2772-9931
https://doi.org/10.1016/j.atssr.2023.05.012

Lung: Short Report

Sex-Specific Effects of Body Composition on Tumor Microenvironment in Non–Small Cell Lung Cancer

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ABSTRACT

BACKGROUND There exists a gap in understanding the interaction between sex and obesity on tumor microenvironment in non–small cell lung cancer (NSCLC). We demonstrate the combined effects of sex and body composition on clinically relevant biomarkers of NSCLC immune response.

METHODS A cohort of 409 patients with NSCLC was subdivided into 4 groups jointly defined by body mass index (BMI; cutoff of 25 kg/m^2) and sex (male–high BMI, male–low BMI, female–high BMI, female–low BMI). Associations between these sex-BMI groups and tumor inflammation (expression of 161 immune response genes), cell proliferation (expression of 10 cell proliferation genes), and programmed cell death ligand 1 (PD-L1; through immunohistochemistry and targeted RNA sequencing) were assessed by Kruskal-Wallis and Wilcoxon rank sum tests for overall and pairwise tests, respectively.

RESULTS We observed an overall significant association of sex-BMI with tumor inflammation ($P < .001$) and cell proliferation ($P = .002$). On pairwise analysis, high-BMI females had significantly higher tumor inflammation compared with low-BMI females ($P = .002$). In addition, women with a high ($P = .004$) and low ($P = .008$) BMI had significantly higher tumor inflammation compared with men with a high and low BMI, respectively. Low-BMI females ($P = .01$) and males ($P = .01$) had more cell proliferation. PD-L1 expression by RNA sequencing ($P = .001$) but not by immunohistochemical analysis differed significantly with sex-BMI. High-BMI females had significantly higher PD-L1 expression compared with low-BMI females ($P = .01$).

CONCLUSIONS We demonstrate that tumor inflammation and PD-L1 expression are more strongly associated with BMI in women than in men. Additional studies are required to better understand the mechanisms underlying these effects and their role in response to checkpoint inhibitors in NSCLC.

(Ann Thorac Surg Short Reports 2023;■:■-■)

The pathophysiologic changes causing the increased incidence and prevalence of many cancers have been shown to be associated with obesity.[1] Alteration in adipokine levels,[1] chronic meta-inflammation,[2] and immune evasion and exhaustion[2] within the tumor microenvironment (TME) are some of the proposed mechanisms explaining this

IN SHORT

- We demonstrate a significant interaction between sex and obesity within the tumor microenvironment.
- Body mass index is more strongly associated with tumor inflammation and programmed cell death ligand 1 expression in women.

Accepted for publication May 16, 2023.

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TABLE Characteristics of the Cohort

Variable	All (N = 409)	BMI ≥25 kg/m² (n = 245)	BMI <25 kg/m² (n = 164)	P Value
Age, y	68 (61-76)	69 (62.5-76.5)	66 (58.2-74.7)	.01
Sex				
Female	232 (56.7)	140 (57.1)	92 (56.1)	.8
Male	177 (43.3)	105 (42.9)	72 (43.9)	
Race				.4
White	362 (88.5)	221 (90.3)	141 (86)	
Black	29 (7.1)	14 (5.7)	15 (9.2)	
Other	9 (2.2)	6 (2.4)	3 (1.8)	
Unknown	9 (2.2)	4 (1.6)	5 (3)	
Smoking status				.01
Current	159 (38.9)	80 (32.7)	79 (48.2)	
Former	183 (44.7)	124 (50.6)	59 (36)	
Never	34 (8.3)	21 (8.6)	1.3 (7.9)	
Unknown	33 (8.1)	20 (8.2)	13 (7.9)	
Insurance				.2
Private	123 (30.2)	73 (29.8)	50 (30.5)	
Medicare only	143 (35)	87 (35.5)	56 (34.1)	
Medicare with private supplement	72 (17.6)	48 (19.6)	24 (14.6)	
Medicaid	36 (8.8)	16 (6.5)	20 (12.2)	
Unknown	35 (8.6)	21 (8.6)	14 (8.5)	
Histologic type				.5
Adenocarcinoma	274 (67)	169 (69)	105 (64)	
Squamous cell carcinoma	67 (16.4)	39 (15.9)	28 (17.1)	
Other	68 (16.6)	37 (15.1)	31 (18.9)	
Stage				.5
I	11 (2.7)	7 (2.9)	4 (2.4)	
II	8 (2)	5 (2)	3 (1.8)	
III	60 (14.7)	41 (16.7)	19 (11.6)	
IV	253 (61.9)	144 (58.8)	109 (66.5)	
Unknown	77 (18.8)	48 (19.6)	29 (17.7)	
TIGS score	52.4 (36.6-68.5)	56.9 (41.2-70.8)	48.7 (34-62.5)	<.001
TIGS group				.001
Strong	146 (35.7)	104 (42.4)	42 (25.6)	
Moderate	124 (30.3)	73 (29.8)	51 (31.1)	
Weak	139 (34)	68 (27.8)	71 (43.3)	
CP score	44.3 (26.2-63.2)	41.7 (22.3-58.7)	49.6 (30-68.6)	.001
CP group				.001
High	84 (20.5)	36 (14.7)	48 (29.3)	
Moderate	189 (46.2)	118 (48.2)	71 (43.3)	
Poor	136 (33.3)	91 (37.1)	45 (27.4)	
PD-L1 RNA	69 (41-87)	74 (47.5-87)	56.5 (36.5-81.2)	
PD-L1 IHC group				.1
PD-L1 +	300 (73.3)	186 (75.9)	114 (69.5)	
PD-L1 −	109 (26.7)	59 (24.1)	50 (30.5)	

Categorical variables are presented as number (percentage). Continuous variables are presented as median (interquartile range). Sociodemographic variables, clinicopathologic variables, and tumor microenvironment biomarkers were compared across the BMI categories by Pearson χ^2 and Wilcoxon rank sum tests for continuous and categorical variables, respectively. BMI, body mass index; CP; cell proliferation; IHC, immunohistochemistry; PD-L1, programmed cell death ligand 1; TIGS, tumor inflammatory gene signature.

association. Literature exploring the effects of obesity on lung cancer incidence and survival showed a protective effect with a high body mass index (BMI). For example, a study of 2641 patients with non-small cell lung cancer (NSCLC) in the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial showed a hazard ratio of 0.78 ($P < .001$) with increasing BMI.[3] Similarly, studies showed that sex is a significant prognostic indicator of immunotherapy outcomes in lung cancer.[4] However, the biologic mechanisms contributing to the interaction between obesity and sex with respect to lung cancer outcomes are not clearly understood. We sought to use previously developed biomarkers of inflammation and cell proliferation within the TME to understand and possibly to explain the differences in clinical outcomes.[5]

PATIENTS AND METHODS

CLINICAL DATA. A retrospective cohort of 409 patients with lung cancer who underwent treatment at Roswell Park Comprehensive Cancer Center in Buffalo, New York, was identified using the institutional thoracic surgery databases and cancer registries. Data elements including patient age, sex, race, smoking status, insurance type, BMI, tumor histology, and stage at the time of patient diagnosis were extracted from electronic health records.

TUMOR GENE PROFILING AND IMMUNOHISTOCHEMISTRY OF LUNG TUMORS. Gene expression profiling was performed by Omniseq Inc (Buffalo, New York) per preexisting operating procedures. Formalin-fixed paraffin-embedded tumor tissue blocks were used for RNA extraction and immunohistochemical (IHC) analysis. IHC analysis was performed to establish programmed cell death ligand 1 (PD-L1) expression in the TME, and RNA extracted from formalin-fixed paraffin-embedded tissue blocks was processed for targeted RNA sequencing to develop a cell proliferation (CP) signature and tumor immunogenic signature (TIGS) score as described in the Supplemental Methods.

DATA ANALYSIS. We divided the cohort into 4 BMI and sex subgroups with a BMI cutoff of 25 kg/m², females with high (n = 140) and low (n = 92) BMI and males with high (n = 105) and low (n = 72) BMI. Analyses were performed with R (version 3.6 and higher; R Foundation for Statistical Computing) and SPSS (version 26; IBM) software. Wilcoxon rank sum test and Kruskal-Wallis rank sum test were used for 2- and 4-group comparisons of continuous variables, respectively, and Pearson χ^2 test was used for categorical variables.

DATA AVAILABILITY. Data generated for this study are available from the authors on request.

RESULTS

The cohort of 409 patients with NSCLC included in this study had a greater proportion of female patients

(56.7%) and a median age of 68 years (interquartile range, 61-76 years). Most patients were White (88.5%), former (44.7%) or current (38.9%) smokers, and covered by either Medicare (35%) or private insurance (30.2%). Adenocarcinoma (67%) was the most common histologic type, followed by squamous cell carcinoma (16.4%). Most of the patients had stage IV disease (61.9%), followed by stage III (14.7%). The median BMI of the cohort was 26.2 kg/m^2 (22.6-29.7 kg/m^2), and 245 patients (59.9%) had a BMI $\geq$25 kg/m^2. The median mean rank expression of TIGS and CP score of the cohort was 52.4 (36.6-68.5) and 44.3 (26.2-63.2), respectively. Almost equal proportions of the cohort were categorized as strongly (35.7%), moderately (30.3%), and weakly (34%) inflamed according to their TIGS score. There were 189 (46.2%) patients categorized as having a moderately proliferative TME according to their CP score; 73.3% of the patients were PD-L1+ on IHC analysis.

On univariate analysis (Table), patients with a higher BMI were more likely to be older ($P = .01$), to be current smokers ($P = .01$), to have higher TIGS ($P < .001$), and to have lower CP score ($P = .001$). Similarly, overweight BMI patients more likely to have a strongly inflamed ($P < .001$) and poorly proliferative ($P = .001$) TME as categorical variables. There was no significant difference with patient sex, race, insurance type, tumor histology, stage, and PD-L1 status between the 2 BMI groups. A comparison of the descriptive statistics of the BMI-sex subgroups is presented in the Supplemental Table.

To better understand the effects of sex on the association between BMI and TME, we performed subgroup analyses. There was a significant difference in TIGS ($P < .001$) and CP score ($P = .002$) across the BMI-sex groups (Figure 1). Pairwise analysis showed that a higher BMI was associated with more inflammation in women ($P = .002$) but not in men. In addition, women had more inflamed tumors compared with men in both high ($P < .001$) and low ($P = .008$) BMI groups. Interestingly, a higher BMI in both women ($P = .01$) and men ($P = .01$) was associated with lower tumor proliferation.

To further understand the immune TME, we compared PD-L1 expression by IHC analysis and RNA sequencing of the *PDL1* gene (CD274) messenger RNA (mRNA). We found that only PD-L1 mRNA expression was significant across the BMI-sex groups ($P = .002$). Both high-BMI females ($P = .001$) and males ($P = .03$) had significantly higher expression than their lower BMI counterparts. Similar pairwise comparison between the sexes showed that only high-BMI females expressed PD-L1 more than high-BMI males ($P = .02$; Figure 2).

We performed similar analysis using BMI of 30 kg/m^2 as a cutoff for BMI and found similar results (Supplemental Figure).

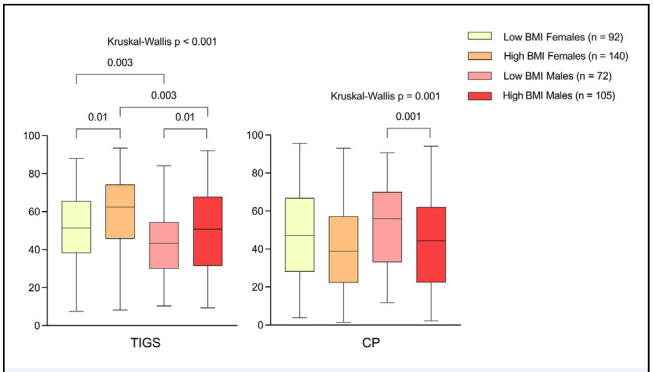

FIGURE 1 Comparison of tumor inflammatory gene signature (TIGS) and cell proliferation (CP) score across body mass index (BMI)–sex subgroups. Four-group and 2-group comparisons were performed by Kruskal-Wallis rank sum test and Wilcoxon rank sum test, respectively.

COMMENT

In this study we performed immune profiling of the TME by RNA sequencing and IHC analysis to gain insights into the differential effects of obesity and sex in NSCLC patients. We found that inflammation, cell proliferation, and PD-L1 gene expression within the TME varied significantly across the 4 BMI-sex groups. On pairwise analysis, we showed that the effect of BMI on the TME was more in women than in men. Female patients with a higher BMI were more likely to have a more inflamed TME. Interestingly, high BMI in women was also associated with higher expression of the gene encoding PD-L1 (*CD274*) but not of surface PD-L1 on IHC analysis. Many studies have independently reported the individual effects of obesity[3] and sex[4] on lung cancer and proposed plausible mechanisms to explain the observed disparities; however, only a few studies have explored the interaction between the 2 in relation to

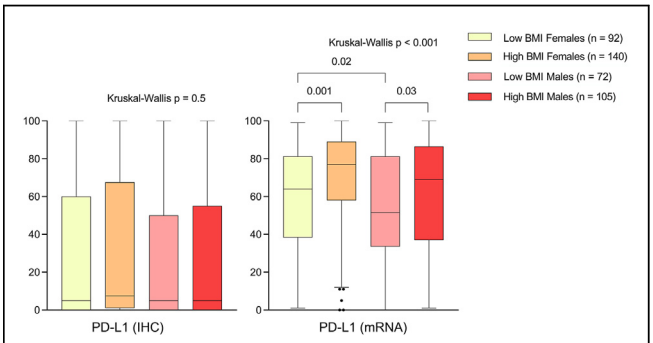

FIGURE 2 Comparison of programmed cell death ligand 1 (PD-L1) immunohistochemistry (IHC) expression and PD-L1 messenger RNA (mRNA) expression across body mass index (BMI)–sex subgroups. Four-group and 2-group comparisons were performed by Kruskal-Wallis rank sum test and Wilcoxon rank sum test, respectively.

lung cancer. A study of around 20,000 patients with NSCLC participating in 16 studies of the International Lung Cancer Consortium found that the interaction between BMI and sex was significant.[6] In the study, the investigators showed that the protective effect of higher BMIs was more in men than in women. Another meta-analysis including 22 clinical trials had similar results.[7] Our study showed that there was a significant difference in expression of biomarkers of tumor inflammation and proliferation across the 4 sex-BMI subgroups. We found that a high BMI in women but not in men was associated with a more inflamed TME. This might partly explain the findings of these studies. The higher baseline inflammation within the TME of high-BMI females may have muted the additional benefit of therapy and thus was associated with no survival benefit, unlike for their male counterparts.

Our study comes with its own set of limitations, including a limited sample size and lack of detailed information on treatment regimens and posttreatment survival outcomes. Another issue with our study might be the use of BMI to define body composition. BMI does not differentiate between fat and lean muscle mass; in addition, BMI is a poor reflection of body fat distribution. Despite these limitations, we believe that the results provide indications of a true interaction between sex, obesity, and the tumor immune microenvironment that deserves further study.

The Supplemental Material can be viewed in the online version of this article [https://doi.org/10.1016/j.atssr.2023.05.012] on http://www.annalsthoracicsurgery.org.

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

FUNDING SOURCES

This work was supported by National Cancer Institute grant (R01CA255515) awarded to Sai Yendamuri and Joseph Barbi. Technical support was offered by Omniseq, a molecular diagnostics company. These and other authors declare no other funding.

DISCLOSURES

Robert Seager, Erik Van Roey, Shuang Gao, Mary Nesline, Jefferey Conroy, and Sarabjot Pabla are employees of Omniseq, a molecular diagnostics company. Roswell Park Comprehensive Cancer Center is a shareholder of Omniseq. These and other authors declare no other conflict of interest.

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