

T Cell and B Cell Infiltration of Primary Head and Neck Squamous Cell Carcinoma is Decreased in Patients with Low Body Mass Index and Low Lymph Node Count from Neck Dissection

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BACKGROUND

Low lymph node count (LNC) from neck dissection (ND) in head and neck squamous cell carcinoma (HNSCC) is associated with poor survival and is considered a potential surgical quality metric. Previously we have shown that patient specific factors including low BMI correlate with low LNC per neck level dissected (normalized LNC). This observation may reflect generalized acquired deficiency in anti-tumor immunity due to undernutrition.

OBJECTIVE

To investigate the effect of low BMI and decreased LNC on primary tumor infiltration by immune cells.

MATERIALS AND METHODS

A retrospective review from UT Southwestern Medical Center and Parkland Hospital identified 384 primary HNSCC patients who underwent ND between 2006 and 2017. Kaplan-Meier survival analysis was performed based on the independent and combined effects of BMI and normalized LNC. Primary cancer specimens from 22 HPV negative, non-oro-pharyngeal HNSCC patients, 6 with low BMI (BMI<23) and low LNC (≤ 5.6 per level), and 16 with normal BMI (BMI ≥ 23) and high LNC (> 5.6 per level), were used to prepare 198 slides from formalin-fixed paraffin-embedded tissue sections. Immunohistochemistry (IHC) was performed using antibodies against CD3, CD4, CD8, CD20, CD21, CD 25, CD56, CD68 and PD1. For each stained slide, 1000x1000 pixel patches were extracted and processed by color deconvolution to separate hematoxylin and IHC staining. Each pixel of the small patch was classified as negative or positive based on IHC intensity relative to a predefined threshold. The positive staining ratio was the total number of positively stained pixels divided by estimated tissue area at the 40x magnification level. CD8 and CD20 stained slides were reviewed for the presence of CD8-rich and CD20-rich aggregates indicating possible tertiary lymphoid structures (TLS).

RESULTS

Both normal BMI ($p < 0.001$) and high normalized LNC ($p = 0.018$) were associated with improved overall survival on cox regression. When combining the effect of BMI and normalized LNC, patients with normal BMI have improved overall survival regardless of normalized LNC. However, low BMI patients with decreased normalized LNC have worse overall survival comparing to high normalized LNC patients ($p = 0.034$).

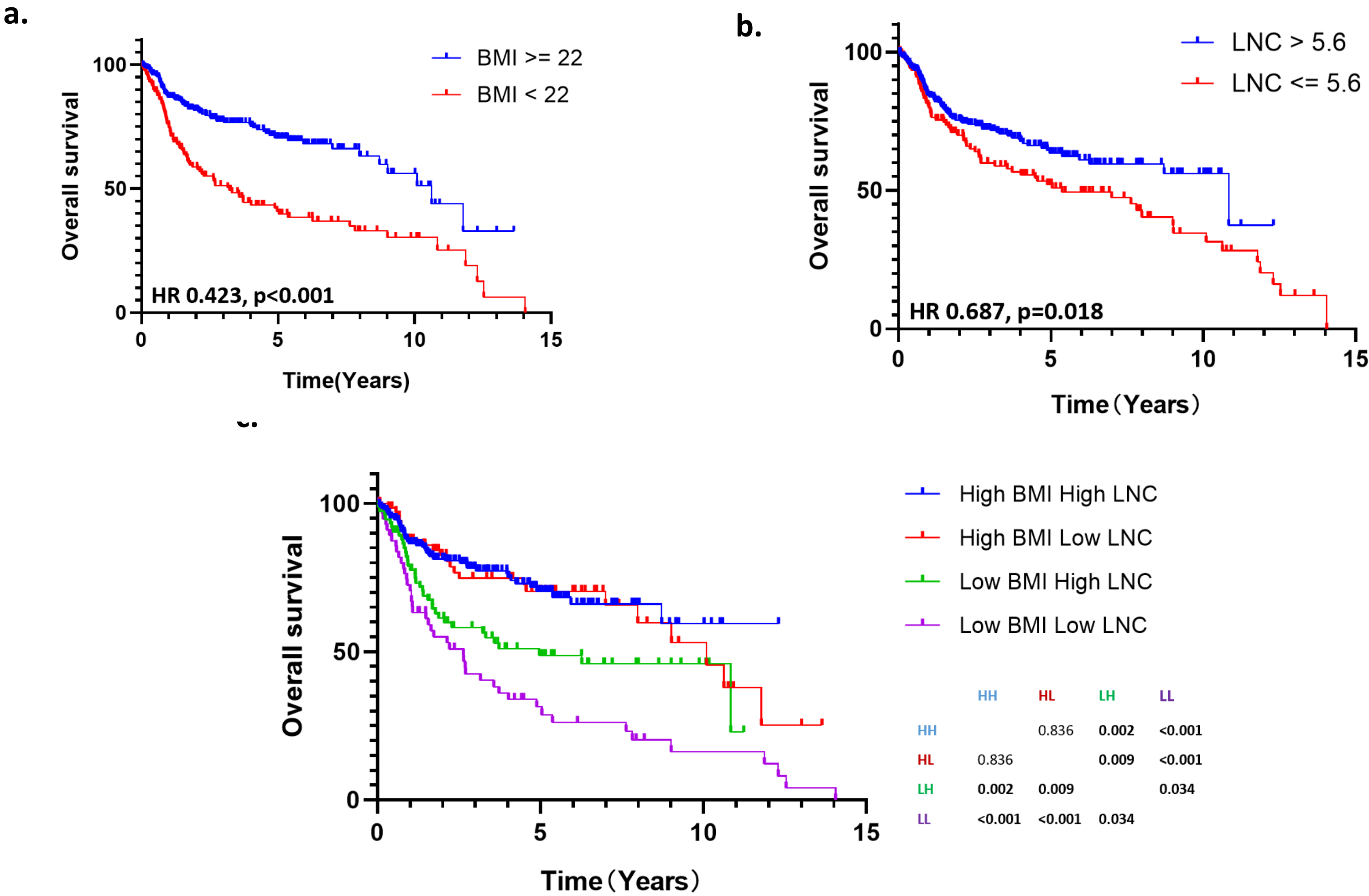


Figure 1. Low BMI and Low LNC are correlated with poor survival

a, Overall survival of patients with BMI $\geq$ or $<$ 23. **b**, Overall survival of patients with normalized LNC $>$ or $\leq$ 5.6. **c**, Overall survival based on combining BMI ($\geq$ or $<$ 23) and Normalized LNC ($>$ or $\leq$ 5.6).

Low BMI patients had significantly fewer CD3 ($p = 0.0136$), CD8 ($p = 0.0003$), and CD20 ($p = 0.0334$) cells associated with their primary tumors compared to normal BMI patients. None of the other markers (CD4, CD21, CD 25, CD56, CD68, and PD1) were significantly different between the primary tumors of low BMI and normal BMI patients. Only 50% (3/6) of low BMI patients had CD20+ and CD8+ aggregates in their tumors, compared to 94% (15/16) of normal-BMI patients.

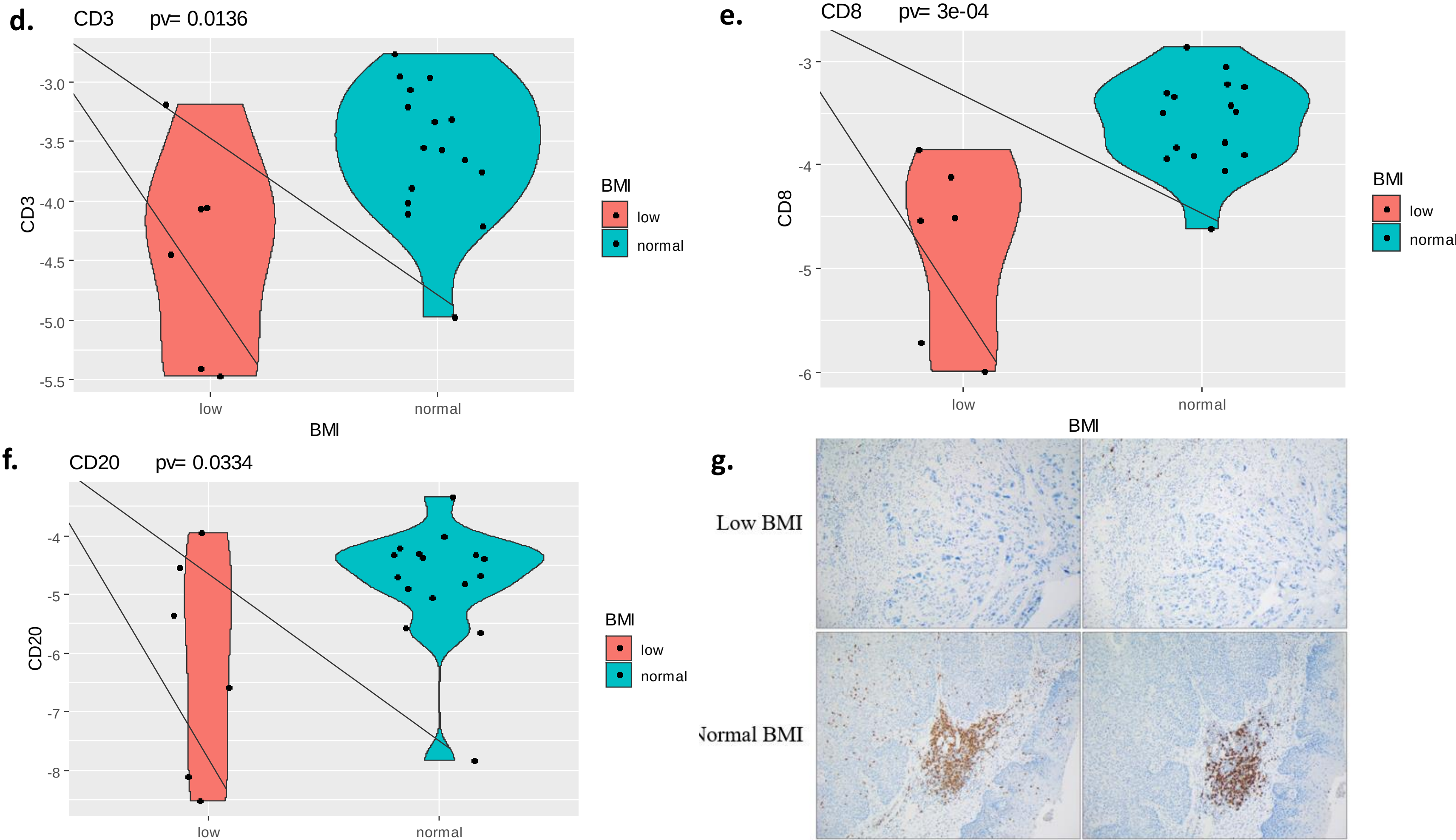


Figure 2. Low BMI and Low LNC are correlated with decreased infiltration of the primary cancers with anti-tumor CD20 and CD8 immune cells.

d-f, Positive IHC staining ratios of CD3 (**d**), CD8 (**e**) and CD20 (**f**) in patients with low BMI low LNC and normal BMI high LNC. Each dot is a single patient. **g**, Density of CD3 and CD20 cells in representative low BMI Low LNC and normal BMI high LNC patients.

CONCLUSION

Primary tumor infiltration by the effector cells of anti-tumor immunity including CD8+ T cells and CD20+ B cells are decreased in patients with low BMI and low LNC and these patients have a worse overall survival compared to patients with high LNC and normal BMI. Low LNC from ND may be a marker for undernutrition and a poor anti-tumor immune response.

DISCLOSURE

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