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Original Article

Pre-treatment serum bicarbonate predicts for primary tumor control after stereotactic body radiation therapy in patients with localized non-small cell lung cancer

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ABSTRACT

Background: Tumor aggressiveness and hypoxia are linked to acidosis in the tumor microenvironment (TME). We hypothesized that low pre-treatment serum bicarbonate, potentially correlating with an acidic and hypoxic TME, predicts for poor outcomes after stereotactic body radiation therapy (SBRT) for non-small cell lung cancer (NSCLC).

Methods: We included patients with localized NSCLC treated to a biologically effective dose (BED) ≥ 100 Gy, with available pre-treatment bicarbonate values within 3 months of treatment. We used receiver operating characteristic analysis to determine the bicarbonate concentration optimally predicting for primary tumor recurrence, and evaluated its association with recurrence and survival. We validated our findings in an independent cohort of patients from three collaborating institutions.

Results: A total of 110 patients and 114 tumors were included in the training cohort, with median follow-up of 15.0 months. Bicarbonate < 26 mEq/L was associated with primary tumor recurrence on univariate (HR = 5.92; 95% CI 1.69–24.88; $p = 0.005$) and multivariate analysis (HR = 5.48; 95% CI 1.37–25.19; $p = 0.020$). The validation cohort consisted of 195 patients and 208 tumors with median follow-up of 27.5 months. In the validation cohort, bicarbonate < 26 mEq/L was again associated with primary tumor recurrence on univariate (HR = 3.38; 95% CI 1.27–9.37; $p = 0.015$) and multivariate analysis (HR = 3.33; 1.18–10.07; $p = 0.023$).

Conclusions: Pre-treatment bicarbonate predicts for primary tumor control in NSCLC treated with SBRT and may be useful for risk stratification. These findings should be confirmed prospectively.

Lung cancer remains the most common cause of cancer-related death in the United States and approximately 85% of lung cancers are non-small cell lung cancers (NSCLCs) [1]. Stereotactic body radiation therapy (SBRT) has emerged as an effective modality and an alternative to surgical resection in the treatment of early-stage NSCLC [2]. While lobectomy or pneumonectomy remains the current standard of care for surgical candidates, SBRT is the preferred treatment for patients who are deemed medically inoperable or refuse surgery [3–5].

Despite the overall efficacy of SBRT, up to 20% of patients may have failure at the site of the treated tumor (primary tumor failure), and even more may have lobar, nodal or disseminated failure of their disease (~30–40%) [6,7]. Furthermore, treatment can entail significant toxicity, in part due to tumor location, but also due to the high doses required to achieve tumor control [8,9]. Identification of independent predictive biomarkers for recurrence in the setting of lung SBRT is warranted in risk stratification, counseling patients, tailoring follow-up, modifying radiation dose via escalation or de-intensification, and directing the use of adjuvant therapies.

Tumor hypoxia may promote radioresistance of malignant cells to ablative doses of radiation [10]. Additionally, tumor hypoxia is known to promote acidosis through the Warburg effect at the level

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of the tumor microenvironment and potentially on a systemic level [11,12]. Conversely, acid-base homeostasis has long been recognized as a vital component of physiological cellular responses and has been implicated in cancer-related immunomodulation and invasiveness [13,14].

While, clinically, systemic acidosis and alkalosis are assessed using arterial or venous blood gas analysis, such tests are invasive and not readily available. On the other hand, serum bicarbonate, readily obtained from peripheral venous blood draw and measured on a routine basic metabolic panel, is nearly ubiquitous in cancer patients for whom co-existing comorbidities and metabolic derangements often necessitate basic serologic evaluation. Given the possible link between acid-base balance and hypoxia and tumor growth, blood-based markers of acid-base balance may furnish simple and valuable information on tumor response to SBRT, and identify high-risk patients to explore personalized adjuvant treatment. We sought to determine whether low levels of pre-treatment serum bicarbonate would be independently associated with recurrence in localized NSCLC patients treated with SBRT, by perhaps serving as a marker of an acidic, hypoxic, and thus "treatment-resistant" tumor microenvironment.

Materials and methods

Patient selection

Under an Institutional Review Board-approved protocol, the records of patients treated at our institution from 2008 to 2017 were retrospectively reviewed using our institutional database. We included any patients with lung-confined, histologically-confirmed, primary NSCLC treated with SBRT alone. This included a small minority of patients with TNM stage T3 or T4 disease secondary to satellite nodules. All patients were either deemed medically inoperable by the referring thoracic surgeon or refused surgery. We included patients treated with up to 8 fractions of hypofractionated "SBRT-like" radiotherapy, due to institutional use of protracted regimens for "central' or "ultracentral" tumors [15]. Patients were only included in the study if they had an available pre-treatment serum bicarbonate value from a basic metabolic panel within 3 months of initiating SBRT. If multiple values were available within this timeframe, the most recent value prior to starting SBRT was used for analysis. To validate our findings in this study, we analyzed an independent cohort of patients treated at three collaborating institutions, using the same inclusion criteria.

Staging and treatment

All patients underwent a free-breathing and four-dimensional (4D) computed tomography (CT) simulation scan with or without contrast, capturing the entirety of the breathing cycle, for radiation treatment planning. The gross tumor volume (GTV) was contoured using the free-breathing scan or 50% phase of the 4D-CT scan and the internal target volume (ITV) was generated using the 4D image set. The ITV was then expanded 5–10 mm in all directions to generate the planning target volume (PTV). Critical organs at risk (OARs) were contoured and dose limits were applied to these OARs per institutional guidelines derived from modern multi-institutional protocols [16–18], as well as published single-institution constraints for 8-fraction treatments [15].

In accordance with institutional and national guidelines, patients were initially seen for follow-up 2–3 months after treatment with a CT or positron emission tomography (PET)/CT scan. They were subsequently followed every 3–6 months for the first 2 years and then every 6 months thereafter. Primary tumor recurrence was defined as recurrence of the treated tumor, as determined by positron emission tomography (PET) scan, biopsy, and/or suspicious imaging findings that led to a change in management when biopsy was unable to be performed. Recurrences were collected regardless of whether or not they were the first recurrence. Additional endpoints included overall survival, defined as the time to death or time to last follow-up from the completion of SBRT, regional nodal recurrence, defined as recurrence in any N1-N3 regional nodes on follow-up imaging, and distant recurrence, defined as recurrences outside of the treated lobe and regional nodes.

Statistical analysis

Statistical analyses were performed utilizing R [19]. Receiver operating characteristic (ROC) analysis was used to identify a cut-off bicarbonate concentration optimally predicting for primary tumor recurrence in the training cohort and this value was used to dichotomize patients into low and high bicarbonate groups in both the training and validation cohorts. Fisher's exact and Mann-Whitney U (Wilcoxon rank-sum) tests were used to compare categorical and continuous variables, respectively, between bicarbonate groups. Logrank tests were conducted to test for difference between Kaplan–Meier curves. Univariate Cox regression analysis was used to estimate hazard ratios between groups. Multivariate Cox regression analysis was performed to confirm the predictability of primary tumor recurrence after adjusting for biologically effective dose (BED), tumor location (central versus peripheral) [18], histology, and gross tumor volume (GTV). These variables were established a priori on the basis of their known predictive role in local recurrence in early-stage NSCLC [20–23]. Due to the low number of primary tumor recurrence events in training and validation cohorts, Cox regression was performed using Firth's penalized maximum likelihood method of bias reduction [24]. Hazard ratios (HR) and 95% confidence intervals (CI) were reported with a two-sided significance threshold of $P < 0.05$. Boxplots and conditional density plots were used to further visualize the association of serum bicarbonate concentration and primary tumor recurrence. All analyses were performed in the same manner for the training and validation cohorts. After confirmation of findings in the validation cohort, a secondary analysis of Charlson comorbidity index (CCI), presence of chronic obstructive pulmonary disease (COPD), and serum creatinine was performed to compare comorbidity of high- and low-bicarbonate groups in the training cohort. Comorbidity data was not available for the validation dataset.

Results

Patient characteristics of the training cohort

A total of 110 patients and 114 treated tumors were included in the initial study cohort with a median post-treatment follow-up time of 15.0 months for living patients (interquartile range [IQR] 8.1–27.0 months) and 13.2 months for all patients (IQR 6.6–25.2 months). Table 1 lists the baseline patient characteristics of this training cohort, including age, gender, and performance status.

The vast majority of tumors were early-stage, with 76 (66.7%) T1 and 28 (24.6%) T2 tumors. T3 and T4 tumors comprised less than 10% of tumors. Squamous cell carcinomas, adenocarcinomas, and unspecified NSCLC represented 36.8%, 54.4%, and 8.8% of the cohort, respectively. All tumors were biopsy-proven. The median SBRT total dose, dose per fraction, and number of fractions was 50 Gy (range 45–60 Gy), 10 Gy (range 7.5–18.7 Gy), and 5 (range 3–8), respectively. All patients had an available basic metabolic panel within 3 months of initiation of SBRT, from which pre-treatment serum bicarbonate was obtained. The median pre-treatment serum bicarbonate was 27 mEq/L (range 20–43 mEq/L), obtained at a median of 1.2 months prior to SBRT (range

Table 1
Baseline patient and treatment characteristics of the training cohort, stratified by pre-treatment bicarbonate < 26 mEq/L and bicarbonate ≥ 26 mEq/L.*

Variable	All Patients (n = 110)	Bicarbonate < 26 mEq/L (n = 34)	Bicarbonate ≥ 26 mEq/L (n = 76)	p
Age				
Median (range)	70.5 (51, 92)	72.5 (54, 87)	70.0 (51, 92)	0.84†
Sex				
Male	71 (64.5%)	26 (40.6%)	45 (59.2%)	0.13‡
Female	39 (35.5%)	8 (23.5%)	31 (40.8%)	
ECOG Performance Status				
0	17 (9.1%)	6 (17.6%)	11 (14.5%)	
1	56 (50.9%)	14 (41.2%)	42 (55.3%)	0.59‡
2	29 (26.4%)	11 (32.4%)	18 (23.7%)	
3	8 (7.3%)	3 (0.09%)	5 (6.6%)	
Smoking (pack-years)				
Median (IQR)	45 (25, 60)	50 (27.5, 70.0)	41.5 (23.8, 60.0)	0.73†
Variable	All Tumors (n = 114)	Bicarbonate <26 mEq/L (n = 35)	Bicarbonate ≥ 26 mEq/L (n = 79)	
Pre-Treatment Bicarbonate (mEq/L)				
Median (range)	27 (20, 43)	24 (20, 25)	29 (26, 43)	<0.001†
Histology				
Squamous Cell Carcinoma	42 (36.8%)	14 (40.0%)	28 (35.4%)	
Adenocarcinoma	62 (54.4%)	16 (45.7%)	46 (58.2%)	
NSCLC, NOS	10 (8.8%)	5 (14.3%)	5 (6.3%)	0.27‡
T-Stage§				
1	76 (66.7%)	23 (65.7%)	53 (67.1%)	
2	28 (24.6%)	8 (22.9%)	20 (25.3%)	
3/4	10 (8.8%)	4 (11.4%)	6 (7.6%)	0.87‡
Tumor Location‖				
Peripheral	47 (41.2%)	11 (33.3%)	36 (44.4%)	
Central	67 (58.8%)	22 (66.7%)	45 (55.6%)	0.38‡
BED (Gy_{10})				
Median (range)	100.0 (100.0, 160.5)	112.5 (100.0, 132.0)	105.0 (100.0, 160.5)	0.19†
GTV (cm^3)				
Median (range)	7.1 (0.5, 158.0)	6.7 (1.8–83.3)	7.7 (0.5–158.0)	0.63†
Number of Fractions for Treatment				
3	10 (8.8%)	2 (6.1%)	8 (9.9%)	
4	42 (36.8%)	16 (48.5%)	26 (32.1%)	
5	52 (45.6%)	10 (30.3%)	42 (51.9%)	
8	10 (8.8%)	5 (15.2%)	5 (6.2%)	0.08‡

* Abbreviations. ECOG – Eastern Cooperative Oncology Group. IQR – interquartile range. NSCLC, NOS – non-small cell lung cancer, not otherwise specified. BED – Biologically effective dose. GTV – Gross Tumor Volume.
† Mann-Whitney U (Wilcoxon rank-sum) test.
‡ Fisher's exact test.
§ American Joint Committee on Cancer (AJCC) 7th edition.
‖ As defined by the Radiation Therapy Oncology Group (RTOG): touching or within the zone (2 cm) of the proximal bronchial tree or adjacent to mediastinal or pericardial pleura.

0–3 months, IQR 0.6–1.7 months). Two-year primary tumor, regional nodal, and distant control of the entire cohort were 84.3%, 70.8%, and 75.2% respectively.

Association of pre-treatment bicarbonate and primary tumor failure

Univariate Cox regression analysis of bicarbonate as a continuous variable indicated lower serum bicarbonate was significantly associated with primary tumor recurrence (HR = 1.32; 95% CI 1.08–1.61, p = 0.008). There was no significant association of low pre-treatment serum bicarbonate with regional nodal recurrence (HR = 1.08; 95% CI 0.96–1.21; p = 0.22), distant recurrence (HR = 1.07; 95% CI 0.95–1.21; p = 0.28), or overall survival (HR = 1.01; 95% CI 0.92–1.07; p = 0.87). Multivariable cox proportional hazards model of bicarbonate as a continuous variable, when accounting for biologically effective dose (BED), central versus peripheral tumor location, histology, and GTV, continued to show lower bicarbonate was significantly associated with primary tumor recurrence (HR = 1.24; 95% CI 1.03–1.56; p = 0.024).

Using receiver operating characteristic curve (ROC) analysis, we determined that a pre-treatment serum bicarbonate concentration of 26 mEq/L was an optimal cutoff for predicting primary tumor recurrence (Fig. 1). Table 1 lists the patient and disease character-

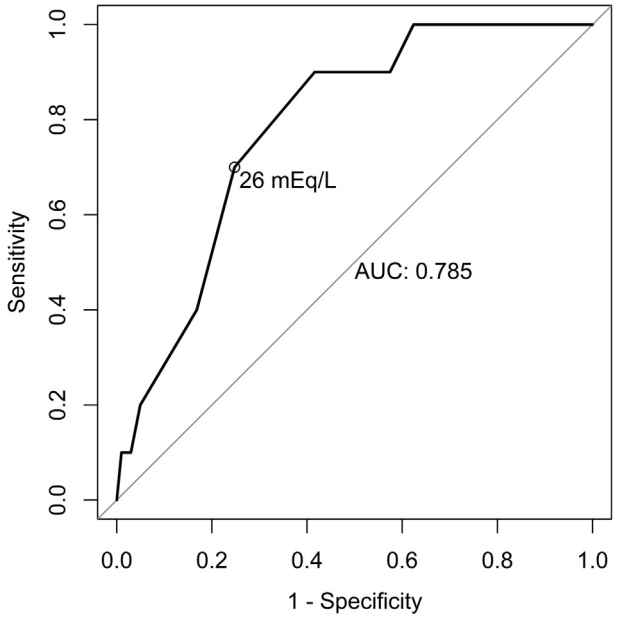

Fig. 1. Receiver operating characteristic curve for pre-treatment serum bicarbonate as a predictor of primary tumor failure.

istics of groups based on dichotomization using a serum bicarbonate concentration of 26 mEq/L. No statistically significant differences between age, gender, ECOG performance status, smoking status, histology, T-stage, central versus peripheral tumor location, BED, number of treatment fractions, or GTV were noted between low and high bicarbonate groups. Cox regression of bicarbonate as a dichotomized categorical variable revealed bicarbonate < 26 mEq/L was significantly associated with primary tumor recurrence on univariate (HR = 5.92; 95% CI 1.69–24.88; $p = 0.005$, Fig. 2) analysis, and remained independently associated with primary tumor recurrence on multivariate analysis (HR = 5.48; 95% CI 1.37–25.19; $p = 0.020$) (Table 2). Supplementary Fig. 1 shows a boxplot of serum bicarbonate values between patients with and without primary tumor recurrence and Supplementary Fig. 2 shows the conditional density plot of primary tumor recurrence as a function of serum bicarbonate concentration. We did not find association of pre-treatment bicarbonate < 26 mEq/L with regional nodal recurrence (HR = 1.37; 95% CI 0.52–3.57; $p = 0.53$), distant recurrence (HR = 1.27; 95% CI 0.45–3.57; $p = 0.65$), or overall survival (HR = 1.24; 95% CI 0.78–1.97; $p = 0.36$) (Supplementary Figs. 3–5).

Validation of pre-treatment bicarbonate and primary tumor failure

Given the significant association of pre-treatment serum bicarbonate with primary tumor failure in our training cohort, we tested this hypothesis using a validation cohort comprised of patients treated at three collaborating institutions. This pooled validation cohort consisted of 195 patients and 208 treated tumors, with a median follow up of 27.5 months for living patients (25.9 months for all patients) (Supplementary Table 1). When analyzed as a continuous variable, lower bicarbonate concentration was again significantly associated with primary tumor recurrence on univariate (HR = 1.22; 95% CI 1.04–1.43; $p = 0.016$) and multivariate analysis (HR = 1.20; 95% CI 1.02–1.42; $p = 0.028$) when accounting for the same covariates analyzed in the training cohort. When analyzed categorically, pre-treatment serum bicarbonate < 26 mEq/L had a statistically significant association with primary tumor failure (HR = 3.38; 95% CI 1.27–9.37; $p = 0.015$) (Fig. 3). On multivariate analysis, bicarbonate < 26 mEq/L continued to be significantly associated with primary tumor failure when accounting for the same covariates analyzed in the training cohort (HR = 3.33; 1.18–10.07; $p = 0.023$) (Table 3). Supplementary Fig. 6 shows a boxplot of serum bicarbonate values between patients

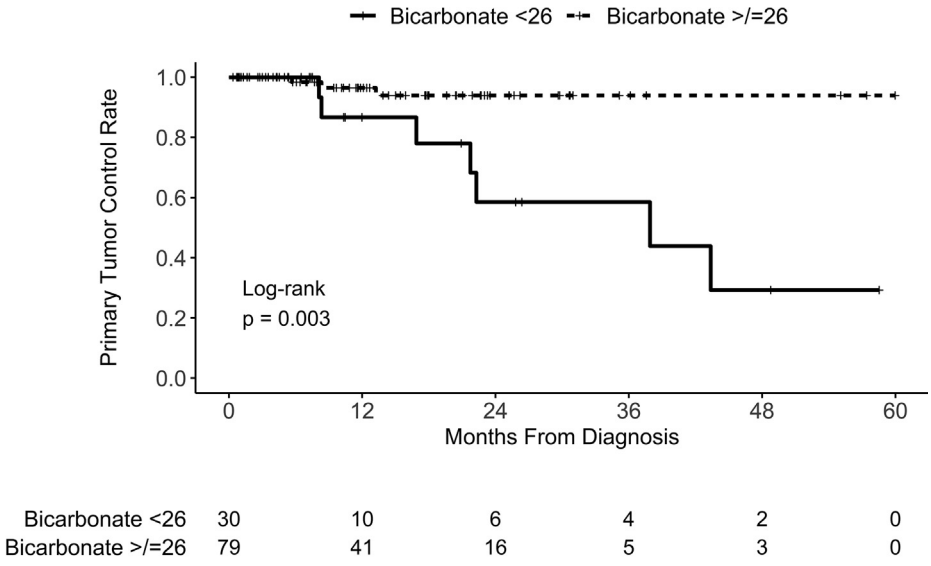

Fig. 2. Kaplan–Meier curves for primary tumor control for the training cohort.

Table 2
Univariate and multivariate hazard ratios for primary tumor recurrence in the training cohort.*

	Univariate Hazard Ratio (95% CI)	p	Multivariate Hazard Ratio (95% CI)	p
Bicarbonate Group				
≥26 mEq/L	1.0	–	1.0	–
<26 mEq/L	5.92 (1.69–24.88)	0.005	5.48 (1.37–25.19)	0.020
BED_{Gy10}	0.99 (0.93–1.04)	0.73	1.01 (0.92–1.08)	0.85
Tumor Location†				
Peripheral	1.0	–	1.0	–
Central	1.64 (0.50–5.94)	0.41	1.48 (0.42–5.70)	0.54
Histology				
Squamous	1.0			
Cell Carcinoma	2.69 (0.58–25.59)	–	1.0	–
Adenocarcinoma	16.41 (0.83–71.43)	0.22	3.59 (0.54–49.39)	0.20
NSCLC, NOS	1.0	0.07	4.22 (0.47–52.52)	0.20
GTV (cm^3)	1.00 (0.96–1.03)	0.74	01.02 (0.96–1.04)	0.45

* Abbreviations: CI – Confidence Interval. BED – Biologically Effective Dose. NSCLC, NOS – Non-small cell lung cancer, not otherwise specified. GTV – Gross Tumor Volume.
† As defined by the Radiation Therapy Oncology Group (RTOG). Central: touching or within the zone (2 cm) of the proximal bronchial tree or adjacent–mediastinal or pericardial pleura.

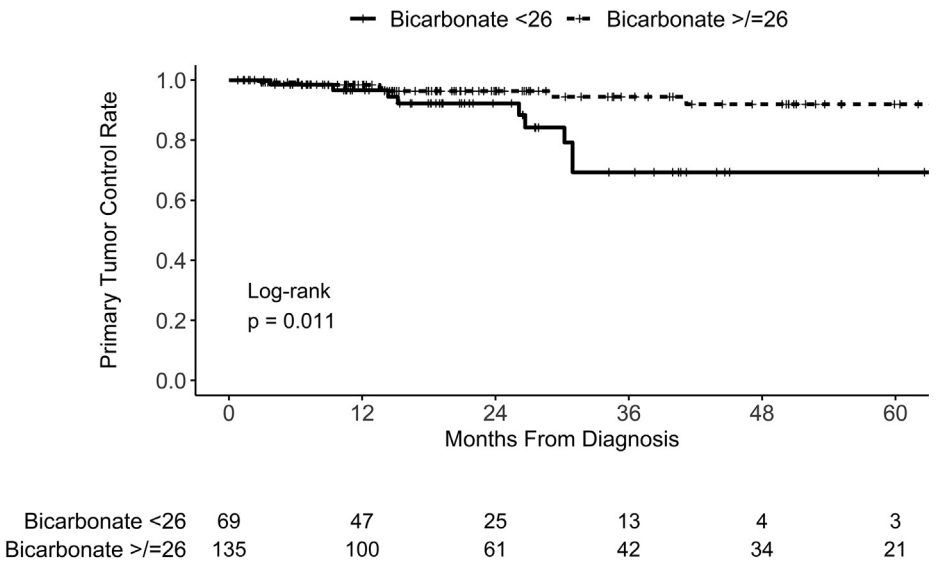

Fig. 3. Kaplan–Meier curves for primary tumor control for the validation cohort.

Table 3
Univariate and multivariate hazard ratios for primary tumor recurrence in the validation cohort.*

	Univariate Hazard Ratio (95% CI)	*p*	Multivariate Hazard Ratio (95% CI)	*p*
Bicarbonate Group				
≥26 mEq/L	1.0	–	1.0	–
<26 mEq/L	3.38 (1.27–9.37)	0.015	3.33 (1.18–10.07)	0.023
BED_{Cy10}	0.99 (0.95–1.03)	0.73	0.99 (0.95–1.03)	0.78
Tumor Location†				
Peripheral	1.0	–	1.0	–
Central	2.14 (0.76–5.60)	0.14	1.55 (0.49–4.43)	0.43
Histology				
Squamous	1.0			
Cell Carcinoma	0.40 (0.14–1.10)	–	1.0	–
Adenocarcinoma	4.82 (0.04–41.48)	0.07	0.44 (0.15–1.24)	0.12
Large Cell	0.31 (0.03–1.40)	0.39	–	–
NSCLC, NOS	1.0	0.14	0.34 (0.04–1.56)	0.18
GTV (cm^3)	1.02 (0.98–1.05)	0.29	1.02 (0.98–1.05)	0.34

* Abbreviations: CI- Confidence Interval. BED- Biologically Effective Dose. NSCLC, NOS- Non-small cell lung cancer, not otherwise specified. GTV- Gross tumor volume.
† As defined by the Radiation Therapy Oncology Group (RTOG). Central: touching or within the zone (2 cm) of the proximal bronchial tree or adjacent to mediastinal or pericardial pleura.

with and without primary tumor recurrence and Supplementary Fig. 7 shows the conditional density plot of primary tumor recurrence as a function of serum bicarbonate concentration.

Additionally, in the validation cohort, there was also a significant association of bicarbonate < 26 mEq/L with regional nodal recurrence (HR = 2.16; 95% CI 0.99–4.70; Cox $p = 0.052$) and distant recurrence (HR = 1.85; 95% CI 0.98–3.48; $p = 0.059$) on univariate analysis, but not overall survival (HR = 1.23; 95% CI 0.787–1.99; $p = 0.38$) (Supplementary Figs. 8–10). On multivariate analysis, bicarbonate < 26 mEq/L trended toward significance for association with regional nodal recurrence (HR = 2.26; 95% CI 0.97–5.25; $p = 0.060$) and distant recurrence (HR = 1.82; 95% CI 0.94–3.50; $p = 0.074$).

Secondary analysis of comorbidity

In order to assess the influence of comorbidity on serum bicarbonate and primary tumor control, we compared the difference in Charlson comorbidity index (CCI), COPD, and serum creatinine between low- and high-bicarbonate groups in a secondary analysis of the training cohort. The median CCI for the cohort with bicarbonate < 26 mEq/L was 7 (range 2–13) and the median CCI for the cohort with bicarbonate ≥ 26 mEq/L was 6 (range 3–11). There was no statistically significant difference in CCI between low- and high-bicarbonate groups (Wilcoxon rank-sum $p = 0.41$). There was a trend toward statistically significant difference ($p = 0.06$) between the median creatinine of the low-bicarbonate (median 1.1; range 0.63–3.5) and high-bicarbonate (median 0.91; range 0.46–3.5) cohorts. There was no statistically significant difference in the proportion of patients with COPD between both groups (bicarbonate < 26 mEq/L: 78.1%, bicarbonate ≥ 26 mEq/L: 71.2%; $p = 0.62$). Additionally, there was no statistically significant association of primary tumor recurrence with CCI (HR = 1.27; 95% CI 0.90–1.72; $p = 0.16$), COPD (HR = 0.77; 95% CI 0.34–1.83; $p = 0.54$), or serum creatinine (HR = 2.90; 95% CI 0.70–9.45; $p = 0.12$).

Discussion

This study is the first to associate serum bicarbonate with disease control in the setting of lung SBRT and, to our knowledge, is the first published study evaluating serum bicarbonate as a predictive biomarker for any cancer therapy. Using a serum bicarbonate concentration of 26 mEq/L as a cutoff established by ROC analysis, we found that decreased serum bicarbonate was significantly associated with higher risk of primary tumor recurrence. We extended

this finding by validating it in a larger, independent cohort of patients treated at three collaborating institutions. While we found no association of pre-treatment serum bicarbonate with overall survival, regional recurrence, or distant metastasis in the training cohort, we found bicarbonate < 26 mEq/L was interestingly also associated with regional nodal and distant recurrence in the validation cohort. It is not clear whether this finding simply reflects improved statistical power to detect differences or longer follow up in the larger validation cohort compared to the training cohort. Nevertheless, pre-treatment serum bicarbonate appears to be useful in stratifying patients with localized NSCLC at high risk for failure of the primary tumor, and potentially regional and distant recurrence, after SBRT.

In the absence of confounders that were not accounted for, two overarching possibilities in associating serum bicarbonate with tumor recurrence. First, it is possible that increased serum bicarbonate directly or indirectly promotes radiosensitization (or conversely, low serum bicarbonate directly or indirectly promotes radioresistance). There is pre-clinical evidence to suggest intracellular and extracellular acidification promote tumor invasiveness [25,26], mitigation of oxidative stress [27], and immunomodulation [28–31], all of which could induce radioresistance. Despite the relative insulation of the tumor microenvironment [32,33], exogenously delivered bicarbonate has been shown to increase tumor extracellular pH, as well as increase chemotherapeutic efficacy, reduce distant metastasis, and reduce tumor growth [34–39]. It is also possible that serum bicarbonate indirectly promotes radiosensitization by inducing systemic changes. Acid-base balance is intricately tied to cardiopulmonary function and, consequently, tissue oxygen delivery [40]. Low pH is known to induce changes associated with cardiac contractility, vasoconstriction, ventilatory drive, oxygen-hemoglobin dissociation, and pulmonary perfusion [40], all of which could theoretically affect oxygen dynamics of the tumor microenvironment. Indeed, the influence of vasoactivity on tumor oxygen delivery formed the basis of early preclinical studies centered on how oxygen affects radiosensitization [41–44]. Nevertheless, several points argue against a causal mechanism for bicarbonate's association with tumor control. First, the relative influence of the potentially indirect physiologic responses are circumstantial [40], and given the significant contribution of locally impaired tissue vascularity in inducing hypoxia [32,33], perhaps it is less likely that subtle pH-induced differences in systemic circulatory or cardiopulmonary dynamics would significantly affect oxygen delivery and radiosensitivity. Moreover, all of the aforementioned findings relate to acute pH changes induced by bicarbonate, whereas laboratory values are more likely to be influenced by the chronic changes in bicarbonate that serve to maintain pH homeostasis [45]. Therefore, it is unclear that serum or tumor pH would be significantly different between low and high bicarbonate cohorts. For these reasons, it is less likely that serum bicarbonate or acidosis itself results in changes in tumor radiosensitivity.

Alternatively, relatively low serum bicarbonate may be a secondary marker of tumor aggressiveness and/or a tumor microenvironment that is conducive to radioresistance. It is well-established that, at the level of the tumor microenvironment, tumor hypoxia induces acidosis through oncogene activation and alteration of cellular metabolism [11,12]. While the milieu of the tumor microenvironment must be distinguished from that of the serum, a number of cases of clinical systemic acidosis secondary to tumor, especially in the setting of metastatic and lymphoproliferative malignancies, have been described [46–53]. Notably, lactic acidosis has been identified as a negative prognostic marker in the setting of metastatic lung and colon cancer [54,55]. Nonetheless, such cases are extreme examples, and although it is possible that aggressive NSCLCs with a propensity for recurrence are characterized by tissue acidosis substantial enough to contribute to detectable changes in serum bicarbonate concentration, we feel it is unlikely that the small, early-stage tumors in our study induced such systemic changes. It is perhaps more likely that low serum bicarbonate is a marker secondary to a host-related process occurring outside of the tumor microenvironment that may lead to radioresistance. For example, pulmonary obstruction, congestive heart failure, pulmonary edema, or pulmonary arterial hypertension all may result in hypoxemia. Although, in healthy individuals such conditions should provoke hypercapnic acidosis and corresponding increase in serum bicarbonate, in states of illness they can be associated with tissue hypoperfusion and/or hypoxia resulting in metabolic acidosis [56]. It is conceivable that lung cancer, which relies on dual bronchial and pulmonary arterial blood supply [56,57], may be similarly affected by such changes, particularly in an SBRT population typified by chronic illness and comorbidity [58]. Retrospective evidence in the setting of advanced non-small cell lung cancer suggests that increased tumor perfusion on CT is associated with improved response to chemotherapy and chemoradiotherapy [59,60]. Thus, decreased serum bicarbonate may be the product of ventilatory, perfusional, or other systemic changes that are associated with decreased radiotherapeutic response of NSCLC.

In addition to primary tumor recurrence, regional and distant recurrence were also associated with low serum bicarbonate in the validation cohort. This is possibly secondary to more patient numbers and longer follow-up in the latter cohort as well as the consequence of local failure leading to development of metastatic disease, rather than pre-existing micrometastatic disease prior to SBRT. Additionally, the absence of an overall survival association in the training and validation cohorts may be due to the overall short follow-up time and/or adequate salvage of primary tumor recurrence [61].

Despite multi-institutional validation of our findings, there are several limitations inherent to the retrospective design of this study. First, despite similar baseline treatment characteristics and multivariable analysis accounting for GTV, histology, central versus peripheral tumor location, and BED, it is possible that heterogeneity between high and low bicarbonate groups exists with regard to other factors, such as patient comorbidities, medications, or pulmonary function. For example, pulmonary or renal comorbidities could potentially have prompted de-escalation of dose or treatment. However, it is worth noting that there was no significant difference in BED, fractionation, or performance status between high- and low-bicarbonate groups, and in a secondary analysis of the training cohort, there was also no significant difference in CCI, COPD, or creatinine. Second, despite the use of penalized regression, the multivariable analysis should be interpreted with caution given the limited number of primary tumor recurrence events. In an attempt to validate bicarbonate's independent association and account for at least some of the effect of known confounders, multiple variables were incorporated. We acknowledge this limits the accuracy of the regression model due to overfitting. Third, it is acknowledged that relative acidemia and alkalemia are best assessed using pH, whereas serum bicarbonate fluctuation is subject to the compensatory influences of renal and pulmonary acid-base mechanisms [62]. Given the potential link between acid-base balance and primary tumor control, directly correlating serum pH with recurrence and survival would be intriguing, although such analysis is limited by the lack of venous and arterial blood gases obtained in our patients during the course of their cancer diagnosis and management. Interestingly, studies in the intensive care unit setting suggest that serum bicarbonate is equally or more accurately predictive of mortality when compared to pH or anion gap, suggesting bicarbonate is a reasonable surrogate of arterial base deficit [63,64]. It is also worth noting that the relative difficulty and invasiveness of blood gas testing would limit the utility

of pH as a routine biomarker. Similarly, while serum lactate would be a useful in exploring some of the theoretical mechanisms for this study's findings, its lack of availability in this retrospective study precludes its assessment as a biomarker. Finally, we acknowledge that serum bicarbonate is dynamic and subject to fluctuation, although this alone should not dismiss its potential as a predictive variable. Indeed, electrolyte measures have been integrated into a number of prognostic scores, such as sodium for liver disease or calcium for metastatic renal cell carcinoma [65,66]. Future prospective studies to validate these findings would ideally obtain a serum chemistry in patients immediately prior to treatment.

In summary, low serum bicarbonate, as measured on a routine serum chemistry panel, is associated with increased primary tumor recurrence after lung SBRT. Thus, serum bicarbonate may be a valuable predictive biomarker that can be obtained both rapidly and inexpensively. These findings merit validation using a prospective cohort, and perhaps merit in-depth preclinical studies to ascertain a clearer mechanistic understanding of our findings.

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Declaration of Competing Interest

None of the authors have conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2019.05.014.

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