Immunoscore combining CD8, FoxP3 and CD68 expression and distribution predicts the prognosis of head and neck cancer patients

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Introduction:

Head and neck squamous cell carcinomas (HNSCC) are among the most prevalent cancers worldwide, setting them in the 6th place. In Belgium, their incidences are higher and such cancers arise at the 4th position in men. Despite advances in therapeutic approaches, the mortality rate has remained relatively constant in recent years, with a 5-year survival rate around 50% and recurrences occurring in 40-60% of treated patients. It appears that the cell composition of the tumour microenvironment (TME) is likely to influence patient outcome. Currently, there is no immune-based classification of head and neck cancer. However, the evaluation of immune cell recruitment to classify HNSCC patients in different immunologic subgroups depending on the TME composition could be helpful to improve patient prognosis. In this study, we propose an immune signature based on CD8+, FoxP3+ and CD68+ count in IT and/or stromal (ST) compartments in a large clinical series of 258 patients with HNSCC.

Patients and Methods

Table 1. Patient population characteristics

Variables	Number of cases (n=258)	Relapse-free survival p-value	HR (95% CI)	Overall survival p-value	HR (95% CI)
Age (years)					
Median (range)	61 (29-90)				
Recurrence (RFS) (months)					
Median (range)	22 (1-245)				
Yes	104				
No	120				
Unknown	34				
Overall Survival (OS) (months)					
Median (range)	33 (1-294)				
Alive	124				
Dead	102				
Unknown	32				
Gender		0,048	0,67 (0,45-0,99)	0,943	0,98 (0,66-1,48)
Male	177				
Female	81				
Anatomical site					
Oral cavity	113				
Oropharynx	80				
Larynx	44				
Hypopharynx	19				
Nasopharynx	2				
Tumor stage		0,274	1,27 (0,99-2,82)	**0,005**	1,91 (1,22-3,00)
I-II	84				
III-IV	180				
Unknown	44				
Histological grade		0,225	0,76 (0,45-0,99)	**0,029**	0,62 (0,40-0,95)
Poorly differentiated	112				
Well differentiated	89				
Unknown	57				
Tumor invasion		0.053	1.68 (0.99-2.82)	0.157	1.42 (0.88-2.30)
Yes	154				
No	59				
Unknown	45				
Risk factors					
Tobacco		0.326	1.32 (0.76-2.29)	0.366	1.29 (0.74-2.24)
Smoker	181				
Non-smoker	36				
Unknown	41				
Alcohol		0.811	1.05 (0.69-1.60)	0.445	1.18 (0.77-1.81)
Drinker	129				
Non-drinker	78				
Unknown	51				
HPV status		0.131	0.65 (0.37-1.14)	0.562	0.86 (0.51-1.44)
Positive	65				
Negative	138				
Unknown	55				
p16 status		0.103	0.57 (0.29-1.12)	0.152	0.62 (0.32-1.19)
Positive	37				
Negative	121				
Unknown	100				

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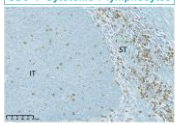
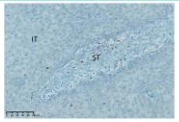
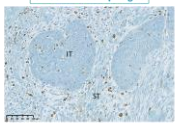
CD8 → Cytotoxic T-lymphocytes



FoxP3 → Regulatory T-lymphocytes



CD68 → Macrophages



Count in 5 randomly fields in the stroma + 5 randomly fields in intratumoral area → Mean → Cut-off value (RStudio) giving the best separation between two groups → Prognostic value of each immune cell type regarding RFS and OS → Combination of most significant marker = **IMMUNOSCORE**

R-1: Correlations between clinical characteristics and RFS/OS

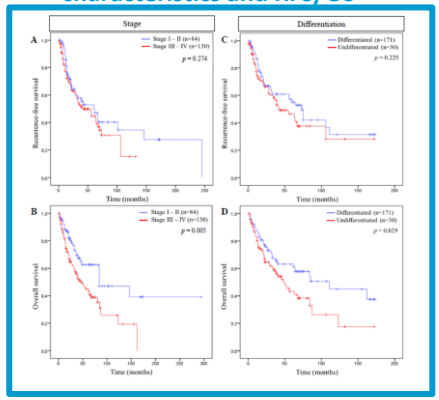


Figure 1: We evaluated the association between tumor stage, histological grade, tumor invasion or risk factors with RFS or OS. Cox regression models highlighted that among such parameters only tumor stage and histological grade correlated with OS.

R-4: Immunoscore as a prognostic marker

Our immunoscore correlated more significantly, and with a greater separation of the two groups, regarding OS (p=0.018) compared to tumor stage (p=NS) and histological grade (p=NS). Multivariate analyses revealed that the immunoscore was the only parameter associated with a strong and independent prognosis value.

R-2: Immune cell number and patient survival

Univariate analysis	Relapse-free survival		Overall survival	
	p-value	HR (95% CI)	*p*-value	HR (95% CI)
CD8 ST 0-1	0.958	0.98 (0.48-2.02)	**0.026**	3.19 (1.15-8.90)
CD8 IT 1-0	**0.011**	3.73 (1.35-10.31)	**0.025**	3.20 (1.16-8.84)
FoxP3 ST 1-0	**0.002**	1.97 (1.28-3.04)	**0.002**	1.95 (1.29-2.96)
FoxP3 IT 1-0	**0.008**	1.84 (1.17-2.87)	**0.001**	2.16 (1.36-3.41)
CD68 ST 1-0	0.206	1.56 (0.78-3.11)	0.076	2.12 (0.93-4.85)
CD68 IT 0-1	**0.004**	1.86 (1.21-2.85)	**0.005**	1.79 (1.19-2.69)

Multivariate analysis	Relapse-free survival		Overall survival	
	p-value	HR (95% CI)	*p*-value	HR (95% CI)
CD8 ST 0-1	0.847	1.10 (0.43-2.78)	**0.028**	5.03 (1.19-21.31)
CD8 IT 1-0	**0.024**	3.36 (1.17-9.67)	**0.038**	3.08 (1.06-8.94)
FoxP3 ST 1-0	0.255	1.87 (0.64-5.49)	0.147	2.46 (0.73-8.28)
FoxP3 IT 1-0	0.056	1.88 (0.98-3.57)	0.109	1.63 (0.90-2.98)
CD68 ST 1-0	0.13	1.63 (0.86-3.09)	0.961	1.02 (0.56-1.83)
CD68 IT 0-1	0.6	1.22 (0.58-2.55)	0.080	1.98 (0.92-4.27)

Univariate and multivariate analysis (Cox regression) were performed for the three immuno-markers CD8, FoxP3 and CD68 in the two compartments for RFS and OS (Table 2). Multivariate analysis showed that the CD8+ cell number was a strong and independent prognostic marker. Using these cut-offs, Kaplan-Meier curves were established for each immune cell in each compartment for RFS and OS. Regarding the ST compartment, longer RFS was significantly associated with a high FoxP3+ cell number, while longer OS correlated with low CD8+ and high FoxP3+ cell numbers. In ST, the CD68+ cell number did not correlate with RFS or OS. In the IT compartment, low CD8+, high FoxP3+ and low CD68+ cell numbers were significantly linked to a longer RFS as well as a longer OS.

R-3: Immunoscore and patient survival

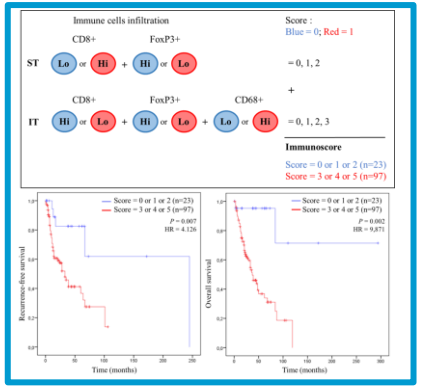


Figure 2: Establishment of the immunoscore in HNSCC tissues based on the immune cell infiltration in the ST and IT compartment. Each tumor is categorized into low (Lo) or high (Hi) density for each immune cells in each tumor region according to the calculated cutoff values. Depending on the immune cells and the tumor compartment, the Lo and Hi classes are associated to the blue or red group which correspond to 0 and 1 score, respectively. According to the total number of the score, each patient is classified in the blue group (low immunoscore) or the red group (high immunoscore). Kaplan Meier curves comparing recurrence-free survival (RFS) and immunoscore and overall survival (OS) and immunoscore are represented.

Conclusion

Our immunoscore represents an efficient and independent prognostic signature that could constitute a novel indicator beyond TNM staging to improve or complement the prediction of clinical outcomes in head and neck cancer patients.

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