



Predictive Biomarkers of Thyroid Dysfunctions Associated with the Treatment of Non-Small Cell Lung Cancer and Cervical Cancer with Immune Checkpoint Inhibitors

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ABSTRACT

BACKGROUND: Treatment with immune checkpoint inhibitors (ICIs) for advanced malignancies has been associated with developing immune-related adverse events (irAEs) severe enough to require the cessation of life-saving tumor immunotherapy.

OBJECTIVES: The present study aimed to identify predictive inflammatory markers of the development of immune-related thyroid dysfunctions in patients with cervical cancer (CC) and non-small cell lung cancer (NSCLC) treated by ICIs.

METHODS: A retrospective study was conducted on twenty-seven patients with CC and NSCLC treated by ICIs. The data were collected before and 12 weeks after treatment. Complete blood count-derived inflammatory markers: dNLR (derived neutrophil to lymphocyte ratio), NLR (neutrophil to lymphocyte ratio), SSI (systemic inflammation index), PLR (platelet to lymphocyte ratio), WHR (white blood cells to hemoglobin ratio) were calculated. In addition, thyroid functional tests were collected. Data statistical analysis was performed by STATISTICA (Stat soft, Inc, USA).

RESULTS: Five patients out of twenty-seven with CC treated by PD-1 and CTLA-4 inhibitors who developed hypothyroidism showed significantly high baseline PLR and low WHR compared to patients without clinical symptoms of hypothyroidism and reference levels TSH and FT4. Association between NLR, dNLR, SSI, and thyroid dysfunction was not observed.

CONCLUSIONS: Our findings show a strong correlation between hypothyroidism and WHR and PLR biomarkers. As a result, using these biomarkers for early identification of hypothyroidism helps treat thyroid dysfunction and improves cancer immunotherapy outcomes.

KEYWORDS: Hypothyroidism; immune checkpoint inhibitors; inflammatory markers; platelet/lymphocyte ratio; white blood cell/lymphocyte ratio.

BACKGROUND

Cancer immunotherapy is a promising treatment strategy that has the potential to improve the overall survival of cancer patients. Novel immunotherapeutic drugs - immune checkpoint inhibitors (ICIs) transfer the immune system from its inhibitory regulatory pathways and upregulate the immune response against tumor cells.^{1,2} In clinical practice, the most widely used agents of ICIs' are cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1) and PD-1 ligand 1(PD-L1), and ligand 2 (PD-L2) inhibitors.

Unfortunately, even though ICIs have demonstrated a remarkable effect in treating cancer, they gave rise to side effects known as checkpoint toxicities or immune-related adverse events (irAEs).^{3,4,5} ICIs have affected almost all organs, but the following systems are more vulnerable: skin, gastrointestinal tract, endocrine system, and joints.

According to the existing evidence, most patients have experienced at least one irAE, and half have experienced at least one grade 3-4 irAE.⁶ Although studies report that the development of checkpoint toxicity is associated with better

outcomes, some immune-related adverse events are severe enough to require treatment discontinuation and, in rare cases, can lead to death. Thus, early recognition and timely diagnosis are crucial.

Our work addresses thyroid dysfunctions caused by ICI medications. The incidence of immune-related hypothyroidism is significantly higher after treatment with combination therapy (anti-CTLA-4 and anti-PD-1), which is often used for the treatment of cervical cancer (CC) and non-small cell lung cancer (NSCLC). Therefore, predicting the onset of toxicity before therapy is critical for choosing patients for ICIs therapy and personalized monitoring.^{3,4}

We aimed to identify the predictive inflammatory markers of developing immune-related thyroid dysfunctions in patients with NSCLC and CC treated with ICIs.

METHODS

The present retrospective study was conducted in the Israel-Georgian Medical Research Clinic "Healthycore" after



the local Ethics Committee approved the study methodology. Immunotherapy-naïve NSCLC and CC patients treated with ICIs were included in the study. Patients with thyroid dysfunctions, autoimmune disorders, and recent steroid treatment were excluded from the study.

We collected the data (baseline and 12 weeks after treatment), which contain the following information for each subject: age, sex, gender, tumor stage, TFTs (thyroid functional tests: thyroid stimulating hormone, TSH, and free thyroxine, FT4), and complete blood count-derived inflammatory markers, such as dNLR (derived neutrophil to lymphocyte ratio, absolute neutrophil count/absolute white blood cell count- absolute neutrophil count), NLR (absolute neutrophil count to absolute lymphocyte count ratio), SII (systemic inflammation index, absolute neutrophil count* platelet count/ absolute lymphocyte count), PLR (absolute platelet count to absolute lymphocyte count ratio), WHR (absolute white blood cells to hemoglobin ratio). In addition, according to physical examination signs and symptoms, we calculated "Zulewski's clinical score for hypothyroidism " (Tab.1).⁷ The reference ranges for TSH and FT4 were determined as follows: 0.4-4 µU/ml and 1-1.8 ng/dL, respectively.

TABLE 1. Zulewski's clinical scoring system

| Evaluation | Description | Score* |
|-----------------------|--|--------|
| Signs | | |
| Slow of movements | Observing patient while walking and sitting | 1 |
| Ankle reflex | Observing delayed relaxation of the ankle reflex | 1 |
| Coarse skin | Dermatologic examination of the hand, forearm, and elbow for thickness and roughness | 1 |
| Periorbital puffiness | Observing periorbital swelling | 1 |
| Cold Skin | Comparing the temperature of the hand with the examiner's | 1 |
| Symptoms | | |
| Diminished sweating | Sweating in a normal or warm room | 1 |
| Voice hoarseness | Change in speaking or singing voice | 1 |
| Paresthesia | Subjective sensations | 1 |
| Dry skin | Dryness of the skin, requiring skin moisturization | 1 |
| Constipation | Bowel habits and use of laxatives | 1 |
| Hearing impairment | Difficulty in hearing | 1 |
| Weight increase | Increase in weight | 1 |

*1 point is assigned when a sign/symptom is present.

Note: A score of 6 and above is defined as hypothyroidism, while 0-2 points are considered euthyroid. A score of 3-5 is defined as intermediate.

For the statistical analysis we used a preliminary descriptive statistics and nonparametric tests. The outcomes were expressed as mean ± standard deviation (M±SD). Finally, to reveal the differences between the two groups, a student's t-test was used. For all tests, p < 0.05 was considered significant.

RESULTS

We retrospectively studied the data of sixty patients, but only twenty-seven patients enrolled according to the inclusion and exclusion criteria. NSCLC and CC patients were treated with a PD-1 inhibitor (n=18), PD-1 and CTLA-4 inhibitors in

combination (n=6), and PD-1/CTLA-4 inhibitors/chemotherapy (n=3).

Out of 27 patients (10 women and 17 men; median age 61 years), 5 developed immune-related hypothyroidism within 12 weeks. They were all females with cervical cancer treated with a combination of PD-1 and CTLA-4 inhibitors (Tab.2).

TABLE 2. Data of five cervical cancer female patients with immune-related hypothyroidism on PD-1/CTLA-4 inhibitors

| Age | Hypothyroidism | Z-score | TSH | | FT4 | | PLR | | WHR | |
|-----|----------------|---------|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
| | | | Baseline | Follow-up | Baseline | Follow-up | Baseline | Follow-up | Baseline | Follow-up |
| 41 | H | 5 | 1.77 | 45.3 | 1.06 | 0.92 | 235.3 | 157.5 | 0.362 | 0.340 |
| 50 | SH | 4 | 0.71 | 4,68 | 1.35 | 1.10 | 495.6 | 247.9 | 0.432 | 0.312 |
| 63 | H | 7 | 2.51 | 227.6 | 1.00 | 0.15 | 355.4 | 369.1 | 0.382 | 0.412 |
| 65 | H | 6 | 1.14 | 22.97 | 1.30 | 0.67 | 354.7 | 429.2 | 0.329 | 0.323 |
| 61 | H | 6 | 2.26 | 29.81 | 1.22 | 0.56 | 684.1 | 369.6 | 0.516 | 0.225 |

Abbreviations: FT4, free thyroxine; H, hypothyroidism; PLR, absolute platelet count to absolute lymphocyte count ratio; SH, subclinical hypothyroidism; TSH, thyroid stimulating hormone; WHR, absolute white blood cells to hemoglobin ratio; Z-score, Zulewski's clinical score for hypothyroidism.

In 12 weeks of treatment, five patients out of 27 revealed a significant increase in TSH (baseline 1.68±0.75, follow-up 66.08±91.46; p<0.0001) and a decrease in FT4 (baseline 1.19±0.15, follow-up 0.68±0.36, p=0.01). In addition, four of the five patients developed severe hypothyroidism, and one had subclinical hypothyroidism. Furthermore, three of the five patients consisted of Zulewski's Clinical score for hypothyroidism. Compared to patients with no clinical signs of hypothyroidism and normal TSH and FT4, all five hypothyroid patients had significantly higher baseline PLR and lower WHR, with p<0.01 and p<0.0001, respectively. No association was found between NLR, dNLR, SSI, and thyroid dysfunction.

DISCUSSION

Over the last few decades, a novel tumor immunotherapy strategy, ICI treatment, has emerged. This unique approach involves breaking the immune silence caused by tumors. Checkpoint regulation is more effective than other treatments for many advanced malignancies, although over-activation of the immune system causes adverse effects, and the thyroid gland is periodically engaged.⁸

Complete blood count-derived inflammatory markers have recently been used as prognostic markers in oncology, cardiovascular, autoimmune, and other diseases.^{9,10}

In addition, there are several studies where ANC (absolute neutrophil count), NLR, and dNLR have been addressed as the independent predictors of survival in cancer patients, prognostic biomarkers of therapeutic response to biologics in autoimmune disease, and predictive role for the risk of preterm delivery, etc.^{11,12,13}

As the symptoms of hypothyroidism mimic those of cancer, the diagnosis of thyroid dysfunction may be missed, leading to life-threatening complications. Thus, identifying biomarkers predictive of immune-related endocrinopathies allows physicians to manage patients accordingly. The use of blood cell counts with prognostic value, high clinical significance, and low cost for the early detection of irAEs may be of great interest to clinicians.

According to our results, CC patients who developed hypothyroidism due to ICIs therapy showed high baseline PLR and low WHR. Reports on the association of peripheral blood markers and immune-related adverse events due to ICIs in patients with different cancers are controversial. It was suggested that a higher baseline and increase in absolute lymphocyte counts (ALC) and eosinophil counts (AEC) after ICI treatment were strongly associated with developing irAEs in patients with solid tumors treated with anti-PD-1 antibodies.¹⁴ A meta-analysis including thousands of patients has shown that a high NLR and PLR at the beginning of ICI therapy was identified as a prognostic marker for developing irAEs.^{15,16,17} Our results correspond to the meta-analysis mentioned above: Patients who developed hypothyroidism due to ICIs therapy showed significantly higher PLR than patients who did not have symptoms of irAEs.

Conversely, Leila Khoja et al.'s study revealed that melanoma patients treated with ipilimumab, NLR, PLR, and ELR (eosinophil to lymphocyte ratio) were not associated with toxicity but only with the response to ICI treatment¹⁸. We also found no association between NLR and developing hypothyroidism after treatment. It has recently been reported that low NLR at baseline is a risk factor for developing irAEs. Multivariate analysis revealed that NLR <2.86 could be an independent predictive factor for the occurrence of irAEs.^{3,19,20} In other studies, unlike our results, low pretreatment PLR value was significantly associated with the development of irAEs.^{3,19} All these results require further validation in additional studies.

The present study has several limitations, such as no data on Anti-TPO (Thyroid peroxidase antibodies), Anti-Tg (Thyroglobulin antibodies) before and after the treatment with ICIs, low numbers of patients, and retrospective design.

CONCLUSIONS

Our findings demonstrate a strong correlation between hypothyroidism and WHR and PLR. As a result, using these biomarkers for early diagnosis of hypothyroidism will help to treat thyroid dysfunction and improve cancer immunotherapy outcomes.

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