Could microcomputed tomography be a new method to distinguish between metastatic and non-metastatic lymph nodes in patients with non-small cell lung cancer as a decision support tool for pathological examination? A pilot study for method validation

Ayten KAYI CANGIR (cangir@medicine.ankara.edu.tr)
Ankara University, University Medical Design Application and Research Center (MEDITAM)

Kaan ORHAN
Ankara University

Süleyman Gökalp GÜNŞ
Ankara University Faculty of Medicine

Hilal ÖZAKINCI
Ankara University Faculty of Medicine

Yusuf KAHYA
Ankara University Faculty of Medicine

Duru KARASOY
Hacettepe University

Serpil SAK
Ankara University Faculty of Medicine

Research Article

Keywords: micro-CT, lung cancer, lymph node metastasis, diagnostic tool, histopathology

Posted Date: June 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3050121/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background:

Patients with non-small cell lung cancer (NSCLC) without lymph node (LN) metastases (pN0) have different survival rates even when the T status is similar. This may be because excised mediastinal and bronchial LNs are currently examined using a 2D method. Because, despite the rules of 2D pathological examination, unfortunately, not all of the removed LN can be sampled, and there may be metastatic foci in these remaining and unsampled LN tissues. Whereas, evaluation with micro-computed tomography (micro-CT) provides detailed information on internal structures of all these LNs as a whole and without damaging the sample.

We used quantitative micro-CT parameters to evaluate the metastasis status of LNs embedded in paraffin blocks.

Methods

Twelve paraffin blocks and the corresponding whole slide images from eight NSCLC patients with pathological mediastinal LN metastases were used. The formalin-fixed paraffin-embedded (FFPE) LN blocks were subjected to micro-CT. Forty-seven regions of interest (ROIs) (17 metastatic foci, 11 normal lymphoid tissues, 10 adipose tissues, and 9 anthracofibrotic areas) were marked. Quantitative structural variables obtained via micro-CT analysis from tumoral and non-tumoral ROIs were analyzed.

Results

Linear density, connectivity, connectivity density, and closed porosity all differed significantly between tumoral and non-tumoral ROIs (kappa coefficients: 1, 0.90, 1, and 1, respectively). Receiver operating characteristic analysis showed that tumoral and non-tumoral ROIs differed in terms of thickness, linear density, connectivity, connectivity density, and percentage of closed porosity.

Conclusions

Quantitative micro-CT parameters can distinguish between tumoral and non-tumoral areas in FFPE blocks of mediastinal LNs. These quantitative micro-CT parameters may facilitate the development of an artificial intelligence algorithm that can detect metastatic foci in the LN in FFPE LN blocks.

1. Background

Non-small cell lung cancer (NSCLC) is an aggressive malignancy, and lymph node (LN) metastasis of resected NSCLC is both an important negative prognostic factor and a determinant of appropriate adjuvant treatment [1]. LN metastases in NSCLC patients greatly affect adjuvant treatment planning and survival [2, 3, 4]. Accurate detection of LN status is critical. However, 30–40% of patients with stage I NSCLC experience postoperative local recurrence and distant metastasis despite histologically confirmed
curative resection and tumor-free LNs (pN0) [2, 5, 6]. Thus, long-term survival in a major subgroup of N0 patients remains unsatisfactory [5, 6]. This may be because of inadequate LN dissection or suboptimal histopathological nodal evaluation [2, 7, 8, 9].

There are two possible explanations for the latter. Micrometastases in haematoxylin and eosin (H&E)-stained sections may have been overlooked during histopathological examination, or a metastatic focus in the paraffin block may be absent in the H&E sections. Although serial sectioning of LN blocks followed by immunohistochemical examination might thus detect LN metastases more accurately, this is both time-consuming and costly [10].

Micro-computed tomography (micro-CT) uses cone-shaped beams for reconstruction and back-projection. The volumetric voxel size is almost 1 million-fold smaller than that of conventional CT, thus approximately 1–50 μm. Micro-CT has many applications given the good resolution and non-invasive nature of the technique [11]. The 3D images allow both quantitative and qualitative evaluation [12]. Micro-CT is non-destructive; further scans and other investigations are possible. Micro-CT is widely employed by bone and dental researchers. Bone biologists have enthusiastically adopted laboratory micro-CT systems because they afford 3D views of the architecture and mechanical competence of the trabecular and cortical bones of both clinical biopsy samples and animal models of disease [13]. In any research field, micro-CT non-destructively and directly yields 3D images [14, 15] with information on the internal structures of materials ranging from industrial equipment to human tissues. Micro-CT is a non-invasive ex-vivo imaging tool that reveals internal 3D structures of opaque samples at sub-micron resolution [16]. Although there is substantial literature on the use of micro-CT to characterize musculoskeletal tissues, especially in the field of dentistry, only a few studies have evaluated other human tissues [11]. Lung studies have commonly focused on small samples, both in vivo and ex vivo. When evaluating lung specimens ex vivo, micro-CT devices operate at very small scales with high radiation doses, yielding digital images with micrometer resolution, enabling the diagnosis of pulmonary diseases that manifest at the microanatomical scale [19, 20, 21]. Micro-CT exhibits many clinical applications across a wide spectrum of pulmonary pathologies, including chronic restrictive and obstructive lung diseases as well as lung cancer; comparative studies of the human lung are rare [22, 23, 24]. Apart from high-resolution images of hard tissue samples, histometric features of the visual microarchitecture can be converted into numerical data; structural variables that can be statistically analyzed are generated from the visual microarchitectural features of tissues.

After surgery, NSCLC patients lacking LN metastases (pN0) have different survival times, even when the T status is the same [2]. Apart from differences in the intrinsic biological characteristics of the tumors, in at least some cases, the variation may reflect the fact that the current histopathological methods cannot fully evaluate all LN tissues. Micro-CT yields 3D images of entire samples in paraffin blocks and numerical values for structural variables; areas within blocks that are not 2D histopathological sections are also evaluated. Thus, we sought differences in micro-CT images between LNs with and without metastases embedded in paraffin blocks and obtained the numerical parameters.
2. Materials and Methods

The study was approved by the Institutional Review Board of the University Faculty of Medicine [IRB no. 16-287-19]. Twelve formalin-fixed paraffin-embedded (FFPE) LN blocks from eight NSCLC patients obtained during routine pathology workup were used. All patients evidenced LN metastases and underwent surgery in the Department of Thoracic Surgery, Faculty of Medicine,... They did not receive neoadjuvant treatment. H&E-stained 4—5-m-thick sections from the 12 paraffin blocks were scanned using the Panoramic 250 Flash digital scanner (3DHISTECH, Budapest, Hungary). Forty-seven ROIs [17 metastatic foci, 11 normal lymphoid tissues, 10 adipose tissues, and 9 anthracofibrotic areas] were marked on the virtual slides by two pathologists. A different random color (red, yellow, green, blue, or grey) was assigned to each ROI on each slide to differentiate the ROIs [Figure 1].

2. 1. Micro-CT

After obtaining the sections, the cassettes were gently removed from the paraffin-embedded tissues using a knife. To ensure the compatibility of the micro-CT images and the virtual histopathological slides, the FFPE tissues were placed at right angles to the blocks using a specimen holder. A high-resolution desktop micro-CT system [Bruker Skyscan 1275, Kontich, Belgium] was used to scan tissues within the blocks. The scan parameters have been described previously [25]. Prior to scanning and reconstruction, the beam-hardening correction and optimal contrast limits were set according to the manufacturer’s instructions.

2. 2. Image analysis

NRecon (ver. 1.6.10.5, SkyScan) and CTAn (ver. 1.19.11.1, SkyScan) were used for visualization and quantitative measurements. We employed a modification of the algorithm described by Feldkamp et al. to obtain axial 2D 1,000 1,000-pixel images [26]. In terms of the reconstruction variables, the ring artifact correction and smoothing were set to zero and the beam artifact correction to 40%. NRecon was used to reconstruct 2D slices of the specimens. Cross-sectional images were reconstructed from the entire volume of each paraffin block. CTAn was employed for analysis. The reconstructed images were further processed by Skyscan CTVox (ver. 3.3.1) to allow visualization by referencing the H&E-stained sections. Guided by both the virtual slides and 3D micro-CT volumes, regions of interest [ROIs] were drawn to include different areas [Figure 1]. Forty-seven ROIs were colored on both the histopathological and micro-CT images. The evaluator of the micro-CT images (KO) was blinded to the nature of the ROIs.

When differentiating malignant from normal tissues, an appropriate threshold is required. To this end, original grayscale images were processed using a Gaussian low-pass filter for noise reduction employing a semi-automatic global threshold method. After thresholding [binarization], the images featured black and white pixels. Then, for each slice, an ROI that contained a single complete object was defined prior to the calculation of various parameters.
A radiologist with 15 years of experience with micro-CT (KO) performed all evaluations. The CTAn software was fully exploited when analyzing the 3D microarchitecture. We measured the percentage object volume, intersection surface, structural thickness, structural linear density, connectivity, connectivity density, and closed and open porosities; all were based on the volume of the ROI.

The percentage object volume relative to the total tissue volume is widely used by pathologists to measure object gain or loss, i.e., the fraction of a volume of interest occupied by tissue.

Structural thickness refers to both the thickness of all tissues and the mean thickness of different tissues. The intersection surface is that of tissue contact. The software measures both the total surface area of the ROI and the extent of the ROI surface intersected by binarized tissue. This allows tissue contact surfaces to be measured at virtual ROI boundaries.

The structural linear density is any value per unit length. The term "linear density" is commonly used when describing the characteristics of 1D objects, although it is also used to describe the density of a 3D quantity in one particular dimension. Just as density is commonly taken to mean mass density, linear density often refers to linear mass density.

Connectivity describes the topology of porous tissue.

Connectivity density is a measure of the extent of tissue connectivity normalized to the total tissue volume.

Several parameters, including the number, volume, total surface, and pore numbers and percentages, can be used to describe porosity. Pores are typically classified as closed or open. An open pore intersects with an ROI boundary and is thus connected to the outside in 2D or 3D; a closed pore does not intersect or connect. Closed pores are black pixels surrounded by a border of white pixels. We measured the percentages of closed and open pores over the entire specimen volume.

2.3. Statistical analysis

Data analyses were performed using IBM SPSS Statistics for Windows ver. 23. The normal distribution of data was assessed by the Shapiro-Wilk test. Two group comparisons [tumoral and non-tumoral ROIs] were performed using the independent t test or the Mann-Whitney U test, depending on whether the data was normally distributed or not. A p-value less than 0.05 is statistically significant. K-means clustering was employed to classify patients into four different clusters and then into two clusters [tumoral and non-tumoral ROIs] based on the relevant variables. The Cohen's Kappa coefficient was used to assess the results obtained from cluster analysis. The optimal cut-off values were detected by receiver operating characteristic (ROC) analysis.

3. Results
Each color represented a different ROI in each block to avoid bias; the data for the 47 ROIs are shown in Supplementary Table 1. Again to avoid bias, four different clusters were blindly created based on the variables subjected to cluster analysis, and the distributions within these clusters of tumor, lymphoid, anthracofibrotic, and adipose tissue areas were determined overall and for each variable (Table 1). Open porosity (100%) and structural linear density (94.1%) for tumor areas; connectivity (100%) and closed porosity (100%) for lymphoid tissues; intersection surface (100%), structural thickness (100%), structural linear density (100%), connectivity density (100%), and open porosity (100%) for anthracofibrotic areas; and structural thickness (100%) for adipose tissues were the parameters with the highest accuracies.

When all variables were included among the four clusters, lymphoid tissue had the highest accuracy and tumor areas the lowest (Table 2). To identify the variables with high accuracy in detecting target areas, eight subgroups were formed for the seven variables in Table 2, and the analyses were repeated. In the subgroup analyses, closed and open porosities best identified tumoral and anthracofibrotic areas, while connectivity and closed porosity best identified lymphoid and adipose areas (Table 2).

### Table 1
Detection percentage of tumor, lymphoid, anthracofibrotic and adipose tissue areas in four different clusters created by K-means clustering.

<table>
<thead>
<tr>
<th></th>
<th>Tumour area (%)</th>
<th>Lymphoid tissue area (%)</th>
<th>Anthracofibrotic area (%)</th>
<th>Adipose tissue area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Intersection surface</td>
<td>82.4</td>
<td>54.5</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>2. Structural thickness</td>
<td>64.7</td>
<td>81.8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3. Structural linear density</td>
<td>94.1</td>
<td>81.8</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>4. Connectivity</td>
<td>58.8</td>
<td>100</td>
<td>88.9</td>
<td>90</td>
</tr>
<tr>
<td>5. Connectivity density</td>
<td>52.9</td>
<td>63.6</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>6. Closed porosity (%)</td>
<td>88.2</td>
<td>100</td>
<td>88.9</td>
<td>80</td>
</tr>
<tr>
<td>7. Open porosity (%)</td>
<td>100</td>
<td>72.7</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100%)</td>
<td>11 (100%)</td>
<td>9 (100%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>
Table 2

For eight subgroups, detection percentage of tumor, lymphoid, anthracofibrotic and adipose tissue areas in four different clusters created by K-means clustering.

<table>
<thead>
<tr>
<th>Lymph node</th>
<th>Set A (%)</th>
<th>Set B (%)</th>
<th>Set C (%)</th>
<th>Set D (%)</th>
<th>Set E (%)</th>
<th>Set F (%)</th>
<th>Set G (%)</th>
<th>Set H (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)(^1)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
<td>(1 + 2 + 3 + 4 + 5 + 6 + 7)</td>
</tr>
<tr>
<td>Tumour area</td>
<td>58.8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>58.8</td>
<td>58.8</td>
<td>58.8</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Lymphoid tissue area</td>
<td>100</td>
<td>72.7</td>
<td>72.7</td>
<td>72.7</td>
<td>72.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Anthracofibrotic area</td>
<td>88.9</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>88.9</td>
<td>88.9</td>
<td>88.9</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Adipose tissue area</td>
<td>90</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

\(^1\) 1: Intersection surface, 2: Structural thickness, 3: Structural linear density, 4: Connectivity, 5: Connectivity density, 6: Closed porosity (%), 7: Open porosity (%)

As our main purpose was to identify tumor areas in LNs, using data from 37 ROIs in the Supplementary Table, k-means clustering analyses were performed by dividing these ROIs into two groups: tumor (n = 17) and non-tumor areas (lymphoid areas = 11 and anthracofibrotic areas = 9) (Table 3). ROC analysis revealed that tumor and non-tumor ROIs were successfully differentiated by the structural linear density, connectivity, connectivity density, and percentage of closed porosity (kappa coefficients: 1, 0.90, 1, and 1, respectively). ROC analysis also revealed that ROIs with a structural linear density 1.67 (sensitivity 1, specificity 1), connectivity 1990 (sensitivity 1, specificity 1), connectivity density 48.22 (sensitivity 1, specificity 1), and/or closed porosity 4.29% (sensitivity 1, specificity 1) were tumor areas (Suppl.Table 1).
Table 3
Results of k-means clustering of tumoural and non-tumoural areas, descriptive statistics and p value.

<table>
<thead>
<tr>
<th>Intersection surface n (%)</th>
<th>Tumour area (n = 17)</th>
<th>Lymphoid tissue + anthracofibrotic area (n = 20)</th>
<th>Kappa coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>10 (50%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Lymphoid tissue + anthracofibrotic area</td>
<td>0</td>
<td>10 (50%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>35.12 (2.8)</td>
<td>47.71 (18.82)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.715</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structural thickness n (%)</th>
<th>Tumour area (n = 17)</th>
<th>Lymphoid tissue + anthracofibrotic area (n = 20)</th>
<th>Kappa coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>10 (50%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Lymphoid tissue + anthracofibrotic area</td>
<td>0</td>
<td>10 (50%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.84 (0.08)</td>
<td>0.56 (0.17)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structural linear density n (%)</th>
<th>Tumour area (n = 17)</th>
<th>Lymphoid tissue + anthracofibrotic area (n = 20)</th>
<th>Kappa coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoid tissue + anthracofibrotic area</td>
<td>0</td>
<td>20 (100%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.99 (0.06)</td>
<td>1.35 (0.09)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Connectivity n (%)</th>
<th>Tumour area (n = 17)</th>
<th>Lymphoid tissue + anthracofibrotic area (n = 20)</th>
<th>Kappa coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>2 (10%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Lymphoid tissue + anthracofibrotic area</td>
<td>0</td>
<td>18 (90%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>2600.9 (350.8)</td>
<td>872.3 (556.8)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Connectivity density n (%)</th>
<th>Tumour area (n = 17)</th>
<th>Lymphoid tissue + anthracofibrotic area (n = 20)</th>
<th>Kappa coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoid tissue + anthracofibrotic area</td>
<td>0</td>
<td>20 (100%)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>65.39 (2.88)</td>
<td>31.36 (3.27)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Closed porosity n (%)</th>
<th>Tumour area (n = 17)</th>
<th>Lymphoid tissue + anthracofibrotic area (n = 20)</th>
<th>Kappa coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoid tissue + anthracofibrotic area</td>
<td>0</td>
<td>20 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumour area (n = 17)</td>
<td>Lymphoid tissue + anthracofibrotic area (n = 20)</td>
<td>Kappa coefficients</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.55 (0.41)</td>
<td>2.22 (0.79)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open porosity n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour area</td>
<td>17 (100%)</td>
<td>11 (55%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Lymphoid tissue +</td>
<td>0</td>
<td>9 (45%)</td>
<td></td>
</tr>
<tr>
<td>anthracofibrotic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>21.18 (1.29)</td>
<td>52.51 (17.92)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

Although NSCLC is fatal, long-term survival is possible for early-stage patients who undergo surgery. Those lacking LN metastasis (pN0) are expected to have better survival rates. Adjuvant therapy is generally not recommended for patients with a tumor diameter of 4 cm and pN0 status. pN1 or pN2 status predicts poor survival and is an indication for adjuvant systemic therapy. Over the last few decades, targeted therapies and immunotherapies have improved long-term survival. Thus, factors determining the decision to commence adjuvant systemic treatment, especially LN metastasis, have become even more important.

Systematic LN dissection is one of the most important components of lung cancer surgery. The LN stations of the lung cancer staging system are evaluated histopathologically. Typically, LNs are sliced to a thickness of approximately 2 mm, macroscopically examined for metastases, and fully embedded in paraffin blocks. 2D H&E-stained sections from the surfaces of the blocks are used to detect metastases. Although macrometastases are very obvious, it is possible to miss micrometastases lying deep in the tissue. This may partially explain why N0 cases with a similar T status have different survival times. In other words, some N0 cases may have metastatic foci in tissues embedded within the paraffin block and thus be missed by the pathologist. Some pN0 cases may be pN1 or even pN2.

Although it is theoretically possible to examine the entire paraffin block using serial sections, this is very expensive and time-consuming. Recently, 3D reconstructions of whole-slide histological data have revealed new diagnostic patterns given the improved correlations between imaging modalities [24]. However, it remains necessary to prepare at least 100–200 serial, glass slide-mounted tissue sections [24]. Until recently, a non-destructive method for evaluating an entire LN in a paraffin block did not exist. Today, micro-CT reveals the internal structures of materials ranging from industrial equipment to human tissues. Micro-CT is a non-invasive ex-vivo imaging tool that reveals the internal 3D structures of opaque samples at sub-micron resolution. Although there is a substantial literature on the use of micro-CT to
characterize musculoskeletal tissues, especially in the field of dentistry, only a few reports have employed micro-CT to evaluate other human tissues [14, 25]. Micro-CT yields high-resolution 3D images along any direction within the volume of a specimen without sectioning of soft or calcified tissues and thus facilitates a better understanding of disease [26, 27]. However, studies on various tissues have reported very different correlations between micro-CT and histopathological data [28, 29–30]. As histomorphometric measurements are 2D and micro-CT assessments are 3D, differences are to be expected.

Micro-CT not only yields high-resolution tissue images; the histometric properties of the visual microarchitecture can be converted into numerical data that can be statistically analyzed. The micro-CT morphological measurements are highly correlated with histomorphometric data that serve as the gold standard when evaluating bone microarchitecture [31]. Statistically analyzed the differences in quantitative values obtained from tumoral versus non-tumoral areas. Our study highlights the capacity of micro-CT imaging to complement—and in terms of specific diagnostic questions [tumor volume and margin], potentially even replace—slide microscopic imaging to guide clinical decisions. Although the approach differs greatly from conventional histopathological evaluation, more innovations and developments are to be expected. Radiomics and artificial intelligence applications are increasingly being used to support medical decisions. The numerical data obtained via micro-CT examination may take visual histopathological evaluation to a level at which artificial intelligence algorithms can be employed. The success of machine learning (ML) algorithms in terms of automating tasks that are not analytically well-defined indicates that such methods engage in automated feature extraction and are thus better than computer vision-based approaches. In the future, after ground truth-based training, an ML algorithm may be able to automatically extract radiomic features that identify lung cancer and metastases in micro-CT images. We identified certain numerical parameters that may serve to train ML models. As the precise geometry, position, and orientation of each feature are known in advance, such an approach may be significantly better than manual data labeling in terms of accuracy and robustness. Ultimately, micro-CT may be capable of automatically detecting NSCLC metastases.

5. Conclusions

Micro-CT was found to be very useful and non-destructive for analyzing FFPE tissue, including LN metastases. We found that the quantitative structural variables of micro-CT distinguished tumoral and non-tumoral areas in FFPE tissue blocks of mediastinal LNs. This will allow the creation of an artificial intelligence algorithm. However, studies with larger sample sizes are necessary.

Abbreviations

CT
Computed tomography
NSCLC
Non-small cell lung cancer
MLN
Mediastinal lymph nodes
LN
Lymph Node
Micro-CT
Micro computed tomography
2D
Two dimensional
3D
Three dimensional
HE
Hematoxylin and eosin
FFPE
Formalin fixed and paraffin embedded
ROI
Region of interest
OV/TV
Object volume
TV
Tissue volume
ST
Structural thickness
IS
Intersection surface
SMI
Structure model index
SLD
Structure linear density
Cn
Connectivity
CnD
Connectivity density

**Declarations**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ayten KAYI CANGIR

No conflict of interest
Kaan ORHAN
Süleymen Gökalp GÜNEŞ
Hilal ÖZAKINCI
Yusuf KAHYA
Duru KARASOY
Serpil Dizbay Sak

No conflict of interest

References


Figures

Figure 1
Whole slide images of three adenocarcinoma cases and the regions of interest [ROIs] that were evaluated with micro-CT for structural parameters [a,b,c]. ROI-Cs,[white circles] to represent carcinoma areas; ROI-Ns [blue circles] to represent non-tumoral pulmonary parenchyma within the same paraffin blocks. Insets show three of the ROI-Cs [HE; original magnification WSIs: 0.4x, insets:10x].