

Role of tumor microenvironment in the prognosis of sinonasal intestinal-type adenocarcinoma (ITAC)

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OBJECTIVES

Sinonasal tumours are uncommon conditions, comprising only 3% to 5% of head and neck malignant neoplasms and 0.2% to 0.8% of all tumours, with striking heterogeneity. In such a complex context, sinonasal intestinal-type adenocarcinoma (ITAC) represents the most common type of sinonasal cancer in Europe and Italy, whereas it is less common in the United States.

The aim of this study is to evaluate the relationship of Overall Survival (OS) and Disease Free Survival (DFS) with clinical aspects (age, type of surgery, relapsing, advanced disease at the diagnosis etc.) and pathological features (subtype, staging, margin status, bone invasion, necrosis, etc.) including tumour microenvironment (TME) characterization.

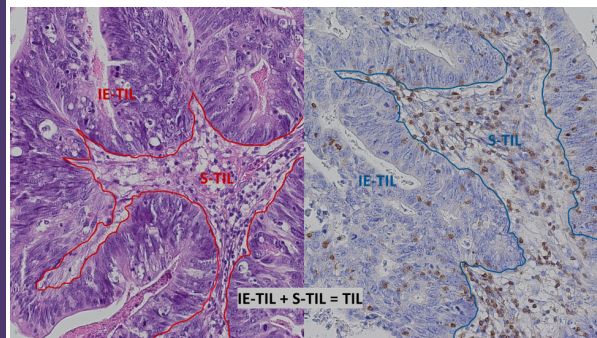
METHODS

Case selection: Consecutive 178 cases with a mean postoperative surveillance time of 66 months (range: 2–206 months) were selected between October 2002 and December 2020 at the University Hospital of Varese.

Pathological analysis: Histological samples were classified according to Barnes' and Kleinsasser-Schroeder's classifications, graded and evaluated for the presence of intestinal metaplasia, dysplasia, intraepithelial adenocarcinoma, necrosis, bone, lympho-vascular and perineural invasion. The characterization of tumour-infiltrating lymphocytes was performed on consecutive sections using CD3, CD8, CD4 (Ventana) and PD-L1 (clone 22C3, Agilent) antibodies on the Benchmark (Roche) automated immunostainer. Tumour-infiltrating lymphocytes (TILs) and Intraepithelial Infiltrating Lymphocytes (IE-TILs) were counted at 400x, using a Leica Laborlux K microscope (field area 0,173 mm²), in 5 consecutive fields (0,85 mm²), selecting areas containing the maximal number of neoplastic cells with minimal reactive stroma and necrosis. The TME was evaluated on 42 patients.

Statistical evaluation: All data were collected and processed with IBM SPSS® Statistics version 25 (IBM Corporation, New York) and Jamovi (The jamovi project 2022, version 2.3, www.jamovi.org). Categorical variables were described through category absolute and percentage distribution, continuous by means of median, mean, standard error and range. Distribution of continuous variables (CD3+ TIL, CD3+ IE-TIL, CD4+ TIL, CD4+ IE-TIL, CD8+ TIL, CD8+ IE-TIL) was assessed through the Shapiro-Wilk test. Association of CD3+ TIL, CD3+ IE-TIL, CD4+ TIL, CD4+ IE-TIL, CD8+ TIL, CD8+ IE-TIL with demographic and clinicopathological information was performed with Mann-Whitney U test, Kruskal-Wallis test or Spearman's correlation test, according to the type of variable.

GRAPHS & TABLES



RESULTS

OS and DFS were 77,4% and 84,6%, respectively, in line with published literature. The percentage of distant metastasis appearing during a mean follow-up time of 10 years was 10.7% (19/178). Univariate analysis confirmed the role pT category ($p < 0.001$), extent of surgical resection (i.e., craniotomoscopic resection, $p < 0.001$), and infiltrated margins ($p < 0.001$) as worse prognostic factors. Surgical margin status appeared to be the strongest prognosticator for this histologic group. The Kleinsasser-Schroeder's classification showed a significant correlation with prognosis ($p < 0.001$), which was not observed using the Barnes' one. The only other histological parameter with statistical significance was bone invasion ($p < 0.001$) negatively influencing OS and DFS, at both univariate and multivariate analysis, while grading did not have a significant prognostic impact ($p = 0.067$).

The TME evaluation showed a great variability in the density of the lymphoid infiltrate, both TILs and IE-TILs; of note, only 1/42 cases did not show CD3+ cell infiltration. The statistical analysis suggested a relationship between TILs, staging, margin status and surgical techniques, namely a higher pT correlated with a lower CD4+ TIL ($p = 0.006$). R+ margins correlated with a lower CD3+ IE-TIL ($p = 0.006$), CD4+ TIL ($p = 0.028$) and CD8 IE-TIL ($p = 0.013$). Bone infiltration correlated with lower CD4+ IE-TIL ($p = 0.048$) and TIL ($p = 0.033$).

The expression of PD-L1 in ITAC was completely negative in mucinous subtypes. It was low in the other subtypes: in 29/42 cases there was not immunoreactivity at all, while in 9/42 cases the CPS value was 1, in 2/42 was 2 and in 2/42 was 5. There was not significant correlation between PD-L1 expression and clinical outcome.

REFERENCES

Castellnuovo P, Turri-Zanoni M, Battaglia P, Antognoni P, Bossi P, Locatelli D. Sinonasal Malignancies of Anterior Skull Base. *Otolaryngologic Clinics of North America*. 2016;49:183–200.
Antognoni P, Turri-Zanoni M, Gottardo S, Molteni M, Volpi L, Facco C, et al. Endoscopic resection followed by adjuvant radiotherapy for sinonasal intestinal-type adenocarcinoma: Retrospective analysis of 30 consecutive patients: Multimodal Treatment for Sinonasal Intestinal-Type Adenocarcinoma. *Head Neck*. 2015;37:677–84.
Organisation mondiale de la santé, Centre international de recherche sur le cancer, editors. WHO classification of head and neck tumours. 4th ed. Lyon: International agency for research on cancer; 2017.
Thompson LDR, Bishop JA. Update from the 5th Edition of the World Health Organization Classification of Head and Neck Tumors: Nasal Cavity, Paranasal Sinuses and Skull Base. *Head and Neck Pathol*. 2022;16:117–18.
Barnes L. Intestinal-Type Adenocarcinoma of the Nasal Cavity and Paranasal Sinuses: The American Journal of Surgical Pathology. 1986;10:192–202.
Kleinsasser O, Schroeder HG. [The pathology and clinical picture of adenocarcinoma of the nose after wood dust exposure]. *Strahlenther Onkol*. 1989;165:437–40.
Fridman WH, Dieu-Nosjean MC, Pagès F, Cremer I, Damotte D, Sautès-Fridman C, et al. The Immune Microenvironment of Human Tumors: General Significance and Clinical Impact. *Cancer Microenvironment*. 2013;6:117–22.
Bruni D, Angelli HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer*. 2020;20:662–80.
Rampinelli V, Ferrari M, Nicolai P. Intestinal-type adenocarcinoma of the sinonasal tract: an update. *Current Opinion in Otolaryngology & Head & Neck Surgery*. 2018;26:115–21.
Sánchez-Fernández P, Ribello C, Costales M, Vivanco B, Cabal VN, García-Marin R, et al. Next-generation sequencing for identification of actionable gene mutations in intestinal-type sinonasal adenocarcinoma. *Sci Rep*. 2021;11:2247.
Szabalewski V, Solasol J, Poizat F, Larrieux M, Crampette L, Mange A, et al. EGFR Expression and KRAS and BRAF Mutational Status in Intestinal-Type Sinonasal Adenocarcinoma. *IJMS*. 2013;14:5170–81.
Franchi A, Fondi C, Paglierani M, Pepi M, Gallo O, Santucci M. Epidermal growth factor receptor expression and gene copy number in sinonasal intestinal type adenocarcinoma. *Oral Oncology*. 2009;45:835–8.

CONCLUSION

- High tumour stage, surgical incomplete removal and bone infiltration are the main worse prognostic factors.
- Histological classification alone is not sufficient to explain the biological behaviour of the neoplasm.
- The Kleinsasser-Schroeder's classification has a significant correlation with prognosis ($p < 0.001$), which is not observed using the Barnes' one.
- The lower TME infiltration is correlated with some of the parameters (margin status, bone invasion, pT) significantly linked to patient's survival.
- PD-L1 expression is low in ITACs and does not correlate with prognosis.