Original Article

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Quantitative Computed Tomography Analyses of Hypothyroidism Induced by PD-1 and PD-L1 Inhibitors

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Abstract

Objective: Anti-programmed cell death ligand 1 (PD-L1) and anti-programmed cell death 1 (PD-1) therapy in cancer treatment cause immune-related adverse events (irAEs), such as hypothyroidism. We investigated the potential of quantitative computed tomography (CT) for early diagnosis and severity assessment of overt hypothyroidism induced by PD-L1/PD-1 treatment.

Methods: Sixty-six patients were enrolled in this study. Patients were divided into two groups: those who developed immune checkpoint inhibitor (ICI) -related hypothyroidism (n=32) and those without thyroid-irAEs (n=34). Using non-contrast CT images, the mean attenuation value of a polygonal region-of-interest delineating the right and left lobes of the thyroid gland was obtained. Longitudinal changes in CT attenuation values of the thyroid gland before and after ICI administration were calculated. Using non-contrast and contrast CT images, the maximum width, length, and anterior−posterior diameter pre- and post-ICI administration were measured to approximate thyroid volume and assess atrophy. Thyrotropin (TSH) levels and levothyroxine doses were analyzed.

Results: The CT attenuation values were significantly decreased in the thyroid-irAE group during or before the TSH levels increase ($p<0.0001$). CT attenuation values decreased 43 days before TSH levels increased. High TSH levels and levothyroxine doses were associated with atrophy ($r=-0.69$, $p<0.0001$ and $r=-0.52$, $p=0.01$, respectively).

Conclusion: Thyroid CT attenuation values decrease before TSH levels increase in overt hypothyroidism induced by ICIs, which may facilitate early diagnosis of hypothyroidism. Maximum width, length, and anteroposterior diameter measurement can reliably detect thyroid atrophy, which is associated with hypothyroidism severity.

Key words: Anti-programmed cell death 1 (PD-1), anti- programmed cell death ligand 1 (PD-L1), hypothyroidism, immune-related adverse events (irAEs)

Introduction

Over the past decade, immune checkpoint inhibitors (ICIs) have markedly improved clinical outcomes in various types of cancer. ICIs activate anti-tumor immune responses by blocking checkpoint proteins. Currently, three main types of ICIs are used in clinical settings: anti-programmed cell death ligand 1 (PD-L1), anti-programmed cell death 1 (PD-1), and anti-cytotoxic T-lymphocyte-associated antigen (CTLA) -4 treatment.

Nevertheless, ICIs can cause endocrine immune-related adverse events (irAEs), with thyroid dysfunction being one of the most common. Thyroid

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dysfunction, such as thyrotoxicosis and hypothyroidism, is a known thyroid-irAE. Painless thyroiditis with transient thyrotoxicosis often precedes hypothyroidism and occurs within 2 weeks to 3 months of the first dose of ICIs. Some patients without prior thyrotoxicosis develop hypothyroidism after ICI treatment[1, 2].

Overt hypothyroidism is characterized by a high level of thyroid-stimulating hormone (TSH) with a low level of serum free thyroxine (FT4) and free triiodothyronine (FT3) or a high level of TSH with symptoms and is treated with levothyroxine (a synthetic T4 hormone). Subclinical hypothyroidism is characterized by high TSH levels with normal serum FT4 levels. In an observational study, the population with subclinical thyroid dysfunction included a high proportion of cases of thyroid-irAEs, and the incidence rate of hypothyroidism varied according to whether subclinical thyroid dysfunction was present (5—50%)[3].

Many patients with transient thyrotoxicosis or thyroid-irAEs are asymptomatic and show mild symptoms. Therefore, patients are diagnosed using routine blood tests. Currently, routine blood tests are the only early diagnostic method for hypothyroidism after transient thyrotoxicosis. If regular blood test intervals are insufficiently short, diagnosis of thyroid-irAEs may be delayed.

Previous studies have reported decreased computed tomography (CT) attenuation values and thyroid gland atrophy in patients with ICI-related hypothyroidism[4]. Previous studies have reported that T cells (particularly cytotoxic $CD4^+$ memory T cells) activated by ICIs could mediate cellular immunity and induce thyroid dysfunction. Follicular cells show high levels of PD-L1 expression; blockade of the PD-1/ PD-L1 axis blunts the protection against immune-mediated thyroid destruction. When thyroid follicular cells are destroyed, loss of iodine concentration within the thyroid glands occurs and leads to decreased CT attenuation values of the thyroid glands[1, 5]. Patients who have low levels of thyroid autoimmunity at baseline are more likely to develop thyroid irAEs. However, no studies have addressed whether CT attenuation values decrease during the initial phase of hypothyroidism. Additionally, whether levothyroxine treatment affects CT attenuation values has not been determined.

Similarly, CT methods for measuring of thyroid gland atrophy have been reported; however, these methods are difficult to perform using non-contrast CT images and cannot be used in clinical practice. Measuring the maximum width, length, and anteroposterior diameter of thyroid glands to calculate the estimated volume is convenient and widely used in ultrasonographic examination in clinical practice. The inter-rater reliability of calculating the estimated volume using 2D ultrasonography is lower than that using 3D ultrasonography. Whether CT is a reliable method for evaluating these measurements to assess thyroid gland atrophy has not been determined. In addition, the patient factors associated with thyroid gland atrophy remain unclear.

To aid the diagnosis of overt hypothyroidism in the early stages, we investigated whether the CT attenuation values of the thyroid glands decrease before TSH levels increase in patients treated with ICIs. Additionally, we evaluated whether CT attenuation values returned to their original level after administration of levothyroxine treatment in these patients. Moreover, we tested whether thyroid atrophy is associated with disease severity.

Materials and Methods

Subjects

This retrospective observational study was approved by the St. Marianna University School of Medicine Ethics Committee (approval number: 5890), which waived the requirement for obtaining informed consent. The inclusion criteria were as follows: 1) Patients with cancer treated with PD-1 or PD-L1 inhibitors; 2) patients who underwent CT before and after ICIs administration at our institution between 2004 and 2022; 3) patients monitored regularly for side effects, mostly using a check sheet for ICIs. Medical staff (such as physicians, nurses, and pharmacists) filled in the check sheet. The contents were: Vital signs (heart rate, body temperature, blood pressure, and oxygen saturation) and performance status and weight. The contents also comprised: Symptoms (cough, shortness of breath, abnormal breath sound, palpitations, hyperhidrosis, headache, dizziness, dry mouth, polyuria, polydipsia, fatigue, depression, tenesmus, constipation, diarrhea, nausea, vomiting, abdominal pain, hematochezia, jaundice, pruritus, rash, redness, nerve pain, numbness, weakness, decreased urine output, edema, vitiligo, photophobia, blurred vision, and defect in vision). Chest X-ray or chest CT and laboratory examinations (sodium, serum FT3, FT4, TSH, TPOAb, TgAb, cortisol, adrenocorticotropic hormone, glucose, hemoglobin A1c, sialylated carbohydrate antigen KL-6, pulmo-

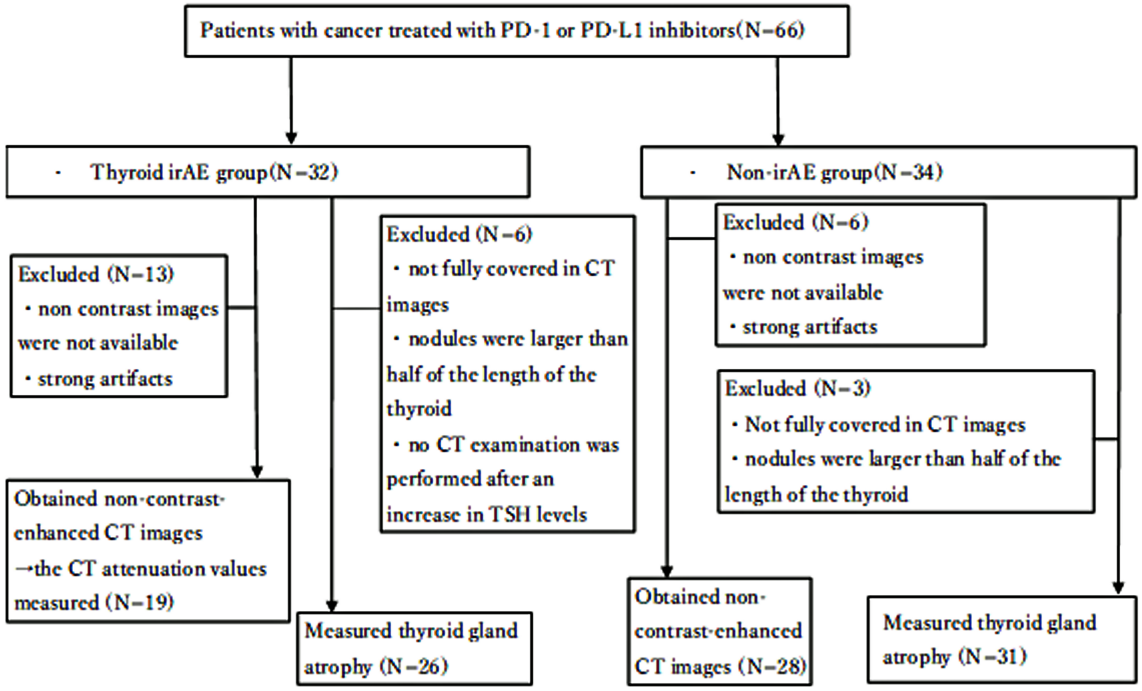


Figure 1. Flow diagram for inclusion and exclusion of studies

nary surfactant protein-D, total bilirubin, creatine kinase, anti-nuclear antibody, and eosinophil) were among the contents. The contents also included testing for hepatitis B and C infection; urine glucose and urinary ketone test; electrocardiography; and referral to other departments.

Sixty-six patients were enrolled in this study. The patients were divided into two groups: those who developed ICI-related thyroid dysfunction (thyroid irAE group, n=32) and those without thyroid-irAEs (non-irAE group, n=34). Non-contrast-enhanced CT images were obtained to measure the CT attenuation values of the thyroid glands from 19 patients in the thyroid-irAE group and 28 patients in the non-irAE group. Thirteen patients from the thyroid-irAE group and six patients from the non-irAE group were excluded for the following reasons: 1) non-contrast images were not available and 2) strong artifacts accounted for more than two-thirds of the thyroid gland on CT images. Thyroid gland atrophy was measured in 26 and 31 patients in the irAE and non-irAE groups, respectively. Six patients in the thyroid-irAE group and three in the non-irAE group were excluded for the following reasons: 1) thyroid glands were not fully covered in CT images, 2) thyroid nodules were larger than half of the length of the thyroid gland, or 3) no CT examination was performed after an increase in TSH levels in the thyroid-irAE group (**Figure 1**).

The definition of the reference standard for thyroid-irAE

Thyroid-irAEs are typically diagnosed by endocrinologists based on many factors, such as anti-thyroid peroxidase antibody (TPOAb) and/or anti-thyroglobulin antibody (TgAb) positivity, correlation with the clinical course of destructive thyroiditis, low free T3/T4 ratio (e.g., <3), and ultrasound diagnosis (decreased blood flow, low echogenicity, and evidence of other diseases) (e.g., Hashimoto's thyroiditis and Graves' disease).

CT scanning

CT examinations were performed using a 64-, 80-, or 320-detector CT scanner (Aquilion-64, Aquilion PRIME, Aquilion One, CANON MEDICAL SYSTEMS CORPORATION, Tokyo, Japan) with the patient in the supine position. Non-ionic contrast material was administered via the antecubital vein using an automatic power injector. Patients received 500 mg I/kg iodine. Contrast medium was injected at a rate of 1.2—2.6 mL/s. The multidetector CT imaging

parameters were as follows: 120 kV; collimation, 0.5 mm; and gantry rotation time, 0.5 s. Most of the images were reconstructed using a standard algorithm with a 1 mm thickness.

Quantitative CT measurements of the longitudinal changes in CT attenuation values of the thyroid gland

Non-contrast CT images obtained before administration of ICIs (proximate to the start date of administration) were transferred to a workstation (ZIO STATION 2 plus, Ziosoft, Inc., Tokyo, Japan). The axial, sagittal, and coronal images were automatically reconstructed with a slice thickness of 5 mm. The presence of artifacts and large thyroid nodules was evaluated. One axial image with a slice thickness of 1 mm (or 5 mm in the absence of slice thicknesses of 1 mm) that was not affected by artifacts and nodules was selected. The distance from the bottom edge of the thyroid to the axial image was measured to determine and select the same location on the CT images examined after using ICIs.

A polygonal region-of-interest (ROI) delineating the right and left lobes of the thyroid was measured and the mean attenuation value of the thyroid gland was obtained (**Figure 2**). Longitudinal changes in the CT attenuation values of the thyroid gland were calculated using the following formula: thyroid mean attenuation value=(right lobe mean attenuation value+ left lobe mean attenuation value)/2, Difference (Delta)=thyroid mean attenuation value after using ICIs-thyroid mean attenuation value before using ICIs. These values were measured independently by two radiologists (TH and MS) who were blinded to the clinical information. The mean values measured by the two radiologists were used for statistical analysis.

CT measurements of thyroid gland atrophy

Non-contrast and contrast-enhanced CT images were transferred to a workstation (ZIO STATION 2 Plus; Ziosoft, Inc). The axial, sagittal, and coronal images were automatically reconstructed with a slice thickness of 5 mm. A longitudinal axis view, which refers to the direction parallel to the thyroid length in the coronal view, was created using multiplanar reconstruction (MPR) techniques. The thyroid gland was fully covered in all scans.

The maximum width, length, and anterior—posterior diameter were measured to approximate the thyroid volume using ultrasonographic assessment methods. The maximum width was assessed in the axial view, length was assessed in the coronal view, and anterior—posterior diameter was assessed in the longitudinal axis view (**Figure 3**). The approximate volume of each lobe was calculated using the following formula: V (ml)=Maximum width (cm)×length×anterior—posterior diameter×π/6. Thyroid gland atrophy was calculated using the following formula: Atrophy=Approximate volume of the right+left lobes determined after using ICIs/approximate volume of the right and left lobes determined before using ICIs.

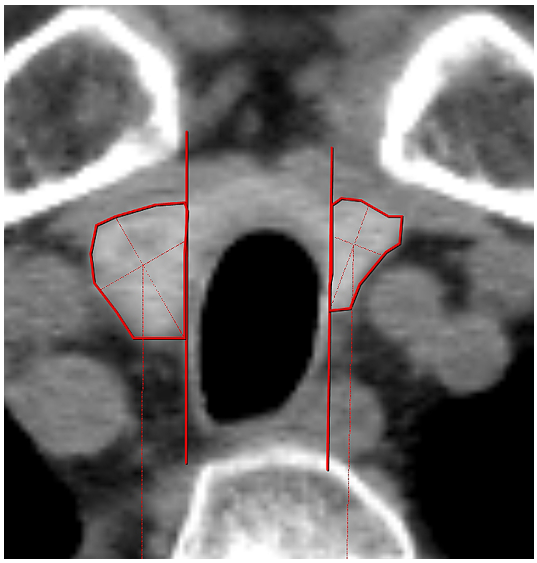


Figure 2. Measurements of thyroid computed tomography attenuation values
A polygonal region-of-interest delineating the right and left lobes of the thyroid was measured. The boundary between the thyroid isthmus and the right/left lobe was defined as the tracheal wall.

MPR techniques were used with non-contrast CT images, and contrast-enhanced CT images of the same patients were referenced to avoid a misalignment of the top of the thyroid and to distinguish the superior thyroid arteries. The values were confirmed independently by two radiologists (TH and MS) who were blinded to the clinical information. The mean values obtained by the two radiologists were used for statistical analysis.

Laboratory examinations

Serum FT3, FT4, and TSH levels were measured using a chemiluminescent immunoassay (CLIA)

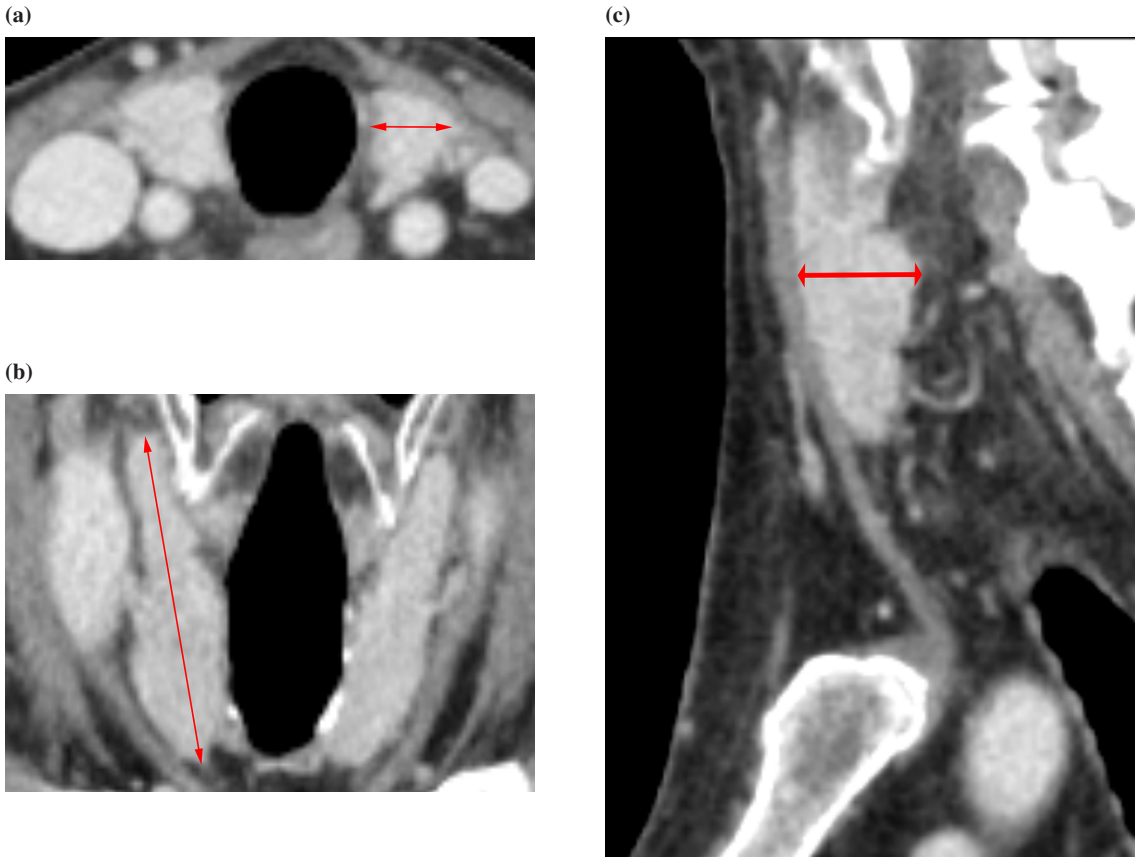


Figure 3. Computed tomography measurements of thyroid gland atrophy
(a) Maximum width was assessed on the axial view, (b) Maximum length was assessed on the coronal view, (c) Maximum antero-posterior diameter was assessed on the longitudinal axis view.

(Roche Diagnostics, Mannheim, Germany; or Fujirebio Inc., Tokyo, Japan). The reference intervals were 2.39—4.06 pg/mL, 0.76—1.65 ng/dL, and 0.61—4.23 μIU/mL, respectively. TPOAb and TgAb were measured using a CLIA (Abbott Japan LLC, Tokyo, Japan). The reference ranges were<5.61 IU/L and<4.11 IU/L, respectively. When TPOAb or TgAb was measured before and after ICI treatment, the values measured after ICI treatment were used to calculate the patient's mean value. When the TPOAb value was over 2000, the mean value was set to 2000.

Statistical Analysis

Mann-Whitney U tests were used to compare the two groups. Spearman's rank correlation coefficient was used for multivariate analyses. All statistical analyses were performed using JMP Pro 15.1.0 (SAS Institute, Cary, NC, USA). Statistical significance was set at P<0.05.

Results

Of the 32 patients with thyroid-irAEs in this study, 23 patients (71.9%) were treated with PD-1 inhibitors (nivolumab 43.8%, pembrolizumab 28.1%), and 9 patients (28.1%) were treated with PD-L1 inhibitors (atezolizumab 12.5%, avelumab 3.1%, durvalumab 12.5%) (**Table 1**). Of these patients, 75% (n=24) were TPOAb- and/or TgAb-positive before and/or after ICI treatment. A history of Hashimoto's thyroiditis or Graves' disease was suggested as the cause of TPOAb- and/or TgAb-positivity in three patients. Except for one patient with subclinical hypothyroidism, the remaining 31 patients with thyroid-irAEs had overt hypothyroidism. Levothyroxine was used in 90.6% of patients with thyroid-irAEs (n=29). The stages of cancer and baseline laboratory examinations related to nutritional condition did not significantly differ between the two groups. The me-

Table 1. Baseline Patient Characteristics

	Thyroid-irAE group	Non-irAE group
Number of patients	**32**	**34**
Gender		
Female	**10 (31.3%)**	**6 (17.6%)**
Male	**22 (68.8%)**	**28 (82.4%)**
Age at the initiation of ICI treatment, mean (SD)	**68.1±10.5**	**71.2±10.4**
Cancer types	**Lung cancer 18 (56.3%) Gastric cancer 4 (12.5%) Others total 10(All 1): Malignant pleural mesothelioma, Uterine cancer, Malignant lymphoma, Hypopharyngeal cancer, Hypopharyngeal + Esophageal+ Tongue cancer, HCC, Renal cancer, Renal pelvis cancer, Malignant melanoma, Breast cancer (31.3%)**	**Lung cancer 22 (64.7%) Renal cancer 2 (5.9%) Bladder cancer 2 (5.9%) Others total 8 (All 1): Malignant pleural mesothelioma, Gum cancer, Nasopharyngeal cancer, Cancer of unknown primary, Esophageal cancer, HCC, Renal pelvis cancer, Malignant melanoma (23.5%)**
Stage		
III	**7 (21.9%)**	**8 (23.5%)**
IV	**25 (78.1%)**	**26 (76.5%)**
Types of ICI		
PD-1 inhibitors	**Total 23 (71.9%) (Nivolumab 14, Pembrolizumab 9)**	**Total 19 (55.9%) (Nivolumab 7, Pembrolizumab 12)**
PD-L1 inhibitors	**Total 9 (28.1%) (Atezolizumab 4, Avelumab 1,**	**Total 15 (44.1%) (Atezolizumab 10, Avelumab 2,**

dian time between ICI treatment and the last follow-up CT scans was not significantly different between the two groups (thyroid-irAE group, 351 days versus non-irAE group, 353 days). The median interval between the CT and laboratory examinations was also not significantly different between the two groups (thyroid-irAE group, 2 days versus non-irAE group, 2 days) (**Table 1**).

Longitudinal changes of CT attenuation values in the thyroid gland before and after PD-1 and PD-L1 inhibitor treatment

In the thyroid-irAE group, the mean CT attenuation values before and after ICI were 100.2±20.1 and 59.3±23.2 HU, respectively. In the non-irAE group, the mean CT attenuation values before and after ICI were 93.7±13.8 and 94.9±14.2 HU, respectively. The mean of the differences in the thyroid-irAE group

	Durvalumab 4)	Durvalumab 3)
Antibody		
Both TPOAb and TgAb negative	**8 (25.0%)**	**32 (94.1%)**
TPOAb and/or TgAb positive	**24 (75.0%) (Hashimoto's thyroiditis 2, Graves' disease 1)**	**2 (5.9%) (Hashimoto's thyroiditis 1)**
Time between pre-ICI CT scans and ICI treatment, median (IQR)	**22 days (8-53)**	**40 days (16-91)**
Time between ICI treatment and last follow up CT scans, median (IQR)	**351 days (238-482)**	**353 days (177-535)**
Interval between the CT and laboratory examinations, median (IQR)	**2 days (0-8)**	**2 days (0-11)**
Frequency of laboratory examinations between ICI administration start date and the day TSH level increased, median (IQR)	**5 times (4-7)**	
Baseline Laboratory examinations related to Nutritional Condition, mean (SD)		
White blood cell count (/μL)	**5250±2023**	**6194±1864**
Red blood cell count (10^6/μL)	**3.72±0.66**	**3.83±0.70**
Hemoglobin (g/dL)	**11.6±1.97**	**11.9±2.08**
Platelet count (10^3/μL)	**263±117**	**271±119**
Total protein (g/dL)	**6.89±0.61**	**6.97±0.54**
Albumin (g/dL)	**3.83±0.53**	**3.78±0.53**
C-reactive protein (mg/dL)	**1.20±1.94**	**1.33±2.09**
Lymphocyte count (/μL)	**1180±547**	**1062±502**

SD: standard deviation, IQR: Interquartile Range

When the CRP value was under 0.03, the mean value was set to 0.03.

was significantly lower than that in the non-irAE group (Mean: -41.0±19.9 versus. 0.5±6.1, respectively, p<0.0001) (**Table 2**). The area under a receiver operating characteristic (ROC) curve (AUC) was 0.99. Moreover, the most accurate cutoff point was -6.6 with sensitivity of 100% and specificity of 99.9%. The lowest value of differences in the non-irAE patients was -17.6. In addition, 89% of patients with thyroid-irAE had differences of -17.6 or below. Inter-rater reliability, which was assessed using the intra-class coefficient (ICC), showed strong agreement (ICC (2,1)=0.89) (**Table 3**).

Moreover, a high TSH level is a criterion for hypothyroidism. Among the patients with thyroid-irAE, CT attenuation values were significantly different (-17.6 or below) at or before the increase in TSH in 79% of patients (**Figure 4**). There were more significant differences in CT attenuation values of the thy-

Table 2. Summary of the Mean CT Attenuation Values and Differences

	Thyroid-irAE group	**Non-irAE group**
Mean CT attenuation values±SD		
Before ICI	**100.2±20.1 HU**	**93.7±13.8 HU**
After ICI (the whole period)	**59.3±23.2 HU**	**94.9±14.2 HU**
At or before the increase of TSH	**67.5±22.1 HU**	
Mean differences±SD		
The whole period	**All patients -41.0±19.9** **Lung cancer patients (n=14) -38.2±18.7** **Non- Lung cancer patients(n=5) -48.7±21.0** **PD-1 inhibitors group (n=14) -44.5±19.0** **PD-L1 inhibitors group (n=5) -31.1±18.9**	**0.5±6.1**
At or before the increase of TSH	**-32.7±16.4**	
Results of mean differences from Bland-Altman Analysis		
Mean Bias (the whole period)	**-1.4**	**1.4**
Limits of Agreement (the whole period)	**-9.5 ~ 6.6**	**-1.3 ~ 4.2**
Mean Bias (at or before the increase of TSH)	**-3.3**	
Limits of Agreement (at or before the increase of TSH)	**-8.5 ~ 1.9**	

SD, standard deviation

Table 3. Summary of the ICC (2,1)

	ICC (2,1)
Differences in CT attenuation values (the whole period)	**0.89**
Differences in CT attenuation values (at or before the increase of TSH)	**0.81**
Thyroid gland atrophy	**0.84**

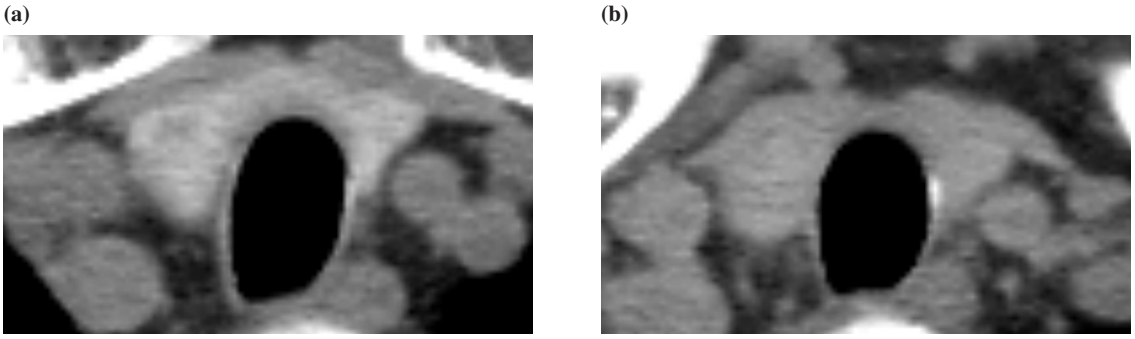


Figure 4. A man in his 70s with a medical history of Hashimoto's thyroiditis was treated with pembrolizumab for lung cancer

(a) Imaging before using pembrolizumab, (b) Follow-up computed tomography (CT) at 56 days before the increase in thyrotropin (TSH). Mean thyroid attenuation values decreased by -33 HU on non-contrast-enhanced CT image obtained 56 days before the increase in TSH (65 days after starting pembrolizumab).

roid-irAE group at or before the increase in TSH than those in the non-irAE group (Mean -32.7±16.4 versus. 0.5±6.1, respectively, p<0.0001). In the thyroid-irAE group, the mean CT attenuation values at or before the increase in TSH was 67.5±22.1 HU. On average, CT attenuation values decreased 43 days before the increase in TSH (**Suppl. 1**). The median frequency of laboratory examinations between the ICI administration start date and the day TSH levels increased was 5. The inter-rater reliability, assessed using the ICC, showed high agreement (ICC (2,1)=0.81) (**Table 3**).

After treatment with levothyroxine, thyroid attenuation values did not return to their original state in any of the patients (average follow-up period 440 days after ICI treatment). The attenuation values remained low in many patients, but some mildly increased from the minimum value (e.g., -35.9 to -11.8; in this case, the levothyroxine dose increased while the thyroid attenuation value increased, indicating a lack of association with thyroid function improvement).

CT measurements of thyroid gland atrophy before and after PD-1 and PD-L1 inhibitor treatment

The ratios of approximate volume of the thyroid glands determined after using ICIs to approximate volume of the thyroid glands determined before using ICIs were significantly lower in the irAE group than in the non-irAE group. (Mean: 0.62±0.32 versus 1.02±0.11, respectively, p<0.0001) (**Table 4**). The AUC of ROC curve analysis was 0.84 and the most accurate cutoff point was 0.91 with sensitivity of 77% and specificity of 100%. The approximate volume of the thyroid gland changed by 0.9 in non-irAE patients. In addition, 18 of the 26 thyroid-irAE patients (69.2%) had atrophy ≤ 0.9 (significant atrophy) (**Figure 5**). Inter-rater reliability, assessed using the ICC, showed strong agreement (ICC (2,1)=0.84) (**Table 3**). The average number of days from the first dose of ICI to ≤ 0.9 atrophy was 218 days (range, 61—441 days). In the significant atrophy group, 13 of 18 patients (72.2%) were TPOAb- and/or TgAb-positive before and after ICI treatment. The mean TPOAb and TgAb levels were 677.6±735.8 IU/L (n=10) and 257.3 ± 353.0 IU/L (n=10), respectively. The mean value of TPOAb was 667.7±857.7 IU/L (n=6) and that of TgAb was 91.4±83.9 IU/L (n=9) in patients in the thyroid-irAE group without significant atrophy.

In addition, the maximum TSH levels (4.66—191.12 μIU/mL) and maximum levothyroxine doses (25—100 μg) at or before the last CT examination were associated with thyroid atrophy (r=-0.69, p<0.0001 and r=-0.52, p=0.01, respectively) (**Table 5**).

Discussion

In this study, we revealed that thyroid gland CT attenuation values significantly decrease in occur before TSH levels increase (i.e., before the hypothyroidism is diagnosed) in patients with overt hypothyroidism. Therefore, a decrease in CT attenuation values in the thyroid gland may be a useful marker for the early detection and therapeutic intervention of overt hypothyroidism induced by PD-1 and PD-L1 inhibitors.

In a previous study, the anti-PD-1 and anti-PD-1 treatments were associated with a higher incidence of

Table 4. Summary of the Thyroid Gland Atrophy

	Thyroid-irAE group	Non-irAE group
Mean of Ratios±SD Approximate Volume of the Thyroid Glands After Using ICIs/Approximate Volume of the Thyroid Glands Before Using ICIs	**All patients 0.62 ± 0.32** **Lung cancer patients (n=15) 0.64±0.33** **Non- Lung cancer patients(n=11) 0.59±0.31** **PD-1 inhibitors group (n=17) 0.63±0.32** **PD-L1 inhibitors group (n=9) 0.60±0.32** **CR+PR group (n=7) 0.79±0.23** **PD group (n=20) 0.63±0.30** **SD group (n=2) 0.95±0.07**	**1.02 ± 0.11**
Results of Bland-Altman Analysis		
Mean Bias	**0.03**	**0.03**
Limits of Agreement	**-0.03 ~ 0.08**	**-0.05 ~ 0.11**

SD, standard deviation; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease.

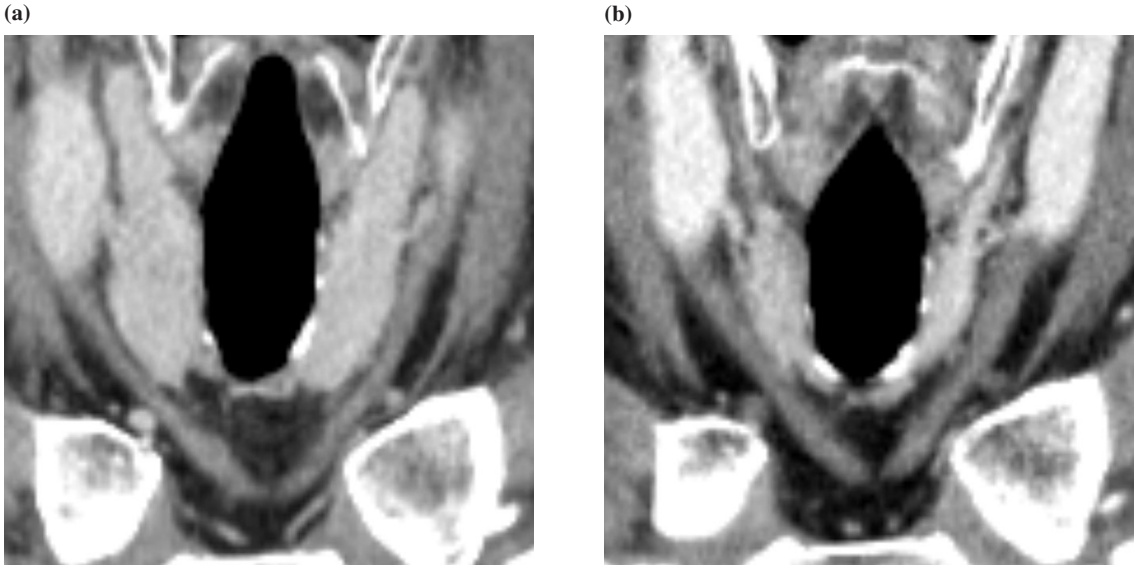
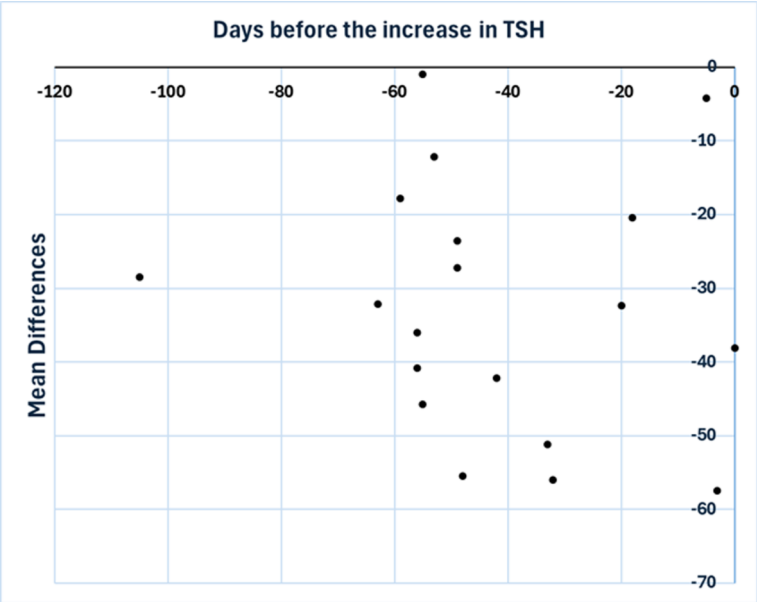


Figure 5. A man in his 70s was treated with avelumab for renal pelvis cancer
(a) Imaging before using avelumab, (b) Follow-up computed tomography (CT) obtained 130 days after starting avelumab. The thyroid was markedly shrunken on contrast-enhanced CT image, obtained 130 days after starting avelumab. Thyroid atrophy was 0.34. TSH (maximum 82.8 μIU/mL) and maximum levothyroxine dose (100 μg) were both high.

Table 5. Association with Thyroid Atrophy

	r	p
Maximum TSH Levels	**-0.69**	**< 0.0001**
Maximum Levothyroxine Doses	**-0.52**	**0.01**



Suppl. 1. Differences in CT attenuation values of the thyroid-irAE group at or before the increase of TSH

thyroid-irAEs than anti-CTLA-4 treatment. The incidence rates of thyroid-irAEs were 4.7% with anti-CTLA-4 treatment (ipilimumab), 8.8% (nivolumab) and 15.6% (pembrolizumab) with PD-1 inhibitor treatment, and 22.2% (atezolizumab) and 13.5% (durvalumab) with PD-L1 inhibitor treatment[6]. The incidence rates of hypothyroidism were 3.8% (ipilimumab) with anti-CTLA-4 treatment, 8.0% (nivolumab) and 8.5% (pembrolizumab) with PD-1 inhibitors, and 6.0% (atezolizumab) and 4.7% (durvalumab) with PD-L1 inhibitors[7].

Decreased CT attenuation values and volume changes in the thyroid glands may reflect anti-tumor effects. Usually, patients with overt hypothyroidism without other serious irAEs continue ICI treatment, without a rest period, if they are treated with levothyroxine. Permanent thyroid dysfunction requiring levothyroxine has been associated with improved cancer outcomes[1, 8]. Thus, thyroid-irAEs may reflect anti-tumor effects. Decreased CT attenuation values and volume changes in the thyroid glands of patients with thyroid-irAEs may be associated with better cancer outcomes. Further studies are needed to verify this hypothesis.

Our findings also suggested that thyroid gland atrophy in overt hypothyroidism can be evaluated with CT by measuring the maximum width, length, and antero—posterior diameter. Moreover, atrophy was associated with the severity of hypothyroidism (maximum TSH level). When atrophy or exacerbation of atrophy is detected on CT images, it would be beneficial to reassess the severity of overt hypothyroidism and the dose of levothyroxine. In a previous study, the volume of the thyroid gland was calculated by adding the areas of the thyroid gland traced on all axial CT images and then using three-dimensional (3D) CT volumetry[7, 9]. In contrast, we measured the maximum width, length, and antero—posterior diameter using ultrasonographic analysis. Thus, this allows the volume of the thyroid gland to be measured more

easily in clinical settings than the method of adding the areas of the thyroid gland traced on all axial CT images and then performing 3D CT volumetry of non-contrast-enhanced CT. In addition, in some cases, the boundary between the thyroid gland and surrounding structures (e.g., the anterior cervical muscle) can be unclear, making it difficult to delineate the thyroid lobe on non-contrast-enhanced CT images because of the decrease in CT attenuation values in the thyroid glands. Our method can be used for calculation in such cases.

In our study, the average days from the first dose of ICI to atrophy of ≤ 0.9 was 218; thus, the duration of the atrophy process was long. This result was consistent with that of a previous study on radiation-induced hypothyroidism, in which the volume of the thyroid gland decreased from 6 months after the completion of radiotherapy[10]. Future studies should consider 3D CT volumetry of contrast-enhanced CT images to confirm our findings with a focus on minor atrophy.

Finally, consistent with previous findings, the TPOAb- and/or TgAb-positivity rates were high in the thyroid-irAE group[1]. Thyroid-irAE patients with atrophy ≤ 0.9 tended to have higher TPOAb and/or TgAb values. This finding is exploratory, and requires further investigation.

The important limitations of our study were the relatively small sample size and large proportion of patients with lung cancer. Thus, further studies with larger sample sizes are required to confirm our findings. Furthermore, the inspection time for CT and laboratory tests was not fixed because of the retrospective nature of the study. Moreover, our thyroid gland atrophy measurement method may have a detection limit. Additional studies should consider performing 3D-CT volumetry using contrast-enhanced CT images to assess minor atrophy. Additionally, we did not analyze the period required for thyroid gland atrophy to stabilize. In a previous study on radiation-induced hypothyroidism, the volume of the thyroid gland decreased from 6 to 30 months after completion of radiotherapy and reached a relatively steady state after 36 months[9]. Future studies should consider more than 36 months of follow-up.

Conclusion

Our data suggest that CT attenuation values decrease before TSH levels increase and remain low in patients with overt hypothyroidism induced by PD-1 and PD-L1 inhibitors who are treated with levothyroxine. These patients were diagnosed with early stage hypothyroidism based on a CT scan. Our analysis demonstrated that thyroid gland atrophy can be assessed using CT by measuring the maximum width, length, and antero—posterior diameter. Thyroid gland atrophy is associated with the severity of hypothyroidism (maximum TSH level). Although CT is often used to evaluate the effects of treatment and screening, it can also be used to estimate thyroid function.

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Author contributions

HT conceived and designed the study. HT and SM collected the data. HT, JH, SY, and RO analyzed and interpreted the data. SM, HM provided overall guidance throughout the research process. All authors contributed to the drafting and revision of the manuscript and approved the submitted version. All authors take responsibility for the accuracy and completeness of the work and are accountable for addressing any related questions regarding its integrity.

Conflicts of Interest

The authors have nothing to disclose.

Supplementary Information

There is one supplementary material. See attached supplementary figure 1.