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ORIGINAL ARTICLE

Relationship Between -2028 C/T *SELP* Gene Polymorphism, Concentration of Plasma P-Selectin and Risk of Malnutrition in Head and Neck Cancer Patients

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Abstract

Until today there is a lack of molecular factors, that could predict either cancer malnutrition or cachexia. Among potential mechanisms, that contribute to development of above syndromes, the systemic inflammatory response with overproduction of cytokines and adhesion molecules is the most likely. Recent papers suggested crucial role of P-selectin adhesion molecule in the initiation of leukocytes recruitment to the site of injury during inflammation, promotion of tumor aggressiveness and contribution to cancer cachexia. The aim of the study was to investigate *SELP* -2028 C/T polymorphism as a risk factor of malnutrition in 66 head and neck cancer (HNC) patients subjected to radiotherapy. Genotyping was conducted by real-time PCR method by means of TaqMan SNP Genotyping Assay. P-selectin Human ELISA Kit was used to determine P-selectin concentration in each extracted plasma samples. CC homozygous subjects had 4-fold higher risk score of being qualified as severely malnourished compared to other genotype carriers ($p = 0.015$). However, the TT homozygous patients were at lowest risk of severe weight loss >10% during the therapy period (OR = 0.20; $p = 0.019$). We also noted, that CC genotype carriers had significantly higher risk of early death incidence compared to CT or TT genotype (median survival time: 29 vs 34 months; HR = 3.02; $p = 0.0085$). Studied *SELP* -2028 C/T seems to be a novel attractive predictive factor of cancer malnutrition in HNC patients, perhaps in a future, patients carrying unfavorable CC genotype could be earlier scheduled for pharmaceutical intervention with parenterall nutrition, therefore they could be prevented from the development of severe malnutrition or even cachexia.

Keywords Malnutrition · Cachexia · Head and neck cancer · SELP · P-selectin

Introduction

Malnutrition and cachexia are frequent events among head and neck cancer (HNC) patients. The prevalence of both syndromes refers to even up to 88% of HNC cases [1, 2]. It seems to caused by anatomic location of developing tumor (oral cavity, throat or larynx), that impedes or inhibits proper nutrition of patients. Problems with ingestion and then developing undernutrition can be a first observable manifestation of the disease while tumor develops latently. Moreover, in some individuals the side-effects of applied therapy with the use of surgery, radiotherapy, chemotherapy or combination of above methods may contribute to development of malnutrition or cachexia [3, 4]. The most frequently demonstrated symptoms related to malnutrition include as follows: dysphagia, anorexia, anemia and weight loss. In contrast to malnutrition, cancer cachexia is multifractional syndrome strongly associated with severe metabolic abnormalities characterized by skeletal muscle loss and increased lipolysis, that cause both weight loss of various degree and significant changes in body composition. Above symptoms probably develop as an effect of complex interaction of tumor and host factors. The notable several

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nutritional deficits contribute to increase in cancer death rate as well as these have a significant negative impact on the patients' quality of life. The malnutrition or cachexia are considered as the prognostic factors in cancer patients – higher risk of an early death incidence and worse therapy outcomes are frequently observed in cachectic patients [5–7].

Although we know more about malnutrition and cachexia, the molecular background of the mentioned is still unknown due to involvement of numerous molecular pathways and accumulation of various genetic alterations in cancer patients. Among potential mechanisms contributing to development of cachexia, the primary initial process is probably the systemic inflammatory response mediated by host and tumor cells followed by release of pro-inflammatory cytokines or cell adhesion molecules, such as P-selectin. P-selectin is encoded by *SELP* gene and belongs to selectins protein family which are expressed on the surface of activated endothelial cells. Upon activation of the endothelial cells by histamine or thrombin during inflammatory response, P-selectin moves from an internal cell location to the endothelial cell surface. The essential function of this adhesion molecule is initiation of leukocytes recruitment to the site of injury during inflammation [8, 9]. The high expression of P-selectin was found on the surface of stimulated endothelial cells in cancer patients, what subsequently promoted invasion of tumor cells into bloodstream for distant metastases formation and enhancement of local growth of the cancer. The altered function of P-selectin was also noted in inflammatory related diseases - rheumatoid arthritis and cardiovascular diseases [9–11]. Limited papers evaluated role of P-selectin in cancer cachexia. However, available literature reports suggested that protein level of P-selectin and hence level of inflammatory response is probably regulated by single nucleotide polymorphisms (SNPs) located within promoter region of *SELP* gene, what can contribute to development of different grade of malnutrition or cachexia [12, 13]. The aim of the study was investigation of *SELP* -2028 C/T (rs3917647) as a malnutrition risk factor in HNC patients. Until today, relationship between studied SNP and malnutrition or cachexia is unknown, however promoter location of studied polymorphism allows to presume its crucial role in P-selectin regulation.

Material and Methods

Study Group

All HNC patients were both enrolled for the study and scheduled for the administration of RTH at the Department of Oncology, Medical University of Lublin during the period between 2014 and 2015. Study group included 52 male and 14 female individuals (median age: 63 years) from whom the following detailed clinical-demographic data regarding performance status (using Eastern Cooperative Oncology Group - World Health Organization (ECOG-WHO) scale), disease stage (using 7th edition of TNM Classification of Malignant Tumours) and alcohol consumption level (using International Statistical Classification of Diseases and Related Health Problems (ICD)) was obtained. Detailed clinical-demographic features of the study group is summarized in Table 1.

The detailed nutritional characteristic of studied HNC patients was assessed by nutritional questionnaires, anthropometric measurements and laboratory tests. At the time of admission all patients were nutritionally evaluated with the use of SGA (Subjective Global Assessment) and the NRS (Nutritional Risk Score, NRS 2002) scales. Taking together the patients' nutritional history (body weight changes, food intake, gastrointestinal syndromes and the appetite) and physical examination conducted by medical professional (assessment of muscle wasting, loss of body fat or the presence of edemas) the scoring of SGA was carried out. Eventually, SGA score was reviewed with a patients' to obtain answers to all questions regarding nutritional status (PG-SGA; Patient-Generated Subjective Global Assessment). Based on above examinations we qualified patients to the following three groups according to SGA score: A (well-nourished), B (moderately malnourished) and C (severely malnourished). The NRS (Nutritional Risk Score, NRS 2002) scale was used to predict risk of malnutrition development in the studied patients. The NRS score < 3 was considered as a low risk of malnutrition, while score > 3 was considered as a high risk of malnutrition. The anthropometric measurements used for the evaluation of patients' nutritional status included Body Mass Index (BMI), body weight and grade of the loss of body weight followed by the results of the laboratory examination. Laboratory test measured concentration of serum total protein (TP), albumin, prealbumin and transferrin. The body weight, BMI, TP and albumin were tested before the commencement of therapy (week -I) and after the termination of therapy (week-VII). Nutritional characteristic of the study group demonstrates Table 1.

All study participants signed an informed consent prior to the study. The study protocol was approved by the Bioethical Commission of the Medical University of Lublin (KE-0254/232/2014).

Genotyping and ELISA

DNA was isolated from peripheral leukocytes of whole blood samples by DNA Blood Mini Kit (Qiagen, USA). Genotyping of *SELP* was conducted using both the TaqMan probes purchased from Thermofisher Scientific (USA) and Genotyping Master Mix (Thermo Fisher Scientific, USA) in the StepOnePlus Real-Time PCR device with allele discriminating computer software (Applied Biosystems, USA). Each step

Table 1 Characteristics ofthe study group

Factor		Study group [$n = 66$]
Gender	Male	52 (78.8%)
	Female	14 (22.2%)
Age, median (range)		63 (42–87)
	> 63	38 (57.6%)
	≤ 63	28 (42.4%)
Histopathological diagnosis	Squamous cell carcinoma	60 (90.9%)
	Other	6 (9.1%)
Tumor location	Upper throat	17 (34.7%)
	Lower throat	49 (65.3%)
	Larynx	37 (56.1%)
	Others	29 (43.9%)
T stage	T1	2 (3.0%)
	T2	9 (13.6%)
	T3	18 (27.3%)
	T4	37 (56.1%)
N stage	N0	18 (27.3%)
	N1	8 (12.1%)
	N2	35 (53.0%)
	N3	5 (7.6%)
M stage	Mx	3(4.5%)
	M0	62 (93.9%)
	M1	1 (1.5%)
Disease stage	I	2 (3.0%)
	III	12 (18.2%)
	IVA	44 (66.7%)
	IVB	3 (4.5%)
	IVC	5 (7.6%)
Performance status (PS)	≤1	59 (89.4%)
	>1	7 (10.6%)
Type of treatment	Surgery + RTH	32 (48.5%)
	Surgery + chemoradiation	15 (22.7%)
	RTH alone	9 (13.6%)
	Induction CHTH + RTH	3 (4.5%)
	Concurrent chemoradiation	7 (10.6%)
Alcohol consumption	Yes	28 (42.4%)
	No	38 (57.6%)
Smoking status	Smoker	54 (81.8%)
	Non-smoker	12 (18.2%)
	Current smoker	48 (88.9%)
	Former smoker	6 (11.1%)
Parenteral nutrition	Yes	11 (16.7%)
	No	55 (83.3%)
Weight (kg)	Mean ± SD	65.03 ± 11.93
BMI	Mean ± SD	23.13 ± 4.44
	≥18.5	54 (81.8%)
	<18.5	12 (18.2%)
SGA	A	10 (15.2%)
	B	30 (45.5%)
	C	26 (39.4%)
NRS	2	44 (66.7%)
	3	19 (28.8%)
	4	2 (3.0%)
	5	1 (1.5%)
Total protein (g/L)	Median ± SD	6.66 ± 0.54
Albumin (g/L)	Median ± SD	3.37 ± 0.26
Prealbumin (g/dL)	Median ± SD	0.24 ± 0.08
Transferrin (g/L)	Median ± SD	2.48 ± 0.61

of genotyping was followed by protocols provided by the kit manufacturer. ELISA technique was used to assess concentration of P-selectin in plasma samples collected from study participants. The dedicated P-Selectin Human ELISA Kit (Thermo Fisher Scientific, USA) with a detection range of 0.63–40.0 ng/mL and the sensitivity equaled to the minimal detectable dose of this kit (<0.20 ng/mL) was used for. The subsequent steps of ELISA were conducted under the conditions included in the protocol provided by the manufacturer.

Statistical Analysis

Statistical analysis was performed with the use of MedCalc v.12.7 computer software (MedCalc Software, Belgium). Fisher's exact test and Chi-squared test were used for testing of the distribution of clinical-demographic and nutritional factors among patients with different genotypes of *SELP*. Differences in the studied factors among individuals with different nutritional status and various *SELP* genotypes were examined by U Mann-Whitney rank sum test and ANOVA Kruskal-Wallis test. Calculation of odds ratio (OR) with 95% Confidence Interval (95% CI) allowed the evaluation of risk of both genetic and clinical-demographic factors on nutritional status of studied patients. Kaplan-Meier Log rank test (univariate analysis) and Cox regression model (multivariate analysis) were applied for selection of factors significantly affecting patients' survival. The results with p value below 0.05 were considered as statistically significant.

Results

Distribution of studied *SELP* -2028 C/T genotype was within the Hardy-Weinberg equilibrium ($p = 0.09$). The following distribution of studied SNP was achieved: CC in 19 patients (28.8%), CT in 26 patients (39.4%) and TT in 21 individuals (31.8%), respectively. The median concentration of plasma P-selectin in the study group was 20.84 ± 6.2 ng/mL.

We evaluated factors affecting the risk of malnutrition and cachexia according to SGA scale. Patients with non-sqamous-cell tumors had significantly lower risk to be qualified as B or C according to SGA scale (OR = 7.57; $p = 0.026$), and patients with PS <1 had significantly lower risk to be classified as severely malnourished according to SGA (OR = 0.08; $p = 0.027$). Moreover, the following factors affected the low risk of malnutrition (B and C) in the study group: weight loss no greater than 5% (OR = 0.15; $p = 0.024$) and TT genotype of *SELP* gene (OR = 0.24; $p = 0.048$). As regards the factors affecting high risk of severe malnutrition (C) the following were found: alcohol consumption (OR = 2.83; $p = 0.046$) and CC genotype of *SELP* (OR = 4.04; $p = 0.015$). The factors affecting the risk of either mild malnutrition or severe malnutrition are presented in Table 2.

Multivariate analysis revealed that histopathological diagnosis and SELP genotype were independent predictive factors of cachexia (C). Diagnosis of squamous-cell carcinoma was related with significantly higher risk (OR = 16.67) of cachexia. On the other hand, TT genotype of the *SELP* was associated with a lower risk of cachexia (approximately 6- fold; OR = 0.17). In multivariate analysis none of the studied factors significantly influenced on the occurrence of malnutrition or cachexia (B or C), however, we noted a trend for higher risk

Table 2 Impact of the clincal-demographic, nutritional and genetic factors on the SGA scoring

SGA							
Factor		A	B and C	*p* OR [95%CI]	A and B	C	*p* OR [95%CI]
Gender	Male	7 (13.5%)	45 (86.5%)	0.465	32 (61.5%)	20 (38.5%)	0.765
	Female	3 (21.4%)	11 (78.6%)	1.75 [0.39–7.89]	8 (57.1%)	6 (42.9%)	0.83 [0.25–2.76]
Age (years)	> 63	8 (21.1%)	30 (78.9%)	0.136	23 (60.5%)	15 (39.5%)	0.988
	≤ 63	2 (7.1%)	26 (92.9%)	0.29 [0.06–1.48]	17 (60.7%)	11 (39.3%)	1.01 [0.37–2.74]
Performance status (PS)	≤1	9 (15.3%)	50 (84.7%)	0.490	39 (66.1%)	20 (33.9%)	**0.027***
	>1	0 (0%)	7 (100%)	0.55 [0.02–6.74]	1 (14.3%)	6 (85.7%)	0.08 [0.01–0.76]
Histopathological diagnosis	Squamous-cell carcinoma	7 (11.7%)	53 (88.3%)	**0.026***	34 (56.7%)	26 (43.3%)	0.123
	Others	3 (50%)	3 (50%)	7.57 [1.27–45.07]	6 (100%)	0	9.98 [0.54–185.27]
Disease stage	I-III	2 (13.3%)	12 (86.7%)	0.936	10 (71.4%)	4 (28.6%)	0.355
	IVA-IVC	7 (13,5%)	45 (86,5%)	0.93 [0.17–5.09]	30 (57.7%)	22 (42.3%)	0.54 [0.15–1.97]
T-stage	T1-T3	6 (20.7%)	23 (79.3%)	0.274	17 (58.6%)	12 (41.4%)	0.7702
	T4	4 (10.8%)	33 (89.2%)	2.15 [0.54–8.49]	23 (62.2%)	14 (37.4%)	0.86 [0.32–2.33]
Tumor location	Upper throat	3 (21.4%)	14 (78.6%)	0.740	12 (70.6%)	5 (29.4%)	0.332
	Lower throat	7 (14.3%)	42 (85.7%)	0.78 [0.18–3.42]	28 (57.1%)	21 (42.9%)	0.56 [0.17–1.82]
	Larynx	6 (16.2%)	31 (83.8%)	0.785	20 (54.1%)	17 (45.9%)	0.221
	Others	4 (13.8%)	25 (86.2%)	0.83 [0.21–3.25]	20 (69,0%)	9 (31,0%)	1.89 [0.68–5.23]
Alcohol consumption	Yes	4 (14.3%)	24 (85.7%)	0.866	13 (46.4%)	15 (53.8%)	**0.046***
	No	6 (15.8%)	32 (84.2%)	1.12 [0.28–4.43]	27 (71.1%)	11 (28.9%)	2.83 [1.02–7.86]
Smoking status	Smoker	8 (14.8%)	46 (85.2%)	0.872	31 (57.4%)	23 (42.6%)	0.267
	Non-smoker	2 (16.7%)	10 (83.3%)	1.15 [0.21–6.26]	9 (75.0%)	3 (25.0%)	2.23 [0.54–9.15]
Concurrent CTH	Yes	0	7 (100%)	0.440	4 (57.1%)	3 (42.9%)	0.843
	No	10 (16.9%)	49 (83.1%)	3.18 [0.17–60.14]	36(61.0%)	23 (39.0%)	1.17 [0.24–5.73]
BMI	<18.5	2 (16.7%)	10 (83.3%)	0.872	8 (66.7%)	4 (33.3%)	0.636
	≥18.5	8 (14.8%)	46(85.2%)	0.87 [0.16–4.73]	32 (59.3%)	22 (40.7%)	0.73 [0.19–2.71]
Weight loss (I vs VII)	<5%	2 (5.4%)	35 (94.6%)	**0.024***	22 (59.5%)	15 (40.5%)	0.830
	≥5%	8 (27.6%)	21 (72.4%)	0.15 [0.03–0.77]	18 (62.1%)	11 (37.9%)	1.12 [0.41–3.02]
	<10%	8 (14.0%)	49 (86.0%)	0.529	34 (59.6%)	23 (40.4%)	0.690
	≥10%	2 (22.2%)	7 (77.8%)	1.75 [0.31–9.97]	6 (66.7%)	3 (33.3%)	1.35 [0.31–5.96]
NRS	<3	7 (15.9%)	37 (84.1%)	0.808	28 (63.6%)	16 (36.4%)	0.477
	≥3	3 (13.6%)	19 (86.4%)	0.83 [0.19–3.60]	12 (54.5%)	10 (45.5%)	0.69 [0.24–1.94]
Genotype of *SELP* gene (−2028 C/T)	TT	6 (28.6%)	15 (71.4%)	**0.048***	14 (66.7%)	7 (33.3%)	0.492
	CT and CC	4 (8.9%)	41 (91.1%)	0.24 [0.06–0.99]	26 (57.8%)	19 (42.2%)	0.68 [0.23–2.02]
	CC	1 (5.3%)	18 (94.7%)	0.184	7 (36.8%)	12 (63.2%)	**0.015***
	TT and CT	9 (19.1%)	38 (80.9%)	4.26 [0.50–36.26]	33 (70.2%)	14 (29.8%)	4.04 [1.31–12.41]

BMI body mass index, *NRS* nutritional risk score, *CTH* chemoradiotherapy

*- statistically significant results [bold]

of worse nutrition status in the case of CC genotype of *SELP* gene (OR = 3.52; $p = 0.0676$). Multivariate analysis of the factors affecting the risk of either mild malnutrition or severe malnutrition is presented in Table 3.

Subsequently, we divided patients into two subgroups regarding the use of parenteral nutrition intervention (parenterally nourished patients (PN) and patients without parenteral nutrition (WPN)) and then we compared the distribution of nutritional and genetic factors between the studied cases. Patients assigned to SGA-C group were more often parenterally treated compared with SGA-A and B patients ($p = 0.032$). During the therapy period, the PN patients increased their BMI comparing with WPN subjects ($p = 0.009$). Moreover, we noted significantly higher P-selectin plasma level in WPN patient in contrast to PN subjects (median: 20.82 ng/mL vs 17.62 ng/mL; $p = 0.044$) (Supplementary Table 1). We also did not find any correlation between clinical-demographic features of studied patients and *SELP* genotype distribution (Supplementary Table 2), however, we noted a correlation between the distribution of *SELP* SNP and the nutritional status of the studied patients. Patients who carried CC genotype had significantly higher P-selectin plasma level in contrast to other genotype carriers (median of 22.91 ng/mL vs 19.29 ng/mL; $p = 0.018$). Homozygous TT subjects had the lowest P-selectin plasma level (median: 17.73 ng/mL) and the highest TP concentration before the commencement of therapy compared with CC and CT patients (median: 6.68 g/L; $p = 0.030$). Genotype distribution of *SELP* -2028 C/T according to patients' nutritional factors is showed in Table 4.

The next goal of the study was examination of the effect of studied SNP on the nutritional status of the studied group including separate analysis for PN and WPN patients (Table 5). Individuals with CC genotype were at significantly higher risk of severe malnutrition comparing with TT or CT patients (OR = 4.04; $p = 0.015$). The same trend was observed in patients who did not undergo parenteral nutrition, such individuals had over 4-fold higher risk of severe malnutrition compared to CT or TT individuals (OR = 4.13; $p = 0.029$). Moreover, parenterally nourished CC subjects prior to therapy demonstrated significantly lower BMI in contrast to other genotype carriers (OR = 39.0; $p = 0.036$). Regarding TT homozygous subjects, they demonstrated both significantly lower risk score to be qualified as B or C according to SGA (OR = 0.20; $p = 0.037$) and lower risk of severe weight loss (>10% during the therapy period) (OR = 0.20; $p = 0.019$) compared to CC and CT patients.

We also examined impact of nutritional, clinical and genetic factors on patients' survival. CC homozygous patients had over 3-fold higher risk of early death incidence and they also demonstrated significantly shorter overall survival (OS) ((29 months vs 34 months (HR = 3.02 [0.89–10.29], $p = 0.0085$)) compared to other genotype carriers (Fig. 1). All factors and their impact on patients' survival are summarized in Table 5. Cox-regression model including all the patients'

Table 3 Multivariate analysis of the impact of the clincal-demographic and genetic factors on the SGA scoring

Factor		*p* OR [95%CI]	
		A vs B and C	A and B vs C
Gender	Male	0.644	0.252
	Female	1.61 (0.21–12.20)	0.39 (0.07–1.95)
Age (years)	> 63	0.092	0.917
	≤ 63	0.19 (0.03–1.31)	0.94 (0.27–3.14)
Performance status (PS)	≤1	0.584	0.987
	>1	2.71 (0.08–95.55)	1.03 (0.02–4.66)
Histopathological diagnosis	Squamous-cell carcinoma	**0.020***	0.182
	Others	16.67 (1.56–169.45)	6.62 (0.41–106.27)
T-stage	T1-T3	0.863	0.946
	T4	1.16 (0.21–6.40)	0.96 (0.28–3.29)
Tumor location	Larynx	0.596	0.132
	Others	0.58 (0.08–4.23)	2.99 (0.72–12.43)
Genotype of *SELP* gene (-2028 C/T)	TT	**0.040***	–
	CT and CC	0.17 (0.03–0.92)	
	CC	–	0.0676
	TT and CT		3.52 (0.91–13.61)
Overall model fit (*p*)		**0.050***	**0.0013***

* - statistically significant results [bold]

Table 4 *SELP* genotype -2028C/T distribution according to clinical-demographic and nutritional factors of studied patients.

Factor(median ± SD)	*SELP*(-2028C > T) genotype					
	TT	CT and CC	*p*	CC	TT and CT	*p*
Weight (kg) (I)	65.81 ± 11.40	64.18 ± 11.91	0.725	67.68 ± 13.95	63.49 ± 10.57	0.185
Weight (kg) (VII)	58.67 ± 7.78	59.40 ± 10.19	0.890	61.89 ± 10.49	58.06 ± 8.86	0.120
BMI (I)	22.79 ± 7,78	23.11 ± 4.65	0.670	24.20 ± 5.64	22.52 ± 3.79	0.197
BMI (VII)	20.17 ± 2.86	21.30 ± 4.21	0.265	22.12 ± 4.56	20.47 ± 3.46	0.135
Transferrin (g/L)	2.54 ± 0.64	2.49 ± 0.60	0.669	2.38 ± 0.57	2.56 ± 0.62	0.226
Prealbumin (g/dL)	0.23 ± 0.10	0.24 ± 0.07	0.562	0.24 ± 0.08	0.24 ± 0.08	0.755
Total protein (g/L) (I)	6.65 ± 0.61	6.66 ± 0.50	0.752	6.82 ± 0.52	6.60 ± 0.53	0.246
Total protein (g/L) (VII)	6.68 ± 0.56	6.26 ± 0.67	**0.030***	6.33 ± 0.79	6.42 ± 0.61	0.876
Albumin (g/L) (I)	3.34 ± 0.22	3.40 ± 0.27	0.685	3.30 ± 0.24	3.41 ± 0.26	0.066
Albumin (g/L) (VII)	3.26 ± 0.42	3.10 ± 0.45	0.259	3.04 ± 0.49	3.19 ± 0.42	0.477
P-Selectin serum level (ng/mL)	17.73 ± 5.96	22.39 ± 5.99	**0.0005***	22.91 ± 6.23	19.29 ± 5.79	**0.018***

BMI body mass index, *TP* total protein

*- statistically significant results [bold]

data discriminated PS, tobacco smoking, SGA-C and CC genotype of *SELP* as the most significant factors affecting shorter OS in the study group (overall model fit $p = 0.006$) (Table 6).

Discussion

Malnutrition and cachexia are unfavorable syndromes related to cancer occurrence and contribute to poor therapy outcomes and higher risk of an early death incidence. Despite the advances in malnutrition and cachexia treatment and prevention, the molecular background of those complex syndromes is still controversial. Moreover, the severe malnutrition may afterwards develops into cancer cachexia. Nowadays, there is difficulty in prediction who patients will develop severe malnutrition or cachexia basing only on clinical factors. The most researchers acquiesce in hypothesis of the crucial role of systemic inflammatory response with the participation of cytokines or adhesion molecules in malnutrition pathogenesis. According to recent papers, genes encoding adhesion molecules are potential candidates involved in the complex immune diseases. Various SNPs of *SELP* are linked to susceptibility towards various inflammatory diseases [14–16].

Until today, only few *SELP* SNPs were thoroughly investigated and among them the 2266 A/C (rs6136) was the most frequently described. In the study of Burkhardt et al. authors explored relationship between rs6136 and the incidence of rheumatoid arthritis. Authors noted, that presence of A allele was associated with a high risk of rheumatoid arthritis as well as led to increased expression of *SELP* mRNA. Authors also suggested, that increased *SELP* expression can promote recruitment of leukocytes to a site of inflamed tissue, such as the synovial lining in rheumatoid arthritis. As a result, the inflammation may be either prolonged or elevated, which might influence disease activity and severity [11]. Other studies also demonstrated, that C allele or CC genotype were associated with decreased level of serum P-selectin concentration [17, 18]. As regards other *SELP* SNPs, the −1969 G/A was found to be associated with increased risk of cardiovascular events in Chinese patients, and C allele of −920 T/C was estimated as a risk factor of systemic lupus erythematosus [19, 20].

There is a little known about the role of *SELP* polymorphisms in cancer-related malnutrition or cachexia. As mentioned above only 2266 A/C was studied as a risk factor and marker of cancer cachexia. In a large study set designed by Tan et al., authors selected rs6136 as a significant risk factor of cachexia in patients with solid tumors. As a primary study result, the P-selectin was found to be upregulated in murine and rats models of cachexia caused by both acute and chronic inflammatory insults after induction of muscle atrophy gene expression. Second, the presence of C allele was found to be significantly associated with severe weight loss of at least 10% in both discovery and validation study set (OR = 0.52 and OR = 0.09, respectively). According to authors identification of P-selectin as relevant in animal models and in cachectic cancer patients supports this as either a risk factor or mediator of cachexia [21]. On the other hand, Avan et al. noted, that rather the AA genotype play crucial role as a unfavorable factor affecting high risk of cachexia in pancreatic cancer patients. In the two studied cohorts, authors found high prevalence of AA homozygous among cachectic subjects ($p = 0.011$ and $p = 0.045$, respectively) [13]. Most recently, Johns et al. in

Table 5 Impact of *SELP* genotype (-2028C > T) on the nutritional status of studied patients

Factor		CC	TT or CT	*p*, OR [95%CI]	TT	CT or CC	*p*, OR [95%CI]
SGA	A	1 (10%)	9 (90%)	0.184	6 (60%)	4 (40%)	**0.048***
All patients	B and C	18 (32.1%)	38 (67.9%)	4.26 [0.50–36.26]	15 (26.8%)	41 (73.2%)	0.24 [0.06–0.99]
	A and B	7 (17.5%)	33 (82.5%)	**0.015***	14 (35%)	26 (65%)	0.492
	C	12 (46.1%)	14 (53.9%)	4.04 [1.31–12.41]	7 (26.9%)	19 (73.1%)	0.68 [0.23–2.02]
SGA	A	0	9 (100%)	0.150	6 (66.7%)	3 (33.3%)	**0.037***
Without parenteral nutrition	B and C	14 (30.4%)	32 (69.6%)	8.48 [0.46–155.68]	13 (28.3%)	33 (71.7%)	0.20 [0.04–0.91]
	A and B	6 (16.2%)	31 (83.8%)	**0.029***	13 (35.1%)	24 (64.9%)	0.895
	C	8 (44.4%)	10 (55.6%)	4.13 [1.15–14.81]	6 (33.3%)	12 (66.7%)	0.92 [0.28–3.03]
SGA	A	1 (100%)	0	0.401	0	1 (100%)	0.944
Parenterally nourished	B and C	4 (40%)	6 (60%)	0.23 [0.01–7.05]	2 (20%)	8 (80%)	0.88 [0.03–29.15]
	A and B	1 (33.3%)	2 (66.7%)	0.620	1 (33.3%)	2 (66.7%)	0.441
	C	4 (50%)	4 (50%)	2.00 [0.12–31.98]	1 (12.5%)	7 (87.5%)	0.29 [0.01–6.91]
NRS	<3	10 (23.8%)	32 (76.2%)	0.241	14 (33.3%)	28 (66.7%)	0.727
All patients	≥3	9 (37.5%)	15 (62.5%)	1.92 [0.65–5.71]	7 (29.2%)	17 (70.8%)	0.82 [0.28–2.45]
NRS	<3	9 (25%)	27 (75%)	0.915	12 (33.3%)	24 (66.7%)	0.795
Without parenteral nutrition	≥3	5 (26.3%)	14 (73.7%)	1.07 [0.30–3.81]	7 (36.8%)	12 (63.2%)	1.17 [0.36–3.73]
NRS	<3	1 (16.7%)	5 (83.3%)	0.056	2 (33.3%)	4 (66.7%)	0.280
Parenterally nourished	≥3	4 (80%)	1 (20%)	20.00 [0.93.429.93]	0	5 (100%)	0.16 [0.01–4.36]
BMI I	<18.5 (UW)	3 (25%)	9 (75%)	0.749	4 (33.3%)	8 (66.7%)	0.901
All patients	>18.5 (N and OW)	16 (29.6%)	38 (70.4%)	1.26 [0.30–5.28]	17 (31.5%)	37 (68.5%)	0.92 [0.24–3.48]
BMI I	<18.5 (UW)	0	9 (100%)	0.150	4 (44.4%)	5 (55.6%)	0.497
Without parenteral nutrition	>18.5 (N and OW)	14 (30.4%)	32 (69.6%)	8.48 [0.46–155.68]	15 (32.6%)	31 (67.4%)	0.60 [0.14–2.58]
BMI I	<18.5 (UW)	4 (100%)	0	**0.036***	0	4 (100%)	0.400
Parenterally nourished	>18.5 (N and OW)	1 (14.3%)	6 (85.7%)	39.0 [1.28–1190.0]	2 (25%)	5 (75%)	4.09 [0.15–108.94]
BMI VII	<18.5 (UW)	17 (33.3%)	34 (66.7%)	0.148	15 (29.4%)	36 (70.6%)	0.441
All patients	>18.5 (N and OW)	2 (13.3%)	13 (86.7%)	0.31 [0.06–1.52]	6 (40%)	9 (60%)	1.60 [0.48–5.29]
BMI VII Without parenteral nutrition	<18.5 (UW)	14 (33.3%)	28 (66.7%)	0.076	13 (30.9%)	29 (69.1%)	0.909
	>18.5 (N and OW)	0	13 (100%)	0.07 [0.01–1.31]	6 (46.1%)	7 (53.9%)	1.083 [0.273–4.293]
BMI VII	<18.5 (UW)	3 (33.3%)	6 (66.7%)	0.186	2 (22.2%)	7 (77.8%)	0.766
Parenterally nourished	>18.5 (N and OW)	2 (100%)	0	9.28 [0.34–252.46]	0	2 (100%)	0.60 [0.02–17.22]
Weight loss	<%5	12 (32.4%)	25 (67.6%)	0.461	10 (27%)	27 (73%)	0.347
(I vs VII)	>%5	7 (24.1%)	22 (75.9%)	0.66 [0.22–1.98]	11 (37.9%)	18 (62.1%)	1.65 [0.58–4.68]
All patients	<10%	19 (34.5%)	36 (65.5%)	0.088	14 (25.5%)	41 (74.5%)	**0.019***
	>10%	0	11 (100%)	0.08 [0.01–1.46]	7 (63.6%)	4 (36.4%)	0.20 [0.05–0.77]
Weight loss	<%5	9 (28.1%)	23 (71.9%)	0.593	10 (31.2%)	22 (68.8%)	0.545
(I vs VII)	>%5	5 (21.7%)	18 (78.3%)	0.71 [0.20–2.49]	9 (39.1%)	14 (60.9%)	1.41 [0.46–4.34]
Without parenteral nutrition	<10%	14 (29.2%)	34 (70.8%)	0.218	15 (31.2%)	33 (68.8%)	0.192
	>10%	0	7 (100%)	0.16 [0.01–2.96]	4 (57.1%)	3 (42.9%)	2.93 [0.58–14.77]
Weight loss	<%5	3 (60%)	2 (40%)	0.383	0	5 (100%)	0.280
(I vs VII)	>%5	2 (33.3%)	4 (66.7%)	0.33 [0.03–3.93]	2 (33.3%)	4 (66.7%)	6.11 [0.23–162.74]
Parenterally nourished	<10%	5 (55.6%)	4 (44.4%)	0.280	1 (11.1%)	8 (88.9%)	0.240
	>10%	0	2 (100%)	0.16 [0.01–4.36]	1 (50%)	1 (50%)	8.00 [0.25–255.77]

BMI body mass index, *NRS* nutritional risk score, *SGA* subjective global assessment, *UW* under weight, *N* normal weight, *OW* over weight

*- statistically significant results [bold]

a large study set enrolling over 1200 individuals with various cancers investigated genetic signature associated with cancer cachexia. Among candidate predictive factors of cancer-cachexia the rs6136 was revealed. Subjects who carried the C allele of the rs6136 SNP in the *SELP* gene were at a reduced risk of cachexia defined by weight loss >5% and > 10% [22].

We were the first, who revealed -2028 C/T of *SELP* as a predictive marker of cancer related malnutrition. We selected the CC genotype as an important risk factor of above syndrome. CC homozygous subjects had 4-fold higher risk score to be qualified as severely malnourished compared to other genotype carriers ($p = 0.015$). This unfavorable trend also concerned patients, that underwent parenterall nutrition during therapy period, because of low BMI at the time of diagnosis. In such patients, the risk of BMI reduction was 39-fold higher in contrast to TT and CT genotype carriers ($p = 0.036$). The TT homozygous patients were at a lowest risk of severe weight loss >10% during the duration of therapy (OR = 0.20; $p = 0.019$) comparing with CC and CT patients. We also noted, that CC patients had a significantly higher risk score of early death incidence compared to CT or TT genotype (median survival time: 29 vs 34 months; HR = 3.02; $p = 0.0085$). The studied SNP also affected plasma P-selectin level, which was the highest in CC homozygous patients, whereas TT genotype carriers had the lowest plasma P-selectin concentration. Studied rs3917647 seems to

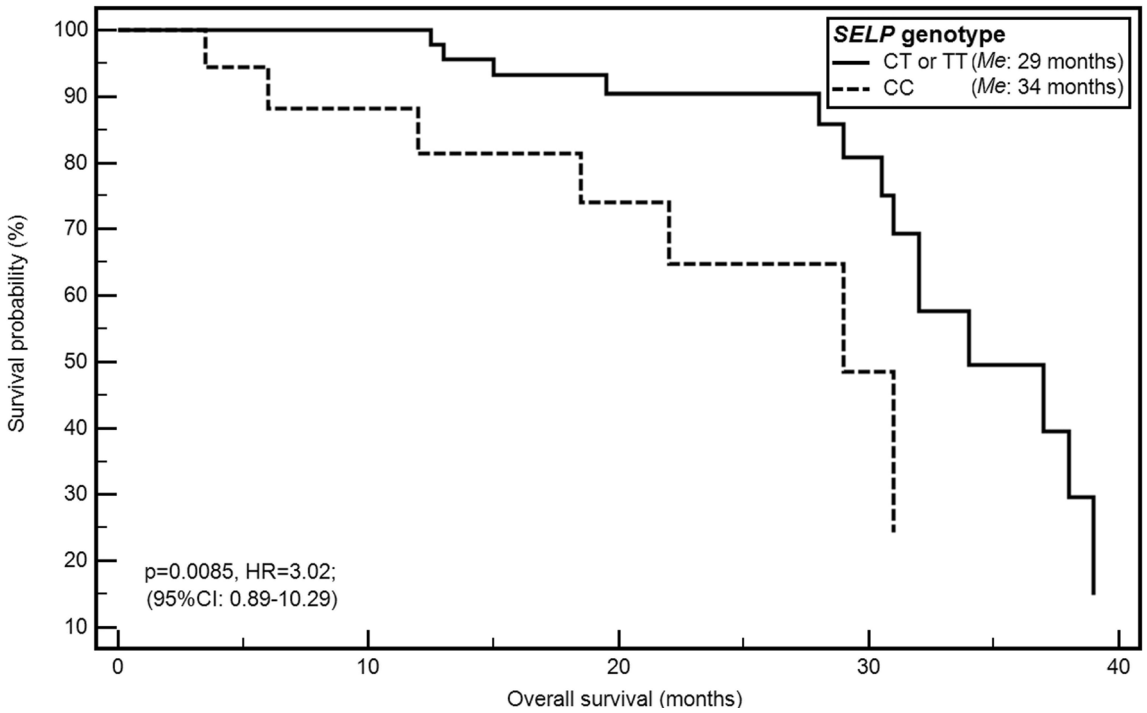

Fig. 1 Impact of *SELP* -2028 C/T on patients' overall survival: differences in overall survival between groups of patients with CC and both TT and CT genotype

Table 6 Factors affecting the overall survival of HNC patients in log-rank test and multivariate Cox logistic regression

Factor	Log-rank test (univariate analysis)	
	HR [95%CI]	*p*
Gender (male)	0.45 [0.14–1.48]	0.082
Age (≥ 63 years)	2.26 [0.95–5.39]	0.083
Smoking history (yes)	2.57 [1.01–6.53]	0.100
Smoking during treatment (yes)	2.68 [1.05–6.85]	0.095
Alcohol consumption (yes)	1.13 [0.46–2.78]	0.774
Performance status (>1)	3.16 [0.59–17.04]	**0.026***
TNM stage (≥IV)	1.31 [0.48–6.61]	0.605
Parenteral nutrition (yes)	1.87 [0.55–6.36]	0.204
SGA (C)	2.87 [0.99–8.23]	**0.008***
NRS (≥ 3)	1.64[0.67–4.01]	0.242
SELP genotype (TT)	0.51 [0.21–1.19]	0.077
SELP genotype (CC)	3.02 [0.89–10.29]	**0.009***
Selectin P serum concentration	0.95 [0.39–2.31]	0.904
Cox proportial-hazard regression model (multivariate analysis)		
Performance status (>1)	7.03 [1.29–38.33]	**0.025***
Tobacco smoking (yes)	8.08 [1.47–44.30]	**0.018***
SGA (C)	6.72 [1.31–34.58]	**0.023***
SELP SNP (CC genotype)	7.10 [1.19–42.19]	**0.032***
Overall model fit $p = 0.006$, stepwise method		

* Cox proportional-hazard regression model include following factors: gender, age (<63 vs ≥ 63 years), smoking history (yes vs no), alcohol consumption (yes vs no), performance status (<2 vs ≥2), TNM stage (<IV vs ≥ IV), parenteral nutrition (yes vs no), SGA status (A and B vs C), NRS status <3 vs ≥3), Selectin P serum concentration (high vs low), SELP genotype (CCvs other, TTvs other), * - statistically significant results [bold]

be a novel attractive predictive factor of cancer malnutrition and potentially cachexia in HNC patients, that should be analyzed along with rs6136 to improve selection of patients with highest risk score of malnutrition. Perhaps in a future, patients carrying unfavorable CC genotype could be earlier scheduled for pharmaceutical intervention with parenterall nutrition, therefore they could be prevented from the development of severe malnutrition or cachexia. One of the limitations of our study was the use of a subjective tool (SGA scale) to nutritional status and occurrence of malnutrition assessment as well as small study group. To confirm important role of -2028 C/T further studies should be conducted on a large cohort of patients with various tumors.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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References

1. O'Neill J, Shaha A (2011) Nutrition management of patients with malignancies of the head and neck. Surg Clin North Am 91:631–639
2. Alshadwi A, Nadershah M, Carlson ER, Young LS, Burke PA, Daley BJ (2013) Nutritional considerations for head and neck cancer patients: a review of the literature. J Oral Maxillofac Surg 71(11):1853–1860
3. Unsal D, Mentes B, Akmansu M, Uner A, Oguz M, Pak Y (2006) Evaluation of nutritional status in cancer patients receiving radiotherapy: a prospective study. Am J Clin Oncol 29:183–188
4. Gorenc M, Kozjek NR, Strojana P (2015) Malnutrition and cachexia in patients with head and neck cancer treated with (chemo)radiotherapy. Rep Pract Oncol Radiother 20(4):249–258
5. Dhanapal R, Saraswathi TR, Govind RN (2011) Cancer cachexia. J Oral Maxillofac Pathol 15(3):257–260
6. Aoyagi T, Terracina KP, Raza A, Matsubara H, Takabe K (2015) Cancer cachexia, mechanism and treatment. World J Gastrointest Oncol 7(4):17–29
7. Donohoe CL, Ryan AM, Reynolds JV (2011, 601434) Cancer Cachexia: mechanisms and clinical implications. Gastroenterol Res Pract 2011:1–13
8. Imhof BA, Dunon D (1995) Leukocyte migration and adhesion. Adv Immunol 58:345–416
9. Geng JG, Chen M, Chou KC (2004) P-selectin cell adhesion molecule in inflammation, thrombosis, cancer growth and metastasis. Curr Med Chem 11(16):2153–2160
10. Reiner AP, Carlson CS, Thyagarajan B, Rieder MJ, Polak JF, Siscovick DS, Nickerson DA, Jacobs DR Jr, Gross MD (2008) Soluble P-selectin, SELP polymorphisms, and atherosclerotic risk in European-American and African-African young adults: the coronary artery risk development in young adults (CARDIA) study. Send to Arterioscler Thromb Vasc Biol 28(8):1549–1555
11. Burkhardt J, Blume M, Petit-Teixeira E, Hugo Teixeira V, Steiner A, Quente E, Wolfram G, Scholz M, Pierlot C, Migliorini P, Bombardieri S, Balsa A, Westhovens R, Barrera P, Radstake TRDJ, Alves H, Bardin T, Prum B, Emmrich F, Cornelis F, Ahnert P, Kirsten H (2014) Cellular adhesion gene SELP is associated with rheumatoid arthritis and displays differential allelic expression. PLoS One 9(8):e103872
12. Fearon KC, Glass DJ, Guttridge DC (2012) Cancer Cachexia: mediators, signaling, and metabolic pathways. Cell Metab 16(2):153–166
13. Avan A, Avan A, Le Large TY et al (2014) AKT1 and SELP polymorphisms predict the risk of developing Cachexia in pancreatic Cancer patients. PLoS ONE 9(9):e108057
14. Kaur R, Singh J, Kaur M (2017) Structural and functional impact of SNPs in P-selectin gene: a comprehensive in silico analysis. Open Life Sciences 12(1):19–33
15. Volcik KA, Ballantyne CM, Coresh J, Folsom AR, Boerwinkle E (2007) Specific P-selectin and P-selectin glycoprotein ligand–1 genotypes/haplotypes are associated with risk of incident CHD and ischemic stroke: the atherosclerosis risk in communities (ARIC) study. Atherosclerosis 195:e76–e82
16. Jacobin VM, Deramchia K, Mornet S et al (2011) MRI of inducible P-selectin expression in human activated platelets involved in the early stages of atherosclerosis. NMR Biomed 24:413–424
17. Miller MA, Kerry SM, Dong Y, Strazzullo P, Cappuccio FP (2004) Association between the Thr715Pro Pselectin gene polymorphism and soluble P-selectin levels in a multiethnic population in South London. Thromb Haemost 92:1060–1065
18. Volcik KA, Ballantyne CM, Coresh J, Folsom AR, Wu KK, Boerwinkle E (2006) P-selectin Thr715Pro polymorphism predicts P-selectin levels but not risk of incident coronary heart disease or ischemic stroke in a cohort of 14595 participants: the atherosclerosis risk in communities study. Atherosclerosis 186:74–79
19. Kou L, Yang N, Chen G et al (2016) Association of SELP genetic polymorphisms and additional gene-smoking interaction on cardiovascular disease in Chinese Han population. Int J Clin Exp Pathol 9(9):9612–9618
20. Morris DL, Graham RR, Erwig LP, Gaffney PM, Moser KL, Behrens TW, Vyse TJ, Graham DSC (2009) Variation in the upstream region of P-selectin (SELP) is a risk factor for SLE. Genes Immun 10:404–413
21. Tan BH, Fladvad T, Braun TP et al (2012) P-selectin genotype is associated with the development of cancer cachexia. EMBO Mol Med 4(6):462–471
22. Johns N, Stretch C, Tan BHL (2017) New genetic signatures associated with cancer cachexia as defined by low skeletal muscle index and weight loss. J Cachexia Sarcopenia Muscle 8(1):122–130