Polymorphism of *TNFRSF1 A* may act as a predictor of severe radiation-induced oral mucositis and a prognosis factor in patients with head and neck cancer

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Objective. The aim of this study was to evaluate the relationship between single nucleotide polymorphism (SNP) (−135 T>C) of *TNFRSF1 A* and the frequency of occurrence and severity of oral mucositis (OM) in patients with head and neck cancer (HNC) treated with radiotherapy (RT).

Study Design. This retrospective, cohort study included 60 patients with HNC treated with intensity-modulated radiation therapy (IMRT). *TNFRSF1 A* SNP analysis (−135 T>C) was performed by using molecular probes (TaqMan, ThermoFisher Scientific, Waltham, MA) in DNA isolated from peripheral blood (QIAamp DNA MiniKit; Qiagen, Germantown, MD).

Results. CC genotype was related to 4.5-fold higher risk of grade 2 OM after the second week of RT. Similarly, CC carriers had a significantly higher risk of severe (grade 3) OM after the fourth (6-fold) and fifth (7.5-fold) weeks of RT. The CC genotype of the *TNFRSF1 A* gene was significantly correlated with a higher risk of shorter overall survival (OS) (> 37 months follow-up period; hazard ratio [HR] = 2.78).

Conclusions. SNP (−135 T>C) of the *TNFRSF1 A* gene may act as a predictor of OM occurrence in patients with HNC treated with IMRT. The studied SNP may also serve as a prognostic factor in such cases. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;130:283−291)

Head and neck cancers (HNC) involving the oral cavity, lip, nasopharynx, oropharynx, hypopharynx, larynx, and salivary glands accounts for almost 900,000 cases worldwide and greater than 450,000 deaths annually.[1] The most common histologic type is head and neck squamous cell carcinoma (HNSCC) diagnosed in almost 90% of cases. Each year, HNSCC leads to nearly 2% of deaths caused by malignant tumors.[2] It is worth mentioning that both the morbidity rate and the mortality rate differ from country to country. Usually, these rates depend on oral cancer-related behaviors, such as alcohol abuse, smoking, chewing tobacco, and, as recent scientific reports show, human papillomavirus (HPV) infections. It is estimated that 70.1% of cases of HPV-related HNC are located in the oropharynx, 32% in the oral cavity, and 20.9% in the larynx.[3] In the multimodal approach, in addition to surgery, which is the first-choice procedure where applicable, radiotherapy (RT), also as part of chemoradiotherapy (CRT), remains one of the most common methods of HNC treatment. RT is not devoid of side effects, such as oral mucositis (OM). Radiation-induced OM occurs in up to 80% of patients with HNC (this rate may even increase to 100% when RT is combined with altered fraction or CRT).[4,5] Over half the patients with HNC suffering from severe OM require reduction of the RT doses and another 35% need to delay or interrupt RT, leading to incomplete oncologic treatment. The Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC) scale is used to determine the extent of damage. Severe injury corresponding to grades 3 and 4 on the RTOG/EORTC scale can occur in almost one-third of treated patients with HNC,[4,6] resulting in many problems, such as deterioration of the quality of the patient's life (including eating problems, resulting in the need for parenteral nutrition); need for additional hospitalizations; and interruptions in RT, as mentioned above.[4,7] The necessity to limit therapy leads to difficulties in local disease control and also to shorter overall survival (OS) because every 5 days of delay in RT increases the risk of progression

Statement of Clinical Relevance

The evaluation of SNP (−135 T>C) of the *TNFRSF1 A* gene may become a promising tool of noninvasive diagnostics in the future and may be helpful in prognosis as well as early detection and monitoring of the oral mucositis in patients with head and neck cancer undergoing radiotherapy.

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by almost 15%.[8] There are a few known risk factors for severe OM: a higher dose of radiation; the volume of tissue treated; exposure to CRT; younger age; tumor location (oral and oropharyngeal); poor functional or poor overall status; and overall treatment time. Yet, no consensus has been established with regard to the influence of various patient characteristics on OM risk (gender, alcohol consumption and smoking, oral intake, low body mass index, pre-existing periodontal disease, advanced stage of disease, and history of severe OM).[4,9-12] Moreover, the significant individual risk of OM caused by genetic predispositions (e.g., single nucleotide polymorphisms [SNPs]) has been described.[13] The occurrence of OM can be caused by the direct impact of radiation on mucosal cells, or it may be the result of the intensification of inflammatory processes regulated, for example, by the TNFR axis (tumor necrosis factor receptor [TNFR])−TNF-α. TNF-α, produced by activated macrophages, is a key factor in the development of the immune response, apoptosis, and cytotoxicity. *TNFRSF1 A* (a member of the TNFR 1 A superfamily) is a gene coding for the TNFR protein. The coding receptor can be located in the cell membrane and react to its ligand, TNF-α, which is also associated with the cell membrane. Their joint action can induce apoptosis or inflammation. In contrast, the soluble form of the receptor may interact with free TNF-α to suppress inflammation.[14] It has been proven that proapoptotic signals leading to acute disorders (e.g., OM) are caused by TNFR1 dysfunction.[15]

Genetic changes, such as SNPs, in regions of gene promoter−encoding TNFRs, may affect their expression and function.[16] Among the SNPs located within the regulatory region of *TNFRSF1 A*, rs767455 was found to be involved in the regulation of TNFR1 protein expression.[17] It can potentially modulate the risk of OM in patients with HNC treated with RT. According to recent findings, there is an association between the SNPs in the genes encoding ligands or receptors and the incidence of OM in patients with HNC undergoing RT.[18-21] Thus, the aim of this study was the evaluation of the relationship between SNP (−135 T>C, rs767455) of *TNFRSF1 A* and the frequency of occurrence and severity of OM in patients with HNC treated with RT.

MATERIALS AND METHODS

The report was prepared in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement checklist (see supplementary material available online).[22]

Study design, setting, participants, variables, and data sources

All participants of our retrospective cohort study were diagnosed between 2014 and 2015 and treated in the Oncology Department in the Medical University of Lublin (Lublin, Poland). The study was conducted in a population of patients who, to a limited extent, had already been included and described in some of our studies.[23,24] The decisive inclusion criteria were age greater than 18 years and a diagnosis of head or neck cancer. In addition, only patients who received a total radiation dose and treated with intensity modulation radiotherapy (IMRT) after surgery or as the final treatment method, with or without sequential and/or concomitant chemotherapy, were included in the study. The exclusion criteria were Sjögren syndrome (to eliminate the impact of immune disorders and salivation on the development of OM); salivary gland tumors (only patients treated solely for head and neck carcinoma in the following locations were included: oral cavity, larynx, and pharynx) or infections (patients with active infection requiring antibiotic therapy were excluded to eliminate the effect of antibiotics on the development of OM); and diagnosed lymphoma, melanoma, or skin cancer or any previous cancers (previously treated head and neck tumors located in the irradiated area were excluded to eliminate the impact of previously used methods, mainly surgery, on the severity of OM). The sources of data were medical history, interview, physical examination, and genetic tests. Patients' performance status was assessed according to the criteria developed by Eastern Cooperative Oncology Group. Alcohol consumption was classified, according to the International Statistical Classification of Diseases and Related Health Problems (ICD) criteria, as "excessive" (F10.1 and F 10.2) or "occasional." Smokers were classified as "former" or "current" smoker. A person who had smoked at least 100 cigarettes in his or her lifetime and who currently smoked was classified as a current smoker. A person who had smoked at least 100 cigarettes in his or her lifetime but who had quit smoking by the time of the interview was categorized as a former smoker. A person who had never smoked or who had smoked less than 100 cigarettes in his or her lifetime was classified as a nonsmoker. The presence of OM was assessed by using the RTOG/EORTC scale before and after each week of RT. OS was counted in months from the beginning of therapy until the end of observation or until the patient's death. Median follow-up was 36 months (range 0.5−40 months).

For radical RT, a linear accelerator ONCOR (Siemens) was used. In all patients, the IMRT technique was applied, with total doses of 54 to 70 Gy (daily dose 2 Gy). Patients with advanced-stage disease were treated with a total dose of 70 Gy in 35 fractions for the tumor and enlarged lymph nodes. Doses of 54 Gy or 60 Gy were used to treat elective lymph nodes. Patients with a high risk who underwent surgery were

given a dose of 66 Gy in 33 fractions, and those with medium and low risks received 60 and 54 Gy, respectively. In addition, some patients were treated with chemotherapy (concurrent or neoadjuvant) based on cisplatin and 5-fluorouracil (PF) regimens. Chemotherapy was given in 1 to 4 courses.

Before the start of RT, 5 mL of whole blood was collected from all study participants (stored in −80˚C until further laboratory analyses were performed). DNA Blood Mini Kit (Qiagen, Toronto, Canada) was used to isolate DNA. The quality and quantity of DNA were assessed by using NanoDrop Lite Spectrophotometer (ThermoFisher Scientific, Waltham, MA). Genotyping was performed by using real-time polymerase chain reaction (RT-PCR), with allelic discrimination software. The Genotyping Master Mix and TaqMan probes (Applied Biosystems, Waltham, MA) specific for the studied *TNFRSF1 A* SNP (ThermoFisher Scientific, Waltham, MA) were used for DNA amplification according to the manufacturer's protocol provided with the kit in the RT7500 RT-PCR device (Applied Biosystems, Waltham, MA). All sample tests were performed in triplicate.

The study group characteristics are presented in Table I. All procedures in studies involving human participants were performed in compliance with the ethical standards of the institutional and/or national research committee (Bioethical Commission in Medical University in Lublin; reference No.: KE-0254/232/2014) and the tenets of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Table I. *TNFRSF1 A* (−135 T>C) genotype distribution, according to patients' clinical−demographic variables

Variable		*n (%)*	*TNFRSF1 A (−135 T>C)*			
		Study group 60 (100%)	*CC (n = 18; 30%)*	*CT (n = 28; 46.7%)*	*TT (n = 14; 23.3%)*	*P*
Gender	**Male**	49 (18.3%)	14 (28.6%)	24 (49%)	11 (22.4%)	.749
	Female	11 (81.7%)	4 (36.4%)	4 (36.4%)	3 (27.2%)	
Age (years)	**≥ 63**	32 (53.3%)	6 (18.8%)	18 (56.2%)	8 (25%)	.115
	< 63	28 (46.7%)	12 (42.9%)	10 (35.7%)	6 (21.4%)	
Performance status	**≤ 1**	53 (88.3%)	15 (28.3%)	24 (45.3%)	14 (26.4%)	.291
	> 1	7 (11.7%)	3 (42.9%)	4 (57.1%)	–	
T stage	**T1**	1 (1.7%)	1 (100%)	–	–	.716
	T2	9 (15%)	2 (22.2%)	5 (55.6%)	2 (22.2%)	
	T3	15 (25%)	3 (20%)	8 (53.3%)	4 (26.7%)	
	T4	35 (58.3%)	12 (34.3%)	15 (42.9%)	8 (22.9%)	
N stage	**Nx**	2 (3.3%)	–	1 (50%)	1 (50%)	.939
	N0	17 (28.3%)	6 (35.3%)	7 (41.2%)	4 (23.5%)	
	N1	6 (10%)	2 (33.3%)	3 (50%)	1 (16.7%)	
	N2	31 (51.7%)	8 (25.8%)	16 (51.6%)	7 (22.6%)	
	N3	4 (6.7%)	2 (50%)	1 (25%)	1 (25%)	
M stage	**Mx**	3 (5%)	–	1 (33.3%)	2 (66.7%)	1.000
	M1	1 (1.7%)	–	1 (100%)	-	
Disease stage	**I**	1 (1.7%)	–	1 (100%)	–	.928
	III	11 (18.3%)	3 (27.3%)	5 (45.5%)	3 (27.3%)	
	IVA	40 (65%)	12 (30%)	18 (45%)	10 (25%)	
	IVB	3 (5%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	
	IVC	5 (10%)	2 (40%)	3 (60%)	–	
Tumor location	**Upper throat**	16 (26.7%)	6 (37.5%)	7 (43.7%)	3 (18.8%)	.724
	Lower throat	44 (73.3%)	12 (27.3%)	21 (47.7%)	11 (25%)	
Tumor location (detailed)	**Larynx**	33 (55%)	11 (33.3%)	12 (36.4%)	10 (30.3%)	.244
	Hypopharynx	11 (18.3%)	1 (9.1%)	9 (81.8%)	1 (9.1%)	
	Nasopharynx	4 (6.7%)	1 (25%)	2 (50%)	1 (25%)	
	Oropharynx	12 (20%)	5 (41.7%)	5 (41.7%)	2 (16.7%)	
Alcohol consumption	**Yes**	27 (45%)	9 (33.3%)	11 (40.8%)	7 (25.9%)	.707
	No	33 (55%)	9 (27.3%)	17 (51.5%)	7 (21.2%)	
Smoking status	**Non-smoker**	10 (16.7%)	3 (30%)	4 (40%)	3 (30%)	.682
	Current smoker	44 (73.3%)	13 (29.5%)	20 (45.5%)	11 (25%)	
	Former smoker	6 (10%)	2 (33.3%)	4 (66.7%)	–	
Prior surgical treatment	**Yes**	44 (73.3%)	14 (31.8%)	19 (43.2%)	11 (25%)	.668
	No	16 (26.7%)	4 (25%)	9 (56.3%)	3 (18.8%)	
Neoadjuvant chemotherapy	**Yes**	10 (16.7%)	2 (20%)	4 (40%)	4 (40%)	.379
	No	50 (83.3%)	16 (32%)	24 (48%)	10 (20%)	
Concurrent chemoradiotherapy	**Yes**	24 (40%)	6 (25%)	13 (54.2%)	5 (20.8%)	.631
	No	36 (60%)	12 (33.3%)	15 (41.7%)	9 (25%)	

Sample size calculation, quantitative variables, and statistical analysis

Statistical analysis was performed by using MedCalc software version 12.7 (MedCalc Software, Belgium). We assessed the size of the sample retrospectively and extrapolated the results to the initial part of the post hoc test chart. We made the calculation on the basis of the study group versus population criteria and continuous primary endpoint. Because of our use of the post hoc retrospective method, we analyzed statistically significant results. The post hoc parameters also included anticipated means, and we defined the types of errors (type I and type II errors). In the majority of studies, the *P* value was set at less than 0.05 to reject the null hypothesis type I (alpha) error of 0.05. In the case of type II (beta) error, the beta cutoff value in the medical literature is mostly established at 20% (0.2), indicating a 20% chance of skipping significant difference; therefore, the type II error was set at 0.2. Considering the incidence of severe OM in the study group (41.9%) and in the general population (based on literature, 60%),[9] the minimum number of samples, to enable credible confirmation of the test hypothesis, was estimated as 58. Because of the changes in the distribution of OM grades in subsequent weeks of RT (the changes were progressive—more severe changes occurred and lower OM grades disappeared) and because of the use of statistical tests that require the analysis of maximum of 2 groups, we adopted several types of comparisons in the analysis: grade 0 versus 1 (after the first week); grade 1 versus 2 (after the second week); and grades 1 and 2 (together) versus grade 3 (for all subsequent weeks). Because the analyzed quantitative variable (age) had nonnormal distribution (evaluated by using the D'Agostino-Pearson normality test), we used median for dichotomizing. Fisher's exact test and the χ^2 test were used to assess the Hardy-Weinberg equilibrium and to compare the variability of the demographic and clinical factors of patients with different genotype variants of the *TNFRSF1 A* gene (SNP: Rs767455; CC, CT, and TT). Odds ratio (OR) with 95% confidence interval (95% CI) were used to assess the OM risk associated with demographic and clinical factors. The Kaplan-Meier estimator and the Cox regression model (to find true, unbiased independent prognostic factors) were used for estimating the factors (with the calculation of the risk coefficient − hazard ratio [HR]) affecting the survival of patients. To address the issue of missing data, we used the pairwise deletion method. Results from $P < .05$ were regarded as statistically significant.

RESULTS

The study group consisted of 60 patients with histologically confirmed advanced HNC (98.3% of them were in stage III or IV of disease, according to 7th edition of TNM [tumor−node−metastasis] classification). The median age of patients was 63 years (range 42−87 years). The distribution of *TNFRSF1 A* genotypes (CC: 30%; CT: 46.7%; TT: 23.3%) was in Hardy-Weinberg equilibrium ($P = .628$; $\chi^2 = 0.231$) and was not significantly different among patients divided on the basis of demographic and clinical factors (see Table I). Starting from the second week of RT, every patient developed OM (varying grades). With each week of RT, intensification of OM reaction was observed. However, in the first 2 weeks, there were no cases with grade 3 OM. In the third week, frequency was 8.3%. After the completion of RT (in the seventh week), grade 3 OM was found in 40% of patients. Most of the studied

Table II. *TNFRSF1 A* (−135 T>C) genotype distribution according to patients' radiation-induced OM severity after subsequent weeks of IMRT

RT week	*OM grade*	*CC (n = 18; 30%)*	*CT (n = 28; 46.7%)*	*TT (n = 14; 23.3%)*	*P*
1	**0** (n = 6; 10%)	-	2 (33.3%)	4 (66.7%)	.022*
	1 (n = 54; 90%)	18 (33.3%)	26 (48.2%)	10 (18.5%)	
2	**1** (n = 35; 58.3%)	6 (17.1%)	18 (51.5%)	11 (31.4%)	.025*
	2 (n = 25; 41.7%)	12 (48%)	10 (40%)	3 (12%)	
3	**1 or 2** (n = 55; 91.7%)	16 (29.1%)	25 (45.4%)	14 (25.5%)	.0,001*
	3 (n = 5; 8.3%)	2 (40%)	3 (60%)	–	
4	**1 or 2** (n = 47; 78.3%)	10 (21.3%)	23 (48.9%)	14 (29.8%)	.008*
	3 (n = 13; 21.7%)	8 (61.5%)	5 (38.5%)	–	
5	**1 or 2** (n = 46; 76.7%)	9 (19.6%)	23 (50%)	14 (30.4%)	.003*
	3 (n = 14; 23.3%)	9 (64.3%)	5 (35.7%)	–	
6	**1 or 2** (n = 45; 75%)	11 (24.4%)	21 (46.7%)	13 (28.9%)	.121
	3 (n = 15; 25%)	7 (46.7%)	7 (46.7%)	1 (6.6%)	
7	**1 or 2** (n = 36; 60%)	8 (22.2%)	17 (47.2%)	11 (30.6%)	.147
	3 (n = 24; 40%)	10 (41.7%)	11 (45.8%)	3 (12.5%)	

IMRT, intensity modulation radiotherapy technique; *OM,* oral mucositis; *RT,* radiotherapy.
*Statistically significant results.

demographic and clinical factors did not influence the risk of grade 3 compared with development of grade 1 or 2 OM. However, there were 3 exceptions related to disease stage and chemotherapy use (concurrent or neoadjuvant). Regardless of the studied SNP of the *TNFRSF1 A* gene, the disease stage was significantly related to the occurrence of severe OM after weeks 1t, 3, 4, and 5 of RT. Regardless of the studied SNP, lack of neoadjuvant chemotherapy was associated with an approximately 50-fold lower risk of developing grade 2 OM after week 2 of RT (odds ratio [OR] = 0.02; $P = .009$) and 100-fold lower risk of grade 3 OM after week 4 of RT (OR = 0.01; $P < .001$). Similarly, regardless of the studied SNP, the lack of concurrent chemotherapy was related to an approximately 5-fold lower risk of grade 3 OM after week 4 of RT (OR = 0.21; $P = .021$) and week 5 of RT (OR = 0.17; $P = .010$) (see Supplementary Tables S1−S3 available online). We found statistically significant differences in the occurrence of severe OM according to *TNFRSF1 A* genotypes in weeks 1 to 2 and 3 to 4 of RT (Table II). The presence of the CC genotype was related to an approximately 4.5-fold (OR = 4.46, $P = .013$) higher risk of grade 2 OM development after week 2 of RT. Similarly, CC carriers had a significantly higher risk of severe (grade 3) OM after week 4 (approximately 6-fold; OR = 5.92; $P = .008$) and week 5 (approximately 7.5-fold; OR = 7.41; $P = .003$). However, the presence of the TT genotype was associated with an approximately 9-fold (OR = 0.11; $P = .020$) lower risk of grade 1 OM development after week 1 of RT (Table III). On the basis of univariate and multivariate analyses (after adjustment for gender, age, stage of the disease [according to the TNM classification], performance status, alcohol consumption, smoking status, concurrent CRT occurrence of grade 3 OM during treatment, and *TNFRSF1 A* genotype), we found that only concurrent CRT and *TNFRSF1 A* genotype significantly affected survival. Introduction of the concurrent CRT significantly decreases the risk of reduced OS (38 vs 32 months; HR = 0.27; $P = .038$). The CC genotype of the *TNFRSF1 A* gene was significantly related to a higher risk of shorter OS (29 vs 37 months; HR = 2.78; $P = .031$; Figure 1). The results of the univariate and multivariate analyses are presented in Table IV.

DISCUSSION

To develop appropriate prophylaxis and an effective treatment, it is necessary to understand the basic molecular mechanisms that determine the development of OM. OM caused by RT indicates damage to normal tissue. Because of the prevalence and severity of OM, it is the main cause for limiting the radiation dose in patients with HNC. The frequency and grades of OM largely affect the continuation and effectiveness of

Table III. Impact of *TNFRSF1 A* (−135 T>C) genotypes on the risk of severe OM after subsequent weeks of IMRT

RT week	*OM grade*	*CC*	*TT or CT*	*P, OR [95% CI]*	*TT*	*CC or CT*	*P, OR [95% CI]*	*CT*	*CC or TT*	*P, OR [95% CI]*
1	**0** (n = 6; 10%)	–	6 (100%)	.207	4 (66.7%)	2 (33.3%)	.020*	2 (33.3%)	4 (66.7%)	.495
	1 (n = 54; 90%)	18 (33.3%)	36 (66.7%)	6.58 [0.35−125.00]	10 (18.5%)	44 (81.5%)	0.11 [0.02−0.71]	26 (48.1%)	28 (51.9%)	1.86 [0.31−11.11]
2	**1** (n = 35; 58.3%)	6 (17.1%)	29 (82.9%)	.013*	11 (31.4%)	24 (68.6%)	.090	18 (51.4%)	17 (48.6%)	.383
	2 (n = 25; 41.7%)	12 (48%)	13 (52%)	4.46 [1.37−14.49]	3 (12%)	22 (88%)	0.30 [0.07-1.21]	10 (40%)	15 (60%)	0.63 [0.22−1.78]
3	**1 and 2** (n = 55; 91.7%)	16 (29.1%)	39 (70.9%)	0.613	14 (25.5%)	41 (74.5%)	.372	25 (45.5%)	30 (54.5%)	.537
	3 (n = 5; 8.3%)	2 (40%)	3 (60%)	1.63 [0.25−10.75]	–	5 (100%)	0.26 [0.01−5.00]	3 (60%)	2 (40%)	1.80 [0.28−11.63]
4	**1 and 2** (n = 47; 78.3%)	10 (21.3%)	37 (78.7%)	0.008*	14 (29.8%)	33 (70.2%)	.095	23 (48.9%)	24 (51.1%)	.505
	3 (n = 13; 21.7%)	8 (61.5%)	5 (38.5%)	5.92 [1.58-22.22]	–	13 (100%)	0.09 [0.00−1.54]	5 (38.5%)	8 (61.5%)	0.65 [0.19−2.29]
5	**1 and 2** (n = 46; 76.7%)	9 (19.6%)	37 (80.4%)	.003*	14 (30.4%)	32 (69.6%)	.082	23 (50%)	23 (50%)	.352
	3 (n = 14; 23.3%)	9 (64.3%)	5 (35.7%)	7.41 [1.99−27.78]	–	14 (100%)	0.08 [0.00−1.39]	5 (35.7%)	9 (64.3%)	0.56 [0.16−1.91]
6	**1 and 2** (n = 45; 75%)	11 (24.4%)	34 (75.6%)	.110	13 (28.9%)	32 (71.1%)	.110	21 (46.7%)	24 (53.3%)	1.000
	3 (n = 15; 25%)	7 (46.7%)	8 (53.3%)	2.70 [0.80-9.17]	1 (6.7%)	14 (93.3%)	0.18 [0.02−1.48]	7 (46.7%)	8 (53.3%)	1.00 [0.31−3.23]
7	**1 and 2** (n = 36; 60%)	8 (22.2%)	28 (77.8%)	.112	11 (30.6%)	25 (69.4%)	.116	17 (47.2%)	19 (52.8%)	.916
	3 (n = 24; 40%)	10 (41.7%)	14 (58.3%)	2.50 [0.81−7.75]	3 (12.5%)	21 (87.5%)	0.32 [0.08−1.32]	11 (45.8%)	13 (54.2%)	0.95 [0.34−2.67]

CI, confidence interval; *IMRT*, intensity modulation radiotherapy technique; *OM*, oral mucositis; *OR*, odds ratio; *RT*, radiotherapy.
*Statistically significant results.

treatment (dose adjustment, limited local effect on the tumor),and, above all, the patient's quality of life.[4,5,6,25,26] It is worth noting that any increase in RT dose is always associated with a greater risk of damage to healthy tissue. The current IMRT technique enables the delivery of a sufficiently high therapeutic dose of radiation directly to the tumor tissue. Although IMRT enables protection of tissues and organs sensitive to ionizing radiation, the toxic effects of radiation cannot be completely eliminated. The main risk factors for OM are RT (including especially CRT, when IMRT is also used; higher dose; the larger volume of tissue treated); age (younger age); tumor location (oral or oropharyngeal); and poor functional status or poor overall status of the patient.[4,9-12] Observation of the differences in the frequency and stage of development of OM in patients with the same risk factors indicates the significant role of genetic predisposition.[27] OM pathophysiology has still not been fully explored. The development of OM occurs in 5 main phases: (1) initiation (predominant inflammatory/vascular processes); (2) the primary damage response; (3) signaling and amplification; (4) ulceration; and (5) healing. In the first phase—that is, inflammation—epithelial, connective, and endovascular tissues that have been damaged by radiation release proinflammatory (e.g., TNF-α, interleukin 1-beta [IL-1 β] and prostaglandins) and anti-inflammatory (e.g., IL-10 and IL-11) cytokines.

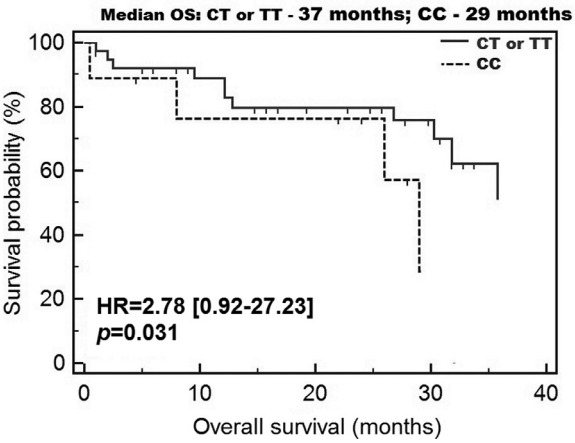

Fig. 1. Reduction in the probability of overall survival, depending on *TNFRSF1 A* gene polymorphism.

Table IV. Overall survival analysis of patients with HNC undergoing IMRT, depending on selected factors

Variable	*Median (months)*	*Univariate P HR (95% CI)*	*Multivariate‡ P HR (95% CI)*
Gender			
Women	32.5	.555	.763
Men	38	1.40 (0.40−4.85)	1.21 (0.24−2.81)
Age (years)			
≥ 63	38	.230	.376
< 63	40	2.09 (0.73−5.97)	1.86 (0.47−7.35)
TNM stage			
I−III	39	.750	.840
IVA−IVC	38	1.22 (0.38−3.99)	1.15 (0.30−4.48)
Performance status			
1	40	.756	.918
2	38	0.73 (0.12−4.36)	1.02 (0.11−8.66)
Alcohol consumption	38	.768	.850
Yes	38	1.16 (0.43−3.09)	1.03 (0.12−2.73)
No			
Smoking history			
Smoker†	37	.219	.468
Nonsmoker	40	3.30 (0.92−11.81)	2.61 (0.33−21.07)
Concurrent chemoradiotherapy			
Yes	38	.019*	.038*
No	32	0.21 (0.06−0.77)	0.27 (0.08−0.93)
Grade 3 OM occurrence during treatment			
Yes	38	.392	.770
No	32.5	0.68 (0.29−1.61)	0.87 (0.36−2.11)
TNFRSF1 A genotype			
CC	29	.027*	.031*
CT and TT	37	2.97 (0.55−14.16)	2.78 (0.92−27.23)

CI, confidence interval; *HNC,* head and neck cancer; *HR,* hazard ratio; *IMRT,* intensity modulation radiotherapy technique; *OM,* oral mucositis.
†Include current and former smokers.
‡Adjusted for all variables from univariate analysis.
*Statistically significant results.

An excess of TNF- α production activates the TNF−TNFR axis causing an increase in inflammation. The imbalance between these mediators can lead to both tissue damage through increased vascular permeability and infiltration of inflammatory cells (proinflammatory) and limit the damage (anti-inflammatory). Epidermal growth factor and keratinocyte growth factor are released in the epithelial phase to accelerate epithelial regeneration after RT-induced apoptosis. The ulceration phase proceeds with the loss of the protective barrier associated with disrupted continuity of the basal membrane. It causes microcoagulations and neutropenia, which is the cause of secondary infections (caused by gram-negative bacteria and yeast).[5,28,29] The inflammatory response is enhanced by bacterial exotoxins that cause the release of more proinflammatory cytokines (e.g., TNF-α and IL-1 β).[27] TNF-α is a ligand of TNFRSF1 A (TNFR1) and TNFRSF1 B (TNFR2) receptors. TNF-α initiates apoptosis by activating TNFR1, and the second pathway—stimulation of TNFR2—induces proliferation of tumor cells and suppressive immune cells.[30] The effects exerted on the oral cavity cells by excessively produced inflammatory cytokines (TNF-α, IL-1 b, IL-6, and IL-8) seem to play a key role in the development of OM. A high concentration of TNF-α and other inflammatory molecules disturbs the homeostasis of the oral mucosa and impairs proper wound healing. These symptoms are caused by a decrease in proliferation, migration, and lower levels of growth.[31,32] So far, several studies have been conducted on the relationship between the severity of OM and the level of TNF-α, but their results have been ambiguous. Some of the studies found a higher level of TNF-α in irradiated patients, whereas this was not observed in other studies.[33-36] A significant correlation between TNF-α levels and the intensity of OM was found by 2 studies. Haddad et al. showed significantly higher plasma TNF-α concentrations in patients with HNC undergoing RT. They also found a significant relationship between increased concentrations of this cytokine and a more severe course of OM.[37] Similar conclusions were demonstrated by Xanthinaki et al.[34] The opposite results were obtained by Meitovitz et al. In their study, there was a decrease in TNF concentration in patients treated with RT and a lack of correlation with OM severity.[35] However, in analyses of the results of studies on animal models, a relationship between the response of irradiated cells and the severity of OM has been noticeable. In the above-mentioned studies, TNF-α levels decreased as a result of administration of benzydamine and IL-11, which reduced the severity of the lesions caused by OM.[38-40] Differences in those findings may result from the collection of various materials in which the level of TNF-α (in saliva and serum) was tested. The ambiguous results of recent studies evaluating plasma TNF-α concentration suggest that genetic predispositions (e.g., SNP of the *TNF-α* gene) may be better predictors of OM and a more accurate prognostic factor in irradiated patients with HNC. It seems that such predispositions can affect both the level of expression and the activity of the encoded protein. In addition, they represent a much more specific indicator compared with the level of cytokines that are produced by both cancerous tissues and normal cells in response to damaging factors. So far, many studies have been carried out to assess the dependence of tissue sensitivity to radiation on genetic predisposition. However, studies on the correlation between SNP and OM have, so far, focused on DNA repair, oxidation and stress reaction, apoptosis, embryogenesis, and inflammation.[18-20,27,41] To the best of our knowledge, 2 of our previous publications were the first reports on the relationship between genetic alterations in the TNF-α−TNFR axis and OM severity in patients undergoing RT.[23,24] Our previous study, which included a group of 58 patients with HNC treated with IMRT, showed a higher risk of grade 3 OM (in weeks 5, 6, and 7 of treatment) in patients with the T-allele of the *TNFRSF1 A* gene (−610 T > G, rs4149570).[23] Also, our recent investigation revealed that people with the CC genotype of the *TNF-α* gene (−1211 T>C) have a higher TNF-α blood concentration compared with those with a T allele. This genotype is, therefore, associated with a more severe course of OM after RT. We also found that TNF-α levels and the presence of the CC genotype are important prognostic factors in patients with HNC treated with RT.[24] Considering our previous and current studies related to the TNF−TNFR axis, SNP−610 T> G seems to have a high predictive value for severe OM. It allows for prediction of onset of severe OM at the end of treatment (weeks 5−7 of RT). However, SNP−135 T>C, discussed earlier in this report, allows prediction of the early onset of severe OM (starting from week 2 of RT). Besides, there is a certain methodologic difference between the 2 studies (in the case of −610 T>G, it was sequencing, and in the case of −135 T>C, it was specific Taqman probes), which could affect sensitivity in the detection of SNPs.[22,24] A meta-analysis conducted by Song et al. (17 studies: 656 patients and 2193 controls) showed a significant correlation between the wild-type variant of the *XRCC3* (x-ray repair cross-complementing 3) gene (722 C>T, P.Thr241 Met, rs861539) and acute irradiation response in patients with various cancers. Of note, in the subgroup of patients with HNC, this SNP was significantly associated with a higher risk of adverse effects caused by radiation. The XRCC3 protein participates in homologous recombination and, thus, plays an important role in the repair of RT-induced DNA double-strand

breaks.[42] SNPs located in the coding sequence (especially when resulting in an amino acid change) can change the functioning of the protein (in the above case, such a change may reduce the efficiency of DNA repair—that is, decrease the ability of wound healing and, thus, increase the risk of more severe OM). In the course of RT, the presence of OM was noted in all patients. The first symptoms typically appeared in the second or third week of RT, and their severity gradually increased. Our study was relatively homogeneous in terms of treatment—that is, IMRT was used in all patients. Similar doses were given to all of the treated patients: 60 to 66 Gy in adjuvant therapy and 70 Gy in RT alone, with fractioning of 2 Gy per day. Also, the volume of irradiated tissues was similar—tumor site or postoperative site and regional lymph nodes. All patients were given a total scheduled dose and completed the course of RT. Interestingly, on weeks 6 and 7, compared with an earlier RT period, there was no significant difference in the risk of worst-grade OM, depending on the genotype of the *TNFRSF1 A* gene. It seems that when the highest dose of RT is achieved (at the end of treatment), the *TNFRSF1 A* gene status no longer influences the risk of development of more severe OM. Both age and gender were not significant factors in the incidence and intensity of OM. Smoking and alcohol abuse, usually indicated as OM risk factors, were not statistically significant. The CC genotype (−135 T>C) of *TNFRSF1 A* was significantly related to a higher risk of reduction of OS. The occurrence of the above SNP may be associated with a higher expression of the encoded receptor (change is located in the regulatory sequence) and, thus, might facilitate the development of inflammation. Long-lasting—particularly, generalized inflammation—may cause exhaustion of the body's immune mechanisms (facilitating escape of cancer cells from immune surveillance) and metabolic imbalance toward catabolism. These changes may lead to systemic cachexia, which significantly increases the risk of reducing survival.[43,44]

Our study has several limitations, including the sample size, the different treatment regimens, and the fact that we did not monitor patients' smoking habits during the study period.

CONCLUSIONS

Our study showed that after IMRT of the head and neck region, patients with the CC genotype of the *TNFRSF1 A* gene have a more severe course of OM. Interestingly, the TT genotype had a reverse-protective effect. Moreover, the CC genotype of the *TNFRSF1 A* gene is an independent prognostic factor of poor OS in patients with HNC undergoing IMRT. Certainly, because of the limitations of our study, as mentioned above, further studies are necessary to confirm our results.

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APPENDIX

Table S1. Impact of demographic and clinical variables on the risk of severe OM after subsequent weeks (1-3) of IMRT.

Variable		*IMRT week and grade of OM*					
		1st week		p, *OR [95%CI]*	*2nd week*		p, *OR [95%CI]*
		0	*1*		*1*	*2*	
Gender	**Male**	5 (10.2%)	44 (89.8%)	0.910	29 (59.2%)	20 (40.8%)	0.778
	Female	1 (9.1%)	10 (90.9%)	1.14 [0.12-10.83]	6 (54.5%)	5 (45.5%)	1.21 [0.32-4.51]
Age	**≥63**	3 (9.4%)	29 (90.6%)	0.863	19 (59.4%)	13 (40.6%)	0.861
	<63	3 (10.7%)	25 (89.3%)	0.86 [0.16-4.66]	16 (57.1%)	12 (42.9%)	1.10 [0.39-3.07]
Disease stage	**I**	1 (100%)	-	0.0011*	1 (100%)	-	0.427
	III	1 (9.1%)	10 (90.9%)		8 (72.7%)	3 (27.3%)	
	IVA	1 (2.5%)	39 (97.5%)		20 (50%)	20 (50%)	
	IV B	1 (33.3%)	2 (66.6%)		2 (66.6%)	1 (33.3%)	
	IV C	2 (40%)	3 (60%)		4 (80%)	1 (20%)	
Tumour location	**Larynx**	2 (6.1%)	31 (93.9%)	0.275	22 (66.7%)	11 (33.3%)	0.151
	Others[#]	4 (14.8%)	23 (85.2%)	0.37 [0.06-2.20]	13 (48.1%)	14 (51.9%)	2.15 [0.76-6.13]
Smoking history	**Smoker**[†]	5 (10%)	45 (90%)	1,000	30 (60%)	20 (40%)	0.560
	Non-smoker	1 (10%)	9 (90%)	1,00 [0,10-9,61]	5 (50%)	5 (50%)	1.50 [0.38-5.86]
Alcohol consumption	**Yes**	2 (7.4%)	25 (92.6%)	0.549	14 (51.9%)	13 (48.1%)	0.358
	No	4 (12.1%)	29 (87.9%)	0.58 [0.10-3.44]	21 (63.6%)	12 (36.4%)	0.61 [0.22-1.73]
Concurrent chemotherapy	**Yes**	1 (4.2%)	23 (95.8%)	0.246	12 (50%)	12 (50%)	0.287
	No	5 (13.9%)	31 (86.1%)	0.27 [0.03-2.47]	23 (63.9%)	13 (36.1%)	0.56 [0.20-1.61]
Neoadjuvant chemotherapy	**Yes**	-	10 (100%)	0.457	-	10 (100%)	0.009*
	No	6 (12%)	44 (88%)	0.33 [0.02-6.25]	35 (70%)	15 (30%)	0.02 [0.01-0.38]

IMRT - intensity modulation radiotherapy technique; OM - oral mucositis
hypopharynx, nasopharynx, oropharynx
† include current and former smokers
* statistically significant results.

Table S2. Impact of demographic and clinical factors on the risk of severe OM after subsequent weeks (3-4) of IMRT.

Factor		*IMRT week and grade of OM*					
		3rd week		p, *OR [95%CI]*	*4th week*		p, *OR [95%CI]*
		1 or 2	*3*		*1 or 2*	*3*	
Gender	**Male**	45 (91.8%)	4 (8.2%)	0.920	38 (77.6%)	11 (22.4%)	0.757
	Female	10 (90.9%)	1 (9.1%)	1.12 [0.11-11.18]	9 (81.8%)	2 (18.2%)	0.77 [0.14-4.09]
Age	**≥63**	29 (90.6%)	3 (9.4%)	0.756	26 (81.2%)	6 (18.8%)	0.559
	<63	26 (92.9%)	2 (7.1%)	0.74 [0.11-4.80]	21 (75%)	7 (25%)	1.44 [0.42-4.96]
Disease stage	**I**	1 (100%)	-	0.007*		1 (100%)	0.005
	III	9 (81.8%)	2 (18.2%)		8 (72.7%)	3 (27.3%)	
	IV A	40 (100%)	-		39 (81.2%)	9 (18.8%)	
	IV B	2 (66.7%)	1 (33.3%)		1 (33.3%)	2 (66.7%)	
	IV C	3 (60%)	2 (40%)		2 (40%)	3 (60%)	
Tumour location	**Larynx**	30 (90.9%)	3 (9.1%)	0.815	26 (78.8%)	7 (21.1%)	0.925
	Others[#]	25 (92.6%)	2 (7.4%)	0.80 [0.12-5.17]	21 (77.8%)	6 (22.2%)	1.06 [0.31-3.64]
Smoking history	**Smoker**[†]	46 (92%)	4 (8%)	0.835	39 (78%)	11 (22%)	0.889
	Non-smoker	9 (90%)	1 (10%)	1.28 [0.13-12.81]	8 (80%)	2 (20%)	0.89 [0.16-4.79]
Alcohol consumption	**Yes**	25 (92.6%)	2 (7.4%)	0.815	20 (74.1%)	7 (25.9%)	0.471
	No	30 (90.9%)	3 (9.1%)	1.25 [0.19-8.08]	27 (81.8%)	6 (18.2%)	0.63 [0.18-2.18]
Concurrent chemotherapy	**Yes**	22 (91.7%)	2 (8.3%)	1.000	15 (62.5%)	9 (37.5%)	0.021*
	No	33 (91.7%)	3 (8.3%)	1.00 [0.15-6.48]	32 (88.9%)	4 (11.1%)	0.21 [0.05-0.79]
Neoadjuvant chemotherapy	**Yes**	10 (100%)	-	0.539	-	10 (100%)	<0.001*
	No	45 (90%)	5 (10%)	2.54 [0.13-49.57]	47 (94%)	3 (6%)	0.01 [0.01-0.07]

IMRT - intensity modulation radiotherapy technique; OM - oral mucositis
hypopharynx, nasopharynx, oropharynx
† include current and former smokers
* statistically significant results.

Table S3. Impact of demographic and clinical factors on the risk of severe OM after subsequent weeks (5) of IMRT.

Factor		*IMRT week and grade of OM*		
		5th week		p, *OR [95%CI]*
		1 or 2	*3*	
Gender	**Male**	39 (79.6%)	10 (20.4%)	0.266
	Female	7 (63.6%)	4 (36.4%)	2.23 [0.54-9.14]
Age	**≥63**	27 (84.4%)	5 (15.6%)	0.138
	<63	19 (67.9%)	9 (32.1%)	2.56 [0.74-8.85]
Disease stage	**I**	-	1 (100%)	0.002
	III	9 (81.8%)	2 (18.2%)	
	IV A	37 (77.1%)	11 (22.9%)	
	IV B			
	IV C	-	3 (100%)	
		2 (40%)	3 (60%)	
Tumour location	**Larynx**	28 (84.8%)	5 (15.2%)	0.105
	Others[#]	18 (66.7%)	9 (33.3%)	2.80 [0.81-9.71]
Smoking history	**Smoker**[†]	39 (78%)	11 (22%)	0.587
	Non-smoker	7 (70%)	3 (30%)	1.52 [0.34-6.87]
Alcohol consumption	**Yes**	20 (74.1%)	7 (25.9%)	0.668
	No	26 (78.8%)	7 (21.2%)	0.77 [0.23-2.55]
Concurrent chemotherapy	**Yes**	14 (58.3%)	10 (41.7%)	0.010*
	No	32 (88.9%)	4 (11.1%)	0.17 [0.05-0.65]
Neoadjuvant chemotherapy	**Yes**	10 (100%)	-	0.152
	No	36 (72%)	14 (28%)	8.34 [0.46-151.86]

IMRT - intensity modulation radiotherapy technique; OM - oral mucositis
hypopharynx, nasopharynx, oropharynx
† include current and former smokers;
* statistically significant results.