3 (95% CI 1.44 -13.0)	**0.0036**
	Kurtosis	57.1%	19.1%	3.0 (95% CI 1.15-7.80)	0.25
	Any 2 CTTA	61.9%	14.3%	4.3 (95% CI 1.44 -13.0)	**0.0036**
	Nodal size on CT	54.6%	32.3%	1.69(95% CI 0.80-3.55)	0.28
>1	Normalised SD	42.9%	14.3%	3.0 (95% 0.94-9.55)	0.086
	Normalised E	47.6%	9.5%	5.0 (95% 1.24-20.1)	**0.015**
	Kurtosis	47.6%	9.5%	5.0 (95% 1.24-20.1)	**0.015**
	Any 2 CTTA	52.4%	4.76%	11.0 (95% CI 1.56 - 77.8)	**0.0014**
	Nodal size on CT	54.6%	19.4%	2.82 (95% CI 1.15 – 6.9)	**0.049**
>2	Normalised SD	19.1%	0%	∞	0.11
	Normalised E	14.3%	4.8%	3.0 (95% 0.34-26.6)	0.61
	Kurtosis	19.1%	0%	∞	0.11
	Any 2 CTTA	19.1%	0%	∞	0.11
	Nodal size on CT	27.3%	3.2%	8.45 (95% CI 0.98 – 73)	**0.048**

HIGHLIGHTS

- CT texture analysis (CTTA) is emerging as a technique for derivation of prognostic biomarkers for patients with NSCLC
- CT texture analysis evaluates quantitatively tumour heterogeneity, which is linked to tumour aggression.
- CTTA in NSCLC is related to nodal status in patients without distal metastases and has potential to inform selection of investigative strategies for the assessment of mediastinal malignancy.