MEDICAL UNIVERSITY – PLEVEN
FACULTY OF MEDICINE

DEPARTMENT OF "DERMATOLOGY, VENEREOLOGY AND ALLERGOLOGY"

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Modern epidemiological, histological and immunohistochemical characteristics of the squamous cell carcinoma of the skin

ABSTRACT OF A DISSERTATION PAPER
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The dissertation is presented on 120 standard typewritten pages. It contains 45 figures, 57 tables and 6 appendices. The bibliography includes 228 literary sources, of which 3 are in Cyrillic and 225 in Latin.

The research on the dissertation was conducted in:

- ✓ Oncology Center of the University Hospital "Dr. Georgi Stranski" - Pleven EAD
- ✓ Clinic of Skin and Venereal Diseases, University Hospital "Dr. Georgi Stranski" - Pleven EAD
- ✓ Clinic of Oncological Surgery, University Hospital "Dr. Georgi Stranski" - Pleven EAD Pleven EAD
- ✓ Laboratory at the Department of Pathology, Faculty of Medicine, Medical University – Pleven
- ✓ Laboratory at the Department of General and Clinical Pathology, Faculty of Medicine, Medical University – Sofia

The epidemiological and morphological studies /histological and immunohistochemical/ in this dissertation work were carried out within the framework of a research project No. 7/2022, funded by the Medical University – Pleven on the topic: “Study of the degree of immunohistochemical expression of PD-1, PD-L1 and CD8 in primary squamous cell carcinoma of the skin and their role in tumor genesis” The conduct of the research under the project was approved by Decision No. 676/31.05.2022 of the KENID at the Medical University – Pleven.

The dissertation work was discussed and directed for public defense at an extended departmental council of the Department of Dermatology, Venereology and Allergology, Faculty of Medicine at the Medical University - Pleven, held on 28.01.2025.

Contents

Abbreviations

NMSC ……..……...Non-melanocytic skin carcinoma
SCC ……..……...……..…… Squamous Cell carcinoma
BCC ……..…… Basal Cell carcinoma
MM……..…….. Malignant melanoma
UV……..……...……. Ultraviolet light
UVA……..……...….. Ultraviolet A-light
UVB……..……...…….. Ultraviolet B-light
EBH……..……. Epidermolysis bullosa hereditaria
EBS……..……..…….. Epidermolysis bullosa simplex
EBJ……..……..……Epidermolysis bullosa junstionalis
EBD……..……Epidermolysis bullosa dystrophica
KS……..……..…… Kindler syndrome
XP……..……..…… Xeroderma pigmentosum
LECD……..……...Lupus erythematosus chronicus discoides
HPV……..……..…… Human papilloma virus
EV……..…… Epidermodysplasia verruciformis
AK ……..……...…….. Actinic keratosis
PAK……..……... Pigmented actinic keratosis
LAK……..……....Lichenoid actinic keratosis
EGFR ……..……... Epidermal growth factor receptor
PD-1 ……..……... Programmed cell death protein - 1
PD-L1 ……..…….. Programmed cell death protein - ligand 1
CD4…..……. Cluster of differentiation 4
CD8……..……...……. Cluster of differentiation 8
AH……..……...……. Actinic cheilitis
BD ……..……..…… Bowen's disease
KA……..……..……... Keratoacanthoma
VK ……..……...……. Verucous carcinoma
KIN……..……... Keratinocyte intraepidermal neoplasia
CIN……..……..... Cervical intraepithelial neoplasia
IHC……..……..…….. Immunohistochemistry
RCM ……..……..... Reflectance confocal microscopy
AJCC……..……..American Joint Committee on Cancer
MMH ……..……..…... Moh's - micrographic surgery
C&E……..……...….... Curettage and electrocoagulation
PDT ……..……...…… Photodynamic therapy
RT ……...…….. Radiotherapy
ESMO……..……...European Association of Medical Oncology
ASCO……..……. American Association of Clinical Oncology
PHTR……...….Pharmaco-therapeutic guidance
5-FU……..……..……5-Fluorouracil
ORR ……..…… Overall response rate
PR ……..…… Partial response

I. INTRODUCTION

Skin cancer is one of the most common neoplastic diseases in the human body. Skin tumors are divided into two groups: melanocytic and non-melanocytic. Malignant Melanoma (MM) is the main representative of melanocytic skin tumors. In the group of non-melanocytic skin carcinomas, the most common is Basal Cell Carcinoma (BCC), followed by Squamous Cell Carcinoma (SCC). The mortality rate from SCC is low, but its treatment is challenging. Small SCCs allow complete cure through surgical excision, unlike larger, locally invasive and metastatic SCCs, which can destroy underlying tissues. They represent a therapeutic challenge for dermatologists, dermatosurgeons, radiation therapists and medical oncologists.

One of the main etiological factors for the development of SCC is chronic exposure to ultraviolet sunlight, especially ultraviolet B – light (UVB) and the use of tanning beds. Other factors include taking certain groups of medications (immunosuppressive, photosensitizing), chronic inflammatory diseases, so-called "precancerous diseases" such as Lupus Erythematosus Chronicus Discoides /LECD/, congenital genetic diseases such as Xeroderma pigmentosum and Epidermolysis bullosa hereditaria (EBH).

Squamous cell carcinoma of the skin is more common among people with a lighter skin phototype (I-III) and can develop in both sun-exposed and non-exposed skin.

Most often, SCC occurs after the age of 65, but recent studies show a trend for more SCC detection in patients under the age of 40.

The diagnosis of SCC of the skin is made by histopathological examination of material from the pathologically altered skin, taken through a skin biopsy. Dermatoscopy is a non-invasive diagnostic method that supports preoperative diagnosis and helps to reduce the number of skin biopsies in cases of suspected SCC. Among the modern methods for diagnosing SCC is Spectral Confocal Microscopy.

The modern clinical classification of SCC includes the following variants: superficial, nodular, ulcerative-infiltrative. According to the histopathological characteristics, SCC are: Nonspecific (classical) type, Keratoacanthoma – like, Verrucous, Clear cell, Acantholytic, Adenosquamous and Spindle cell types.

The gold standard for treating SCC is surgical excision. In very small SCC, with low risk of recurrence, electrocautery, curettage, local 5-fluorouracil or imiquimod cream can also be applied. A modern therapeutic method that is safe for surrounding tissues and organs is the so-called Moh’s surgery. In locally advanced and inoperable SCC, radiotherapy is applied with good results. Metastatic SCC are referred for systemic treatment. Until recently, classical chemotherapeutic agents with insufficient to moderate efficacy were used in medical oncology. Modern checkpoint inhibitors of the programmed cell death receptor-1 (PD-1) and its ligand-1 (PD-L1)

are agents for the treatment of metastatic and inoperable SCC, which demonstrate a significantly better therapeutic effect.

II. AIM AND TASKS

The aim of this dissertation work is to study the contemporary aspects of epidemiology, morphological manifestations (histological and immunohistochemical characteristics), surgical and medical treatment in patients diagnosed with Squamous Cell Carcinoma of the Skin.
To achieve this goal, the following tasks have been developed:

Task 1: To study the incidence of non-melanocytic skin cancers in patients registered in the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region from 2000 to 2023.

Task 2: To study the prevalence and specific features of SCC of the skin in patients kept on dispensary registration in the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region from 2016 to 2023.

Task 3: To study the morbidity by gender, age, place of residence in patients diagnosed with skin SCC, kept on dispensary registration in the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region from 2016 to 2023.

Task 4: To specify the clinical forms of the disease in patients diagnosed with skin SCC, kept on dispensary registration in the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region from 2016 to 2023.

Task 5: To perform a detailed histopathological examination of 100 invasive SCC to determine prognostic histopathological criteria for recurrence and metastasis.

Task 6: To perform a detailed immunohistochemical study of the markers PD-1, PD-L1, CD8 in 100 invasive SCC to determine prognostic immunohistochemical criteria determining the behavior of the disease and its prognosis.
Task 7: To perform a detailed immunohistochemical study of the markers Ki-67, CD4, the CD4:CD8 ratio in 70 invasive SCCs to determine prognostic immunohistochemical criteria determining the behavior of the disease and its prognosis.

Task 8: To analyze the therapeutic options and their effectiveness during the different stages of the disease.

III. MATERIAL AND METHODS

1. Material

To study the incidence of non-melanocytic skin cancer (NMSC) in Pleven District, the study group consisted of 5748 patients registered in the Oncology Registry at the Oncology Center of the University Hospital "Dr. Georgi Stranski" Pleven EAD, with a diagnosis according to ICD-10 C44.0 - C44.9, during the period January 2000 - December 2023.

To study the prevalence and specific features of SCC, the study group consisted of 355 patients with histologically verified SCC, kept on dispensary registration in the Oncology Registry at the Oncology Center of the University Hospital "Dr. Georgi Stranski" Pleven EAD, during the period January 2016 - December 2023. Patients were registered after surgical treatment performed at the Ear, Nose and Throat Clinic, Plastic and Reconstructive Surgery Department, Purulent-septic Surgery Department, Ophthalmology Clinic, and Oncological Surgery Clinic.

2. Methods

2.1. Prospective epidemiological study of patients with SCC for the period January 2020 - December 2023

2.1.1. Analysis of anamnestic data and clinical presentation

Filling the “Patient Information Sheet” form – by the patient and the doctor.

Individual survey (Survey method) – anamnesis, data on gender, age, duration of the disease, concomitant diseases, concomitant therapy, family history, etc. of each of the detected patients with SCC.

Clinical assessment (Clinical examination) of SCC in each patient, according to the diagnostic criteria for classification of the disease – localization, description of macroscopic characteristics of the lesion (size, presence of infiltration and surface).

2.1.2. Paraclinical and instrumental examinations of patients with SCC (Complete blood count, biochemical tests, Ro-graphy of the lung, Fine needle aspiration biopsy).

2.1.3. Surgical excision of SCC in patients within the framework of CP No. 199.1 (199) "Treatment of skin and mucous membrane tumors - malignant neoplasms" at the Clinic of Oncological Surgery at the University Hospital "Dr. Georgi Stranski" Pleven EAD.

2.1.4. Histological examination of the materials obtained from the excision of pathologically altered skin of each patient with SSc in the Laboratory of the Department of Pathology, Faculty of Medicine, Medical

University - Pleven. Filling in a detailed histological sheet specific to SSc.

A specially designed Questionnaire is filled out, which includes data from the individual survey, the clinical signs of the disease, the histological and immunohistochemical examination, and the treatment.

2.2. Retrospective epidemiological study of patients with SCC registered in the period 2016 - 2019

The data is obtained from the personal files of patients with SSC, kept on dispensary registration in the Oncology Register at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD. The above-mentioned patients have mandatory filled out the form "Declaration of informed consent of the patient regarding the procedure for admission and conduct of diagnostic tests and treatment procedures", before conducting their surgical treatment within the framework of CP No. 199.1 (199). The form is unified for the University Hospital "Dr. G. Stranski" Pleven EAD and is attached to the History of the disease - current version valid from 2019 to the present.

3. Laboratory methods

3.1. Histopathological study

Search for histological materials from patients with SCC who underwent surgical treatment at the University Hospital "Dr. G. Stranski" Pleven EAD for the period 2016-2023 in the Department of General and Clinical Pathology at the University Hospital "Dr. G. Stranski" Pleven EAD

Histological examination is performed using standard techniques. After surgical treatment, the excision materials are fixed in formalin (10% formaldehyde solution). The preparations are embedded in paraffin blocks, cut on a microtome, fixed on slides and stained with Hematoxylin and Eosin. The results are observed under a light microscope.

We selected 100 SCC to examine in detail. We divided the tumors into 2 groups:

Group 1 – low-risk for recurrence and metastasis SCC: diameter up to 2 cm; depth of invasion up to 4 mm; suitable for surgical treatment – 50 carcinomas;
Group 2 – high-risk carcinomas for recurrence and metastasis: recurrent and/or metastatic; diameter over 2 cm; depth of invasion over 4 mm; locally advanced carcinomas; suitable or unsuitable for surgical treatment; SCC developed against the background of genetic diseases such as Xeroderma pigmentosum, Epidermolysis bullosa hereditaria – 50 carcinomas

For the purpose of the histopathological study, we created a detailed histological sheet specific to SCC.

3.2. Immunohistochemical study

When performing the Immunohistochemical analysis, the Immunohistochemistry (IHC) method is applied, which selectively identifies antigens in the cells of the selected tissue area using antibodies that bind specifically to the antigens in the tissue sample.

From the previously selected patient tissue samples, fixed in formalin and embedded in paraffin, 3-micron-thick sections are prepared, which are mounted on silanized slides. The tissue samples are deparaffinized and rehydrated with xylene and alcohol in a descending order of 90%, 80% and 70% EtOH.

After heat-mediated antigen retrieval in Dako EnVision FLEX TRS, Low pH for 20 min/ 95°C and blocking of endogenous peroxidase with 3% H2O2, for assessment of PD-1 expression we applied **mouse monoclonal [NAT105] anti-PD1 antibody, Abcam** at a working concentration of 1/50 for 30 min incubation time.

After heat-mediated antigen retrieval with Dako EnVision FLEX, High pH buffer for assessment of PD-L1 expression we used **recombinant rabbit anti-PD-L1 antibody [SP142] - C-terminal** for 30 min. incubation time. For assessment of CD8 expression we applied **Dako FLEX Mono Mo a Hu CD8, cl C8/144B, RTU** for 30 min. incubation time.

After heat-mediated antigen retrieval in Dako EnVision FLEX TRS, Low pH for 20 min/ 95°C and blocking of endogenous peroxidase with 3% H2O2, for assessment of Ki-67 expression we applied **mouse monoclonal [Clone MIB-1] anti-Ki-67 antibody** for 30 min incubation time.

After heat-mediated antigen retrieval in Dako EnVision FLEX TRS, Low pH for 20 min/ 95°C and blocking of endogenous peroxidase with 3% H2O2, for assessment of CD4 expression we applied **mouse monoclonal [Clone 4B12] anti-CD4 antibody** for 30 min incubation time.

For control and quality assurance we included positive tissue controls from tonsillar carcinoma, in parallel with patient tissue samples.

Visualization of the complexes was performed with the Dako EnVision FLEX+ Mo (LINKER) detection system for 30 + 20 min. incubation time. Counterstaining of the cell nuclei was performed with Mayer's Hematoxylin.

PD-1 and PD-L1 expression was assessed by the “tumor proportion score” (TPS) (DAKO), defined as the percentage of all tumor cells showing partial or complete membrane staining (intensity ≥1+).

Ki-67, CD4, CD8 expression and the CD4:CD8 ratio were assessed by the so-called “eyeballing global score”, and the results are in %. No hot spots of expression were selected, but the entire preparation was viewed.

Ki-67 expression was counted outside the stratum basale of the epidermis (only tumor cells outside the stratum basale, since the basal layer of the epidermis normally shows increased proliferative activity). % of at least 500 cells was reported.

CD4/CD8 expression was reported in the peri-tumor infiltrate (only in the stromal reaction). CD4 is expressed not only by T-lymphocytes but also by macrophages. Cells showing dendritic processes were subtracted from % CD4.

Immunohistochemical staining for each antibody was scored as positive and negative, and a “tumor proportion score” (TPS) was then applied.

PD-L1 expression was scored as follows: no expression (TPS≤1%); low expression (TPS 2-10%); moderate expression (TPS 11-49%) and high expression (TPS>50%).

PD-1 expression was scored as: no expression (TPS≤1%); low expression (TPS 2-10%); with moderately high expression (TPS 11-20%) and with high expression (TPS 21-30%) and with very high expression (TPS>30%).

CD8 expression was also assessed as follows: no expression (≤1%); with low expression (2-10%); with moderately high expression (11-39%) and with high expression (>40%).

Ki-67 expression was assessed as: low (<10%); moderate (10-25%) and high (>25%).

We performed IHC studies for PD-1, PD-L1 and CD8 on materials from 100 patients and for Ki-67, CD4 and the CD4:CD8 ratio on materials from 70 patients diagnosed with invasive SCC who underwent surgical treatment at the University Hospital "Dr. G. Stranski" Pleven EAD for the period 2019 -2023 inclusive.

The studies of PD-1, PD-L1 and CD8 were performed in the Laboratory of the Department of Pathology, Faculty of Medicine, Medical University – Pleven, using the AS480 Autostainer Link 48 (AS Link 48), manufactured by DAKO/Agilent - an open automated system for immunohistochemical IHC staining.

The studies and readings of Ki-67, CD4 and the CD4:CD8 ratio were performed in the Department of General and Clinical Pathology, Faculty of Medicine, Medical University – Sofia.

The tumors were again divided into 2 groups:

Group 1 – low-risk carcinomas for recurrence and metastasis – 50 patients;

Group 2 – high-risk carcinomas for recurrence and metastasis – 50 patients

For the purpose of the histopathological study, we created an immunohistochemical sheet for reporting the expression of PD-1, PD-L1, CD8 in patients with SCC.

4. Statistical methods

To prove the reliability of the obtained results, establish statistical patterns and relationships between the studied characteristics, the following statistical methods were used:

A) Descriptive statistics

- Quantitative variables are presented through the summary statistical characteristics - arithmetic mean (Mean) and standard deviation (SD);

- Categorical variables are summarized through absolute (n) and relative (%) frequencies;

B) Chi-square test - when studying dependencies between descriptive (categorical) data with two or more categories.

C) Kaplan-Meier analysis - for assessing the curve (function) of survival until the occurrence of the studied events.

D) Log Rank test - for comparing survival curves in two or more independent groups.

The adopted threshold level of significance is α=0.05. Statistical significance is accepted when the p value is less than α.

The specialized statistical package SPSS (Statistical Package for the Social Sciences) version 20.0 was used to process the data from the study

5. Ethical aspects

The reagents used to achieve the scientific goals and objectives were obtained under **project No. 7/2022**, approved by the Research Ethics Committee (REC) of the Medical University of Pleven. All patients from the prospective epidemiological study signed the **"Patient Information Sheet"** form, an integral part of the research project No. 7/2022. The conduct of the research under **this project was approved by official Decision No. 676/31.05.2022 of the REC** at the Medical University of Pleven.

IV. RESULTS OF OWN STUDIES

1. Epidemiological study of Squamous Cell Carcinoma of the skin

1.1. Epidemiological study of non-melanocytic skin cancer in patients registered at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region from 2000 to 2023

For the study period (January 2020 - December 2023), the registered patients with NMCC in the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region are 5748. Male patients are 3055 (53%), and female patients are 2693 (47%), as shown in Figure 1.

Figure 1. Distribution of patients with NMSC by gender for the period 2000 – 2023.

The average age of diagnosis is 71 years. Men have an average age of 70.6 years, and women – 71.5 years.

The number of registered patients during the years 2000 - 2023 is shown in Figure 2.

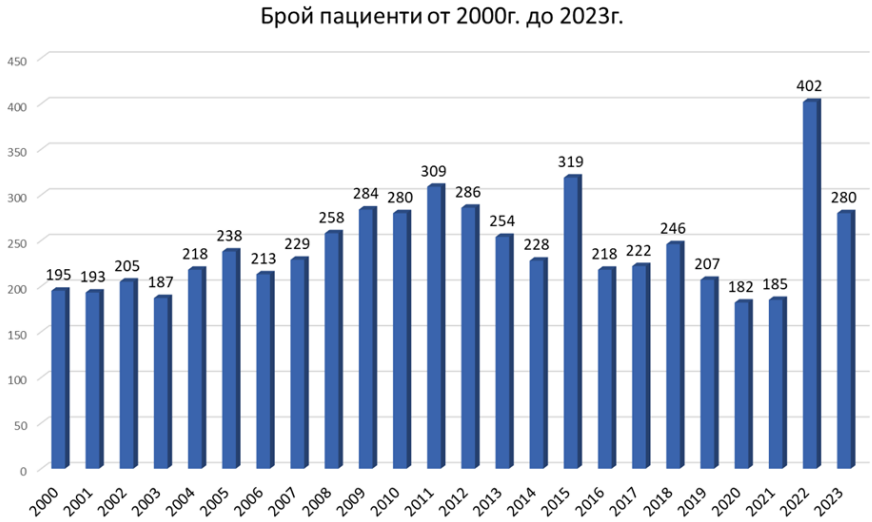

Figure 2. Registered patients with NMCC at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region from 2000 to 2023

The data analysis shows that an average of 243.2 patients with skin SCC are registered at the Pleven District Oncology Center each year. A decrease in the number of registered patients was found in 2020 and 2021 and a subsequent increase in patients with skin SCC in 2022.

Data from the Oncology Registry provide information on the histopathological diagnosis of patients with NSCLC, presented in Figure 3.

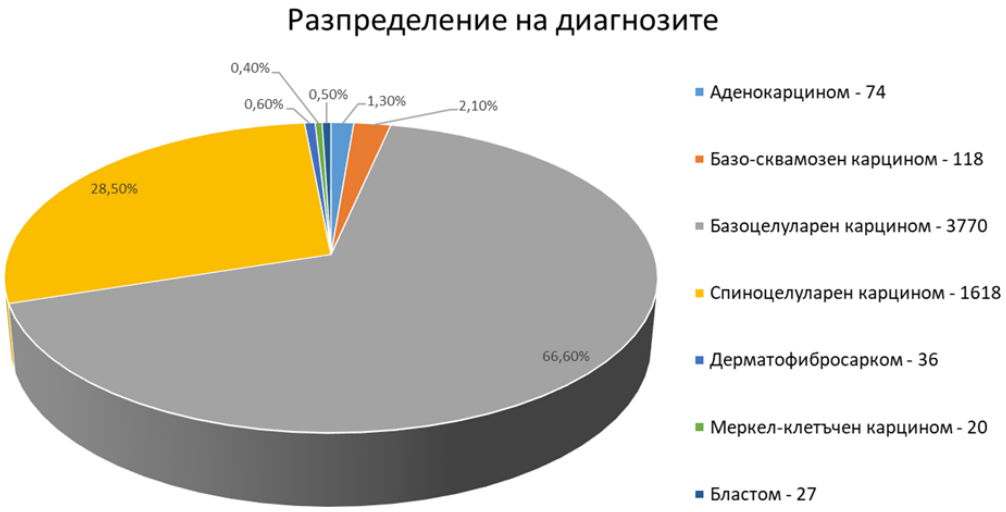

Figure 3. Histopathological diagnosis of patients with NSCLC registered for the period 2000 to 2023

The highest relative proportion is of patients with BCC – 66.6%, followed by those with SCC – 28.5%, Basal-squamous carcinoma – 2.1%, Primary cutaneous adenocarcinoma – 1.3%, Dermatofibrosarcoma – 0.6%, Blastoma – 0.5% and Merkel cell carcinoma – 0.4%. The distribution of carcinomas according to TNM classification and staging is demonstrated in Table 1, Table 2, Table 3 and Table 4.

Table 1. Distribution of tumors according to T-stage (Macroscopic diameter)

T	N	%
T1	3781	66,6
T2	1594	28,1
T3	223	3,9
T4	81	1,4
Total	5679	100,0

Table 2. Distribution of tumors according to N – stage (Lymph node metastasis)

N	N	%
N0	5578	98,9
N1	60	0,9
N2	2	0,04
N3	2	0,04
Total	5640	100,0

Table 3. Distribution of tumors according to M – stage (Metastasis to internal organs)

M	N	%
M0	5657	99,8
M1	7	0,1
M2	2	0,03
Total	5666	100,0

Table 4. Distribution of patients diagnosed with NSCLC according to Clinical Stage of the disease

Stage	N	%
Stage I	3659	64,8
Stage II	1737	30,8
Stage III	222	3,9
Stage IV	27	0,5
Total	5645	100,0

The highest relative proportion of tumors in T1 – macroscopic diameter <20mm (66.6%), N0 – without involvement of lymph nodes (98.9%), M0 – without the presence of metastatic lesions in internal organs (99.8%).

According to the clinical stage of the disease, the largest relative proportion of patients is in Stage I – 64.8%. The lowest proportion is in patients with metastatic lesions in internal organs – Stage IV (0.5%).

1.2. Epidemiological study of Squamous Cell Carcinoma of the Skin in the Pleven District for the period 2016 - 2023

For the period we examined (2016-2023) in Pleven District, we identified 355 patients, aged 40 to 96 years, diagnosed with skin SCC. There were 185 male patients (52.1%) and 170 female patients (47.9%), as shown in Figure 4.

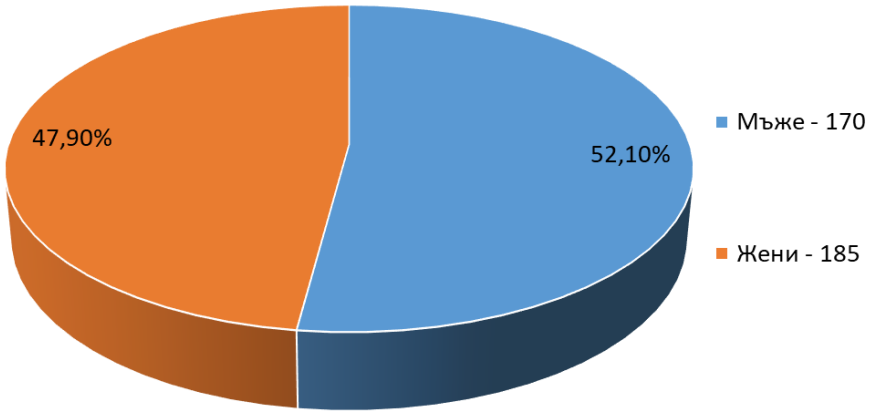

Figure 4. Distribution of patients with skin SCC by gender.

The mean age of the patients was 75.02 years with a standard deviation of ±10.74 years. The mean age of men was 74.11 years (±10.57) and that of women was 76.01 (±10.87). The results are shown in Figure 5.

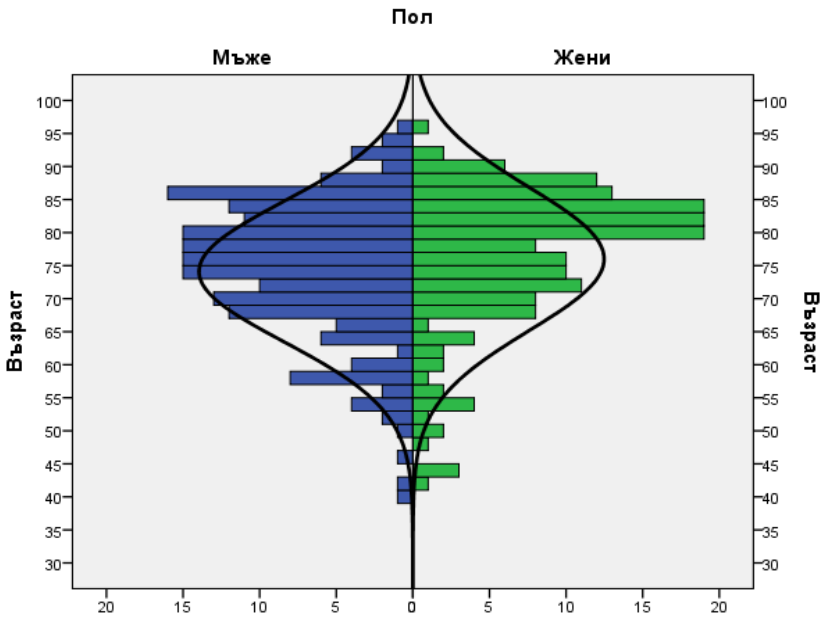

Figure 5. Age distribution of male and female patients with SCC.

In the study group, 41 patients (11.5%) developed SCC before the age of 60, 60 patients (16.9%) were in the 61-70 age group, 128 patients (36.1%) were in the 71-80 age group, and 126 patients (35.5%) were over 80 years of age, as shown in Figure 6.

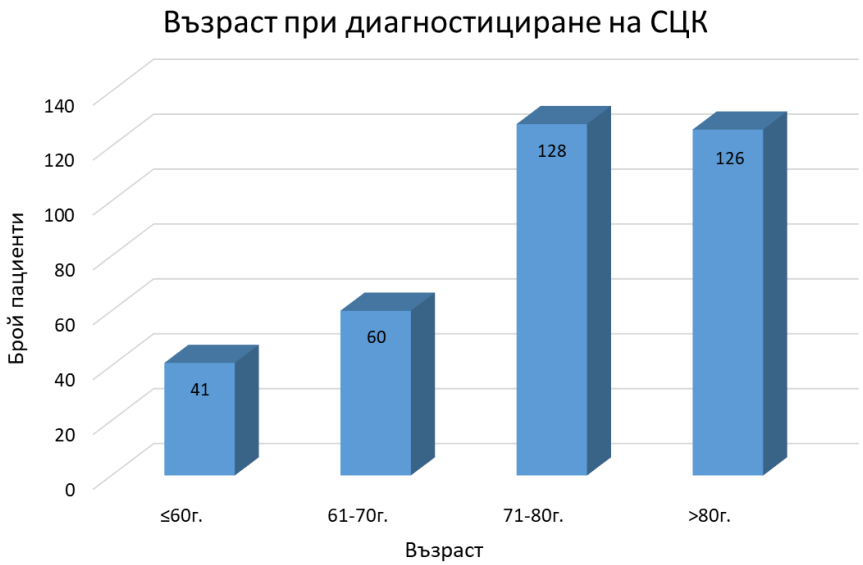

Figure 6. Age at which the patient was diagnosed with skin SCC

There is a tendency for the disease to be more common in male patients with an average age of 75 years.

A study on the profession of patients according to the National Classification of Professions and Positions in Bulgaria from 2011. The results are presented in Table 5.

Table 5. Professional referral of patients with skin SCC

Profession	N	%
Managers	2	0,6
Technicians and applied professions	7	2,0
Administrative support staff	1	0,3
Public service, trade and security staff	11	3,2
Skilled workers in agriculture, forestry, hunting and fishing	3	0,9
Skilled workers and related tradespeople	3	0,9
Machine operators and assemblers	1	0,3
Non-specialized occupations	3	0,9
Pensioners	312	91,0
Total	343	100,0

According to the place of residence of patients with skin SCC, we identified 194 patients (54.6%) living in rural areas and 161 patients (45.4%) living in urban areas.

2. Clinical Studies

A clinical-morphological and histopathological analysis was performed in a retrospective and prospective plan, covering all 355 patients with Squamous Cell Carcinoma of the Skin.

The photoexposure of the patients in their daily lives was studied. 238 patients (67%) work in agriculture, crop production and animal husbandry. Of the patients surveyed, 260 (73.2%) reported having experienced severe sunburns in their lifetime.

Two patients (1.13%) were receiving immunosuppressive therapy for concomitant onco-hematological diseases before the onset of SCC.

Regarding precancerous conditions that led to the development of SCC, 2 patients developed SCC on the background of Lupus erythematosus discoides (Figure 7), 2 patients – on the background of Epidermolysis bullosa hereditaria dystrophica (Figure 8 and 9) and 2 patients with Essential Thrombocythemia (Figure 10), treated with Hydroxyurea for many years, were found.

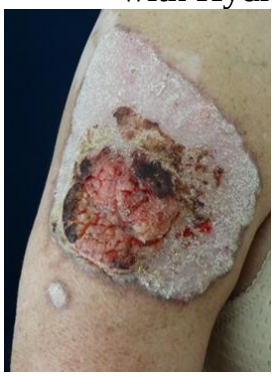

Figure 7: SCC after Lupus erythematosus discoides

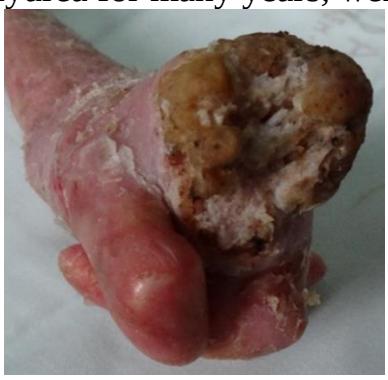

Figure 8: SCC on the background of Epidermolysis bullosa hereditaria dystrophica

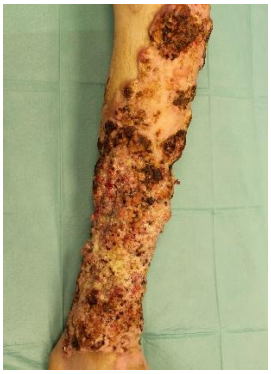

Figure 9: SCC on the background of Epidermolysis bullosa hereditaria dystrophica

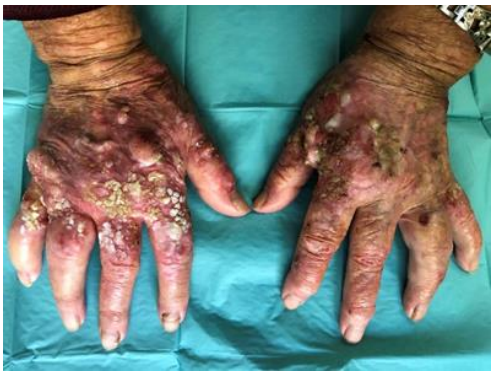

Figure 10: SCC in the setting of Essential Thrombocythemia

The present study investigated the incidence of multiple skin carcinomas. In 21 people (5.9%) more than one SCC was found, and in 15 (4.3%) – SCC and BCC. In 5 patients (1.4%) non-melanocytic carcinoma (NMC) and malignant melanoma (MM) developed simultaneously. In the remaining 314 people, 1 tumor was diagnosed, so 1 patient was considered 1 case.

The topographic localization is presented in Figure 11.

Figure 11. Distribution of SCCs according to topographic location

The high relative proportion of tumors affecting the facial area (206 SCCs – 58.02%) is striking. The specific facial localization of SCCs is presented in Figure 12.

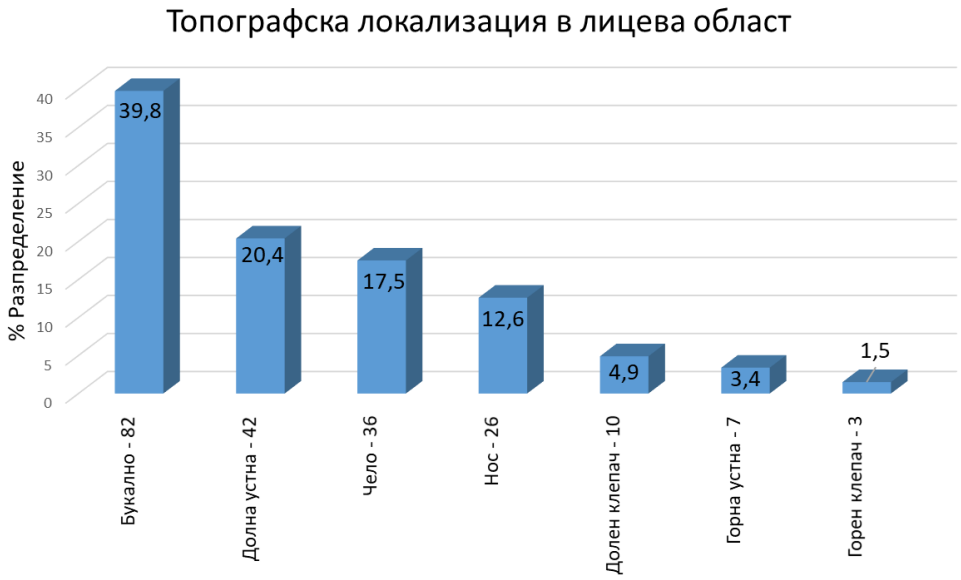

Figure 12. Specific localization of SCC in the facial area

The average macroscopic diameter of the tumors was 19.86 mm (± 14.34 mm). The minimum tumor diameter was 4 mm, and the maximum – 100 mm. Statistical analysis of the data showed that the difference between the tumor diameter in the different age groups was significant: $P<0.05$ (Actual value 0.028). A statistical correlation was also found between the tumor diameter and overall survival in the studied group of patients: $P<0.05$ (Actual value $p<0.001$). The result is shown in Figure 13.

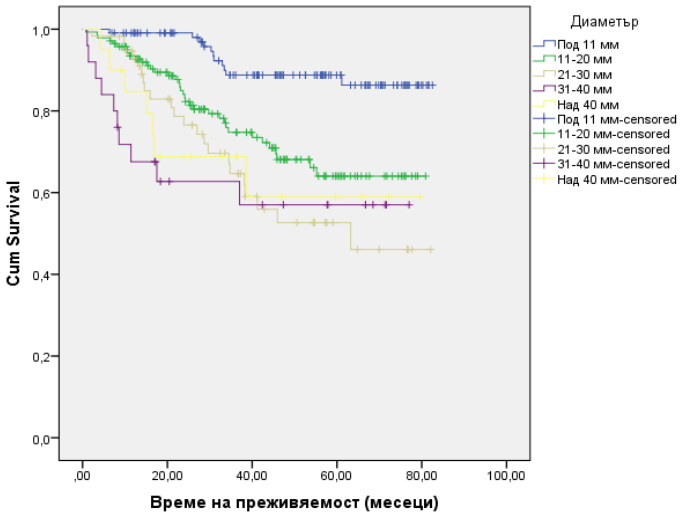

Figure 13. Relationship between tumor macroscopic diameter and overall survival of patients with cutaneous SCC

The staging of the cases was done according to the AJCC TNM classification, 8th version from 2017. The distribution of tumors according to their macroscopic diameter, category T, is presented in Table 6.

Table 6. Distribution of tumors by T-category

T	N	%
T1	214	60,3
T2	107	30,1
T3	25	7,0
T4	9	2,5
Total	355	100,0

The largest relative share of tumors in category T1, with a diameter of up to 20 mm – 60.3%. Tumors in category T2 with a size of 20-40 mm are twice less common – 30.1%.

The distribution of cases according to the presence of metastases in the regional lymph nodes, category N, is presented in Table 7.

Table 7. Distribution of tumors by N-category

N	N	%
N0	343	96,6
N1	8	2,3
N2	2	0,6
N3	2	0,6
Total	355	100,0

Lymph node metastases were found in only 3.4% of the cases examined.

The distribution of cases according to the presence of visceral metastases, Category M, is given in Table 8.

Table 8. Distribution of tumors according to M category

M	N	%
M0	351	98,9
M1	2	0,6
M2	2	0,6
Total	355	100,0

Distant metastases were found in only 1.2% of the studied patients.

The distribution of cases with cutaneous SCC, depending on the clinical stage defined according to the TNM classification, is shown in Table 9.

Table 9. Staging of tumors

Stage	N	%
Stage I	214	60,3
Stage II	125	35,2
Stage III	10	2,8
Stage IV	6	1,7
Total	355	100,0

The highest relative proportion of cases was in Stage I (60.3%), and the lowest in Stage IV (1.7%).

The histological features of the tumors were analyzed. Preparations obtained from the tumors, stained with standard hematoxylin-eosin, were examined. The distribution of primary skin SCCs depending on the tumor thickness according to the Breslow scale is shown in Figure 14.

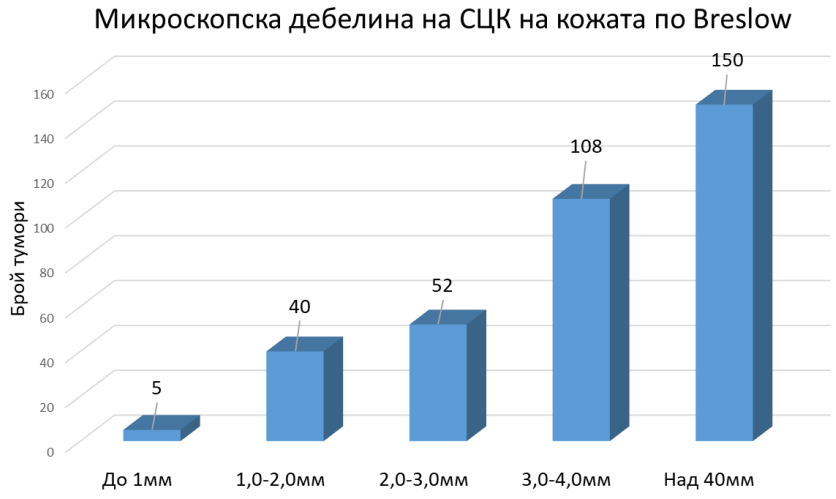

Figure 14. Microscopic thickness of the skin SCC

The largest number of tumors with a Breslow thickness of over 40 mm, and the lowest of tumors with a thickness of <1 mm. The analysis of the data shows a statistically significant relationship between the microscopic thickness of the tumors

and the age of the patients: P<0.05 (Actual value 0.001). The tumors are thicker in the group of elderly patients – 71-80 years.

The analysis of the macroscopic diameter of the SCC and the microscopic thickness also reveals a statistically significant relationship: P<0.05 (Actual value P<0.001). The larger the tumor diameter, the greater the tumor thickness. The distribution of tumors according to diameter and tumor thickness is presented in Table 10.

Table 10. Relationship between macroscopic diameter and microscopic thickness of the SCC (Chi-Square Tests)

Diameter		Microscopic thickness of carcinoma					Total	p
		Up to 1,0mm	1,0-2,0mm	2,0-3,0mm	3,0-4,0mm	Over 4,0mm		
Up to 11 mm	N	5	31	32	39	2	109	<0,001
	%	100,0%	77,5%	61,5%	36,4%	1,3%	30,8%	
11-20 mm	N	0	8	16	60	57	141	
	%	0,0%	20,0%	30,8%	56,1%	38,0%	39,8%	
21-30 mm	N	0	0	4	8	46	58	
	%	0,0%	0,0%	7,7%	7,5%	30,7%	16,4%	
31-40 mm	N	0	1	0	0	25	26	
	%	0,0%	2,5%	0,0%	0,0%	16,7%	7,3%	
Over 40 mm	N	0	0	0	0	20	20	
	%	0,0%	0,0%	0,0%	0,0%	13,3%	5,6%	
Total	N	5	40	52	107	150	354	
	%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	

Invasive carcinoma was detected in 302 (85.1%) of the tumors, and in 53 (14.9%) - “in situ” carcinoma. The distribution of invasive SCC of the skin according to histological subtypes according to data from the “History of the disease” is presented in Table 11.

Table 11. Distribution of histological types of invasive SCC according to data from the “History of the disease”.

Histological type	N	%
Acantholytic	8	2,3
Spindle cell	1	0,3
Adenosquamous	1	0,3
No data	345	97,2
General	355	100,0

A very high proportion of SCC for which there is no data on histological subtype (97.2%) from the "history of the disease" is established.

The distribution of primary SCC depending on tumor differentiation (grading) was evaluated in 159 cases and is presented in Table 12.

Table 12. Histological grading of tumors

Histological grading	N	%
G1	83	52,2
G2	64	40,3
G3	11	6,9
G4	1	0,6
Total	159	100,0

Keratinization was assessed in 349 carcinomas – 309 (88.5%) of them were keratinizing, and 40 (11.5%) – non-keratinizing. Keratinizing skin SCCs predominated in the studied group.

The assessment of 23 penile carcinomas, as tumors with a more specific biological course, shows that the most frequent localization is on the glans penis - 82.6% of cases, and less often on the shaft of the penis - 17.4%. According to the histopathological subtype, the tumors are divided into: 8 cases (34.8%) with Erythroplasia of Queyrat, 3 cases (13%) with Bowenoid papulosis, and 12 invasive carcinomas.

The distribution of cases according to the treatment performed is as follows: in 302 of the patients (85.1%) surgical treatment was applied (Figure 1 A-D; Appendix 6), and in 53 patients (14.9%) – a combined approach (surgical excision + radiotherapy).

In 303 of the cases (85.4%) surgical excision led to complete removal of the carcinoma. In the remaining 52 patients (14.6%) after surgical excision radiotherapy was applied due to tumor persistence. In 41 patients the applied dose was 50 Gy, in 10 - 60 Gy, and in 1 patient a 70 Gy radiotherapy dose was applied.

In 11 (3.09%) systemic therapy was applied for the treatment of advanced and metastatic SCC. In 7 patients, the treatment was represented by a classic polychemotherapy regimen, and 4 patients were treated with immunotherapy with the PD-1 inhibitor Cemiplimab. Two of the latter patients developed inoperable tumors on the background of Epidermolysis bullosa hereditaria, and 1 – on the background of long-term treatment of Essential Thrombocythemia with Hydroxyurea.

The applied Chi-Square Test for data analysis found that there is a statistically significant relationship between the macroscopic diameter of the primary carcinoma and the frequency of radiotherapy use: $P<0.05$ (Actual value $P<0.001$). The need for radiotherapy is higher in carcinomas with a macroscopic diameter of more than 20 mm.

The occurrence of local recurrence and metastases after treatment were analyzed. During the follow-up of the treated patients with skin SCC, the occurrence of local recurrence was found in 17 patients (4.8%). The time to recurrence was divided into 3 groups – up to 1 year (7 patients – 41.2%), from 1 to 2 years (8 patients – 47.1%) and more than 3 years (2 patients – 11.8%) after surgical treatment. Statistical analysis of the data showed a statistically significant relationship between the macroscopic diameter of the tumor and the time to recurrence $P<0.05$ (Actual value $P<0.001$), demonstrated in Table 13.

Table 13. Correlation between time to local recurrence and macroscopic diameter of the primary SCC (Log Rank test, p=0.001).

Diameter	Time (Months)	95% CI	
Under 11 mm	81,80	0,69	80,45
11-20 mm	83,22	1,65	79,99
21-30 mm	74,52	2,91	68,80
31-40 mm	68,62	4,55	59,70
Over 40 mm	61,67	7,54	46,90

A statistically significant relationship was found between the time at which the patient sought medical help after the tumor appeared and the time to recurrence: P<0.05 (Actual value P<0.001).

A statistically significant relationship was found between the stage of the SCC and the time to recurrence: P<0.05 (Actual value P<0.007). The higher the stage of the primary tumor, the shorter the time to local recurrence.

Lymphatic biopsy (excisional/fine needle aspiration biopsy) was performed in 44 patients with histologically verified primary SCC of the skin and enlarged regional lymph nodes. **In 18 of them (5.1%) loco-regional metastases in lymph nodes were found, and in 8 patients (2.25%) distant metastatic lesions in internal organs were registered**. A statistically significant relationship was found between the development of metastases and the macroscopic diameter of the primary SCC: P<0.05 (Actual value P<0.001). The result is demonstrated in Table 14.

Table 14. Statistically significant relationship between macroscopic diameter of SCC and development of metastatic lesions (Chi-Square Tests)

Diameter		Did the patient develop loco-regional metastases in lymph nodes		Total	P
		No	Yes		
Under 11 mm	N	108	1	109	<0,001
	%	32,1%	5,6%	30,8%	
11-20 mm	N	136	5	141	
	%	40,5%	27,8%	39,8%	
21-30 mm	N	56	2	58	
	%	16,7%	11,1%	16,4%	
31-40 mm	N	23	3	26	
	%	6,8%	16,7%	7,3%	
Over 40 mm	N	13	7	20	
	%	3,9%	38,9%	5,6%	
Total	N	336	18	354	
	%	100,0%	100,0%	100,0%	

The analysis of the relationship between the age of the patients and the time of recurrence did not show a statistically significant correlation between the two indicators: P>0.05 (Actual value P=0.656).

Oncological comorbidity was assessed in the group examined by us. 14 patients (3.94%) were found to develop another malignant disease before the development of skin SCC. One patient (0.3%) developed lung carcinoma, 1 patient – brain carcinoma, 2 patients (0.6%) – breast carcinoma, 3 patients – gastrointestinal tract carcinoma and 7 patients (2.0%) – prostate carcinoma.

The analysis of the causes of death from the disease in a retrospective aspect shows that a total of 70 patients (19.7%) died. Two patients (0.56%) died from severe Covid-19 infection, 42 patients (11.83%) from acute vascular event (heart attack/stroke), 13 patients (3.66%) died from chronic cardiovascular diseases, 6 patients (1.69%) lost their lives due to other malignant diseases, and **7 patients (1.97%) died due to advanced or metastatic SCC**. The analysis of the median survival by gender of patients with SCC of the skin showed no statistically significant differences between male and female gender: P>0.05 (Actual value P=0.216). A statistically significant relationship was found between the age of patients diagnosed with SCC in Stage IV and the time to death: P>0.05 (Actual value P=0.001). In patients ≤60 years, the median survival to death was 78.26 months, and in those >80 years. – 53.30 months. The results are shown in Figure 15.

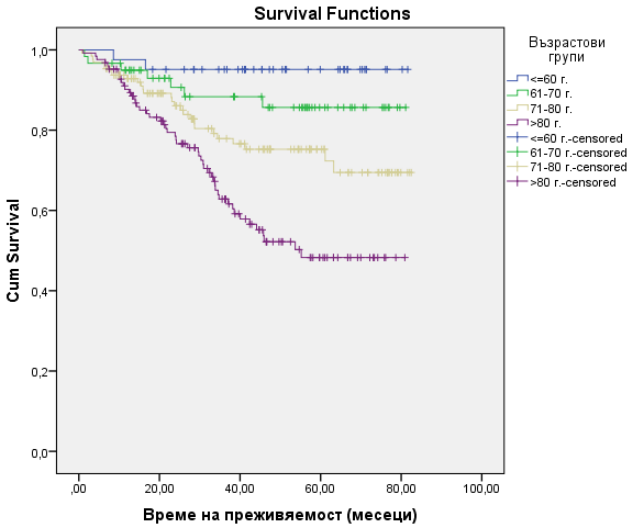

Figure 15. Correlation between age of patients with Stage IV CVD and time to death (Log Rank test, p<0.001)

3. Histopathological study

A detailed histopathological examination of 100 invasive SCCs was performed. The tumors were divided into two groups according to their macroscopic diameter. Group 1 - with macroscopic diameter <20mm and low risk of recurrence and metastasis - 50 pcs. and Group 2 - macroscopic diameter >20mm and high risk - 50 pcs. The localization, microscopic thickness of the tumor, cellular differentiation of tumor cells, perineural and lymphovascular invasion, peritumoral lymphocytic

stromal reaction were studied. The distribution of tumors by localization is shown in Figure 16.

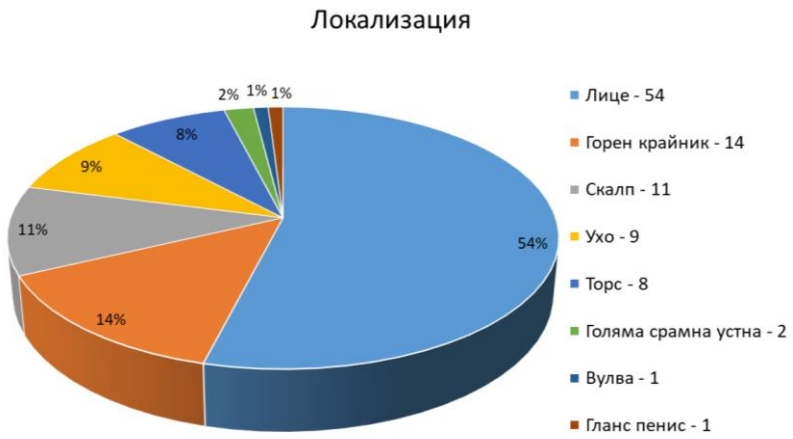

Figure 16. Localization of the 100 SCCs

Again, the higher proportion of tumors in the head and neck region is striking. The tumors examined are located in different areas of the face. Figure 17 shows the specific localization.

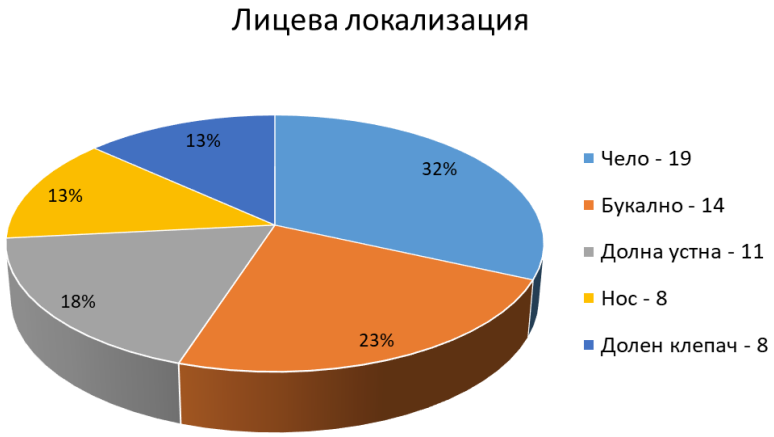

Figure 17. Specific facial localization of the 100 SSCs

The distribution of tumors according to histopathological subtype is as follows: 76 are non-specific (classic) type of SCC (Figure 18), 15 – Keratoacanthoma-like invasive SCC (Figure 19), 4 – Acantholytic (Figure 20), 3 – Verrucous (Figure 21), 1 is defined as Clear-cell carcinoma (Figure 22) and 1 – Pigmented SCC (Figure 23).

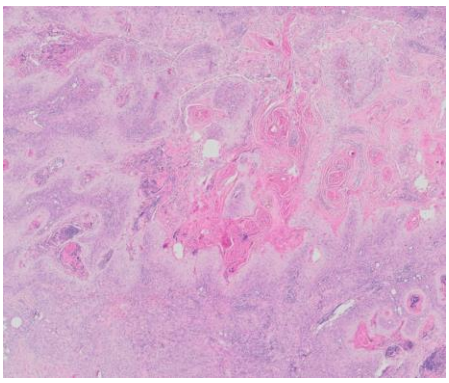

Figure 18. Classical (non-specific) SCC, Hematoxylin and Eosin (x20)

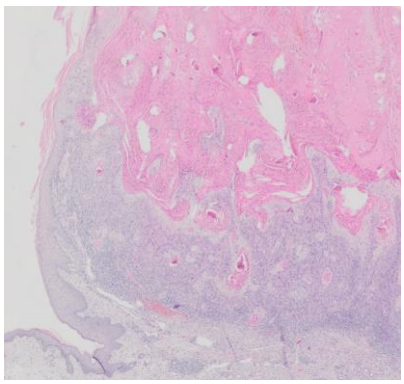

Figure 19. Keratoacanthoma-like invasive CCC, Hematoxylin and Eosin (x20)

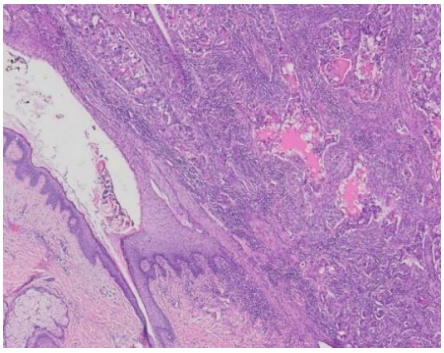

Figure 20. Acantholytic CC, Hematoxylin and Eosin (x50)

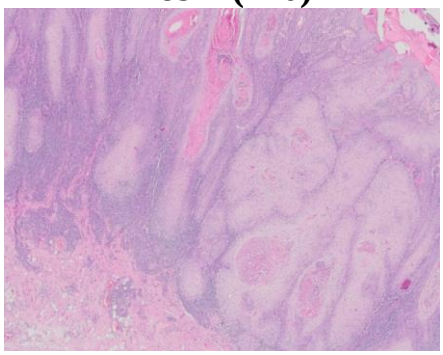

Figure 21. Verrucous CCC, Hematoxylin and Eosin (x20)

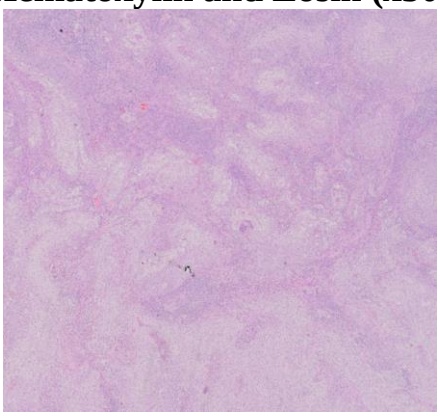

Figure 22. Clear-cell carcinoma, Hematoxylin and Eosin (x20)

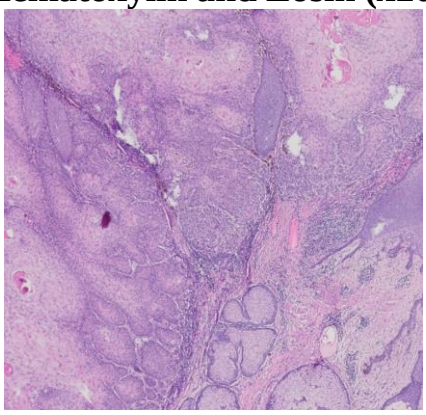

Figure 23. Pigmented SCC, Hematoxylin and Eosin (x20)

Morphological assessment of the tumors revealed a predominance of well- to moderately differentiated tumors: 71% were well-differentiated, 19% were moderately differentiated, and 10% were poorly differentiated. Data analysis did not show a statistically significant relationship between the histopathological subtype of SCC and the cellular differentiation of the tumor cells: $P<0.05$ (Actual value 0.069). The average histological thickness of the examined tumors according to the Breslow scale was 5.92 mm (±3.08 mm), with a minimum value of 1 mm and a maximum of 18 mm.

Data analysis showed a statistically significant difference between the thickness of the tumors in Group 1 and Group 2: P<0.05 (Actual value <0.001) with thicker tumors in the SCC group with a macroscopic diameter >20 mm. The average thickness in the low-risk carcinoma group was 4.93 (±2.34), and in the high-risk group – 7.13 (±3.44). (Table 15).

Table 15. Difference between the histological thickness of the SCC in Group 1 and Group 2 (Mann-Whitney Test)

Indicator	Macroscopic diameter	N	Mean	SD	Min	Max	p
Histological Breslow thickness	Up to 20 mm	55	4,93	2,34	1,00	11,00	<0,001
	Over 20 mm	45	7,13	3,44	1,00	18,00	

Data analysis also demonstrated a statistically significant difference between the Breslow level of invasion in the group of classical (non-specific) SCC and Keratoacanthoma-like invasive SCC: P<0.05 (Actual value <0.001) with thicker classical SCC (Table 16).

Table 16. Statistically significant difference between the histopathological Breslow thickness in classic SCC and in Keratoacanthoma-like invasive SCC (Mann-Whitney Test)

Indicator	Histological type	N	Mean	SD	Min	Max	p
Histological Breslow thickness	Non-specific (classical)	76	6,47	3,20	2,00	18,00	<0,001
	Keratoacanthoma-like	15	3,53	1,41	1,00	6,00	

The level of invasion of the examined group of 100 primary skin SCCs was studied and 56 tumors reaching the dermis, 36 - the hypodermis and 8 tumors affecting the underlying tissues under the hypodermis (5 - muscles and 3 - perichondrium) were found. The lymphovascular and perineural invasion of the examined SCCs was assessed. Lymphovascular invasion was found in 7 of the tumors, and perineural in 5. The analysis of the data shows that there is a statistically significant relationship between the level of invasion and lymphovascular invasion: P<0.05 (Actual value 0.001). The greater the invasion, the higher the probability of lymphovascular invasion. No significant relationship was found between the macroscopic diameter in Group 1 and Group 2 and lymphovascular invasion: P<0.05 (Actual value 0.238) (Table 17).

Table 17. Relationship between macroscopic diameter of SCC (Group 1 and Group 2) and lymphovascular invasion (Fisher's Exact Test)

Lymphovascular invasion			Macroscopic diameter		Total	p
			Uo to 20 mm	Over 20 mm		
Yes		N	2	5	7	0,238
		%	3,6%	11,1%	7,0%	
No		N	53	40	93	
		%	96,4%	88,9%	93,0%	
Total		N	55	45	100	
		%	100,0%	100,0%	100,0%	

No statistically significant difference was found between the histopathological subtype and lymphovascular invasion: P<0.05 (Actual value 0.795) (Table 18).

Table 18: Histopathological subtype of SCC versus lymphovascular invasion (Fisher's Exact Test)

Histological type		Lymphovascular invasion		Общо	p
		Yes	No		
Nonspecific (classical) type	N	7	69	76	0,795
	%	100,0%	74,2%	76,0%	
Verrucous	N	0	3	3	
	%	0,0%	3,2%	3,0%	
Acantholytic	N	0	4	4	
	%	0,0%	4,3%	4,0%	
Keratoacanthoma-like	N	0	15	15	
	%	0,0%	16,1%	15,0%	
Other	N	0	2	2	
	%	0,0%	2,2%	2,0%	
General	N	7	93	100	
	%	100,0%	100,0%	100,0%	

No statistically significant relationship was found between tumor cell differentiation and lymphovascular invasion: P<0.05 (Actual value 0.320) and between macroscopic diameter and lymphovascular invasion: P<0.05 (Actual value 0.129) (Table 19).

Table 19. Relationship between SCC cell differentiation and lymphovascular invasion (Fisher's Exact Test)

Tumor grading		Lymphovascular invasion		Total	p
		Yes	No		
Well differentiated	N	4	67	71	0,320
	%	57,1%	76,1%	74,7%	
Moderately differentiated	N	3	16	19	
	%	42,9%	18,2%	20,0%	
Poorly differentiated	N	0	5	5	
	%	0,0%	5,7%	5,3%	
Total	N	7	88	95	
	%	100,0%	100,0%	100,0%	

Examining perineural invasion, a statistically significant relationship was found with the level of tumor cell invasion: P<0.05 (Actual value 0.006). (Table 20).

Table 20. Relationship between the level of SCC invasion and the presence of perineural invasion (Fisher's Exact Test)

Level of invasion		Perineural invasion		Total	p
		Yes	No		
Dermis	N	0	56	56	0,006
	%	0,0%	58,9%	56,0%	
Hypodermis	N	3	33	36	
	%	60,0%	34,7%	36,0%	
Under Hypodermis	N	2	6	8	
	%	40,0%	6,3%	8,0%	
Total	N	5	95	100	
	%	100,0%	100,0%	100,0%	

No statistically significant relationship was found between perineural invasion and the macroscopic diameter of the SCC: P<0.05 (Actual value 0.086), as well as between the histological subtype of the SCC and perineural invasion: (Actual value 0.744).

The peritumoral lymphocytic stromal reaction was also assessed. It was high - in 68%, moderately expressed - in 21%, low-expressed - in 10% and absent in 1% of the examined tumors. No statistically significant relationship was found between the histological thickness of the tumor and the lymphocytic reaction: P<0.05 (Actual value 0.382), as well as between the two groups of tumors: P<0.05 (Actual value 0.224). (Table 21).

Table 21. Correlation between lymphocyte stromal reaction and macroscopic diameter in Group 1 and Group 2 (Fisher's Exact Test)

Lymphocytic stromal reaction		Macroscopic diameter		Total	p
		Up to 20 mm	Over 20 mm		
Absent	N	1	0	1	0,224
	%	1,8%	0,0%	1,0%	
Mild	N	5	5	10	
	%	9,1%	11,1%	10,0%	
Moderate	N	15	6	21	
	%	27,3%	13,3%	21,0%	
High	N	34	34	68	
	%	61,8%	75,6%	68,0%	
Total	N	55	45	100	
	%	100,0%	100,0%	100,0%	

The data analysis showed a statistically significant relationship between surgical success and histological Breslow thickness of the SCC: P<0.05 (Actual value <0.001), as well as macroscopic tumor diameter: P<0.05 (Actual value 0.038). The analysis is presented in Table 22.

Table 22. Relationship between success of surgical treatment, histological tumor thickness according to Breslow and macroscopic diameter of the SCC (Mann-Whitney Test).

Indicator	Is it completely excised	N	Mean	SD	Min	Max	p
Histological Breslow thickness	Yes	85	5,44	2,76	1,00	18,00	<0,001
	No	15	8,67	3,42	2,00	16,00	
Macroscopic diameter of the tumor in mm	Yes	85	22,87	13,20	8,00	100,00	0,038
	No	15	38,67	26,98	10,00	100,00	

The larger the macroscopic diameter of a skin SCC, and the thicker the tumor according to Breslow, the greater the likelihood that it will not be completely surgically removed.

4. Immunohistochemical study

Immunohistochemical (IHC) examination of PD-L1, PD-1 and CD8 expression was performed on the histological materials used in the histopathological study. Again, the SCCs were divided into two groups according to their macroscopic diameter. Group 1 - with a size <20mm and low risk of recurrence and metastasis and

Group 2 - with a size >20mm and high risk. Of the examined tumors, 45 SCCs without PD-L1 expression (≤1%), 35 tumors with low expression (2-10%), 12 SCCs with moderately high expression (11-49%) and 8 cases with high expression (> 50%) were found. The different degree of IHC expression of PD-L1 is shown in Figures 24, 25, 26 and 27. The antibody used was Anti-PD-L1 antibody [SP142] - C-terminal, Abcam. Statistical analysis of the data did not establish a statistically significant relationship between PD-L1 expression and histological Breslow thickness: P<0.05 (Actual value P=0.134) The results of the analysis are shown in Table 23.

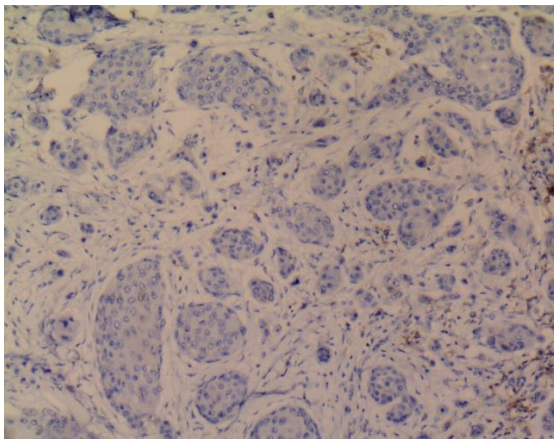

Figure 24. Absence of PD-L1 expression in primary SCC ≤1%

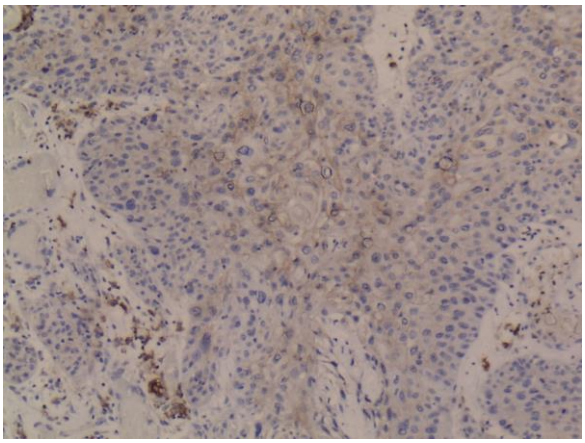

Figure 25. Low PD-L1 expression 2-10%

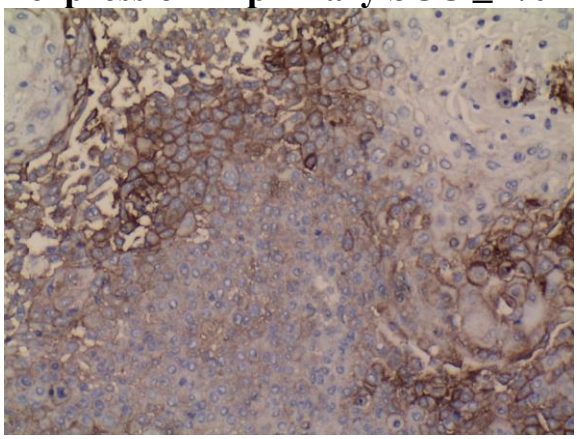

Figure 26. Moderate expression of PD-L1 11-49%

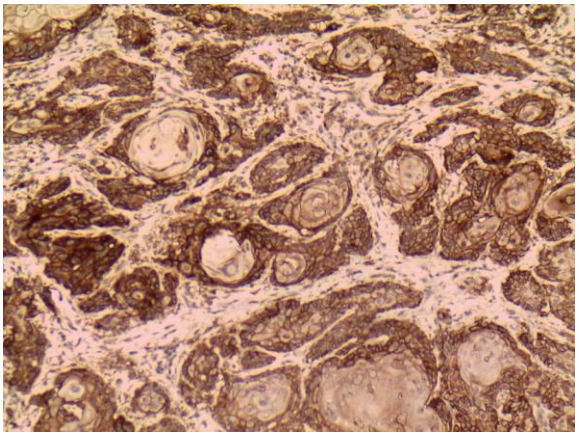

Figure 27. High PD-L1 expression > 50%

Regarding the IHC expression of PD-1: 10 tumors were without expression (≤1%), 33 were with low expression (2-10%), 27 SCC – with moderately high expression (11-20%), 22 cases – with high expression (21-30%) and 8 – with very high (>30%).

Examples of the degree of PD-1 expression are presented in Figures 28, 29, 30, 31 and 32. The antibody used was Anti-PD1 antibody [NAT105], Abcam. Data analysis did not reveal a statistically significant relationship between PD-1 expression and histological Breslow thickness: P<0.05 (Actual value P=0.453) (Table 23).

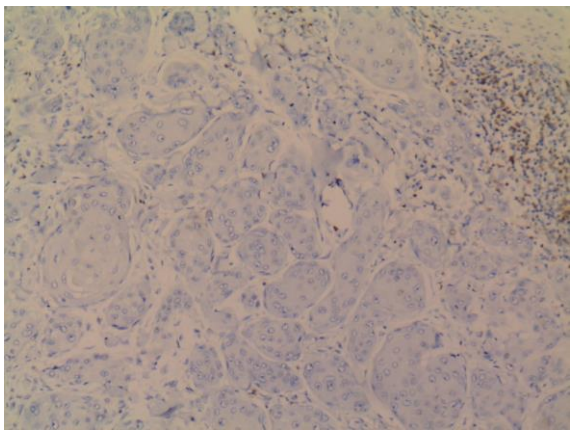

Figure 28. Lack of PD-1 expression in primary SCC ≤1%

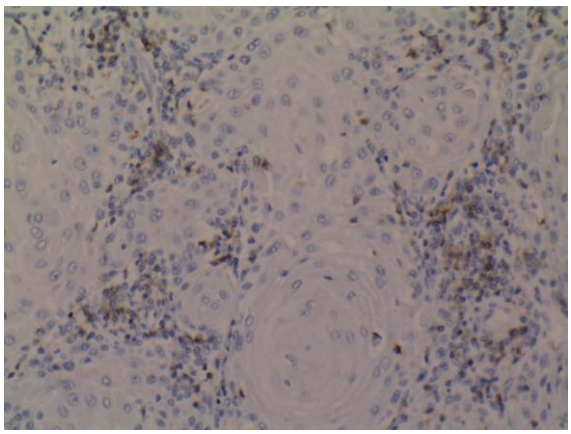

Figure 29. Low PD-1 expression 2-10%

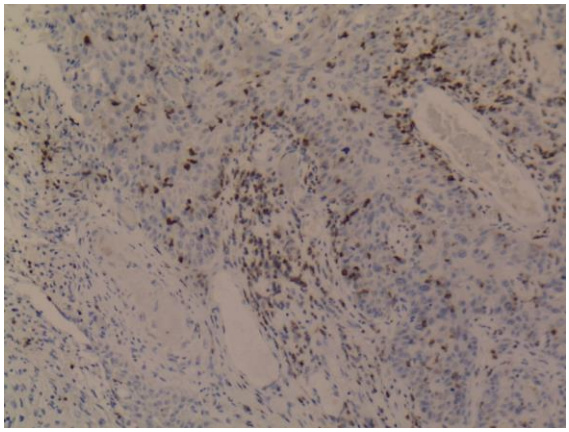

Figure 30. Moderately high PD-1 expression 11-20%

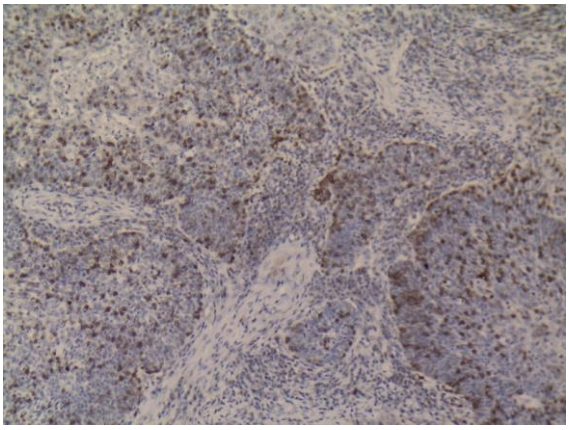

Figure 31. High PD-1 expression 21-30%

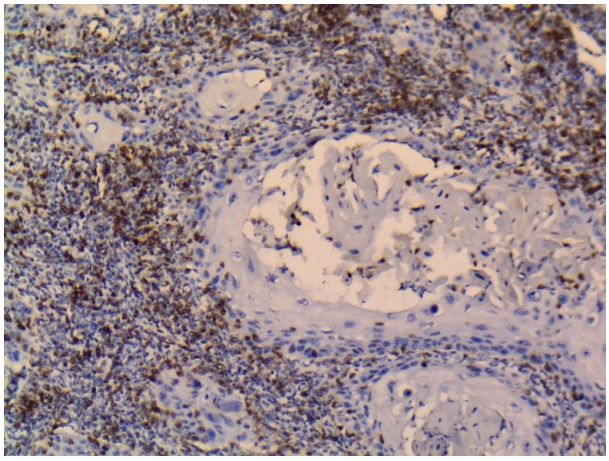

Figure 32. Very high PD-1 expression >30%

Examining CD8 expression, 1 SCC with no expression (≤1%), 13 tumors with low expression (2-10%), 48 cases with moderate expression (11-39%) and 37 with high expression (>40%). The degree of IHC expression of CD8 is shown in Figures 33, 34, 35 and 36. The results demonstrate that there is no statistically significant relationship between the degree of CD8 expression and the histopathological thickness of the primary SCC: $P<0.05$ (Actual value $P=0.146$) (Table 23).

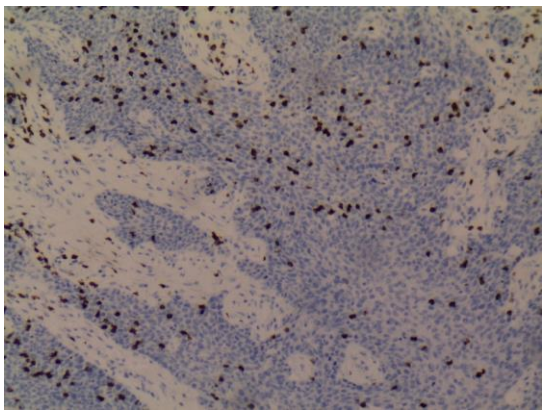

Figure 33. CD8 expression in primary SCC: No expression ≤1%

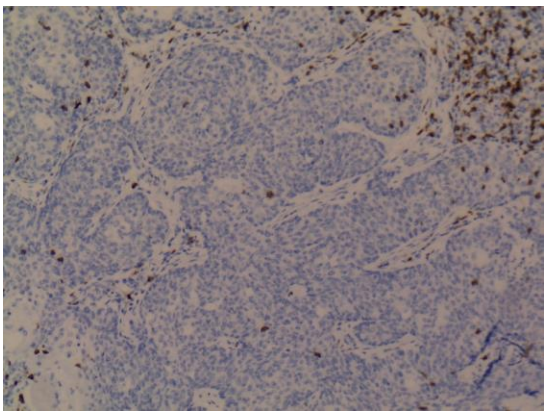

Figure 34. CD8 expression in primary SCC: Low expression 2-10%

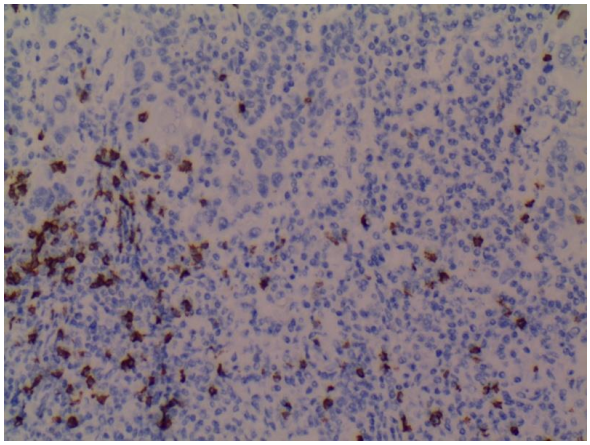

Figure 35. CD8 expression in primary SCC: Moderately high expression 11-39%

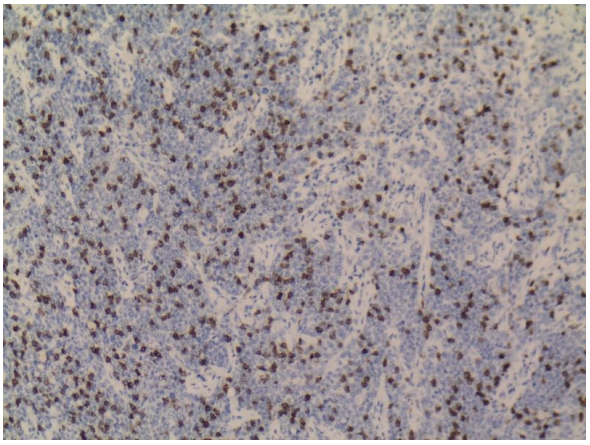

Figure 36. CD8 expression in primary SCC: High expression > 40%

Table 23. Correlation between immunohistochemical expression of PD-1, PD-L1 and CD8 and histological Breslow thickness of primary SCC

PD-L1	N	Breslow дълбочина на инвазия				
		Mean	SD	Min	Max	
Без експресия ≤1%	45	6,16	3,3	1	18	P=0,134
Ниска експресия 2-10%	35	5,37	2,24	2	10	
Умерено висока експресия 11-49%	12	6,83	3,41	2	11	
Висока експресия > 50%	8	6,5	4,85	3	16	
PD-1						
Без експресия ≤1%	10	5,9	3,28	2	11	P=0,453
Ниска експресия 2-10%	33	6,12	2,58	3	13	
Умерено висока експресия 11-20%	27	5,37	2,44	1	11	
Висока експресия 21-30%	22	6,32	4,54	1	18	
Много висока експресия >30%	8	5,71	2,06	4	9	
CD8						
Без експресия ≤1%	2	10	0	10	10	P=0,146
Ниска експресия 2-10%	13	7,15	2,79	4	13	
Умерено висока експресия 11-39%	48	5,6	3,17	1	18	
Висока експресия > 40%	37	5,78	3,03	2	16	

SCCs with greater depth of invasion showed higher PD-L1 and PD-1 IHC expression, and thinner carcinomas demonstrated higher CD8 expression. Comparing Group 1 and Group 2, there was no statistically significant difference between the macroscopic diameter of the SCC and the expression of PD-L1 (P=0.464), PD-1 (P=0.696) and CD8 (P=0.390). The results are shown in Tables 24, 25 and 26.

Table 24. Correlation between PD-L1 expression of tumor cells in Group 1 and Group 2

PD-L1 expression		Macroscopic diameter		Total	p
		Up to 20 mm	Over 20 mm		
Without expression ≤1%	N	23	22	45	0,464
	%	43,4%	48,9%	45,9%	
Low expression 2-10%	N	24	11	35	
	%	45,3%	24,4%	35,7%	
Moderately high expression 11-49%	N	3	9	12	
	%	5,7%	20,0%	12,2%	
High expression > 50%	N	3	3	6	
	%	5,7%	6,7%	6,1%	
Total	N	55	45	100	
	%	100,0%	100,0%	100,0%	

Table 25. Lack of statistical difference between PD-1 expression of cytotoxic lymphocytes in Group 1 and Group 2

PD-1 expression on cytotoxic lymphocytes		Macroscopic diameter		Total	p
		Up to 20 mm	Over 20 mm		
No expression ≤1%	N	6	4	10	0,696
	%	11,1%	8,9%	10,1%	
Low expression 2-10%	N	18	15	33	
	%	33,3%	33,3%	33,3%	
Moderately high expression 11-20%	N	12	15	27	
	%	22,2%	33,3%	27,3%	
High expression 21-30%	N	13	9	22	
	%	24,1%	20,0%	22,2%	
Very high expression >30%	N	5	2	7	
	%	9,3%	4,4%	7,1%	
Total	N	55	45	100	
	%	100,0%	100,0%	100,0%	

Table 26. Difference in CD8 expression on SCCs relative to macroscopic tumor diameter

CD8 expression on cytotoxic lymphocytes		Macroscopic diameter		Total	p
		Up to 20 mm	Over 20 mm		
No expression ≤1%	N	1	0	1	0,390
	%	1,9%	0,0%	1,0%	
Low expression 2-10%	N	5	8	13	
	%	9,3%	17,8%	13,1%	
Moderately high expression 11-39%	N	29	19	48	
	%	53,7%	42,2%	48,5%	
High expression > 40%	N	19	18	37	
	%	35,2%	40,0%	37,4%	
Total	N	55	45	100	
	%	100,0%	100,0%	100,0%	

Ten of the examined SCCs were poorly differentiated, 19 moderately differentiated and 71 tumors were well differentiated. The data analysis did not reveal a statistically significant difference between tumor grading and PD-L1 expression (P=0.277), PD-1 (P=0.552) and CD8 (P=0.889). Of the well-differentiated SCCs,

only 49% showed PD-L1 expression, while 90% of the poorly differentiated SCCs were PD-L1 positive. Analyzing lymphovascular invasion, no statistically significant relationships were found with PD-L1 (P=0.065), PD-1 (P=0.825) and CD8 immunohistochemical expression (P=0.548). The results are shown in Table 27, Table 28 and Table 29.

Table 27. Lack of statistical correlation between PD-L1 expression on tumor cells and lymphovascular invasion

PD-L1 expression on tumor cells		Lymphovascular invasion		Total	p
		Yes	No		
No expression ≤1%	N	3	42	45	0,065
	%	42,9%	46,2%	45,9%	
Low expression 2-10%	N	1	34	35	
	%	14,3%	37,4%	35,7%	
Moderately high expression 11-49%	N	3	9	12	
	%	42,9%	9,9%	12,2%	
High expression > 50%	N	0	6	6	
	%	0,0%	6,6%	6,1%	
Total	N	7	91	98	
	%	100,0%	100,0%	100,0%	

Table 28. Lack of statistical correlation between PD-1 expression on cytotoxic lymphocytes and lymphovascular invasion

PD-1 expression on cytotoxic lymphocytes		Lymphovascular invasion		Total	p
		Yes	No		
No expression ≤1%	N	0	10	10	0,825
	%	0,0%	10,9%	10,1%	
Low expression 2-10%	N	2	31	33	
	%	28,6%	33,7%	33,3%	
Moderately high expression 11-20%	N	2	25	27	
	%	28,6%	27,2%	27,3%	
High expression 21-30%	N	2	20	22	
	%	28,6%	21,7%	22,2%	
Very high expression >30%	N	1	6	7	
	%	14,3%	6,5%	7,1%	
Total	N	7	92	99	
	%	100,0%	100,0%	100,0%	

Table 29. Lack of statistical correlation between CD8 IHC expression and lymphovascular invasion.

CD8 expression on tumor (lymphocytes)		Lymphovascular invasion		Total	p
		Yes	No		
No expression ≤1%	N	0	1	1	0,548
	%	0,0%	1,1%	1,0%	
Low expression 2-10%	N	2	11	13	
	%	28,6%	12,0%	13,1%	
Moderately high expression 11-39%	N	2	46	48	
	%	28,6%	50,0%	48,5%	
High expression > 40%	N	3	34	37	
	%	42,9%	37,0%	37,4%	
Total	N	7	92	99	
	%	100,0%	100,0%	100,0%	

Examining the IHC expression of PD-L1 and perineural invasion, no statistically significant relationship was found (P=0.284). The result is demonstrated in Table 30.

Table 30. IHC expression of PD-L1 versus presence of perineural invasion

PD-L1 expression on tumor cells		Perineural invasion		Total	p
		Yes	No		
No expression ≤1%	N	4	41	45	0,284
	%	80,0%	44,1%	45,9%	
Low expression 2-10%	N	0	35	35	
	%	0,0%	37,6%	35,7%	
Moderately high expression 11-49%	N	1	11	12	
	%	20,0%	11,8%	12,2%	
High expression > 50%	N	0	6	6	
	%	0,0%	6,5%	6,1%	
Total	N	5	93	98	
	%	100,0%	100,0%	100,0%	

No association was found between perineural invasion and PD-1 expression by cytotoxic lymphocytes: P=0.710 (Table 31).

Table 31. IHC expression of PD-1 versus presence of perineural invasion

PD-1 expression on cytotoxic lymphocytes		Perineural invasion		Total	p
		Yes	No		
No expression ≤1%	N	0	10	10	0,710
	%	0,0%	10,6%	10,1%	
Low expression 2-10%	N	3	30	33	
	%	60,0%	31,9%	33,3%	
Moderately high expression 11-20%	N	1	26	27	
	%	20,0%	27,7%	27,3%	
High expression 21-30%	N	1	21	22	
	%	20,0%	22,3%	22,2%	
Very high expression >30%	N	0	7	7	
	%	0,0%	7,4%	7,1%	
Total	N	5	94	99	
	%	100,0%	100,0%	100,0%	

No statistical correlation was found between perineural invasion and also CD8 expression of peritumoral lymphocytes: P=0.327. The results are shown in Table 32.

Table 32. IHC expression of CD8 versus presence of perineural invasion

CD8 expression on tumor (lymphocytes)		Perineural invasion		Total	p
		Yes	No		
No expression ≤1%	N	0	1	1	0,327
	%	0,0%	1,1%	1,0%	
Low expression 2-10%	N	2	11	13	
	%	40,0%	11,7%	13,1%	
Moderately high expression 11-39%	N	2	46	48	
	%	40,0%	48,9%	48,5%	
High expression > 40%	N	1	36	37	
	%	20,0%	38,3%	37,4%	
Total	N	5	94	99	
	%	100,0%	100,0%	100,0%	

Analyzing the inflammatory reaction around the primary SCC, it was found that the lymphocytic stromal reaction was absent in 2 SCCs, weak in 9, moderate in 21 tumors and high in 68. The data analysis did not reveal a statistically significant relationship with PD-L1 (P=0.637) and PD-1 (P=0.491) IHC expression (Table 33 and Table 34).

Table 33. Relationship between PD-L1 expression on tumor cells and lymphocyte stromal response

PD-L1 expression on tumor cells		Lymphocytic stromal reaction				Total	p
		Absent	Weak	Moderate	Severe		
No expression ≤1%	N	2	5	13	27	47	0,637
	%	100,0%	55,6%	61,9%	38,8%	45,9%	
Low expression 2-10%	N	0	2	6	27	35	
	%	0,0%	22,2%	28,6%	40,3%	35,7%	
Moderately high expression 11-49%	N	0	2	1	9	12	
	%	0,0%	22,2%	4,8%	13,4%	12,2%	
High expression > 50%	N	0	0	1	5	6	
	%	0,0%	0,0%	4,8%	7,5%	6,1%	
Total	N	2	9	21	68	100	
	%	100,0%	100,0%	100,0%	100,0%	100,0%	

Table 34. Relationship between PD-1 expression on cytotoxic lymphocytes and lymphocyte stromal response

PD-1 expression on cytotoxic lymphocytes		Lymphocitic stromal reaction				Total	p
		Absent	Weak	Moderate	Severe		
No expression ≤1%	N	0	1	3	6	10	0,491
	%	0,0%	10,0%	15,0%	8,8%	10,1%	
Low expression 2-10%	N	1	6	9	17	33	
	%	100,0%	60,0%	45,0%	25,0%	33,3%	
Moderately high expression 11-20%	N	0	1	5	21	27	
	%	0,0%	10,0%	25,0%	30,9%	27,3%	
High expression 21-30%	N	0	2	2	18	22	
	%	0,0%	20,0%	10,0%	26,5%	22,2%	
Very high expression >30%	N	0	0	1	6	7	
	%	0,0%	0,0%	5,0%	8,8%	7,1%	
Total	N	1	10	20	68	99	
	%	100,0%	100,0%	100,0%	100,0%	100,0%	

The analysis established a statistically significant relationship between CD8 IHC expression and lymphocyte stromal reaction: P<0.05 (Actual value P=0.011), which is shown in Table 35.

Table 35. Statistically dependent relationship between CD8 expression on peritumoral lymphocytes and lymphocyte stromal reaction

CD8 expression on tumor (lymphocytes)		Lymphocitic stromal reaction				Total	p
		Absent	Weak	Moderate	Severe		
No expression ≤1%	N	0	0	1	0	1	0,011
	%	0,0%	0,0%	4,8%	0,0%	1,0%	
Low expression 2-10%	N	1	3	4	5	13	
	%	100,0%	30,0%	19,0%	7,5%	13,1%	
Moderately high expression 11-39%	N	0	7	10	31	48	
	%	0,0%	70,0%	47,6%	46,3%	48,5%	
High expression > 40%	N	0	0	6	31	37	
	%	0,0%	0,0%	28,6%	46,3%	37,4%	
Total	N	1	10	21	67	99	
	%	100,0%	100,0%	100,0%	100,0%	100,0%	

IHC examination of Ki-67, CD4 and CD4:CD8 expression was performed on 70 of the histological materials used in the histopathological study. Again, the SCCs were divided into Group 1 and Group 2. The study aimed to determine whether there was a statistically dependent relationship between the expression of Ki-67, CD4 and the CD4:CD8 ratio in primary SCC and clinicopathological prognostic factors for recurrence, metastasis and mortality.

Examining Ki-67 expression, it was found that of the low-risk carcinomas, 21 showed low expression (<10%), 6 had moderate expression (10-25%) and 8 had high expression >25%. None of the high-risk carcinomas showed low expression, 11 had moderate expression and 25 had a very high proliferative index >25%. The degree of IHC expression of Ki-67 is demonstrated in Figures 37, 38, 39, 40, 41, 42 and 43. The data analysis revealed a statistically significant difference between Ki-67 expression in the two groups: P<0.05 (Actual value P<0.001). (Table 36). It is interesting that we found heterogeneous SCCs, which simultaneously have tumor nuclei with a very high proliferative index and those with a very low one.

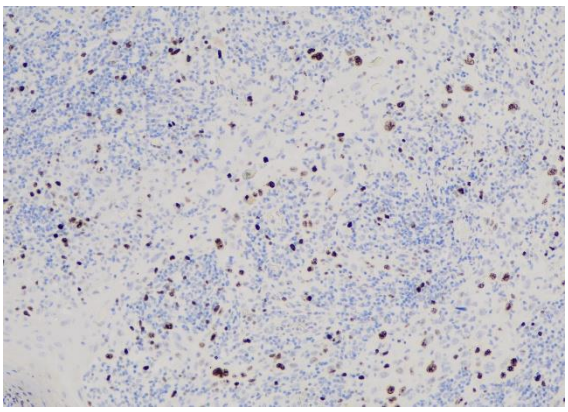

Figure 37. Low Ki-67 expression – 12%

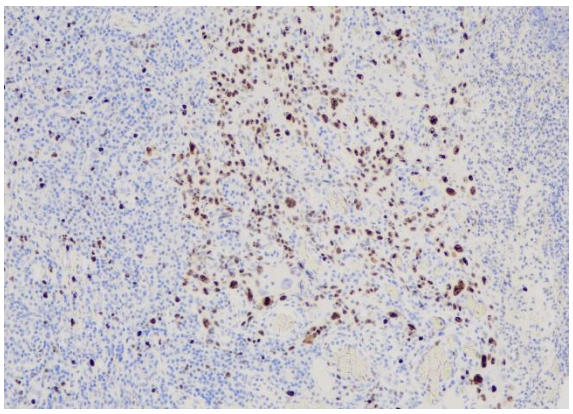

Figure 38. Moderate Ki-67 expression – 20%

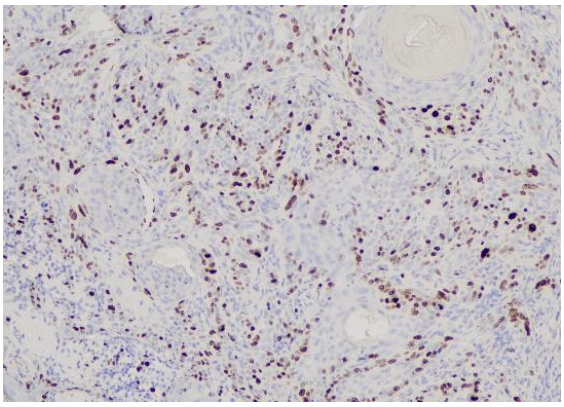

Figure 39. High Ki-67 expression – 25%

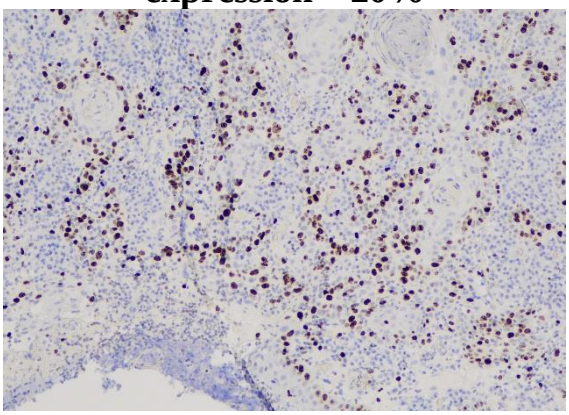

Figure 40. High Ki-67 expression – 40%

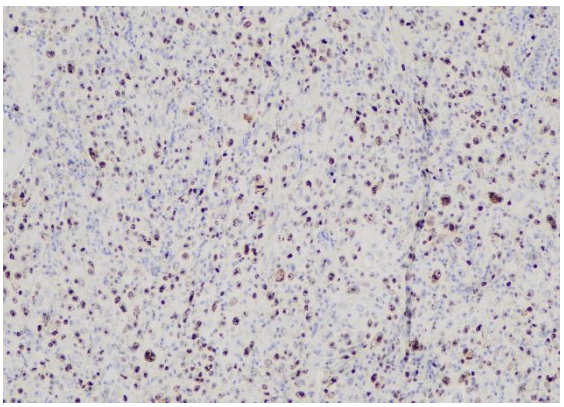

Figure 41. Very high Ki-67 expression – 70%

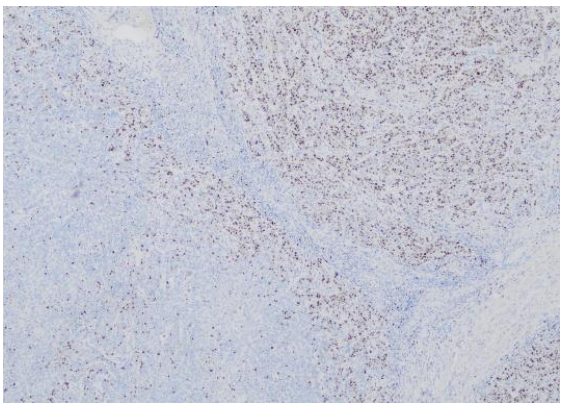

Figure 42. Heterogeneous tumor: Nuclei with very high and nuclei with low Ki-67 expression

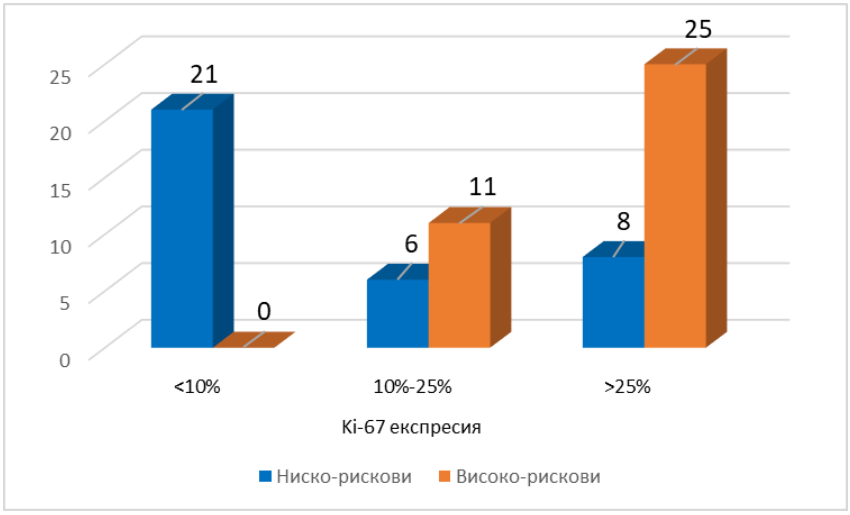

Figure 43. Distribution of IHC expression of Ki-67 from low-risk and high-risk SCCs

Table 36. Statistically significant difference between Ki-67 expression in the two groups

Indicator	SCC type	N	Mean	SD	Min	Max	p
What is the proliferative index relative to Ki67 expression in %?	Low risk	35	14,49	15,60	1,00	50,00	<0,001
	High risk	35	45,71	25,45	15,00	90,00	

Regarding CD4 expression, we found a minimum value of 10% and a maximum of 60% in low-risk patients, and a minimum value of 5% and a maximum of 60% in high-risk patients. The different degree of IHC expression of PD-L1 is shown in Figures 44, 45, 46 and 47. Statistical analysis of the data did not establish a statistically significant relationship between CD4 expression in the two groups: P<0.05 (Actual value P=0.355). The results of the analysis are shown in Table 37.

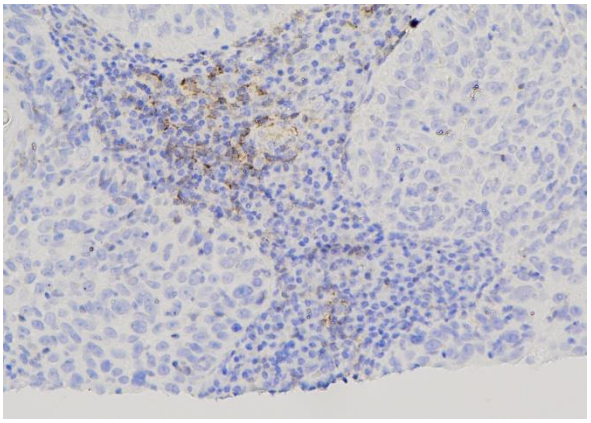

Figure 44. Low CD4 expression – 10%

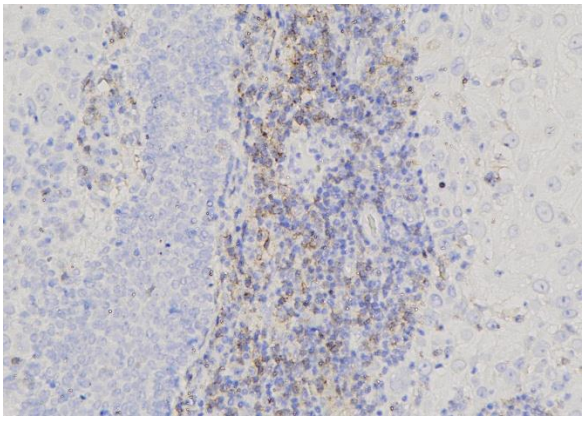

Figure 45. Moderate CD4 expression – 20%

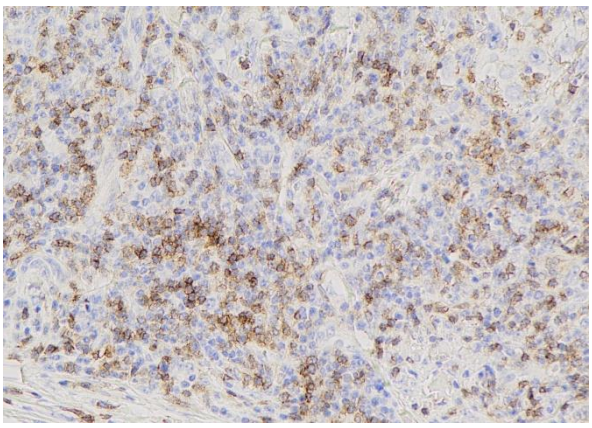

Figure 46. High CD4 expression – 40%

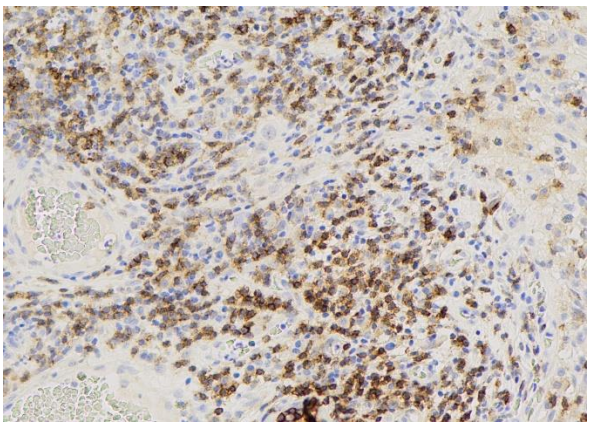

Figure 47. Very high CD4 expression – 60%

Table 37. Lack of statistically significant difference in CD4 expression between the two groups

Indicator	SCC type	N	Mean	SD	Min	Max	p
What is the CD4 expression of the lymphocyte infiltrate around the tumor in %?	Low risk	35	32,29	18,60	10,00	60,00	0,355
	High risk	35	28,43	15,89	5,00	60,00	

Examining the CD4:CD8 ratio of the selected SCCs, a mean value of 1.54 was found in the low-risk tumors and 1.12 in the high-risk ones. The data analysis revealed a statistically significant difference between the two tumor groups: $P<0.05$ (Actual value $P=0.022$). The results of the analysis are shown in Table 38.

Table 38. Statistically significant difference in CD4:CD8 ratio in low-risk and high-risk SCCs

Indicator	SCC type	N	Mean	SD	Min	Max	p
What is the CD4/CD8 ratio of lymphocytes around the tumor?	Low risk	35	1,54	0,85	0,30	3,00	0,022
	High risk	35	1,12	0,86	0,30	4,00	

Examining the IHC expression of Ki-67, CD4 and the CD4:CD8 ratio and tumor differentiation, no statistically significant difference was found between well-differentiated, moderately differentiated and poorly differentiated SCC. The result is presented in Table 39.

Table 39. Lack of statistically significant difference between Ki-67 expression, CD4 and CD4:CD8 ratio and tumor differentiation.

Indicator	SCC grading	N	Mean	SD	Min	Max	p
What is the proliferative index relative to Ki67 expression in %?	Well differentiated	55	28,15	24,08	1,00	75,00	0,088
	Moderately differentiated	8	33,00	33,54	5,00	85,00	
	Poorly differentiated	4	62,50	31,75	35,00	90,00	
What is the CD4 expression of the lymphocyte infiltrate around the tumor in %?	Well differentiated	55	31,09	15,86	10,00	60,00	0,852
	Moderately differentiated	8	31,25	20,13	10,00	50,00	
	Poorly differentiated	4	37,50	25,98	15,00	60,00	
What is the proliferative index relative to Ki67 expression in %?	Well differentiated	55	1,39	0,93	0,30	4,00	0,514
	Moderately differentiated	8	1,20	0,58	0,30	1,70	
	Poorly differentiated	4	1,60	0,12	1,50	1,70	

Comparing Ki-67, CD4 and the CD4:CD8 ratio with the histological Breslow thickness, we found a statistically significant relationship with Ki-67 expression: P<0.05 (Actual value P<0.001). We did not find a similar correlation with CD4 expression (Actual value P=0.624) and the CD4:CD8 ratio (Actual value P=0.097). The result is presented in Table 40.

Table 40. Statistically significant relationship between histological Breslow thickness and Ki-67 expression

What is the exact histological Breslow thickness of the tumor?	What is the proliferative index relative to Ki67 expression in %?	What is the CD4 expression of the lymphocyte infiltrate around the tumor in %?	What is the CD4/CD8 ratio of lymphocytes around the tumor?
R	0,735	-0,060	0,200
p	<0,001	0,624	0,097
N	70	70	70

Analyzing the lymphocytic stromal reaction around the SCC, a statistically significant relationship was found with the expression of CD4: P<0.05 (Actual value P=0.020) and the ratio of CD4:CD8: (Actual value P=0.010), but no correlation with the expression of Ki-67: (Actual value P=0.577). The result is presented in Table 41.

Table 41. Statistical correlation between CD4 expression and CD4:CD8 ratio with lymphocyte stromal reaction around the SCC

Indicator	Lymphocyte Stromal Reaction Assessment	N	Mean	SD	Min	Max	p
What is the proliferative index relative to Ki67 expression in %? What is the CD4 expression of the lymphocyte infiltrate around the tumor in %?	Weakly Expressed	5	30,60	40,53	1,00	75,00	0,577
	Moderately Expressed	6	38,33	37,24	5,00	85,00	
	Strongly Expressed	59	29,22	24,00	2,00	90,00	
Indicator	Weakly Expressed	5	17,00	2,74	15,00	20,00	0,020
	Moderately Expressed	6	46,67	13,66	30,00	60,00	
	Strongly Expressed	59	29,83	17,19	5,00	60,00	
What is the proliferative index relative to Ki67 expression in %?	Weakly Expressed	5	1,30	0,27	1,00	1,50	0,010
	Moderately Expressed	6	2,23	0,61	1,70	3,00	
	Strongly Expressed	59	1,24	0,88	0,30	4,00	

Statistical analysis of the data shows a cutoff level of the CD4:CD8 ratio of 1.3 in the studied tumor group with the largest statistically significant difference between the two groups: P<0.05 (Actual value P=0.002). The result is presented in Table 42.

Table 42. CD4:CD8 ratio cut-off level in SCC

CD4/CD8		SCC type		Общо	p
		Low risk	High risk		
<=1.3	N	11	24	35	0,002
	%	31,4%	68,6%	50,0%	
>1.3	N	24	11	35	
	%	68,6%	31,4%	50,0%	
Общо	N	35	35	70	
	%	100,0%	100,0%	100,0%	

V. DISCUSSION OF THE RESULTS

Epidemiological and clinical study

Squamous cell carcinoma of the skin is a representative of the group of non-melanocytic skin carcinomas and accounts for about 30% of tumors in it. It is also the second most common neoplastic disease in the United States, with over 1 million cases per year. Most epidemiological studies show that the male gender is more affected. Over the past 40 years, a trend has been established for an increase in the incidence of SCC. Men develop SCC more often in the head and neck area. In Australia, the incidence of SCC was found to be 499/100,000 men and 291/100,000 women. Data from the European Union demonstrate that the incidence of SCC varies from 9 to 96 per 100,000 in men and from 5 to 68 per 100,000 in women. Squamous cell carcinoma of the skin is a disease characteristic of the population with a light Fitzpatrick phototype (I-III). Therefore, the incidence is higher among Caucasians and in areas with strong sunlight.

Our results on registered patients with NMCC in the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region for the period 2000 - 2024 present 5748 patients. Similar to the data of Hussain et al. (2010), the male gender prevails in the studied cohort - 3055 men and 2693 women. For the 24-year study period, an average of 243.2 patients with NMCC were diagnosed annually. A sharp decline in the number of newly registered patients in 2020 and 2021 and a subsequent sharp increase in 2022 is striking. These results correlate with the results of Jennyfa K. Ali and John C. Riches (2020), who describe the impact of the Covid-19 pandemic on the diagnosis and follow-up of oncological patients. Their results show a 42% decrease in newly diagnosed patients in 2021, which is fully consistent with our results. Among the 5748 patients registered, the highest relative share of BCC is striking – 66.6%, followed by SCC – 28.5%, which is consistent with the results of Chorchado-cobos et al. from the USA (2020). They describe an increase of almost 30% of SCC and almost 70% of BCC in the group of NMCC.

According to the TNM classification, the highest relative proportion of tumors in T1 – 66.6%, N0 – 98.9% and M0 – 99.8% is striking, and according to the clinical stage of the disease - Stage I – 64.8%. The lowest is the proportion of patients with metastatic lesions in internal organs – Stage IV (0.5%). These results fully correspond to the results of Ciuciulete et al. (2022).

According to the address registration of patients, it is striking that in Pleven district, patients from almost every district in the Republic of Bulgaria, as well as one patient of Greek origin, were diagnosed, surgically and medically treated. This is an example of centralization of onco-dermatological specialized care in large regional cities, similar to that described in the study by Kodumudi et al. (2021).

Our results from the detailed epidemiological and clinical study for the period 2016 - 2023 present 355 patients, aged 40 to 96 years, who developed SCC for the period 2016 - 2023. Similar to the data of Karagas et al. (1999), the male gender prevails in the cohort studied by us - 185 male patients and 170 female patients.

Although most affected patients were male, no statistically significant difference was found between the two sexes with SCC: P>0.05 (P=0.057). The highest incidence of SCC was found at 75 years of age (±10.74 SD), which is consistent with the data of Music et al. (2017) – the highest incidence of the disease between 64 and 84 years. All patients with SCC examined by us were of the Caucasian race, with skin Phototype I-III, similar to the data of Marks et al. (1993) and Karia et al. (2013). According to their origin, 194 patients (54.6%) were found living in rural areas and 161 patients (45.4%) – in urban areas, consistent with the data of Corchado-Cobos et al. (2020).

Regarding the risk factors for the development of SCC, the photoexposure of the patients was studied. Of these, 238 patients (67%) practiced plant and animal husbandry as a hobby or profession and 1 patient practiced sports. Of all the patients studied, 260 patients (73.2%) admitted to having experienced severe sunburns in their lifetime. The results regarding photoexposure and sunburns are consistent with the results of Zanetti et al. (2006). Our results confirm the association of skin neoplastic diseases with sun exposure, as patients developed both SCC and MM (1.4%) and BCC (4.3%). Two patients were also found to have received immunosuppressive therapy for concomitant onco-hematological diseases before the onset of SCC, which confirms immunosuppression as a cause of SCC development, similar to that described by Euvrard et al. (2003) and Silverberg et al. (2013). Of the precancerous lesions that led to the development of SCC, 2 patients developed the disease on the background of Lupus erythematosus chronicus discoides disseminatus, 2 patients – on the background of Epidermolysis bullosa hereditaria dystrophica and 2 patients with Essential Thrombocythemia, treated with Hydroxyurea for many years, whose results fully correspond to the data from Condorelli et al. (2019), Bhat et al. (1012) and Tao et al. (2012).

The time it takes for the patient to seek medical help for the skin formation was studied. The data analysis shows a statistically significant relationship between the time to diagnosis and the macroscopic diameter of the SCC: P<0.05 (Actual value P=0.001). The longer the time to diagnosis, the larger the tumor.

Regarding the localization of the SCC, our results correlate with those of Brantsch et al. (2008). The highest incidence of tumors was found in the head and neck region, followed by carcinomas of the torso, upper limb and penis. Of the specific facial localization, the most common are SCCs in the cheek region, followed by tumors of the lower lip, forehead and nose.

The average macroscopic diameter of the tumors examined by us is 19.86 mm (± 14.34 mm). The minimum tumor diameter is 4 mm, and the maximum – 100 mm. Statistical analysis of the data shows that the difference between the tumor diameter in different age groups is significant: P<0.05 (Actual value 0.028), i.e. larger tumors are found in older patients. The statistically significant relationship between tumor diameter and overall survival found in our study: P<0.05 (Actual value p<0.001), corresponds to the data of Thompson et al. (2016). The larger the tumor diameter, the lower the survival of the patients.

According to the histopathological examination of all 355 tumors, the smallest number of tumors with a histological thickness of up to 1 mm and the largest number with a thickness of more than 4 mm were found, which is a characteristic of high-risk SCC, described by Schmults et al. (2013). Statistical analysis showed a statistically significant relationship between the microscopic thickness of the tumors and the age of the patients: $P<0.05$ (Actual value 0.001) with thicker tumors in the group 71-80 years. When comparing the macroscopic diameter of the SCC and the microscopic thickness, a relationship was also found: $P<0.05$ (Actual value $P<0.001$). The larger the tumor diameter, the greater the tumor thickness, which correlates with the results of Schmults et al. [113]. Of the tumors studied, 302 (85.1%) were invasive SCC of the skin, and 53 (14.9%) were "in situ" carcinoma. This ratio corresponds to that presented by Katalinic et al. (2003).

Similar to Brantsch et al. (2008), the localization and clinical appearance of the SCC of the penis were studied and 23 tumors with this localization were identified. Of these, 82.6% involved the glans penis, and 17.4% - the skin of the body of the penis. According to the type of tumor, 8 of them were Erythroplasia of Queyrat, 3 were Bowen-oid papulosis, and the remaining 12 were invasive SCC. These results fully correspond to the data published by the above-mentioned authors.

Studying the methods of treatment of SCC, in 85.1% of patients surgical treatment was applied alone, and in 14.9% - a combined approach (surgical excision + radiotherapy). Standard surgical excision was successful (complete removal of the carcinoma) in 85.4% of cases. The results of success of surgical treatment fully correspond to the results of Lansbury et al. (2013). In the remaining 14.6% of patients after surgical excision, radiotherapy was applied due to persistence of the disease. In 11 patients (3.09%) systemic therapy was applied. In 7 patients a classic polychemotherapy regimen was used, and 4 patients were treated by immunotherapy with the PD-1 inhibitor Cemiplimab. Two of the latter patients developed inoperable tumors on the background of Epidermolysis Bullosa Congenita, and 1 – on the background of long-term treatment of Essential Thrombocythemia with Hydroxyurea. In all three patients, treatment with Cemiplimab was discontinued – in the patients with Epidermolysis Bullosa Congenita due to disease progression with an increase in SCC or the appearance of new SCC on the skin, and the patient with Essential Thrombocythemia was discharged.

A statistically significant relationship was established between the macroscopic diameter of the primary carcinoma and the frequency of radiotherapy use: $P<0.05$ (Actual value $P<0.001$). The need for radiotherapy was higher in carcinomas with a macroscopic diameter of more than 20 mm, similar to the results of Stewart et al. (2022).

Following the tendency for tumor recurrence, local recurrence was established in 17 patients (4.8%). Statistical analysis of the data showed a statistically significant relationship between the macroscopic diameter of the tumor and the time to recurrence $P<0.05$ (Actual value $P<0.001$), and that in tumors with a diameter of more than 40 mm this period is significantly shorter. A statistically significant relationship was also found between the stage of the SCC and the time to recurrence:

P<0.05 (Actual value P<0.007). The higher the stage of the primary tumor, the shorter the time to local recurrence. These results are similar to those presented by Pickering et al. (2014).

In 18 patients (5.1%) after performing a lymph node biopsy (excisional or fine needle aspiration biopsy), loco-regional metastases in lymph nodes were found. In 8 of these patients (2.25%) distant metastatic lesions in internal organs were also registered. A statistically significant relationship was found between the development of metastases and the diameter of the primary SCC: P<0.05 (Actual value P<0.001), similar to the results of Leibovitch et al. The larger the diameter, the greater the possibility of developing metastases (2005).

Our results for recurrence and mortality are close to those of Karia et al. (2013), who reported the occurrence of local recurrence in 5.4% of cases and a mortality of 1.5-2% in the USA, reaching 4% in some countries.

The analysis of mortality from the disease in our retrospective study shows that 7 patients (1.97%) died due to advanced or metastatic SCC. The median survival does not show statistically significant differences between male and female sex: P>0.05 (Actual value P=0.216). A statistically significant relationship was found between the age of patients in Stage IV and the time to death: P>0.05 (Actual value P=0.001). In older patients, the average life expectancy is significantly shorter.

This leads to the conclusion that it is necessary to follow up patients with invasive skin SCC for at least 4 years after surgical excision of the tumor, as recommended by the European Society for Medical Oncology (ESMO).

Histopathological study

Numerous studies have been conducted to establish the relationship between certain histopathological characteristics of SCC and a higher risk of recurrence, metastasis, and mortality.

Ramirez et al. (2005) described that a tumor diameter of more than 20 mm carries a higher risk of disease progression. Clayman et al. (2005) described a 40 mm tumor diameter with a worse prognosis, but reported that SCC localized in the lip and auricle can metastasize even with a diameter of <20 mm. The same authors also described tumor thickness in mm, as well as the level of invasion as prognostic factors. Their results demonstrated that SCC with a thickness of >4 mm have a 45.7% higher risk of recurrence and metastasis. In contrast, SCC with a thickness of <2 mm carries a minimal risk of progression. They also described that skin SCC invading tissues below the level of subcutaneous fat (fascia, muscle, cartilage, bone) is associated with higher mortality.

The results of our histopathological study show that SCC with a macroscopic diameter >20 mm have a statistically significant deeper invasion compared to tumors with a smaller diameter P<0.05 (Actual value <0.001), similar to the results of Mullen et al. (2006). The mean thickness in the group of low-risk carcinomas is 4.93 (±2.34), in contrast to the thickness of high-risk – 7.13 (±3.44).

A significantly greater histopathological thickness was found for classic (non-specific) SCC compared to Kerathoacanthoma-like invasive SCC: P<0.05 (Actual

value <0.001). The average thickness of classic SCC was 6.47 mm and that of Kerathoacanthoma-like invasive SCC was 3.53 mm, similar to the results presented by Kwiek et al. [86].

Similar to Mullen et al. (2006), our study found that the degree of differentiation of SCC has a significant effect on the risk of progression. The above authors described complete cure in 37% of poorly differentiated SCC, 59% of moderately differentiated SCC, and 88% of well-differentiated SCC after 2 years of follow-up.

Similar to the results of Fernandez-Figueras et al. (2015), our results did not show a statistically significant relationship between the histopathological subtype of SCC and the cellular differentiation of the tumor cells: P<0.05 (Actual value 0.069): 71% were well-differentiated, 19% were moderately-differentiated and 10% were poorly-differentiated.

Similar to Mendenhall et al. (2007), who reported perineural and lymphovascular invasion in 7% of cases and described the association with a high risk of recurrence, metastasis and mortality, we found lymphovascular invasion in 7% of cases and perineural invasion in 5% of the examined SCC. A statistically significant relationship was found between the level of invasion and lymphovascular invasion: P<0.05 (Actual value 0.001). Similar to the above authors, we find that the greater the invasion, the higher the probability of lymphovascular invasion. In contrast, we do not find a relationship between macroscopic diameter (<20mm or >20mm) and lymphovascular invasion: P<0.05 (Actual value 0.238).

Our results show a relationship between the level of invasion and the presence of perineural invasion: P<0.05 (Actual value 0.006). The deeper the structures involved by the SCC, the greater the risk of perineural involvement. Unlike Mendenhall et al. (2007), we do not describe a relationship between perineural invasion and the macroscopic diameter of the SCC: P<0.05 (Actual value 0.086) and the histological subtype: P<0.05 (Actual value 0.744).

According to Maimela et al. (2018) the presence of a large number of tumor-infiltrating lymphocytes around the tumor is a good prognostic factor, which is associated with a lower risk of recurrence and metastasis. In the SCCs examined by us, the peritumoral lymphocytic stromal reaction was high in 68%, moderate in 21%, low in 10% and absent in 1%. No statistically significant relationship was found between the microscopic thickness of the tumor and the lymphocytic reaction: P<0.05 (Actual value 0.382). We also did not find a statistical relationship with the diameter of the carcinoma (<20mm and >20mm): P<0.05 (Actual value 0.224).

Similar to the study by Carter et al. (2013), our results demonstrate the importance of a detailed histopathological report containing data on SCC differentiation, aggressive subtypes (acantholytic, adenosquamous), tumor depth and thickness, presence or absence of perineural and lymphovascular invasion, invasion of fascia, muscle, cartilage or bone, presence or absence of inflammatory reaction and status of the excision margins (involved or not by tumor cells).

Analyzing the success of surgical treatment, 85% of tumors were completely removed after primary surgical excision, and in 15% of tumors persistence was detected – involvement of a peripheral or deep excision margin. A relationship was

found between surgical success and Breslow histological thickness of the SCC: P<0.05 (Actual value <0.001), as well as macroscopic tumor diameter: P<0.05 (Actual value 0.038), as described by Thompson et al. (2016). In larger tumors in width and depth, the chance of incomplete removal with single surgical excision is significantly higher.

The histopathological characteristics of the skin SCC are extremely important for the patient's future. They can change the patient's follow-up periods, as well as subsequent therapy after surgical treatment (adjuvant surgery, radiotherapy or systemic therapy).

According to Zhang et al. (2021) postoperative radiotherapy is performed in case of tumor persistence – incomplete removal with involvement of the bottom or side resection lines by tumor cells or in case of negative prognostic signs – poorly differentiated SCC, presence of lymphovascular and perineural invasion, Breslow thickness over 6mm. Radiation therapy can also be used as a stand-alone therapy, without surgical excision. After histological confirmation of the tumor, in older patients, with more concomitant diseases, who would not tolerate surgical excision.

According to Lee et al. (2020) patients with frequently recurring SCC, with large inoperable tumors or those in whom surgical treatment would disfigure them with a significant decrease in quality of life, or would lead to the removal of a certain part of the body, or who have already developed metastases in lymph nodes or internal organs, are suitable for systemic chemotherapy therapy or immunotherapy. The patients we present, who underwent therapy with Cemiplimab, belong to the group with large inoperable tumors or those in which surgical treatment would disfigure them with a significant decrease in quality of life, or would lead to the removal of a certain part of the body.

Immunohistochemical study

PD-L1, also called B7 homologous protein (B7-H1) or cluster of differentiation 274 (CD274), was first described in 1999. It is a very important costimulatory molecule of the immune response that induces immune tolerance in the tumor microenvironment. PD-L1 is rarely expressed by normal non-pathogenic tissues. It is found in tumors such as malignant melanoma, lung carcinoma, breast carcinoma, pancreatic carcinoma, kidney, bladder, esophageal, colon and rectal tumors. The binding between PD-L1 and PD-1 induces apoptosis of T lymphocytes and leads to a worse prognosis for patients. Monoclonal antibodies targeting PD-L1 and/or PD-1 block the binding between the two receptors, which in addition to blocking apoptosis, also leads to longer survival of T lymphocytes and increased antitumor activity. As a result of these actions, differentiation and proliferation of tumor cells are suppressed. Immunohistochemical expression of PD-L1, PD-1 and CD8 in primary invasive SCC shows that they can be used as biomarkers, predictors of response to immunotherapy with PD-L1/PD-1 inhibitors. Tumors that respond to immunotherapy usually have high expression. They could also determine the risk of recurrence and metastasis. On the other hand, high expression of CD8 in SCC may be a positive prognostic marker. The prognostic value of PD-L1/PD-1 is controversial. A number of studies have

shown that high expression of PD-L1 correlates with poor prognosis in gastric carcinoma, hepatocellular and renal carcinoma, but in Merkel cell carcinoma and breast cancer it is a marker of better prognosis.

There are not many studies in the literature on the IHC expression of PD-L1, PD-1 and CD8 in non-melanocytic skin carcinomas. Slater and Googe (2016) in their study presented PD-L1 positive expression in 20% of low-risk and 70% of high-risk SCCs. They analyzed 40 primary SCCs and found a statistically significant association between PD-L1 expression and histopathological characteristics for high-risk SCC: tumor thickness >6mm, macroscopic diameter >20mm and low tumor differentiation. In contrast to their published data, our results did not demonstrate a relationship between PD-L1/PD-1 expression and the aforementioned parameters, but found a tendency for SCCs with greater histopathological thickness and deeper level of invasion to show higher PD-L1 and PD-1 expression, and tumors with less histopathological thickness to show higher CD8 expression.

Similar to the results presented by Oh et al. (2019), our results show a statistically significant association between PD-L1 and PD-1 expression and tumor differentiation.

Roper et al. (2017) found that high PD-L1 expression is a prognostic factor for longer disease-free survival in high-risk head and neck SCC. Their results, using the sp263 antibody clone, demonstrated the association when PD-L1 expression was >5%. On the other hand, Garcia-Diez et al. (2018) showed that higher PD-L1 expression is associated with increased metastatic risk. They used the same antibody clone as us in our study, SP142. A study by Varki et al. (2018), using the SP142 anti-PD-L1 clone, demonstrated positive expression (>5%) in 26% of 66 primary SCCs. In contrast to these results, our study demonstrated that 64% of PD-L1 positive cases had expression 2–10% and 36% showed expression >10% PD-L1, i.e. in our study the immunohistochemical expression of PD-L1 was significantly higher than presented.

Schaper et al. (2017) found a statistically significant association between the inflammatory reaction around the SCC and PD-L1 expression. In contrast to these authors, our results showed that 98 of the tumors had a diverse inflammatory reaction, but no statistically significant association with PD-L1 expression was found. Similar to Schaper et al., our study did not find a statistically significant association between PD-L1 expression, tumor diameter, and cellular differentiation.

Investigating the difference in PD-L1 expression between primary SCC and lymphatic metastases in SCC, Amoils et al. (2017) demonstrated a significantly higher expression in metastatic tissues. Their results did not show a statistically significant relationship between PD-L1 expression and the clinicopathological characteristics of the primary SCC. Our results fully correlate with the results of the above authors.

Alferraly et al. (2019), in their study on SCC, did not find a statistically significant relationship between Ki-67 expression and tumor grading. Our results also did not find a similar correlation. Our results on Ki-67 expression show that high-risk SCC

have significantly higher expression and correlate with those of many studies in the field of oral squamous cell carcinoma.

The results of Ehidiamhen et al. (2024) demonstrate that aggressive subtypes of BCC and SCC have a significantly higher proliferative index Ki-67. They show that all subtypes of SCC show higher Ki-67 expression compared to BCC, as SCC is a tumor with a more aggressive behavior. Our results correlate with theirs.

Bauer et al. (2018) studied the expression of CD4 and CD8 in actinic keratosis, keratoacanthoma and invasive SCC. They found statistically significant differences with the highest expression of CD4 in keratoacanthomas and the highest CD8 expression in invasive SCC. Similarly, we found higher expression of CD4 in low-risk SCC and higher CD8 expression in high-risk SCC, as well as a lower CD4:CD8 ratio in high-risk SCC.

Studying the CD4:CD8 ratio in gastric carcinoma, Skubleny et al. (2023) found that carcinomas with a higher ratio had a better prognosis in terms of survival and response to therapy, and described correlations with the histopathological characteristics of the tumor. Similarly, we found a significantly higher CD4:CD8 ratio in low-risk SCC. The threshold level we reached was 1.3, with a statistical relationship between lower levels of the CD4:CD8 ratio and high-risk SCC.

Studying the CD4:CD8 ratio in breast carcinoma, Wang et al. (2017) found much higher expression of CD4 and CD8 in the tumor periphery than in the tumor stroma. They demonstrated a statistically significant association between lower levels of the CD4:CD8 ratio and more aggressive subtypes of breast carcinoma. Like these authors, we reach the same results for skin SCC – a lower level (below 1.3) in high-risk SCC and a higher level (above 1.3) in low-risk SCC.

VI. CONCLUSION

Non-melanocytic skin carcinomas, and in particular BCC and SCC, are the most common tumors in the human body. Worldwide, their number exceeds all other neoplastic diseases combined. Delay in diagnosis, as well as the “rejuvenation” of the population developing SCC of the skin, leads to significant challenges in subsequent treatment and economic losses.

Among the predisposing factors for the development of SCC are male gender, light skin type, sun exposure without photoprotection, use of solariums, smoking. Additional risk factors are the intake of photosensitizers, toxic substances, immunosuppressants, as well as suffering from Psoriasis vulgaris, Epidermolysis bullosa hereditaria, Xeroderma pigmentosum, Lupus erythematosus discoides, Human papilloma virus, facultative and obligate precancerous lesions.

SCC has the richest palette of gene mutations compared to all other tumors, which is also the basis for constant study and development of various new therapeutic models for the treatment of locally advanced and metastatic forms of the disease.

The conducted epidemiological study places NSCLC and SCC among the most common tumors in the Bulgarian population. Stratification of risk factors provides guidelines for future research on the causes of SCC development. Data on the

frequency of recurrence, metastasis, as well as lethal outcome, correspond to data from the world literature, which indicates the high quality of the Bulgarian, and in particular Pleven, medical service.

Clinical studies clarify the age of the most affected population, the predilection sites for SCC development, the specific localization, TNM classification, staging and treatment of the tumor, as well as factors worsening the success of surgical treatment alone.

Histological study clarifies the histological types of SCC and proves how important is the detailed histopathological analysis of SCC of the skin and what are the characteristics of high-risk tumors for recurrence and metastasis. It can change the behavior of the dermatologist, dermatosurgeon, radiation therapist and oncologist in choosing the most appropriate therapy for the patient.

Immunohistochemical study of PD-1, PD-L1 and CD8 of SCC of the skin is one of the few performed in the world. The lack of statistically significant relationships with certain high-risk histological characteristics of SCC shows that IHC-study is important, but in certain cases. It is most useful for patients with frequently recurring, inoperable and metastatic SCC of the skin. IHC - study of PD-1, PD-L1 and CD8 can be a prognostic factor. On the one hand, it determines the probability of developing metastases from skin SCC to lymph nodes and internal organs, and on the other hand, the probability of complete, partial or no response to therapy with modern PD-1 inhibitors. IHC-study is most informative when studying metastatic tissues.

Studies on IHC-expression of Ki-67, CD4 and the CD4:CD8 ratio in skin SCC are also rare. The statistically dependent difference in Ki-67 expression has been proven in many types of tumors, which is also confirmed by our results. Studies of CD4 expression and the CD4:CD8 ratio are currently very modern and are performed on many types of tumors, but in NSCLC they are still rare. Our results show that CD4 and the CD4:CD8 ratio can be used as prognostic immunohistochemical criteria determining the behavior of the disease and its prognosis.

We hope that all these data will contribute to a better understanding of SCC among the Bulgarian population, to the search for and timely diagnosis, to better surgical treatment, radiotherapy and the application of systemic therapy.

VII. CONCLUSIONS

1. NMSC are the most common tumors among the Bulgarian population. In the Oncology Registry at the Oncology Center at the University Hospital "Dr. G. Stranski" Pleven EAD in the Pleven region, 5748 patients with NSCLC were registered for a period of 24 years.
2. There is an equivalent distribution according to the sex of patients with SCC. A slightly higher frequency of SCC development is found in male patients - 53%, while in female patients the frequency is 47%.
3. The highest relative share of patients who developed SCC of the skin is in the age group between 71 - 80 years. In men, the average age is 70.6 years, and in women - 71.5 years.
4. SCC of the skin is the second most common carcinoma in the group of NSCLC with 28.5%. In the first place in the group is BCC with 66.6%, and in the third place – mixed basal-squamous carcinoma with 2.1%.
5. The relative share of patients living in rural areas and practicing plant and animal husbandry is higher, i.e. they have higher photoexposure. The relationship between chronic photoexposure and sunburn of the skin and the increased frequency of development of skin SCC has been established
6. The longer the time until the correct diagnosis and referral for treatment, the larger, difficult to surgically treat and recurrent/metastatic skin SCC develop.
7. The most frequently affected topographic area by SCC is the face, followed by the scalp, ear, torso, upper limb, glans penis and lower limb. The highest relative proportion of tumors is in the buccal region, followed by those of the lower lip, forehead, nose, lower eyelid, upper lip and upper eyelid.
8. A statistically significant relationship is established between the tumor diameter of the primary skin SCC and the frequency of recurrence and metastasis in lymph nodes and internal organs.
9. A statistically significant relationship is established between the macroscopic diameter, localization, histological thickness according to Breslow, tumor differentiation, the presence of perineural and lymphovascular invasion and the frequency of tumor persistence, recurrence and metastasis.
10. No statistically significant relationship is established between PD-1, PD-L1 and CD8 – IHC expression and high-risk histopathological characteristics in skin SCC. Based on our results and the literature, it is clear that the study of PD-1, PD-L1 and CD8 is more informative and provides information about patient survival and response to immunotherapy with checkpoint inhibitors when performed on metastatic tissues.
11. A statistically significant relationship was found between Ki-67, CD4 and the ratio of CD4:CD8 T-lymphocytes and high-risk histopathological characteristics of skin SCC. CD4 markers and the CD4:CD8 ratio can be used as prognostic immunohistochemical criteria determining the behavior of the disease and its prognosis.

VIII. CONTRIBUTIONS OF THE SCIENTIFIC WORK

Priority contributions with an original nature

1. A large-scale epidemiological study covering a 24-year period was conducted in a large group of patients with cutaneous SCC in Bulgaria.
2. Clinical and morphological data of a large number of patients with cutaneous SCC were analyzed.
3. A systematic histopathological study was conducted in a prospective series of patients with cutaneous SCC.
4. For the first time in Bulgaria, the immunohistochemical expression of PD-1, PD-L1 and CD8 in primary cutaneous SCC was studied and their prognostic value for recurrence, metastasis and response to therapy with checkpoint inhibitors was determined.
5. For the first time in Bulgaria, the immunohistochemical expression of Ki-67, CD4 and the ratio of CD4:CD8 T-lymphocytes in primary cutaneous SCC was studied and a statistically significant difference between low-risk and high-risk tumors was demonstrated.
6. The epidemiology, diagnostic methods, treatment, recurrence and metastasis rates of skin SCC for the Pleven region have been studied.
7. A case of locally advanced skin SCC in a patient suffering from Epidermolysis bullosa hereditaria dystrophica, successfully treated with immunotherapy – Cemiplimab, is described.
8. A case of locally advanced skin SCC induced by long-term intake of Hydroxyurea in a patient suffering from Essential Thrombocythemia, treated with immunotherapy – Cemiplimab, is described.

Contributions with a confirmatory and theoretical nature

1. The spectrum of diseases associated with skin SCC among the Bulgarian population was studied - "in situ" carcinomas, facultative and obligate precancers, concomitant diseases and concomitant therapy.
2. The relationship between chronic sun exposure and sunburns and the development of skin SCC was studied.
3. The relationship between high-risk histopathological characteristics of skin SCC and the frequency of recurrence, metastasis and mortality was studied.
4. The relationship between high-risk histopathological characteristics of skin SCC and their influence on the reduction of the frequency of radical single surgical excisions was studied.
5. The relationship of IHC-expression of PD-1, PD-L1 and CD8 in primary skin SCC was studied and the lack of influence on the frequency of recurrence, metastasis and mortality was established.

Contributions of a scientific and applied nature

1. Въведен е специфичен хистопатологичен фиш при СЦК на кожата.
2. Създаден е алгоритъм за промяна на терапевтичния подход при пациенти със СЦК на кожата при определени хистопатологични характеристики.
3. Въведени са определени отстояния от видимия периферен ръб на тумора, според неговия диаметър, при извършване на хирургична ексцизия.
4. Въведен е cutoff от 1,3 за съотношението CD4:CD8. Туморите с по-високо ниво са с по-благоприятно протичане, докато по-агресивните се характеризират с ниво на CD4:CD8 под 1,3

List of scientific priduction on the topic of the dissertation

I. Publication activity

1. **Vasilev P.**, Karaivanov M., Karamanliev M., Dimitrov D., Troyanova P., Yordanova I. Squamous cell carcinoma of the skin: epidemiology, diagnosis, management, recurrence and mortality rates for the Bulgarian population. Journal of Biomedical and Clinical Research, 2023, 16, 2: 180-185, DOI:10.2478/jbcr-2023-0024, ISSN: 1313-9053, Web of Science
2. **Vasilev P.**, Popovska S., Karaivanov M., Dimitrov D., Yordanova I., Squamous cell carcinoma of the skin: a study of clinicohistopathological correlations predictive for recurrence, metastasis and mortality. Journal of IMAB–Annual Proceeding Scientific Papers, 2023, 29(4), pp.5139-5144, DOI: 10.5272/jimab.2023294.5139, ISSN: 1312-773X, Q4, Web of Science, IF 0,1
3. **Василев П.**, Калев Д., Микински А., Стефанова Н., Караиванов М., Киров В., Йорданова И. Лечение на Carcinoma spinocellulare при тежка рецесивна Epidermolysis bullosa dystrophica – представяне на клиничен случай и обзор на литературата. Дерматология и Венерология, 2021, 3: 3-10, ISSN 0417-0792
4. **Василев П.**, Караманлиев М., Караиванов М., Димитров Д., Йорданова И. Модифицирана Tenzel пластика - оперативно лечение и реконструкция при пациент с инвазивен спиноцелуларен карцином в лява зигоматична област и ляв долен клепач, Окулопластична и реконструктивна хирургия, 2024, стр. 18-22, ISBN 978-954-756-340-7

II. Participation in scientific forums in Bulgaria

1. **Василев П.**, Киров В., Караиванов М., Микински А., Стефанова Н., Калев Д., Йорданова И. Лечение със Cemiplimab на спиноцелуларен карцином на кожата при пациент с генерализирана автозомно-рецесивна дистрофична булозна епидермолиза. Симпозиум Дерматолози „Диагностика и възможности за лечение на пациенти с кожни неоплазми“, Българска асоциация по Дермато-онкология Правец, 25-27 Юни 2021, /oral presentation/
2. **Василев П.**, М. Караманлиев, М. Караиванов, Д. Димитров. Инвазивен спиноцелуларен карцином на лицето – хирургично лечение и тъканна реконструкция, XXIX Софийски Дерматологични Дни „Проф. Асен Дурмишев“ София, 4 – 7 Ноември 2021, /oral presentation/
3. **Василев П.**, М. Караманлиев, М. Караиванов, Д. Димитров, И. Йорданова, Д. Господинов, П. Троянова. Авансирал спиноцелуларен карцином на кожата при пациентка с Polycythemia vera и дълготрайно приложение на Hydroxyurea, XXX-ти Софийски Дерматологични дни „Проф. Асен Дурмишев“, София, 03–06 Ноември 2022, /oral presentation/
4. **Василев П.**, Д. Калев, М. Караиванов, М. Караманлиев, Д. Димитров, И. Йорданова, Д. Господинов, П. Троянова. Имунотерапия със Cemiplimab – клиничен опит. Втори Национален Конгрес по Дермато-онкология, София, 11 – 13 Ноември 2022, /oral presentation/
5. Петракиева М., **Василев П.,** Господинова К. Гинчева В. Йорданова И., Господинов Д. Кожен лупус и спиноцелуларен карцином на кожата, Х-ти Национален конгрес на българските дерматовенеролози, София, 02 – 05 Ноември 2023, /oral presentation/
6. **Василев П.**, М. Караиванов, Д. Димитров, П. Троянова, И. Йорданова. Хистологични варианти и особености на спиноцелуларния карцином на кожата. Варненски Дерматологични дни, Варна, 21-22 Април 2023, /oral presentation/
7. **Василев П.**, Караманлиев М., Караиванов М., Димитров Д., Йорданова И. Модифицирана tenzel пластика - оперативно лечение и реконструкция при пациент с инвазивен спиноцелуларен карцином в лява зигоматична област и ляв долен клепач. Окулопластична и реконструктивна хирургия, Плевен, 19-20 Април 2024, /oral presentation/
8. **Василев П.** Лечение със Cemiplimab на плоскоклетъчен карцином на кожата при пациенти с булозна епидермолиза. XIV Научна конференция по Онкология, Боровец 14-16 Юни 2024, /oral presentation/

III. Participation in scientific forums abroad

9. Yordanova I., **Vasilev P.**, Trashlieva M., Gospodinov D. Squamous cell carcinoma of the skin in patients with congenital bullous epidermolysis. XIII ASKED Kongres, Belgrade, 3 – 4 December 2021, /oral presentation/
10. **Vasilev P.**, Karaivanov M., Kalev D., Dimitrov D., Gospodinov D., Troyanova P., Yordanova I. Advanced cutaneous squamous cell carcinoma induced by long-term

Hydroxyurea treatment – a case report, 25th World Congress of Dermatology, Singapore, 03-08 July 2023, /poster/

11. **Vasilev P.**, Kalev D., Karamanliev M., Dimitrov D., Karaivanov M., Troyanova P., Yordanova I. Cutaneous squamous cell carcinoma in a patient with dystrophic epidermolysis bullosa –a case report, 25th World Congress of Dermatology, Singapore, 03-08 July 2023, /poster/
12. **Vasilev P.**, Popovska S., Kraevska E., Karaivanov M., Yordanova I. PD-L1, PD-1 and CD8 expression in 100 primary invasive cutaneous squamous cell carcinomas. EADV Spring Symposium. Malta 16-18 May 2024, /poster/