Translational Oncology 14 (2021) 101220

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Original Research

Peripheral blood values as predictors of autoimmune status in oral cavity squamous cell carcinoma

Anjali Pillai [a], Cristina Valero [a], Kathleen Navas [b], Quaid Morris [b,1], Snehal G. Patel [a,1,*]

[a] Department of Surgery, Head and Neck Service, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, United States
[b] Memorial Sloan Kettering Cancer Center, Sloan Kettering Institute, New York, NY, United States

ARTICLE INFO

Keywords:
Autoimmune disease
Oral squamous cell carcinoma
Inflammatory response
Head and neck cancer
Immune dysregulation

ABSTRACT

Background: Recent literature has highlighted the role of the host in prognosis in oral squamous cell carcinoma (OSCC). Autoimmune (AI) disease represents a macroscopic depiction of host status. The goal of this study was to predict an AI "status" and to analyze the utility of this "status" as a prognostic indicator in OSCC.
Methods: From a departmental database of OSCC patients (n = 1377), 125 patients with an AI disorder were identified. PBL values were obtained and standardized for analysis. A LASSO regression model was used to determine the best predictors of AI status and an AI score was developed. The score was then analyzed across various survival endpoints.
Results: When AI score was divided into a binary variable, patients in the highest quartile had a significantly worse overall survival (OS), local recurrence-free (LRFP) and distant recurrence-free probability (DRFP). Survival curves showed significant differences for OS, DSS, LRFP, and DRFP.
Conclusions: AI diseases are immune dysregulations that could play a role in prognosis. Therefore, development of an AI score is necessary to depict host status in a ubiquitous manner. AI score as a binary variable may be more utilitarian in a clinical setting, compared to the continuous score. This novel tool needs validation and integration into more tumor and host characteristics. This investigation showed utility of such a score, similar to PBL data in OSCC prognosis. Future studies should incorporate other relevant variables known to affect outcome and implement a more comprehensive predictive model.

Introduction

In oral cavity squamous cell carcinoma (OSCC), host factors have been proven to affect outcomes in patients treated with primary surgery. When ascertaining risk, it is prudent to identify predisposing factors outside of tumor criteria. Previous studies published by our group have shown how host factors such as peripheral blood leukocytes (PBLs), hemoglobin, and albumin are independently associated with outcomes in OSCC [1,2].

Immune surveillance has been recently given a key role in oncology. On occasion, tumors deemed to be low risk do poorly, and conversely, tumors deemed to be high risk have extended survival. The host is probably the key difference in these situations and more specifically, the host's immune system. Tumorigenesis occurs by evading angiogenesis suppressors, apoptotic signals, other defense mechanisms [3,4], and most significantly, tumor-infiltrating-lymphocytes (TIL) [5–7].

Specific Autoimmune (AI) diseases have also been associated with predisposing individuals to and increasing cancer risk as seen in a broad review [8]. A myriad of studies that included oral cancer patients saw an increased risk of oral malignancies due to the presence of AI disease, while a few dissented and contradicted those findings [9–24].

AI disease represents a larger consequence of a dysregulated immune system. However, in the absence of a confirmed diagnosis, the host's immune system may still depict similar phenomena in ways that can potentially be objectively identified. The goal of this study was to identify discernible trends in pre-treatment blood values that could

Abbreviations: ai, autoimmune; Cbc, complete blood count; Cmp, complete metabolic panel; Drfp, distant recurrence-free probability; Dss, disease-specific survival; Lrfp, local recurrence-free probability; Msk, memorial sloan kettering cancer center; OS, overall survival; OSCC, oral cavity squamous cell carcinoma; PBL's, peripheral blood leukocytes; RRFP, regional recurrence-free probability.

* Corresponding author.
E-mail address: patels@mskcc.org (S.G. Patel).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.tranon.2021.101220
Received 18 July 2021; Received in revised form 15 August 2021; Accepted 8 September 2021

predict an AI "status". Secondly, we aimed to analyze the value of this "status" as a predictor of outcomes in OSCC patients.

Materials and methods

In this study, we analyzed patients with OSCC treated with surgery at Memorial Sloan Kettering Cancer Center (MSK) between 1998 and 2015. The cohort was selected from our departmental database of patients who had a biopsy-proven invasive OSCC treated with primary surgery. Exclusion criteria were synchronous HNSCC, prior treatment of the reference carcinoma, distant metastasis at presentation, and prior history of non-endocrine head and neck cancer. The final cohort consisted in 1377 patients. The retrospective study design was approved by MSK's Institutional Review Board, which determined informed consent was not necessary due to the retrospective, deidentified characteristics of the investigation.

The main variables investigated included past medical history of an autoimmune (AI) disease and peripheral blood values found in complete blood count (CBC) and comprehensive metabolic panel (CMP) tests. History of an AI disease was assessed using a query of International classification of disease (ICD) codes with subsequent chart review to confirm. Only blood tests drawn within a month prior to surgery were included, selecting for analysis the closest to surgery date. Any lab value missing for more than 10% of the cohort was excluded ($n = 6$), leaving 36 lab values remaining for analysis. Urinalysis values were additionally collected, however 47% of the cohort had missing data. Therefore, we excluded urinalysis values from the study. The Supplementary Table 1 shows the breakdown of mean, standard deviation, and percent missing for each lab value in the overall cohort as well as within the group of patients without an autoimmune disease ($n = 1252$), and the group of patients with an autoimmune disease ($n = 125$).

Normality of the lab values was assessed using Kolmogorov-Smirnov and Shapiro-Wilk non-parametric tests. The results depicted significance values below 0•05 for both, showing non-normal distributions for all lab parameters. Initial attempts were made to normalize the data by utilizing a square root and logarithmic transformation. However, adjuvant analyses using the same non-parametric tests yielded significant values again for all parameters, suggesting the distribution was still skewed.

To standardize all lab values for further analysis, and to simplify filling in missing data, we performed a z-score transformation individually on each value. Afterwards, we replaced missing values by zeros, corresponding to filling in missing values with their means. Transformed values over 3 were replaced by 3 and those less than −3 were replaced by −3. In the following, we call these the standardized lab values.

Using the standardized lab values, we trained a LASSO regression model with cross-validation to determine the best predictors of autoimmune status. The model with the lambda that minimized the average cross-entropy loss on held-out data. Our model identified fourteen predictors (albumin, alkaline phosphatase (ALKP), aspartate transaminase (AST), blood urea nitrogen (BUN), CO2, glucose, potassium, protein, absolute lymphocyte and monocyte counts, percentage of eosinophils, platelet and red blood cell (RBC) counts, and prothrombin time). These were then used to score patients based on the likelihood of having a medical history of autoimmune disease with a logistic regression, with a higher score indicating more likely to have this history. The score was then transformed from a probability to a logOdds to create the variable known as AI Score.

A multivariable analysis with overall survival (OS) as dependent variable was conducted including the newly derived AI score and the clinicopathologic characteristics that were significant in the univariate analysis as independent variables. A second multivariable analysis was performed including the AI Score and an additional variable indicating presence/absence of an AI disease diagnosed and reported in the patient's charts. We additionally conducted multivariable analyses for disease-specific survival (DSS), local recurrence-free probability (LRFP), regional recurrence-free probability (RRFP) and distant recurrence-free probability (DRFP).

Next, after analyzing the AI Score as a continuous variable, we categorized the AI Score into High vs Low using top quartile as cutoff. Post hoc, we analyzed if there were differences within the Low AI Score group (AI Score bottom 75%). Comparison of AI Score using tertiles and quintiles in relation to OS, DSS, LRFP, RRFP, and DRFP was used.

Hazard ratios were calculated according to Cox's proportional hazard regression model, also used to perform the multivariable analyses. A nominal P value of less than 0•05 was considered statistically significant. All hypothesis tests were two-sided. All statistical analyses were conducted using Stata (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Results

Characteristics of the cohort ($n = 1377$)

The patient́s characteristics are shown in Table 1. The mean age was 62 (range, 18–100) and 56•4% of the patients were men. Tobacco and alcohol usage were present in 62•5% and 69•6% of patients, respectively. Based on Washington University Head and Neck Comorbidity Index (WUHNCI), 28•8% of patients had a score equal or greater than one [25]. The most common subsite was the tongue (53•5%). An advanced pathological stage (stages III-IV) was seen in 47•5% of the patients based on the 8th edition of the American Joint Committee on Cancer (AJCC) TNM classification [26].

A comparison of host and tumor characteristics based on AI status is additionally shown in Table 1. The AI disease group had a higher percentage of females compared to the group without AI disease (60% vs 42%, $P <$ •001) and a higher percentage of patients with comorbidities (38% vs 28%, $P =$ •013). No significant differences in the remaining characteristics were seen between the groups.

Prognostic capacity of AI score as continuous variable

Using AI Score as a continuous variable, we observed significant differences in OS, where higher scores were associated with poorer survival (HR = 1•64; 95% CI, 1•44–1•86; $P <$ •001) in the univariate analysis. In the multivariable analysis, the AI Score maintained independent prognostic capacity (HR = 1•28; 95% CI, 1•11–1•47; $P =$ •001). When analyzing DSS, a higher AI Score was associated with poorer survival in the univariate analysis (HR = 1•45; 95% CI, 1•18–1•78; $P <$ •001), but it did not maintain significance when analyzed in the multivariable analysis (HR = 1•14; 95% CI, 0•90–1•44; $P =$ •269). The results of the uni- and multivariable analysis for OS and DSS are shown in Table 2.

Table 3 shows the results of the uni- and multivariable analyses for LRFP, RRFP, and DRFP. AI Score as a continuous variable showed prognostic capacity for LRFP and DRFP endpoints in the univariate analysis (HR=1•41; 95% CI, 1•16–1•71; $P =$ •001 and HR=1•39; 95% CI, 1•04–1•85; $P =$ •025, respectively), and it maintained independent prognostic capacity in the multivariable analysis for LRFP (HR=1•35; 95% CI, 1•09–1•68; $P =$ •006), but it did not for DRFP (HR=1•32; 95% CI, 0•98–1•78; $P =$ •065). No significant differences were seen for RRFP in the univariate analysis nor in the multivariable analysis (HR=1•08; 95% CI, 0•86–1•35; $P =$ •523; HR=0•998; 95% CI, 0•79–1•27; $P =$ •988).

When the AI Status (Yes/No) was included in the multivariable analysis with the AI Score, the AI Status (Yes/No) did not show significance in either of the analyses, while the AI Score maintained significance (see Supplementary Table 2).

Prognostic capacity of AI score as categorical variable

To better reflect how AI Score may be used in a clinical setting and to better characterize the relationship between the AI Score and various

Table 1
Clinicopathologic characteristics of the cohort ($n = 1377$) and comparison of patients with and without an autoimmune (AI) disease.

Characteristics	Overall No. of patients (%)	No AI Disease ($n = 1252$) No. of patients (%)	AI Disease ($n = 125$) No. of patients (%)	*P*
Age, mean (SD, range)	61•9 (14•4, 18•3–100•4)	62•0 (14•5, 18•3–100•4)	61•6 (13•3, 26•6–93•7)	•808
Sex				<0•001
Male	776 (56•4)	726 (58•0)	50 (40•0)	
Female	601 (43•7)	526 (42•0)	75 (60•0)	
Tobacco use				•240
Never	517 (37•6)	464 (37•1)	53 (42•4)	
Ever	860 (62•5)	788 (62•9)	72 (57•6)	
Alcohol use				•224
Never	419 (30•4)	375 (30•0)	44 (35•2)	
Ever	958 (69•6)	877 (70•1)	81 (64•8)	
WUHNCI				•013
0	980 (71•2)	903 (72•1)	77 (61•6)	
≥ 1	397 (28•8)	349 (27•9)	48 (38•4)	
Subsite				•648
Oral Tongue	737 (53•5)	665 (53•1)	72 (57•6)	
Lower Gum	181 (13•1)	163 (13•0)	18 (14•4)	
Floor of Mouth	165 (12•0)	154 (12•3)	11 (8•8)	
Buccal Mucosa	107 (7•8)	96 (7•7)	11 (8•8)	
Upper Gum	93 (6•8)	84 (6•7)	9 (7•2)	
Retromolar Trigone	68 (4•9)	65 (5•2)	3 (2•4)	
Hard Palate	26 (1•9)	25 (2•0)	1 (0•8)	
pT classification (AJCC 8th edition)				•006
pT1	447 (32•5)	394 (31•5)	53 (42•4)	
pT2	346 (25•1)	313 (25•0)	33 (26•4)	
pT3	260 (18•9)	249 (19•9)	11 (8•8)	
pT4	257 (18•7)	232 (18•5)	25 (20•0)	
Not recorded	67 (4•9)	64 (5•1)	3 (2•4)	
pN classification (AJCC 8th edition)				•860
pN0	941 (68•3)	851 (68•0)	90 (72•0)	
pN1	124 (9•0)	112 (9•0)	12 (9•6)	
pN2	135 (9•8)	126 (10•1)	9 (7•2)	
pN3	158 (11•5)	145 (11•6)	13 (10•4)	
Not recorded	19 (1•4)	18 (1•4)	1 (0•8)	
pOverall Stage (AJCC 8th edition)				•127
Stage I	407 (29•6)	359 (28•7)	48 (38•4)	
Stage II	244 (17•7)	220 (17•6)	24 (19•2)	
Stage III	211 (15•3)	198 (15•8)	13 (10•4)	
Stage IV	444 (32•2)	408 (32•6)	36 (28•8)	
Not recorded	71 (5•2)	67 (5•4)	4 (3•2)	
Grade				•381
Well differentiated	234 (17•0)	212 (16•9)	22 (17•6)	
Moderately differentiated	873 (63•4)	788 (62•9)	85 (68•0)	
Poorly differentiated	205 (14•9)	193 (15•4)	12 (9•6)	
Not recorded	65 (4•7)	59 (4•7)	6 (4•8)	
Perineural Invasion				•172
Absent	864 (62•8)	776 (62•0)	88 (70•4)	
Present	420 (30•5)	389 (31•1)	31 (24•8)	
Not recorded	93 (6•8)	87 (7•0)	6 (4•8)	
Lymphovascular Invasion				•545
Absent	1098 (79•7)	994 (79•4)	104 (83•2)	
Present	186 (13•5)	171 (13•7)	15 (12•0)	
Not recorded	93 (6•8)	87 (7•0)	6 (4•8)	
Margin Status				•834
Negative	415 (30•1)	376 (30•0)	39 (31•2)	
Close	815 (59•2)	744 (59•4)	71 (56•8)	
Positive	141 (10•2)	126 (10•1)	15 (12•0)	
Not recorded	6 (0•4)	6 (0•5)	0 (0•0)	
Treatment				•281
Surgery	857 (62•2)	771 (61•6)	86 (68•8)	
Surgery and Adjuvant Radiotherapy	395 (28•7)	365 (29•2)	30 (24•0)	
Surgery and Adjuvant Chemoradiotherapy	125 (9•1)	116 (9•3)	9 (7•2)	

Abbreviations: AI, autoimmune; WUHNCI, Washington University Comorbidity Index; p, pathological; AJCC, American Joint Committee on Cancer.

Table 2
Univariate and Multivariable analysis for overall survival (OS) and disease-specific survival (DSS) with Autoimmune (AI) Score as continuous.

Characteristic	Overall Survival				Disease-Specific Survival			
	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1•038 (1•032–1•044)	<0•001	1•031 (1•024–1•038)	<0•001	1•013 (1•004–1•022)	•007	1•008 (0•997–1•018)	•151
Sex		•733		••		•009		•002
Female	1		••		1		1	
Male	0•973 (0•831–1•139)				0•714 (0•556–0•918)		0•651 (0•498–0•850)	
Tobacco use		•001		•053		•391		••
Never	1		1		1		••	
Ever	1•326 (1•121–1•568)		1•194 (0•997–1•429)		0•895 (0•694–1•154)			
WUHNCI	1•254 (1•191–1•319)	<0•001	1•142 (1•072–1•217)	<0•001	1•114 (1•007–1•232)	•036	1•092 (0•970–1•23)	•145
LVI		<0•001		•017		<0•001		•003
Absent	1		1		1		1	
Present	1•999 (1•626–2•460)		1•315 (1•050–1•647)		3•140 (2•363–4•173)		1•609 (1•180–2•193)	
PNI		<0•001		<0•001		<0•001		•002
Absent	1		1		1		1	
Present	2•128 (1•807–2•506)		1•544 (1•279–1•864)		3•333 (2•572–4•318)		1•578 (1•182–2•107)	
Margin Status								
Negative	1		1		1		1	
Close	1•532 (1•267–1•853)	<0•001	1•139 (0•924–1•403)	•224	1•829 (1•313–2•548)	<0•001	1•218 (0•849–1•749)	•284
Positive	3•023 (2•354–3•884)	<0•001	1•922 (1•455–2•538)	<0•001	5•047 (3•392–7•509)	<0•001	2•199 (1•424–3•397)	<0•001
Histologic grade								
Well diff	1		1		1		1	
Moderately diff	1•560 (1•238–1•966)	<0•001	1•063 (0•814–1•388)	•653	4•308 (2•399–7•736)	<0•001	1•795 (0•954–3•380)	•070
Poorly diff	2•155 (1•632–2•846)	<0•001	1•147 (0•824–1•595)	•416	7•128 (3•828–13•274)	<0•001	1•774 (0•889–3•540)	•104
pStage AJCC8								
I	1		1		1		1	
II	1•699 (1•292–2•235)	<0•001	1•480 (1•105–1•981)	•009	2•760 (1•498–5•087)	•001	2•469 (1•280–4•764)	•007
III	1•827 (1•380–2•418)	<0•001	1•475 (1•085–2•007)	•013	4•413 (2•472–7•877)	<0•001	3•428 (1•807–6•500)	<0•001
IV	4•245 (3•401–5•299)	<0•001	2•543 (1•957–3•303)	<0•001	13•394 (8•118–22•099)	<0•001	7•572 (4•239–13•526)	<0•001
AI Score, Continuous	1•639 (1•443–1•861)	<0•001	1•276 (1•106–1•473)	•001	1•445 (1•177–1•775)	<0•001	1•140 (0•904–1•439)	•269

Abbreviations: HR, Hazard ratio; CI, confidence interval; WUHNCI, Washington University Comorbidity Index; LVI, lymphovascular invasion; PNI, perineural invasion; diff, differentiated; pStage, pathological overall stage; AJCC8, American Joint Committee on Cancer 8th Edition; AI, Autoimmune.

outcomes, we dichotomized the AI Score to generate a binary variable.

First, to dichotomize AI Score, to label patients likely and unlikely to have a history of autoimmune disease, we selected the highest quartile, to allow for some false positives in the assignment of AI status and under the assumption that our 9% rate of AI in the cohort was an underestimate of the actual prevalence of disease.

Patients in the highest quartile had a significantly worse OS than patients in the bottom three quartiles both in the univariate analysis (HR = 1•73; 95% CI, 1•46–2•06; $P < •001$) and the multivariable analysis (HR = 1•40; 95% CI, 1•16–1•69; $P < •001$). For DSS, significant differences were seen in the univariate analysis (HR = 1•39; 95% CI, 1•05–1•84; $P = •023$), but not in the multivariable analysis (HR = 1•20; 95% CI, 0•88–1•63; $P = •249$).

We analyzed the dichotomized AI Score for LRFP, RRFP, and DRFP. For LRFP and DRFP, the AI Score as a binary variable was significant in the multivariable analysis (HR=1•43; 95% CI, 1•08–1•91; $P = •014$ and HR=1•73; 95% CI, 1•17–2•54; $P = •006$). For RRFP, the AI Score was not significant in the multivariable analysis (HR=1•16; 95% CI, 0•84–1•60; $P = •376$). The results of the multivariable analyses for all the endpoints of interest are shown in Table 4. Moreover, survival curves comparing AI Score top 25% vs bottom 75% for the endpoints of interest are shown in Fig. 1.

We repeated the analyses first using tertiles and then using quintiles as cutoffs to analyze possible differences within the lowest 75% of the population. The results validated that only the highest AI Scores had significant differences in outcomes compared to the rest of the cohort (see Supplementary Tables 3 and 4).

Discussion

AI diseases in other retrospective studies, are limited by clinical ICD verifications [9–24]. While the reason for this is to avoid including any ineligible patients, symptomatic individuals without confirmed diagnoses are therefore left out of these analyses and adverse risk is not assessed. If there were widely accessible lab parameters that stratified patients based on an overarching immune status, especially in the realm of difficult-to-diagnose AI diseases, outcomes could be assessed beyond the scope of pre-existing, diagnosed conditions. Therefore, we believe that AI status as a unique indicator compared to AI disease diagnoses can provide a broader reach and more generalizable metrics.

AI diseases could partly reflect host immune characteristics that play a role in oncologic outcomes [3–7]. However, they are rarely taken into account. While our group and others have identified the significant role host status plays especially in relation to peripheral blood leukocytes, AI diseases and status has not been assessed to the same degree [1,2]. Therefore, the initial goal of this study was to identify any trends in lab values that could formulate an AI status and subsequently apply this to the scope of OSCC prognosis.

As stated in the results section, AI diseases primarily affect women and this was also validated in the differences seen between the AI and no-AI groups [27]. As is known, women are at lower risk for developing oral cancer particularly due to their limited environmental exposures to the known carcinogens compared to men, so an underlying autoimmune or immunomodulatory condition is a potential explanation for this phenomenon. However, most of the other parameters were not significantly dissimilar between the two. While the clinicopathological differences were not stark between the AI and no-AI groups, there were differences in survival outcomes noted. As discussed previously, an AI score was established as a binary variable comparing the top quartile to the bottom three quartiles. Overall survival (OS) was significantly different between the patients in the highest quartile of AI score and the lower three quartiles (Fig. 1). Additionally, disease-specific survival (DSS), local recurrence-free probability (LRFP), and distant recurrence-free probability (DRFP) were all significantly different between the two groups. These results suggest that an elevated AI status, or dysregulated immune system outside of an AI disease or diagnosis can

Table 3
Univariate and Multivariable analysis for local recurrence-free probability (LRFP), regional recurrence-free probability (RRFP) and distant recurrence-free probability (DRFP) with Autoimmune (AI) Score as continuous.

Variable	LRFP				RRFP				DRFP			
	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1•009 (1•000–1•017)	•053	••	••	1•005 (0•995–1•015)	•333	••	••	0•862 (0•608–1•224)	•407	••	••
Sex		•630		••		•161		••		•359		••
Female	1		••		1		••		1		••	
Male	0•943 (0•744–1•200)				0•825 (0•630–1•080)				1•006 (0•993–1•019)			
Tobacco use		•001		••		•255		••		•979		••
Never	1		••		1		••		1		••	
Ever	1•134 (0•887–1•448)				0•854 (0•650–1•121)				0•995 (0•695–1•424)			
WUHNCI	1•041 (0•934–1•162)	•466			0•999 (0•878–1•136)	•985			0•898 (0•732–1•103)	•305		
LVI		•004		•388		<0•001		•095		<0•001		•036
Absent	1		1		1		1		1		1	
Present	1•611 (1•167–2•223)		1•165 (0•823–1•649)		2•141 (1•540–2•977)		1•356 (0•948–1•940)		3•520 (2•393–5•176)		1•563 (1•030–2•371)	
PNI		<0•001		•003		<0•001		•378		<0•001		•006
Absent	1		1		1		1		1		1	
Present	1•913 (1•493–2•451)		1•545 (1•155–2•068)		1•942 (1•469–2•567)		1•153 (0•840–1•580)		3•757 (2•609–5•410)		1•783 (1•184–2•688)	
Margin Status												
Negative	1		1		1		1		1		1	
Close	1•514 (1•137–2•014)	•004	1•424 (1•021–1•987)	•037	1•643 (1•176–2•297)	•004	1•252 (0•871–1•800)	•225	1•883 (1•169–3•033)	<0•001	1•236 (0•729–2•095)	•431
Positive	2•886 (1•959–4•252)	<0•001	2•226 (1•403–3•529)	•001	2•854 (1•809–4•505)	<0•001	1•775 (1•075–2•928)	•025	6•178 (3•573–10•681)	<0•001	2•593 (1•415–4•751)	•002
Histologic grade												
Well diff	1		1		1		1		1		1	
Moderately diff	1•344 (0•953–1•900)	•092	0•963 (0•640–1•449)	•856	2•689 (1•604–4•508)	<0•001	2•052 (1•143–3•686)	•016	4•983 (2•021–12•291)	<0•001	1•732 (0•678–4•434)	•251
Poorly diff	1•717 (1•126–2•618)	•012	0•926 (0•556–1•542)	•776	4•466 (2•543–7•844)	<0•001	2•712 (1•409–5•219)	•003	10•450 (4•099–26•639)	<0•001	2•029 (0•745–5•524)	•166
pOverall Stage (AJCC 8th Ed)												
I	1		1		1		1		1		1	
II	1•339 (0•908–1•973)	<0•001	1•336 (0•876–2•038)	•178	1•900 (1•204–2•997)	•006	1•535 (0•949–2•485)	•081	1•456 (0•603–3•513)	•404	1•226 (0•481–3•123)	•670
III	1•439 (0•972–2•132)	•069	1•266 (0•811–2•038)	•300	1•671 (1•025–2•725)	•040	1•189 (0•701–2•017)	•518	2•512 (1•125–5•607)	•025	1•663 (0•682–4•056)	•264
IV	2•593 (1•897–3•544)	<0•001	1•842 (1•247–2•47)	•002	3•665 (2•504–5•363)	<0•001	2•131 (1•363–3•331)	•001	10•856 (5•792–20•345)	<0•001	5•306 (0•983–1•783)	•065
AI Score,												
Continuous	1•408 (1•158–1•711)	•001	1•352 (1•091–1•676)	•006	1•077 (0•858–1•351)	•523	0•998 (0•786–1•267)	•988	1•386 (1•042–1•845)	•025	1•324 (0•983–1•783)	•065

Abbreviations: HR, Hazard ratio; CI, confidence interval; WUHNCI, Washington University Comorbidity Index; LVI, lymphovascular invasion; PNI, perineural invasion; diff, differentiated; p, pathological; AJCC, American Joint Committee on Cancer; AI, Autoimmune.

Table 4
Multivariable analysis with Autoimmune (AI) Score categorized as top 25% vs bottom 75% for all the endpoints of interest.

Variable	OS Multivariable analysis		DSS Multivariable analysis		LRFP Multivariable analysis		RRFP Multivariable analysis		DRFP Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1•032 (1•025–1•039)	<0•001	1•008 (0•997–1•018)	•145	••	••	••	••	••	••
Sex		••		•001		••		••		••
Female	••		1		••		••		••	
Male			0•646 (0•496–0•841)							
Tobacco use		•046		••		••		••		••
Never	1		••		••		••		••	
Ever	1•200 (1•003–1•437)									
WUHNCI	1•151 (1•081–1•226)	<0•001	1•098 (0•976–1•234)	•121	••	••	••	••	••	••
LVI		•023		•003		•417		•095		•034
Absent	1		1		1		1		1	
Present	1•299 (1•037–1•626)		1•610 (1•182–2•193)		1•154 (0•816–1•632)		1•357 (0•949–1•941)		1•570 (1•035–2•382)	
PNI		<0•001		•002		•003		•380		•004
Absent	1		1		1		1		1	
Present	1•558 (1•290–1•880)		1•582 (1•185–2•111)		1•546 (1•156–2•069)		1•152 (0•840–1•579)		1•808 (1•202–2•721)	
Margin Status										
Negative	1		1		1		1		1	
Close	1•138 (0•923–1•403)	•225	1•139 (0•924–1•403)	•224	1•430 (1•025–1•995)	•035	1•242 (0•864–1•786)	•241	1•229 (0•725–2•082)	•445
Positive	1•944 (1•472–2•568)	<0•001	1•922 (1•455–2•538)	<0•001	2•236 (1•410–3•545)	•001	1•772 (1•074–2•934)	•025	2•638 (1•441–4•829)	•002
Histologic grade										
Well diff	1		1		1		1		1	
Mod diff	1•065 (0•816–1•390)	0•643	1•793 (0•953–3•377)	•070	0•961 (0•639–1•445)	•849	2•051 (1•142–3•683)	•016	1•699 (0•665–4•341)	•268
Poorly diff	1•137 (0•817–1•582)	0•447	1•761 (0•882–3•517)	•109	0•929 (0•558–1•546)	•776	2•679 (1•392–5•158)	•003	1•952 (0•716–5•318)	•191
pOverall Stage AJCC 8th Ed										
I	1		1		1		1		1	
II	1•480 (1•105–1•981)	0•008	2•482 (1•286–4•791)	•007	1•334 (0•874–2•035)	•181	1•563 (0•965–2•532)	•069	1•278 (0•502–3•258)	•607
III	1•476 (1•085–2•007)	0•013	3•440 (1•813–6•526)	<0•001	1•262 (0•808–1•971)	•306	1•215 (0•717–2•062)	•469	1•715 (0•703–4•183)	•235
IV	2•560 (1•971–3•325)	<0•001	7•635 (4•273–13•641)	<0•001	1•887 (1•278–2•786)	•001	2•147 (1•373–3•357)	•001	5•446 (2•594–11•437)	<0•001
AI Score										
Lowest 75%	1		1		1		1		1	
Highest 25%	1•397 (1•159–1•685)	<0•001	1•198 (0•881–1•628)	•249	1•434 (1•076–1•912)	•014	1•157 (0•838–1•598)	•376	1•725 (1•170–2•543)	•006

Abbreviations: OS, Overall Survival; DSS, Disease-specific survival; LRFP, local recurrence-free probability; RRFP, regional recurrence-free probability; DRFP, distant recurrence-free probability; HR, Hazard ratio; CI, confidence interval; WUHNCI, Washington University Comorbidity Index; LVI, lymphovascular invasion; PNI, perineural invasion; diff, differentiated; Mod, moderately; p, pathological; AJCC, American Joint Committee on Cancer; AI, Autoimmune.

impact outcomes in OSCC.

As a continuous variable, AI score maintained independent prognostic capacity for OS and LRFP in both the univariate and multivariable analyses (Tables 2 and 3). AI score as a binary variable was incorporated as an option that could increase utility in a clinical setting. The patients were divided into two categories: the highest quartile of AI score against the lowest three quartiles. In this case, patients had worse OS in the highest quartile compared to those in the lowest three. For LRFP and DRFP, the AI score as a categorical variable was also shown to be an independent prognostic factor in the multivariable analysis (Table 4).

As AI score is a novel concept and has yet to be validated in any manner of its kind in a clinical setting, both the continuous and categorical avenues were explored. The division of AI score into a binary variable may be more applicable in a clinical setting. The binary variable would help stratify patients more easily for risk analysis.

The patients in this cohort were selected because they had not been treated prior to surgery at MSK and peripheral blood values submitted were prior to surgery as well. In a less controlled setting, there may need to be a wider stratification in order to accurately determine which patients are at an elevated risk for poorer outcomes, although this was seen with OS in both continuous and binary analyses. However, the AI score as a binary variable additionally identified poorer prognosis and outcomes in the LRFP and DRFP settings. This may allude to a better stratification, as mentioned, of a dysregulated immune system and its effects.

It is prudent to note that the patients in this cohort had no prior treatment to presentation at MSK. The AI score is reflective of an underlying dysregulated immune system and adds to the literature investigating the effects of this on cancer outcomes [1–7]. Of note, most of the patients in the cohort used to derive significant PBL values were female as more females have AI disease compared to men in both our cohort and in the general population, which may represent referral bias.

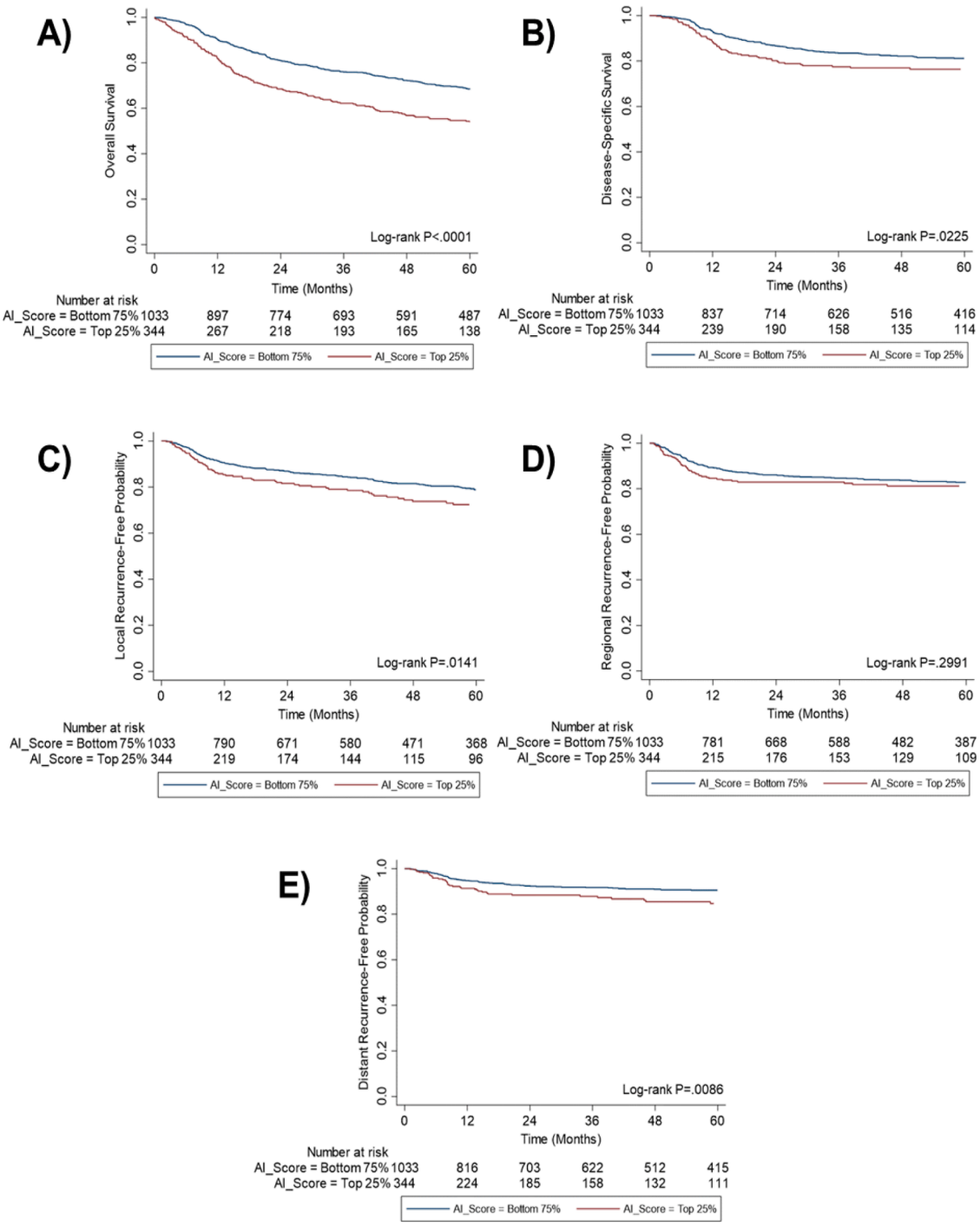

Fig. 1. Survival curves according to Autoimmune Score (AI Score)
(A) Overall Survival, (B) Disease-Specific Survival, (C) Local Recurrence-Free Probability, (D) Regional Recurrence-Free Probability and (E) Distant Recurrence-Free Probability comparing top 25% AI Score to bottom 75% AI Score.

Additionally, only 49.6% of females in the cohort were smokers compared to 72.4% of males ($p < .001$). However, our overall cohort sex distribution is consistent with the current paradigm shift seen in increasing incidence of oral cancer in the US [28]. While the score as a binary variable is showing prognostic potential based on these results, it is only one parameter in the grand scheme of factors that affect and predict oncological outcomes. This tool is novel and can therefore be made more accurate with validation and extension into more tumor and host characteristics. The role of this investigation was to discover whether this score had a role at all, like pre-treatment peripheral blood values [1,2]. Future studies should incorporate other relevant variables known to affect outcome and implement a more comprehensive predictive model.

As discussed earlier, AI (Y/N) was also included in this analysis but did not maintain significance in the multivariable analysis while AI score did (Supplementary Table 2). An AI diagnosis is often not documented, misdiagnosed or underdiagnosed, and overall provides a poorer method by which to stratify patients. In addition, the dysregulation that may result from an AI diagnosis is not limited to that realm. We believe that with the AI score, we can better determine who has an altered

immune status than the presence or absence of a diagnosed AI disease to stratify patients into limited binary groups.

The peripheral blood data is a non-invasive, universally utilized, and cost-effective way to determine if a patient may be at higher risk of poorer prognosis, or even local or distant disease failure. While discernible symptoms may seem like the gold standard especially in a clinical setting, there is evidence to show that it is simply not enough to truly quantify how these patients are adversely affected. Additionally, patients are often included in studies investigating this association between prognosis and AI diseases who are currently on immunosuppressive treatment. In these scenarios, it is difficult then to parse out the true causative agent: the disease or the treatment for the disease [9–24].

To further explain how AI status matters clinically, it has already been documented that pre-treatment blood values within the OSCC patient population is associated with poorer prognosis [1,2]. Additionally, the role that tumor-infiltrating lymphocytes, T cells, and other immunological markers play in cancer risk and outcomes has been established [3–5]. However, the phenomenon of dysregulation at the molecular and blood serum level has not been affiliated with what is seen in AI patients at a diagnostic level. To link the two, fourteen lab values were included as those associated with an AI disease diagnosis (Supplementary Table 1). After linking these lab values to an AI diagnosis, an AI status outside of a definitive diagnosis was developed that could be utilized to stratify risk for patients in the future. The applicability of this pre-operatively as well as pre-treatment is tantamount as our understanding of the host immune system escalates.

As a binary variable, clinically the utility to place patients in a high-risk top quartile is relatively easy and is not difficult to interpret. Currently, pre-operative blood draws are done routinely but the multitude of lab values obtained afterward is difficult to consolidate, utilize, and practically implement as a risk assessment tool in the real world. Having a preset algorithm to stratify patients based on these lab values would do the work for physicians and clinicians attempting to plan treatment for patients with some covert level of immune dysregulation. The ease of utility, cost-effectiveness, and universal use of blood draws makes this prognostic tool an incredibly feasible opportunity to provide more information for treating physicians to help decide and mold treatment plans.

While the findings in the study with this novel prognostic tool are tantalizing, there are limitations to this study. First and foremost, this study is retrospective in nature and was done only in one cohort treated at the same center. Additionally, the statistical power of the AI patients is low since only 124 patients within the OSCC database had a diagnosed and documented AI condition to compare peripheral blood data to. The limited number and therefore low power may have had an impact on the determination of which fourteen lab values to use for the AI status prognostic tool. While the database was limited to OSCC patients, this phenomenon is likely not specific to this disease and subsite and therefore should be further analyzed in other cancers. Lastly, this is a novel method of calculating association between specific lab values and a categorical variable (AI disease status), so there may be others pertinent to an “AI Score” that this analysis was unable to capture.

In conclusion, our investigation yielded significant results in that a novel AI Score that can eventually be used in a clinical setting with prognostic potential was formulated using easily obtained lab parameters. AI Score as a binary variable found significant poorer outcomes across OS, LRFP, and DRFP. This signal contributes to the knowledge about the host’s role in cancer outcomes. The significance of the analysis lies in both the development of the AI Score itself as well as its utility in cancer care. Additionally, host factors have been identified through this study with prognostic value that can be incorporated into statistical analyses such as nomograms later on. Future studies will need to validate the AI Score in other cohorts, expand to other cancer types and to then compare in population-based studies to the general population.

CRediT authorship contribution statement

Anjali Pillai: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Cristina Valero:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Kathleen Navas:** Methodology, Writing – review & editing. **Quaid Morris:** Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Snehal G. Patel:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Snehal G. Patel has a patent PCT/US2016/026717 Methods of Cancer Detection Using PARPI-FL pending, holds equity in Summit Biomedical Imaging, has a patent US 10,016,238 B2 Apparatus, system and method for providing laser steering and focusing for incision, excision and ablation of tissue in minimally-invasive surgery, holds equity in ColdSteel Laser Inc, has a patent PCT/US2014/073053 Systems, methods, and apparatus for multichannel imaging of fluorescent sources in real time, has a patent PCT/US2015/065816 Cyclic peptides with enhanced nerve-binding selectivity, nanoparticles bound with said cyclic peptides, and use of same for real-time *in vivo* nerve tissue imaging and has a patent PCT/US2016/066969 Imaging systems and methods for tissue differentiation, e.g., for intraoperative visualization.

Acknowledgment

Funding: This study was partly funded by Fundación Alfonso Martín Escudero and the NIH/NCI Cancer Center Support Grant P30 CA008748 and NCI R25 CA233208.

Data sharing

The data that support the findings of this study are available from the corresponding author, SP, upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101220.

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