Medical University - Plovdiv City

Faculty of Medicine

Department of Special Surgery

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Contemporary Practical Approach in Surgical Treatment and Follow up of Patients with Malignant Melanoma of Skin

Author's Summary of
His Dissertation for Conferment of Educational and Scientific Degree PhD

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Patients, who were included in the study with reference to the dissertation, were operated mainly at the Clinical Department of Plastic, Reconstructive and Esthetic Surgery, and were monitored at the Cabinet of Oncological Dermatology of University General Hospital for Active Treatment "Dr. Georgi Stranski" EAD, Pleven City (Bulgaria). The histologic pathology examinations were performed at Clinical Department of Anatomic Pathology of UGHAT "Dr. Georgi Stranski" EAD, Pleven City.

The dissertational work consists of 246 pages along with the two applications and is illustrated with 38 tables, 51 color figures, and 22 pictures. The References include 299 literature sources - three of them are in Cyrillic alphabet, and 296 - in Latin alphabet.

The defense of the dissertation work will be held on September 30, 2019 from 14:00 at Second Auditorium of Complex of Auditoriums, Medical University - Plovdiv City.

The materials in connection with the defense are presented available at the site of MU - Plovdiv City: www.mu-plovdiv.bg

Table of Contents

Used Abbreviations

ABCDE - dermatoscopic criteria for melanoma growth

ANOVA - analysis of variance

AIDS - acquired immunodeficiency syndrome

AM - achromatic melanoma

BAD - British Association of Dermatologists

BRAF - a proto-oncogene

CAT - computed axial tomography

CBC - complete blood count

CNS - central nervous system

ENT - ear, nose, and throat specialist

ESMO - European Society for Medical Oncology

ESR - erythrocyte sedimentation rate

GIT - gastrointestinal tract

LDH - lactate dehydrogenase

MM - malignant melanoma

MRI - magnetic resonance imaging

NCCN - National Comprehensive Cancer Network

PET/CT - positron emission tomography-computed tomography

PM - pigmented melanoma

SLNB - sentinel lymph node biopsy

SLN - sentinel lymph node

TNM - classification of malignant tumors

UGHAT - University General Hospital for Active Treatment

^{99}**Tc** - Technetium-99

Preface

The incidence rate and prevalence rate of malignant melanoma of skin continuously increases on a global scale. The leader in that regard is New Zealand with incidence rate of 50/100 000. A tendency of disease occurrence more early in lifetime in the groups involved is also observed, which still further increases the severity of the problem. That - along with the exceptional aggressiveness in the course of disease - puts it in the leading places of significance among the oncological diseases. All stated so far, is also completely valid for Bulgaria - the following statistical data were found: prevalence rate for the year 2013 - 57.4/100 000; prevalence rate for the year 2014 - 59/100 000; and prevalence rate for the year 2015 - 61/100 000; incidence rate for the year 2013 - 5.8/100 000; incidence rate for the years 2014 and 2015 - 6.5/100 000; while the mortality rate for the year 2016 on its part was within 2.1/100 000 subjects.

We have to especially underline that Bulgaria is at one of the last places by incidence rate of primary melanoma of skin in Europe, but the mortality rate in the group of subjects is one of the highest of all European countries. This results from the fact that the disease in most of the cases is found in an advanced stage, and the treatment with medications is very expensive. That is why the proper and timely medical care of patient with malignant melanoma is of huge significance for improvement of the outcome of therapy of that insidious disease. It must be unified and consistent with the most recent world tendencies.

Unfortunately - at the present moment in Bulgaria - there is not a uniform opinion upon prevention, diagnostics, therapy, and follow up of those patients; there are only some more general guidelines created upon consideration of the matter by dermatologists, anatomic pathologists, radiologists, and oncologists.

Literature Review

Introduction

The word "melanoma" was used for the first time by Rene Laënnec, who in a manuscript of his in the year 1812 described a case with dissemination of the disease. The malignant melanoma develops after malignant transformation of pigment-producing melanocytes. The major part of them are localized in the basal layer of epidermis of skin, but such ones are also found in the eyes, ears, GIT, genitourinary system, meninges.

Etiopathogenesis

1. Ultraviolet radiation
2. Skin phototype
3. Pigmented nevi
4. Use of pesticides
5. Continuous exposure to sun and sunburn
6. Genetic factors
7. Geographic location

8. Familial predisposition
9. Immunosuppressive conditions
10. Non-melanoma skin carcinomas

Clinical Picture

The color of melanoma usually varies from pink in the amelanotic variant, to black-blue - the most frequently found one - in the pigmented variants. The form of lesion is quite different and with uneven borders. It may be raised, covered with crusts or with secondary changes as erosions and ulcerations, while the size in diameter is usually more than 5 mm.

The early diagnostics has enormous significance for improvement of malignant melanoma prognosis. The **ABCDE** criteria for malignant change of existing nevi were introduced on that occasion. They include:

1. **A**symmetry - irregular form;
2. **B**orders - irregular borders with multiple angles;
3. **C**olor - the change of color and its appearance is a characteristic feature of melanomas;
4. **D**iameter - most frequently their diameter is more than 5 mm, however cases with lesser diameter have recently become more frequent;
5. **E**volving over time - the malignant lesions are never unchanged in time; they change their characteristics (size, color, form, itching, bleeding) usually for the worse.

Clinical Subtypes of Malignant Melanoma

1. Superficial spreading melanoma
2. Lentigo maligna and lentigo maligna melanoma
3. Nodular melanoma
4. Acral lentiginous melanoma
5. Amelanotic melanoma
6. Desmoplastic melanoma
7. Nevoid melanoma
8. Mucosal melanoma
9. Juvenile melanoma

Diagnosis

1. Medical history
2. Physical examination

- Inspection
- Dermatoscopy

3. Laboratory examinations
    - CBC and ESR
    - Examination of S100 protein in serum

- Examination of LDH

4. Examinations that use specialized equipment
   - Ultrasound examination
   - Radiograph of lung and heart
   - CAT, MRI, PET/CT
5. Biopsy of the primary lesion
   - Excisional (total) biopsy
   - Partial biopsy
   - Fine-needle aspiration biopsy
6. Anatomic pathology examination of the biopsic material
   - Breslow's thickness
   - Clark's level
7. Staging according to TNM classification

Treatment

1. Radical repeated excision of tumor bed
2. Performing of sentinel biopsy of regional lymph nodes
3. Lymph node dissection of regional lymph nodes
4. Additional surgical treatment
   - Removal of distant metastases
   - Removal of local relapses
5. Conservative treatment
   - Local therapy
   - Systemic therapy
   - Radiotherapy
6. Follow up

Aim

A study of the differences based on the main prognostic indexes and the associated with them survival rate and mortality rate in our patients with malignant melanoma of skin for the period from 2012 to 2016, with presentation of the newest practical aspects in their surgical treatment and subsequent creating of a working algorithm for diagnostics, treatment and follow up, that is adapted to the conditions in Bulgaria.

Tasks

1. To be clarified the differences by gender, age, location, histological variant, tumor thickness, depth of invasion, lymph node dissection, local relapse, distant metastases, stage of disease, and presence of concomitant oncological and non-oncological diseases in patients with malignant melanoma of skin in the groups without and with sentinel lymph biopsy.
2. To be compared the survival rate and mortality rate of patients with malignant melanoma of skin in the groups without and with sentinel lymph biopsy, and also to be evaluated their dependence on gender, location of disease, histological variant of melanoma, Breslow's thickness of tumor, Clark's level of invasion, presence and type of lymph node dissection, presence of local relapse, presence of distant metastases, and stage of disease.
3. To be investigated and described the contemporary indications, methods, and the ensuing from them results in performing of repeated excision, sentinel biopsy, and lymph node dissection in patients with malignant melanoma of skin.
4. To be created an algorithm - adapted to the conditions in Bulgaria - for diagnostics, treatment, and follow up of patients with malignant melanoma of skin, which to be presented in an individual printed form named "Medical Form for a Patient with Malignant Melanoma of Skin".

Material and Methods

1. Patients
2. Recent guidelines for treatment and follow up of patients with malignant melanoma of skin in countries that lead in the management of this problem on a global scale.
3. Surgical methods
4. Statistical methods

Patients

A total of 151 patients with malignant melanoma of skin, treated surgically in the period from 2012 to 2016 - mainly at the Clinical Department of Plastic and Reconstructive Surgery of UGHAT "Dr. Georgi Stranski" EAD, Pleven City - were included. Among them 78 in number were men (51.7%), and women - 73 (48.3%), while the age range was between 17 and 91 years. They were divided into two groups depending on whether sentinel biopsy of regional lymph nodes was performed or not, while lymph node dissection was performed in patients of both groups.

1. The group of patients with sentinel biopsy consisted of 58 individuals, of which 26 were men, and 32 - women.
2. The group of patients with no sentinel biopsy consisted of 93 individuals, of which 52 were men, and 41 - women.

Recent Guidelines for Treatment and follow up of Patients with Malignant Melanoma of Skin

The most recent guidelines for management of malignant melanoma of skin were studied - namely the ones of the National Comprehensive Cancer Network (NCCN) of the USA, the European Society of Medical Oncology (ESMO), the British Association of Dermatologists (BAD), as well as the dermatological societies of Germany, Switzerland, Australia, and New Zealand, etc.

Surgical Methods

Sentinel Lymph Node Biopsy and Repeated Excision

For sentinel biopsy performing we undertook a combined method of dyeing of nodes by means of the radiopharmaceutical ^{99}Tc sulphur colloid and the dye Patent Blue V. The operative technique included incision of skin at a preliminarily marked location after scintigraphy, with following finding of a sentinel lymph node dyed in blue, its removal and sending for histologic pathology examination.

Reexcision of the scar from the previous biopsy of tumor followed, while the distance of the surrounding incision is in direct dependence on the thickness of melanoma. In our cases it usually has a radius of 2 cm. The removed skin flap is sent again for stable histological preparation, in which residual tumor is to be looked for.

Lymph Node Dissection

It represents surgical removal of the regional lymph node basin involved by the metastatic process. A priority for our medical team were the axillary and inguino-femoral lymph node dissections, while the cervical lymph node dissections were performed by ear, nose, and throat specialists. It has to be noted that the latter were applied only in four patients.

- Axillary lymph node dissection

According to the most recent guidelines, it must be performed at three levels, which are determined in relation to the position of smaller pectoral muscle.

- Inguino-femoral lymph node dissection

Removal of the entire complex of lymphatic and adipose tissue - which lies on and below the inguinal ligament next to the apex of femoral triangle (a.k.a. Scarpa's triangle) as well as around the femoral blood vessels in itself - is performed.

Removal of Local Relapses, Muscular and Skin Metastases

The surgical technique in these cases manifests itself by excision as far as non-affected tissues (a "safety margin" of healthy-looking tissues is included) and - if possible - at a distance of 2 cm from all lesions found.

Statistical Methods

The data were entered and processed with statistical software IBM SPSS Statistics 24.0. For a level of significance - at which the zero hypothesis is rejected - was accepted $p < 0.05$.
The following statistical methods were applied:

1. Descriptive analysis - in table mode was presented the frequency distribution of the reviewed signs, divided into groups of examination.
2. Cross-tabulation - for searching of relationship between some categorical signs.
3. Graphical analysis - for visualization of the results obtained.
4. Test $\chi 2$ - for testing of hypotheses for presence of relationship between some categorical variables.
5. Fisher's exact test - for searching of dependence between two categorical variables.
6. Parametric Student's *t*-test - for testing of hypotheses for any difference between the average arithmetical values of two independent samples.
7. ANOVA - for comparing of the averages of more than two variables.
8. Correlational analysis - for finding of relationship between two variables.
9. Variational analysis - for assessment of the characteristics of the central tendency and sparsity of data.
10. Alternative analysis - for comparison of the relative portions.

11. Test of Kaplan-Meier and Cox regression for assessment of the differences in survival rate and factors, which have some influence.
12. Criteria for validity of tests.

Results and Consideration

I. To be clarified the differences by gender, age, location, histological variant, thickness of tumor, depth of invasion, lymph node dissection, local relapse, distant metastases, stage of disease, and presence of concomitant oncological and non-oncological diseases in patients with malignant melanoma of skin in groups without and with sentinel lymph biopsy.

I.1 Distribution of patients with MM by gender

- Totally

Out of 151 patients with malignant melanoma of skin, 78 in number (51.7%) were men, and 73 (48.3%) were women (Fig. 1).

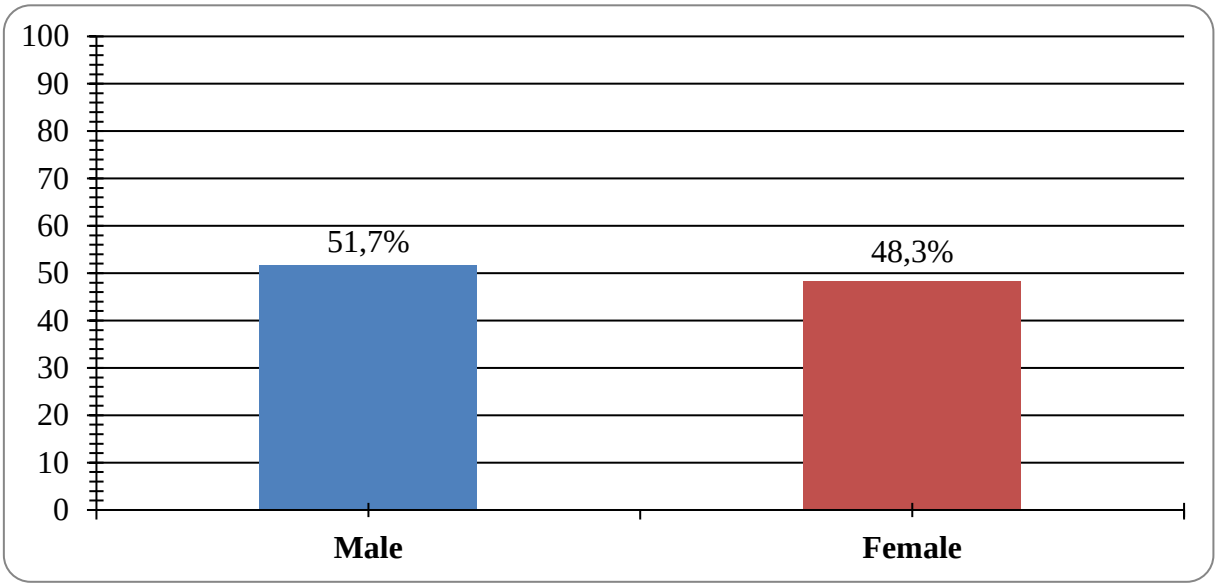

Figure 1. Distribution of patients with MM by gender (%)

Our results demonstrated that the malignant melanoma of skin is more common in men (51.7%) than in women (48.3%), which completely corresponds to the data of foreign scientific sources.

- Distribution in the groups with and without sentinel biopsy

- Of all 151 studied patients with MM, sentinel lymph biopsy was performed in 58 of them, while 26 (44.8%) were men, and 32 (55.2%) were women. Significant differences by gender were not found in this study.
- 93 individuals were included in the group of patients without sentinel biopsy, out of which 52 (55.9%) were men, and 41 (44.1%) were women.
- In the group of men, 52 individuals (66.7%) were without sentinel biopsy, and 26 (33.3%) underwent such a biopsy. In the group of women, the results were respectively 41 in number (56.2%) and 32 (43.8%) (Fig. 2).

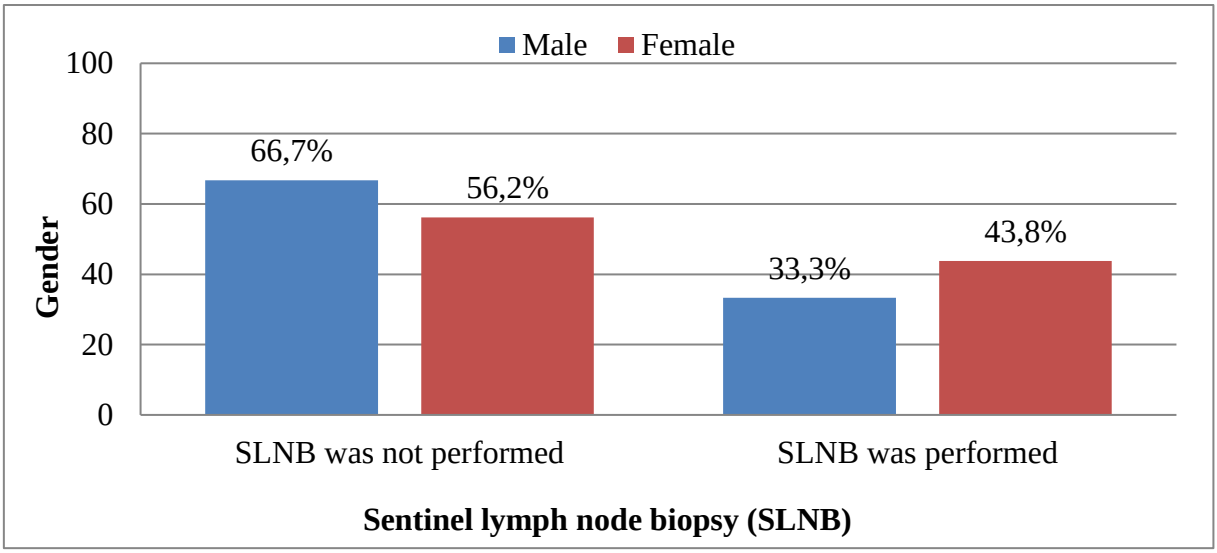

Figure 2. Distribution of patients with MM without and with SLNB by gender (%)

The data analysis showed us that in the group of patients with sentinel biopsy predominated women (55.2%), while in the group without such one predominated men (55.9%). Comparing our results with the ones of Gershenwald et al., we found that in the group with performed sentinel biopsy in their study predominated men (57.5%).

I.2 Distribution of patients with MM by histological variant

- Totally

Patients with pigmented variant represented 90.7% of all patients with MM of skin (Fig. 3).

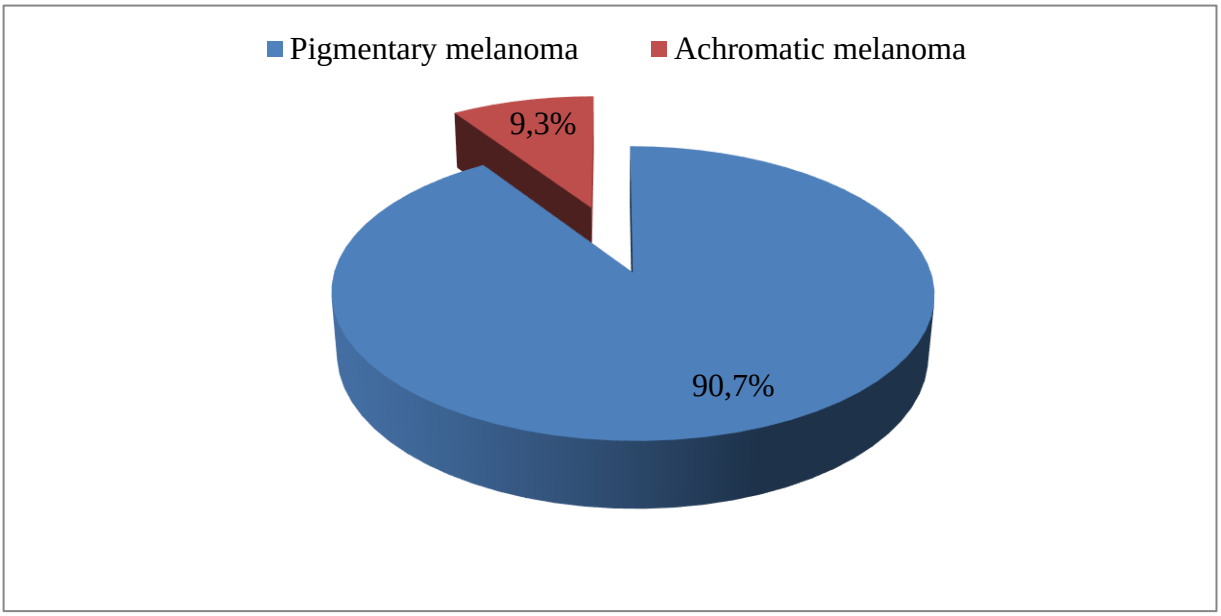

Figure 3. Distribution of patients with MM according to histological variant (%)

We ascertained - on analysis of our results - that the percentage level of achromatic melanoma of 9.3% found by us was a little higher than the average levels of 8% described in the literature.

➢ Distribution in the groups with and without sentinel biopsy

In the group with sentinel biopsy barely 3.4% comprised patients with achromatic MM, while in the group without such one, they were 12.9% (Fig. 4).

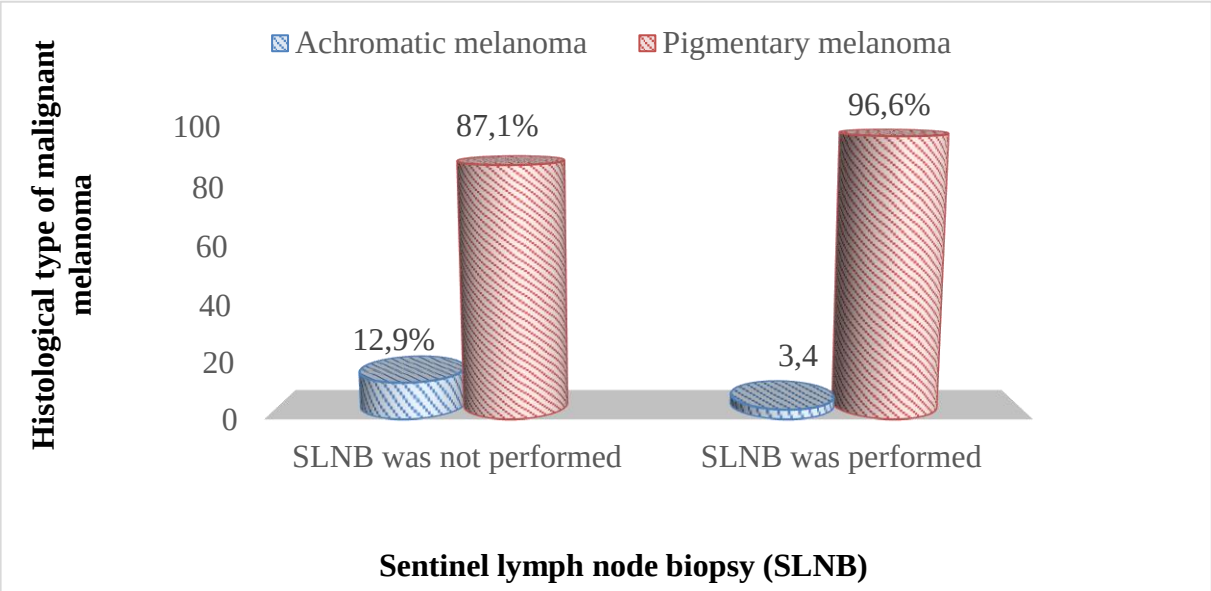

Figure 4. Distribution of MM according to the histological variant in the groups without and with SLNB (%)

The frequency of performed SLNB is about three times higher in patients with pigmented melanoma (40.9%) compared to those with achromatic melanoma (14.3%), while the differences in the groups were significant ($\chi^2 = 3.796$, $p = 0.051$, $N = 151$).

The ascertained by us significant difference ($\chi^2 = 3.796$, $p = 0.051$, $N = 151$) in the frequency of performed SLNB in patients with pigmented melanoma (40.9%) compared to those with achromatic one (14.3%) is due to the fact that making of diagnosis of the latter occurs in a late stage, which in most of the cases does not allow performing of sentinel biopsy.

I.3 Distribution of patients with MM according to Breslow's thickness of tumor

➢ Totally

With highest frequency were patients with Breslow's thickness of MM between 2.1 and 4.0 mm - 27.8%, followed by patients with thickness of more than 4.1 mm (Fig. 5).

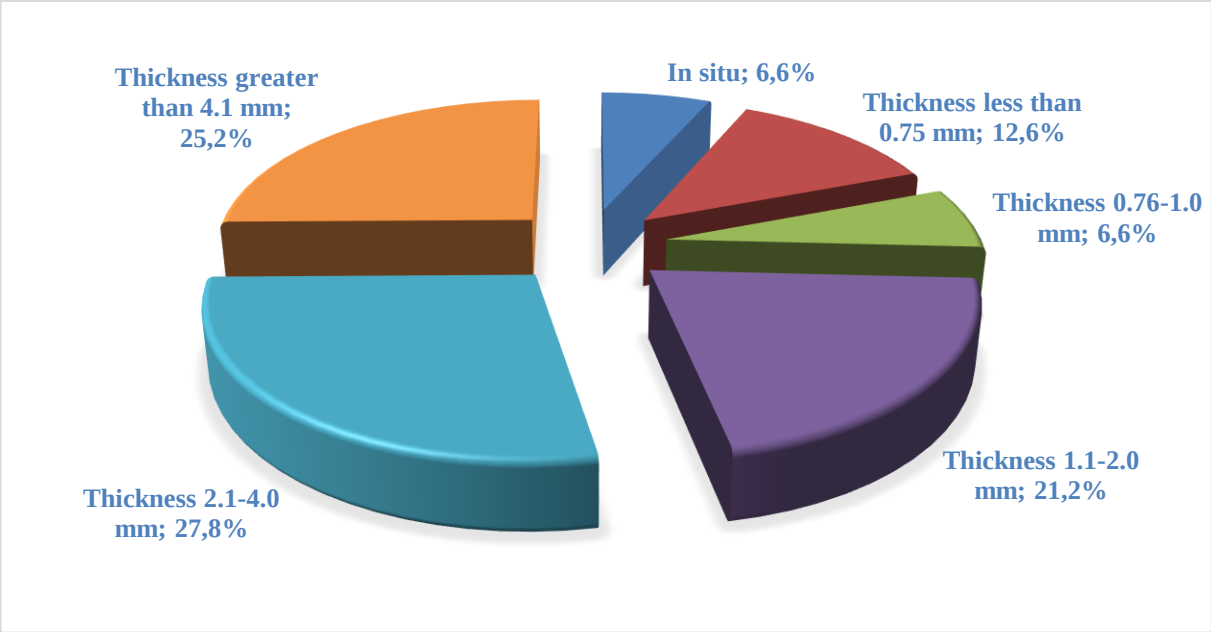

Figure 5. Distribution of patients according to Breslow's thickness of MM (%)

The mean Breslow's thickness of MM in the whole group of patients was 2.4 mm with values between 0 and 11 mm.

The highest frequency (27.8%) - found by us - of patients with Breslow's thickness of MM between 2.1 and 4.0 mm significantly differed from the results reported by Moris Anger et al., that presented highest frequency (39.3%) in patients with Breslow's thickness of tumor of less than 1 mm. This shows that the making of a primary diagnosis of MM in our conditions is realized at a later stage, when the thickness of melanoma is bigger.

- Distribution in the groups with and without sentinel biopsy

In the group without sentinel biopsy predominated patients with Breslow's thickness of melanoma of more than 4.1 mm - 32.2%, while in the group with SLNB predominated patients with thickness of tumor between 1.1 and 2.0 mm - 39.7%.

The mean Breslow's thickness of MM in the group without sentinel biopsy was 2.5 mm with variation between 0 and 11 mm, while the one in the group with SLNB was 1.8 mm with values between 1 and 5 mm.

We found higher portion of patients with MM of skin and SLNB performed, in which the tumor invasion was within the ranges of 0.76 - 1 mm, 1.1 - 2.0 mm, and 2.1 - 4.0 mm. An exception make the two endmost groups (respectively invasion of less than 0.75 mm and invasion of more than 4.1 mm), in which the portion of patients with MM without SLNB performed was higher, while the differences were significant ($\chi^2 = 29.563$, $p = 0.001$, $N = 151$) (Fig. 6).

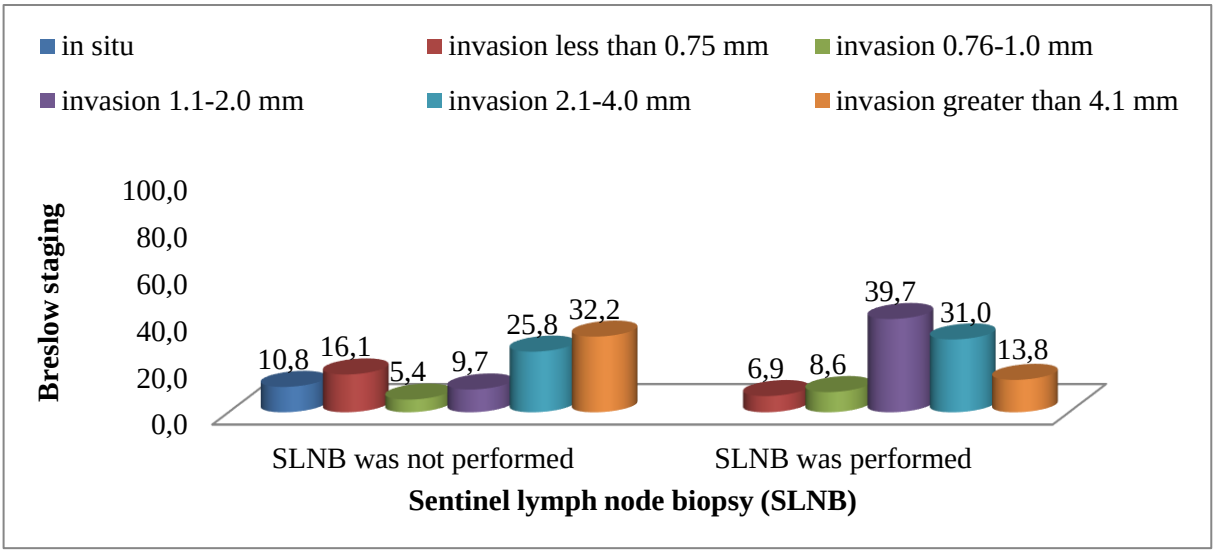

Figure 6. Distribution according to the Breslow's thickness of MM in the groups without and with SLNB (%)

The evaluated by us statistically significant difference (χ^2 = 29.563, p = 0.001, N = 151) in the groups with invasion of less than 0.75 mm and of more than 4.1 mm, where the portion of patients with MM without SLNB performed was higher compared to the group with SLNB performed, corresponded to the indication for performing of SLNB, namely Breslow's thickness of MM between 0.75 and 4.1 mm.

I.4 Distribution of patients with MM according to the presence of local relapse

- Totally

Local relapses of MM were observed in 13 patients, which represents 8.6% of all patients (Fig. 7).

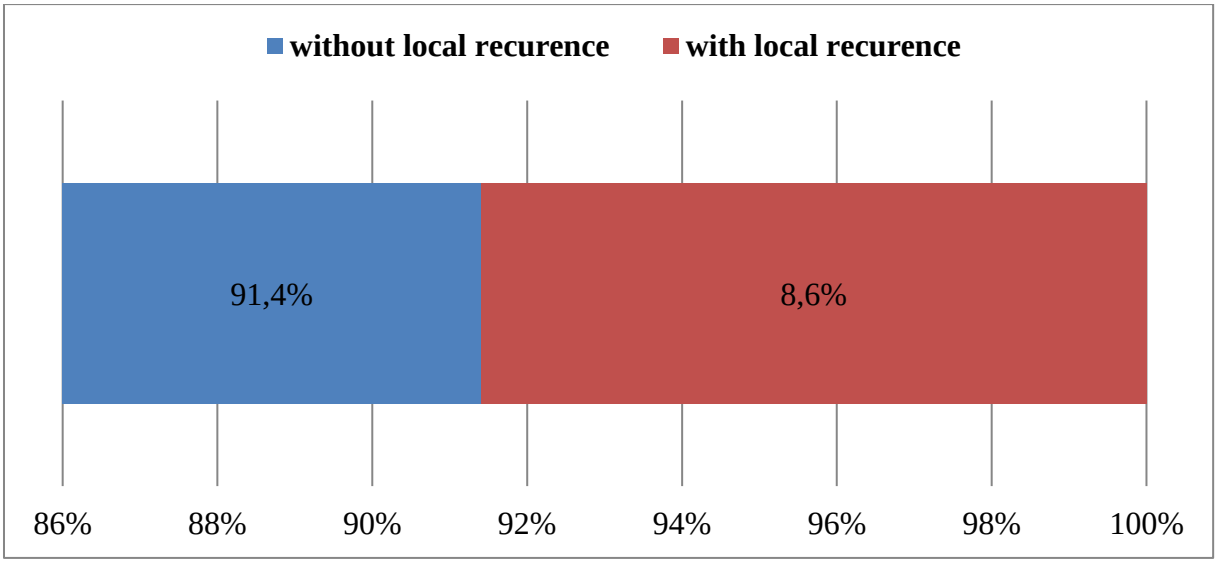

Figure 7. Distribution of patients with MM according to the presence of local relapse (%)

The level of local relapses - found by us in statistical analysis of the data - of 8.6% was about two times higher than the quoted in the literature level between 3 and 5%.

Those results show again that the primary diagnosis of MM of skin of our patients was made on relatively advanced tumors.

- Distribution in the groups with and without sentinel biopsy

The analysis of the results of patients without SLNB showed that local relapses were found in ten individuals (10.8%), while in the group with SLNB they were three (5.2%). This results in their frequency being two times higher in patients of the first group (Fig. 8).

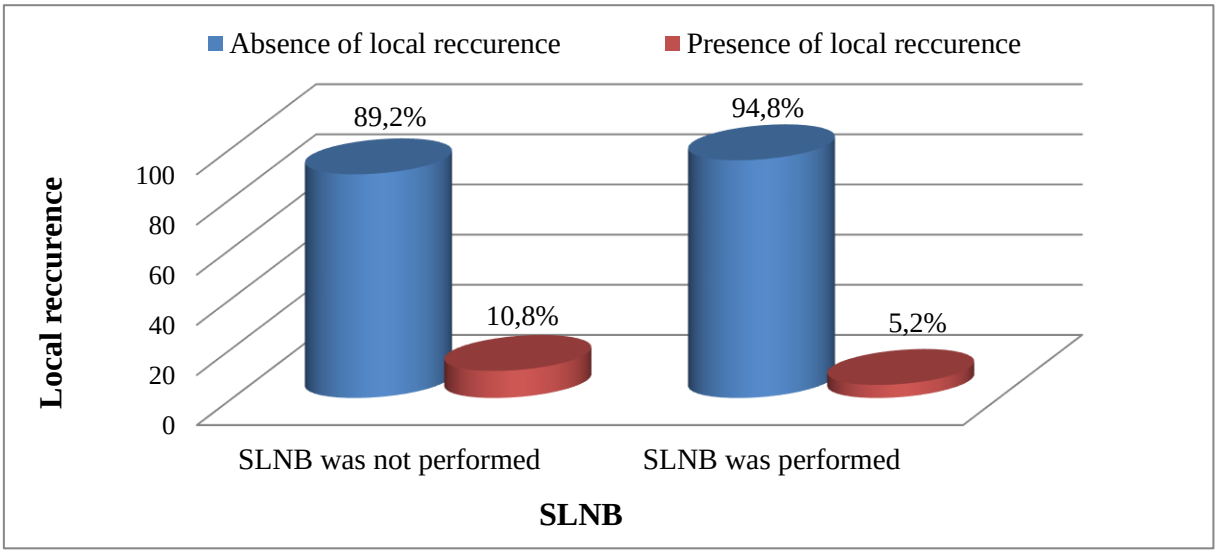

Figure 8. Distribution of patients with MM according to presence of local relapse in the groups without and with SLNB (%)

Unfortunately - in these cases - explicit significance cannot be deduced, because the number of subjects is too small (less than 15) in order statistically correct calculation of it to be carried out.

Our statistical results demonstrated 5.2% local relapses in the group with SLNB, which completely corresponds with the demonstrated levels of 5.3% in the study of Sakovska et al.

The lower levels of local relapses, which were observed in the group with SLNB, probably were due to the earlier stages of disease of patients included it.

I.5 Distribution of patients with MM according to the stage of disease

- Totally

One is left with the impression that in the majority of studied patients the disease was in an advanced stage: 39 (25.8%) were in stage IV, 12 (7.9%) were in stage III, while 19 (12.6%) -

in stage IIC. Better prognosis had about 37% of patients - 26 (17.2%) were in stage IB, 21 (13.9%) - in stage IA, while 9 (6.1%) - in stage 0 (Fig. 9).

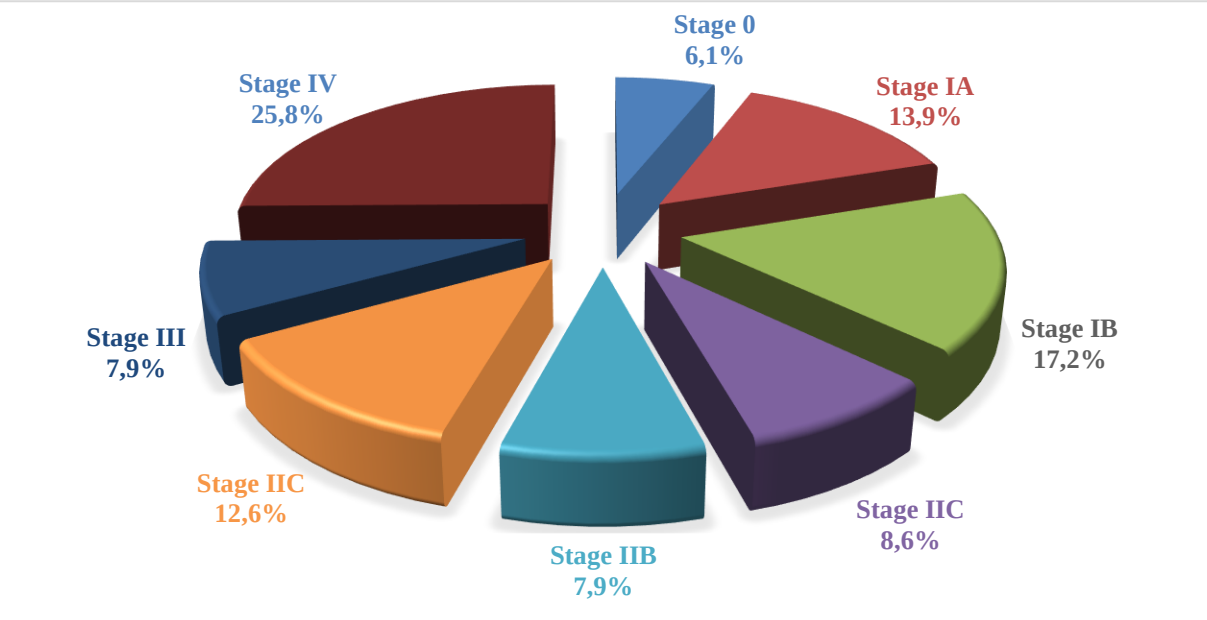

Figure 9. Distribution of patients with MM according to the stage of disease (%)

It was found - by analysis of data in relation to the stage of MM in which our patients were - that most of them (33.7%) were in stages III and IV. This is in absolute contrast with the data of many collectives who performed large scale studies on this matter, showing that their patients in lowest percentage were in those two stages. It must be heavily scored that this result of ours is a very negative tendency in making of diagnosis and treatment of patients with malignant melanoma of skin in Bulgaria.

- Distribution in the groups with and without sentinel biopsy

In analysis of the distribution of patients with MM of skin by stages, in the groups without and with sentinel biopsy, it turned out that in the first group a predominating part of patients were in stage IV - 29% (27 patients), while in the second group - patients in stage IB - 29.3% (17 patients) (Fig. 10).

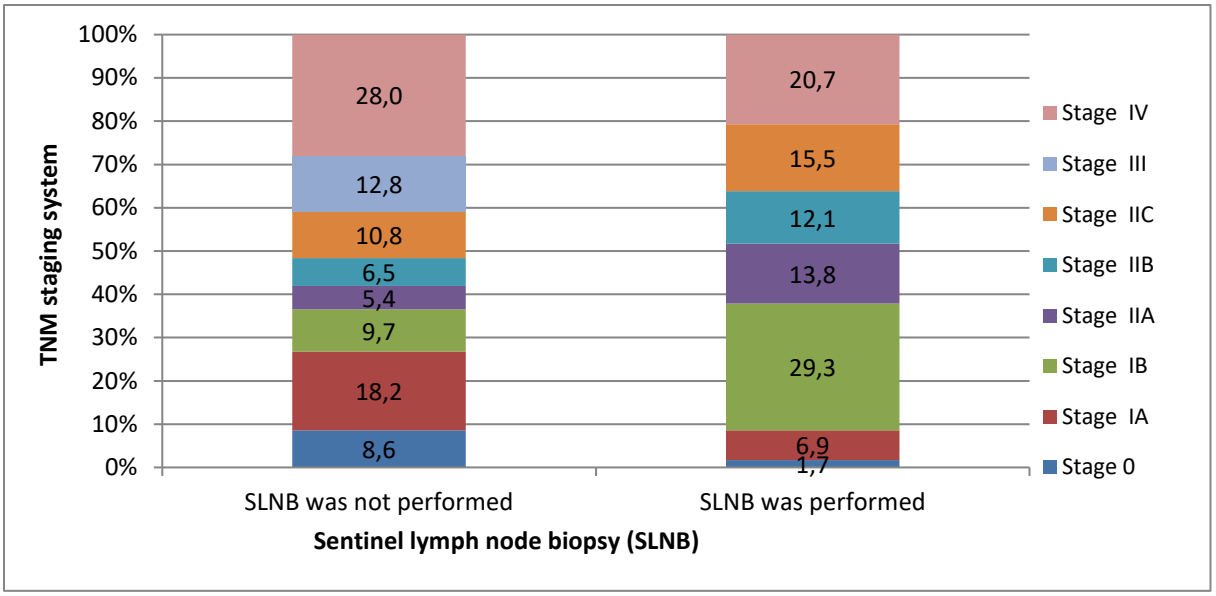

Figure 10. Distribution of patients with MM according to the stage of disease in the groups without and with SLNB (%)

There is presence of significant differences in the groups according to the performance of SLNB and determination of the stage of disease. The portion of patients with MM in stage IB was three times higher with SLNB performed (29.3%) than when none was carried out (9.7%). About three times higher was the portion of patients with SLNB, in whom MM was in stage IIA (13.8%) compared to those in whom no SLNB was performed (5.4%). In 29% of patients without SLNB performed, MM was in stage IV compared to 20.7% of patients with SLNB performed, while the differences were significant ($\chi^2 = 28.204$, $p = 0.001$, $N = 151$).

Upon comparison of our data, with respect to the stage of MM in the group with SLNB, with those in the group without SLNB, we found three times higher portions of patients in stages IB and IIA in the former, as well as significantly lower portion of patients in the last two stages of the disease, while the differences in this case were significant ($\chi^2 = 28.204$, $p = 0.001$, $N = 151$). That is due to the fact that we strictly observed the indications for performing of SLNB, which on principle exclude patients in the most advanced stage, because in this case the probability of local and systemic metastasis is enormous.

II. To be compared the survival rate and mortality rate in patients with malignant melanoma of skin in the groups without and with sentinel lymph biopsy, and also to be evaluated their dependence on gender, location of disease, histological variant of melanoma, Breslow's thickness of tumor, Clark's level of invasion, presence and type of lymph node dissection, presence of local relapse, presence of distant metastases and stage of disease.

II.1 Mean survival rate in months depending on gender

The median survival rate in case of malignant melanoma is higher in women ($\bar{x}$ = 72 months, SE = 0.0) than in men ($\tilde{x}$ = 45 months, SE = 6.2), while the differences are statistically significant (log rank = 5.975; df = 1; p = 0.015). The probability of a man with melanoma to survive one year after making of diagnosis is 88%, and it is lower than the survival rate in women for the same period of time (t = 11) (90%). Unfortunately the presence of many of the so called censored cases does not allow making of parallel comparison of survival rate between men and women after the second, third, and following years since making of diagnosis of the malignant disease (Fig. 11).

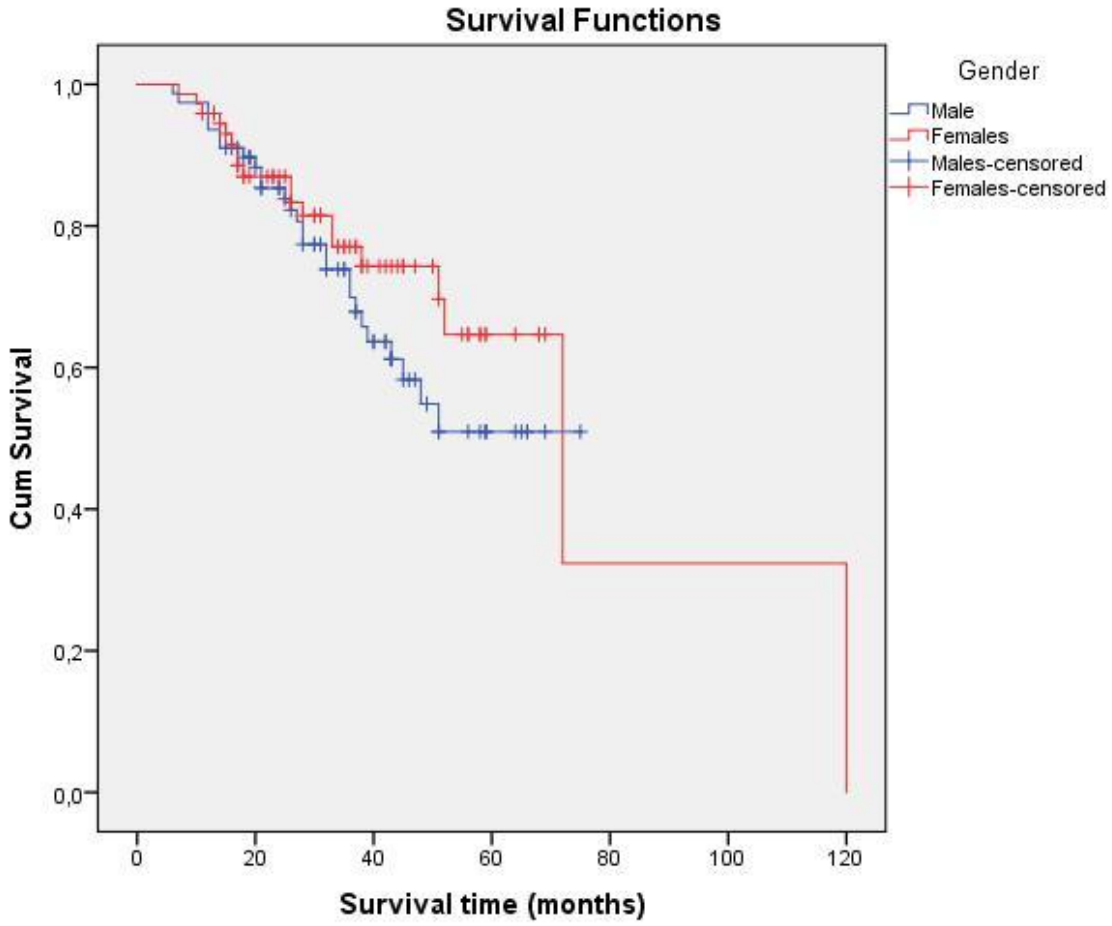

Figure 11. The Kaplan-Meier curve of survival rate in patients with MM according to gender.

The significant difference found by us (log rank = 5.975; df = 1; p = 0.015) in the median survival rate of malignant melanoma of skin in women ($\bar{x}$ = 72 months, SE = 0.0) and men ($\tilde{x}$ = 45 months, SE = 6.2) completely coincides with the data given by Jose et al. in a population-based study of 11 774 patients with MM performed in Munich (Germany) for the period from 1978 to 2007, and this probably denotes biological differences between the two genders in development of the disease or different, depending on the gender, interaction between disease and host.

II.2 Mean survival rate in months according to the histological variant of MM

The mortality rate of achromatic melanoma (AM) is about three times higher (71.4%) compared to the one from pigmented melanoma (PM) (26.4%). The probability of patient with AM to survive one year after making of diagnosis of the disease is 92.9%, and it is lower than the one of patient with PM (94.9%). Patient with AM has 62.5% probability of 28-month survival (81.1% in patient with PM), 41.7% probability for 36-month survival (76.6% in patient with PM) and barely 13.9% probability to survive 48 months (69.2% in patient with PM). The median survival rate is twice higher in patients with PM ($\tilde{x}$ = 72 months) in relation to 36-month survival rate in patients with AM, while the differences in the two groups were statistically significant (log rank = 8.634; df = 1; p = 0.003) (Fig. 12).

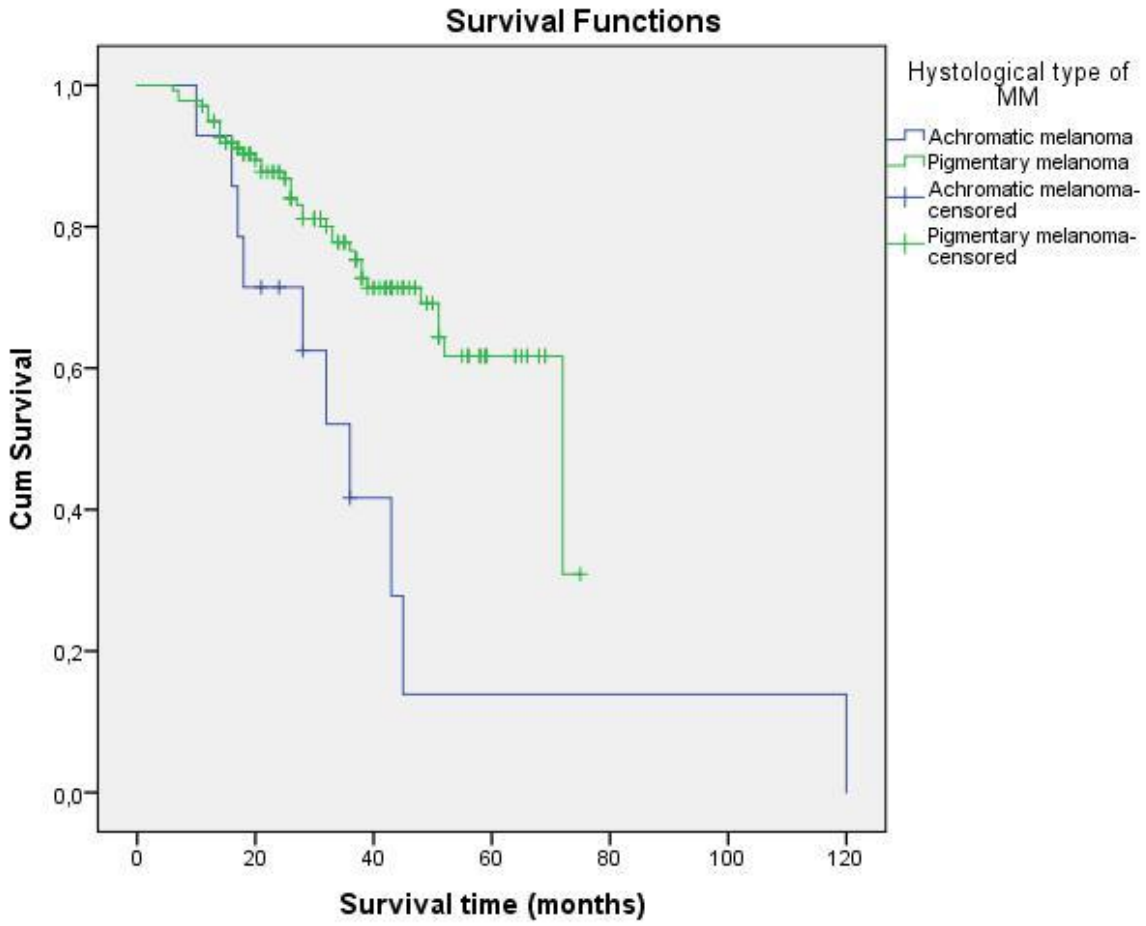

Figure 12. Kaplan-Meier curve of survival rate in patients with MM according to the histological variant of tumor

When analyzing the statistical data with respect to the survival rate in months in case of achromatic histological variant of melanoma of skin compared to the pigmented one, we found that it is two times less in the former than in the latter - $\tilde{x}$ = 36 months compared to $\tilde{x}$ = 72 months, while the differences in the two groups were statistically significant (log rank = 8.634; df = 1; p = 0.003). This completely corresponds to the results of other similar studies on that subject. The reasons for that lie in the facts, that the amelanotic variant has a more aggressive course, giving distant metastases more early and local relapses more often, and furthermore its diagnostics is realized too late due to the not typical clinical picture.

II.3 Mean survival rate in months according to the presence of local relapse in patients with MM

The Kaplan-Meier curve of survival rate has a more steep incline in patients in whom a local relapse of disease was found, which is related to poorer prognosis of MM. The probability of 12-month survival is 92.3% on local relapse (94.9% upon absence of relapse), 24-month survival in presence of local relapse has probability of 60.6% (85.1% upon absence of relapse), 36-month survival in presence of local relapse is with probability of 40.4% (76.7% upon absence of relapse). The median survival rate is more than three times higher upon absence of local relapse of MM (x̃= 120 months) than upon presence of one (x̃= 36 months), while the differences in the groups are significant (log rank = 9.618; df = 1; p = 0.002) (Fig. 13).

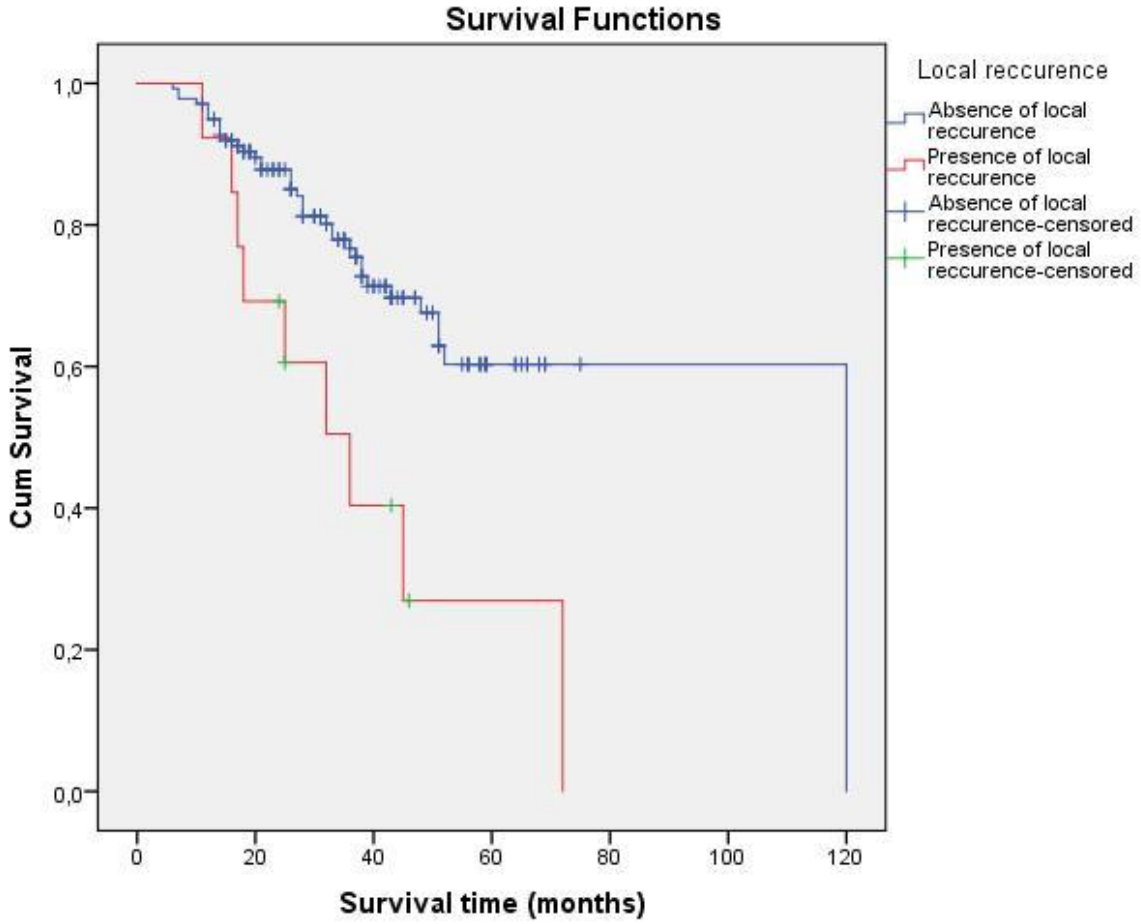

Figure 13. Kaplan-Meier curve of survival rate in patients with MM according to the presence or absence of local relapse.

Evaluating the results from the mean survival rate of patients with MM of skin - with presence or absence of local relapse - we found that Kaplan-Meier curve is more steep in patients with presence of such one, which is related to poorer prognosis of disease. The probability of 12-month survival is 92.3% in patients with local relapse and 94.9% in patients without such one, the probability of 24-month survival is respectively 60.6% to 85.1%, and the probability of 36-month survival is respectively 40.4% to 76.7%. The median survival rate is three times higher upon absence of local relapse of MM (x̃= 120 months) than upon presence of such one (x̃= 36

months), while the differences in the groups are significant (log rank = 9.618; df = 1; p = 0.002). The data completely coincide with those from other similar studies and show that the presence of local relapse is one of the main factors that worsen the survival rate in patients with MM of skin.

II.4 Mean survival rate in months according to the stage of disease in patients with MM

The specific type of each of the curves of Kaplan-Meier confirms the significance of stage of disease for the prognosis of MM. The probability of 12-month survival in patients in stage IV is 84.6%, in patients in stage III - 91.7%, and it is lower than that in patients with more early stage of disease (94.4% in patients in stage IIC). The probability of patient with MM in stage IV to survive the second year after making of diagnosis is 65.8% and it is lower than that of patient in stage III (70.7%). The three-year survival of MM has 31.4% probability in patients in stage III and 47.6% in patients in stages IV and IIC (Fig. 14).

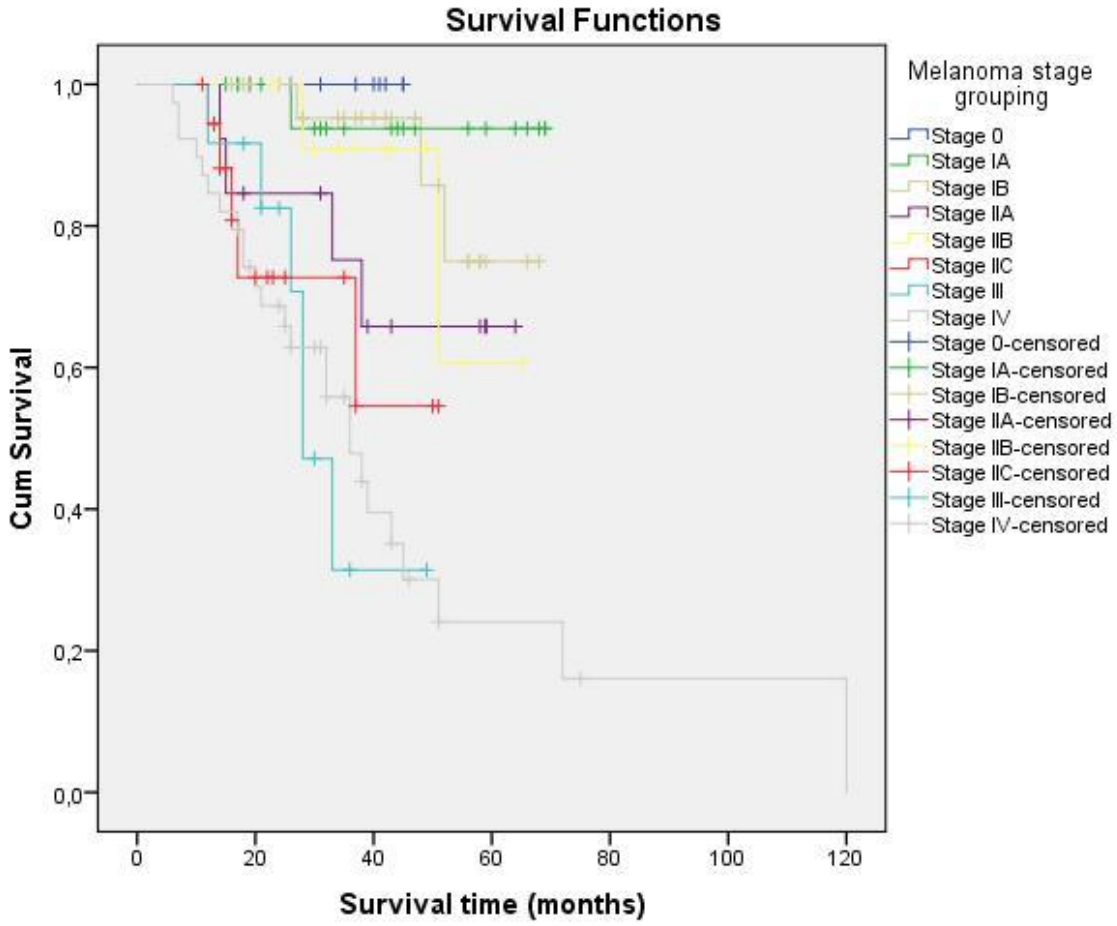

Figure 14. Kaplan-Meier curve of survival rate in patients with MM according to the stage of disease

The statistical analysis of the results with respect to the mean survival rate according to the stage of disease in patients with cutaneous MM showed that there is a specific type of every of

the curves of Kaplan-Meier. This confirms the significance of stage for prognosis of the disease. The probability of 12-month survival in patients in stage IV and stage III is respectively 84.6% and 91.7%, while it is lower in patients with earlier stage of disease - 94.4% in patients in stage IIC. The dependencies are similar in evaluating of two- and three-year survival rate as well. Those results are completely comparable with data published in literature by other collectives of authors.

The above-mentioned facts are evidence, that the stage of disease also pertains to the main prognostic factors with strong influence on the survival rate.

III. To be studied and described the up-to-date indications, methods and ensuing from them results in performing of repeated excision, sentinel biopsy and lymph node dissection in patients with malignant melanoma of skin.

III.1 Sentinel biopsy

- The most modern indications, which were studied and used by us in performing of sentinel biopsy were:
  - Negative regional lymph nodes found upon clinical examination
  - Breslow's thickness of cutaneous melanoma of more than 1 mm
  - In cases of melanomas with thickness from 0.76 to 1 mm performing of sentinel biopsy must beforehand be considered and precised, while individual approach is to be applied in relation to the particular case.
  - For melanomas with thickness of less than 0.75 mm: the biopsy of sentinel lymph node is performed only as an exception, and at that with presence of additional factors as concomitant ulcerations of tumor, increased mitotic index, young age, etc.

- Method of sentinel biopsy

We used double-detection method in which we initially applied radiopharmaceutical ^{99}Tc sulphur colloid - strictly intradermally around the scar from the previous biopsy of melanoma. We prepared lymphoscintigraphic map of the regional lymph node basin (Fig. 15) by placement of skin markers at the locations of sentinel lymph nodes after examination in gamma-ray chamber (Fig. 16).

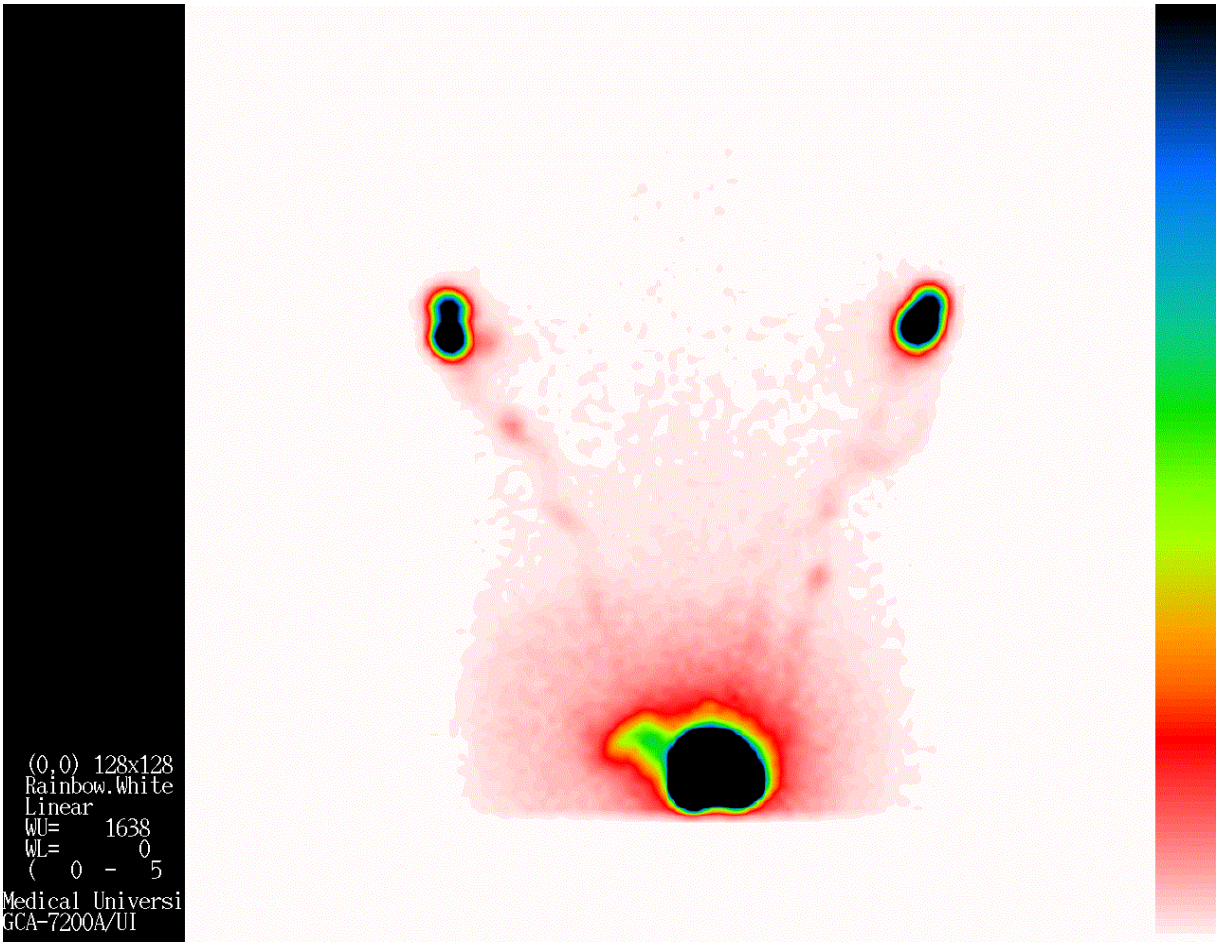

Figure 15. Lymphoscintigraphic map

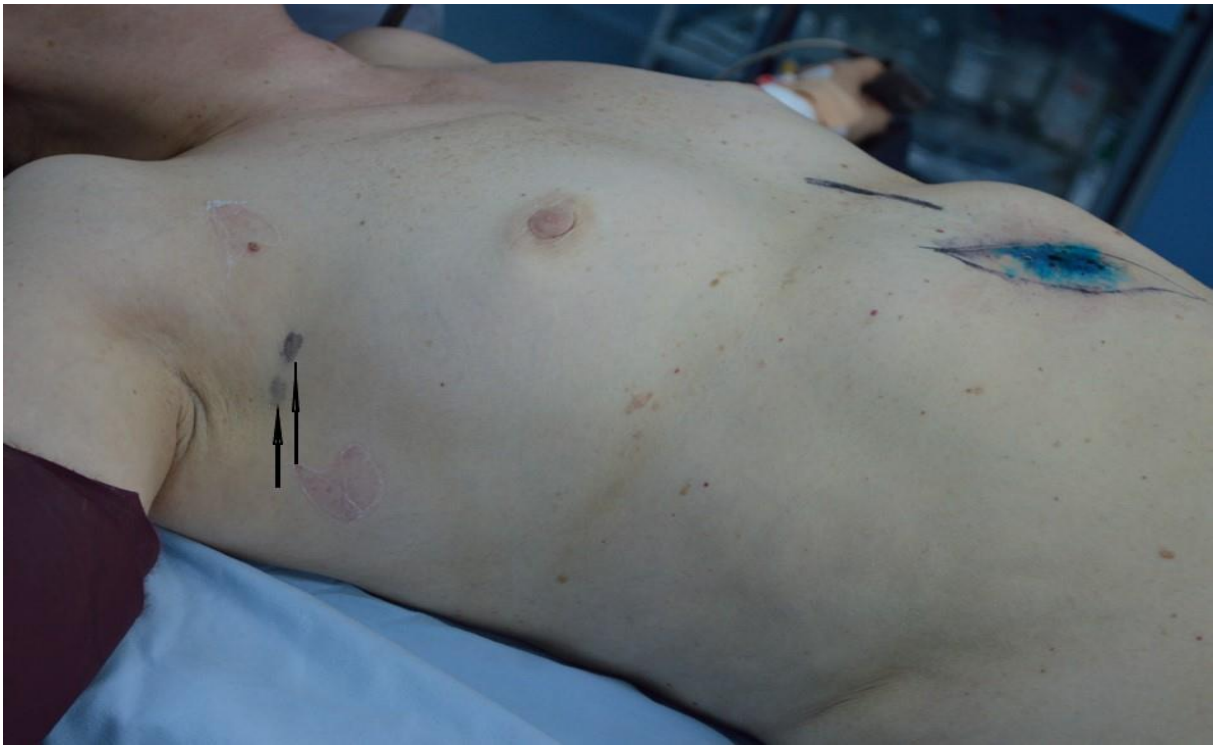

Figure 16. Markers on the skin compared to the lymphoscintigraphic map of sentinel lymph nodes

At a following stage in the operating theater, after negative skin allergic sample - again strictly intradermally around the scar from biopsy - we applied the dye Patent Blue V in a dose of 1-2 ml at about 8-10 locations (Fig. 17).

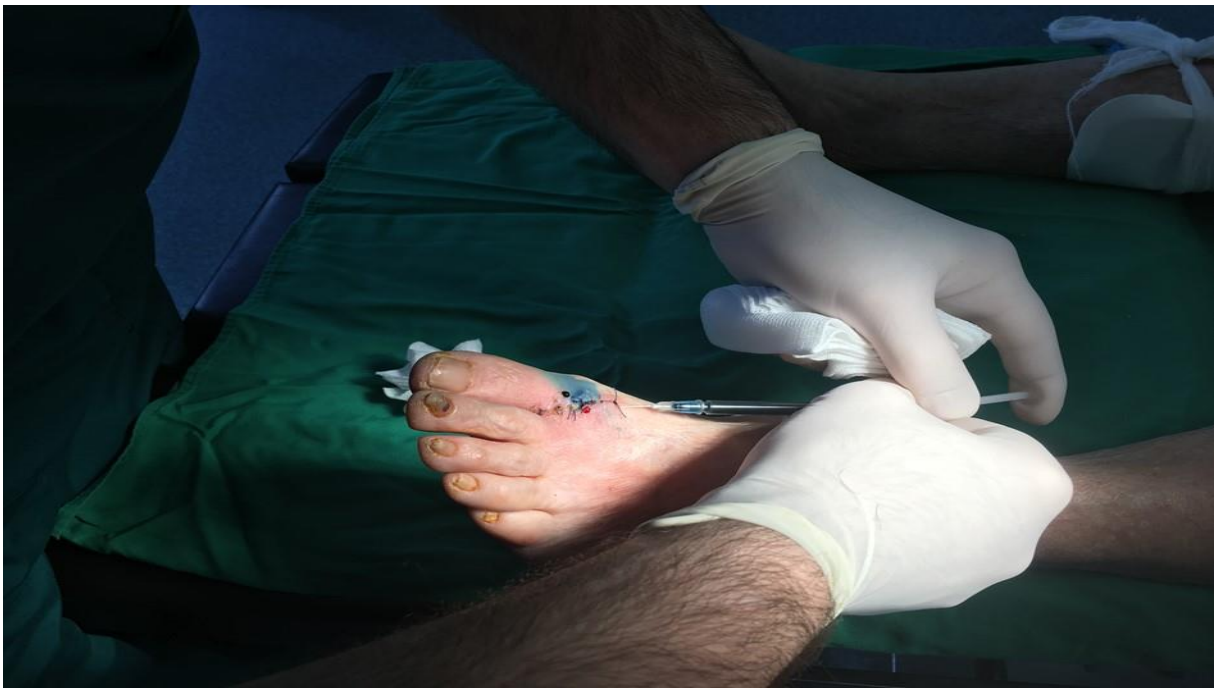

Figure 17. Injection of Patent Blue V

The search of a sentinel lymph node begins after 6-8 minutes and is realized by means of standard operative technique, while it is in conformity with the skin markers and the presence of dyed lymphatic, leading to a blue lymph node (Fig. 18).

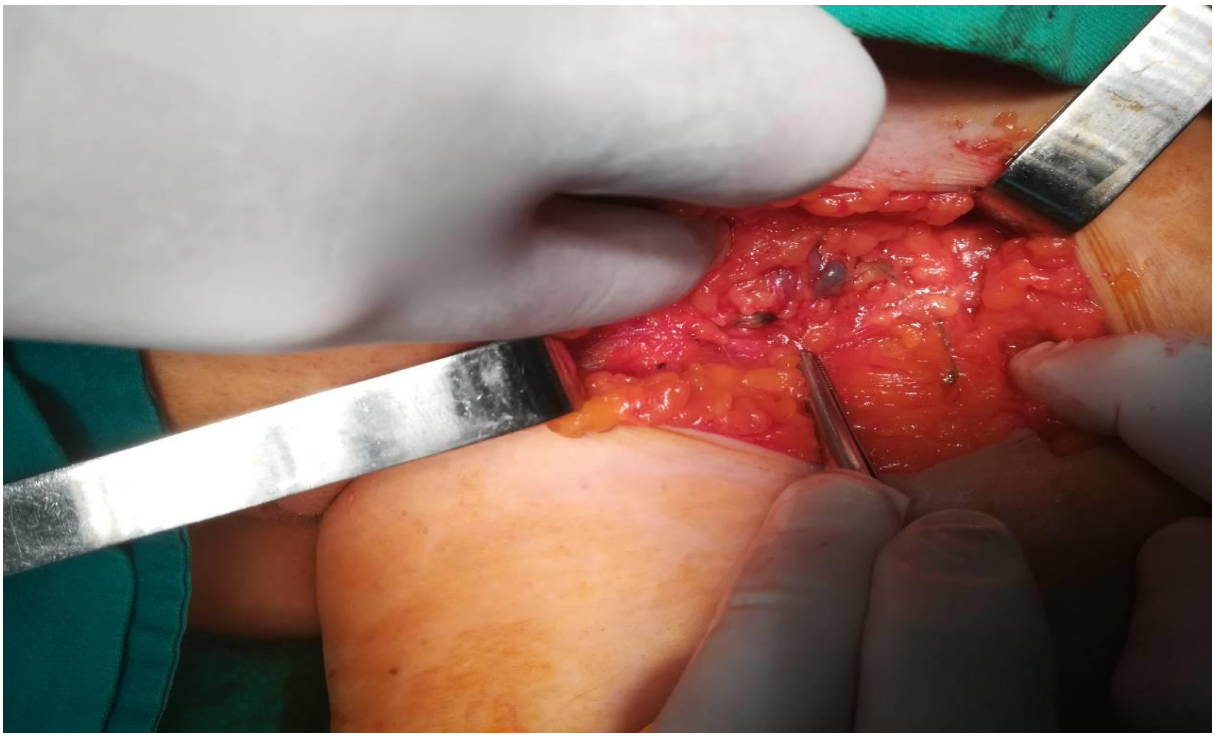

Figure 18. Dyed in blue lymphatic and sentinel lymph node.

- Results

The mean duration of sentinel biopsy was 30 minutes with variation between 10 and 60 minutes, while we did not observe any complications connected with disturbances in the regional lymph flow in any of the cases (Fig. 19).

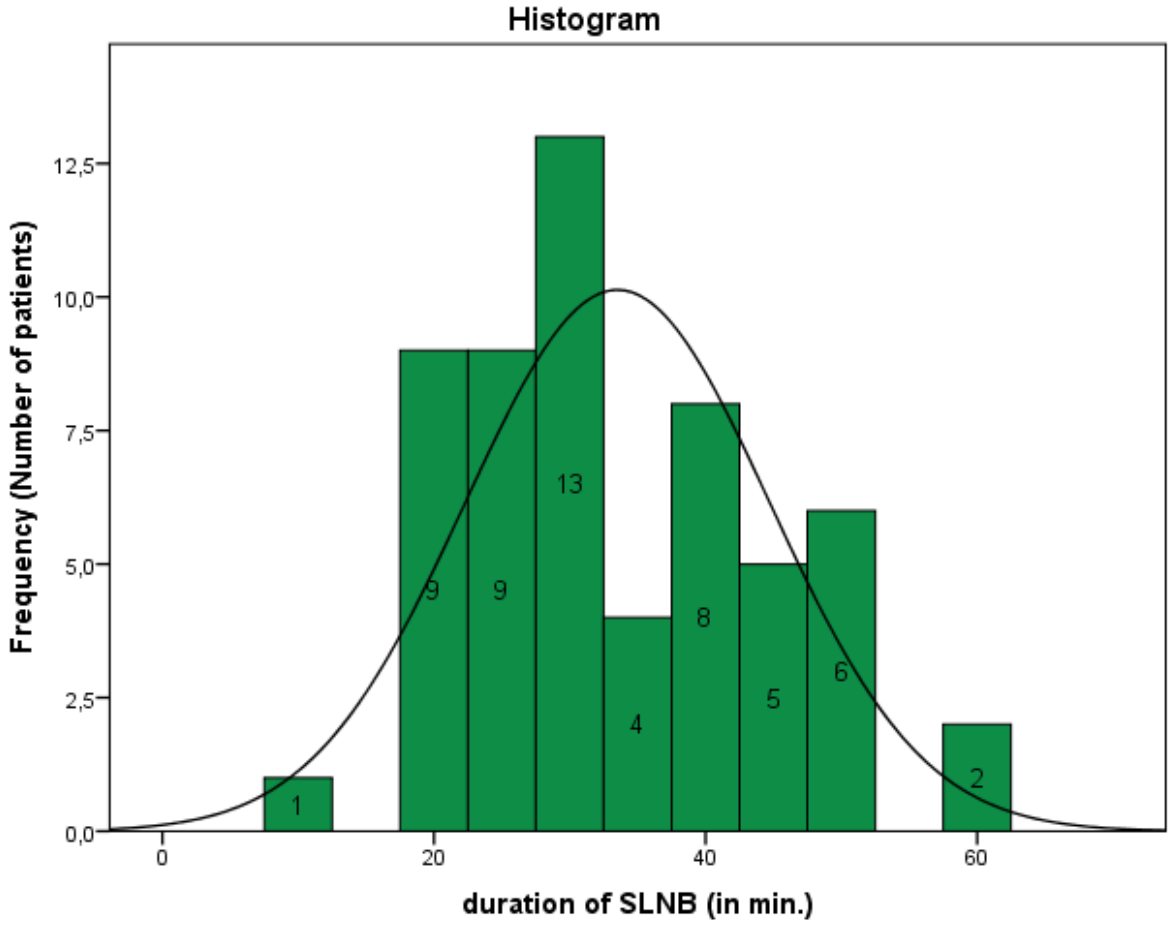

Figure 19. Duration of SLNB

Our medical team implemented a detailed investigation of the most modern indications and contraindications for performing of sentinel biopsy on malignant melanoma of skin in literature, and strictly adhered to them.

We used routinely for the first time in Bulgaria double-detection method with ^{99}Tc sulphur colloid and Patent Blue V in connection with finding of sentinel lymph nodes, by the methods as described in the literature. At first, we performed obligatorily a skin allergic sample in order to test the hypersensitivity of organism to the dye. In two of our patients we found hypersensitivity with concomitant urticaria of huge perimeter around the sample, as well as vesicles over it itself. No biopsy in these patients was carried out - only repeated excision at the location of tumor. We presumed to perform also several modifications of the method of our own: bigger number of locations of injection of Patent Blue V (8-10 sites); additional injection of the dye, if we did not initially reach dyed lymphatic or a node in the exploration of the

regional lymph node basin; as well as heating of the injection area with the purpose of acceleration of dyeing of nodes.

It is important to note that the achieved by us average duration of the sentinel biopsy was 30 minutes. During this period of time, we used possibly the most sparing operative technique with the purpose of maximum avoiding of impairment of blood vessels with concomitant hemorrhage in the operative field, which would hinder the more easy finding of sentinel lymph nodes. This is the reason why we did not observe in any of the cases complications connected with extreme blood loss and disturbances in the regional lymph flow. Our success rate with regard to finding of the sentinel lymph node by means of double-dyeing method was about 96%, which is a little more than the rate described in the literature of 88-90%. Upon calculation of the monetary value of method, it turned out that it is quite less expensive compared to the other technique extensively used for search of the sentinel lymph node with manual gamma probe, having in mind the price of the latter, while in addition the final results were commensurable. The only thing, which may be described as a disadvantage of the double-dyeing method, is the little longer training of the medical team engaged with it.

III.2 Reexcision at the site of previous biopsy of cutaneous malignant melanoma

When performing of reexcision, we accepted as a rule the borders of skin incision to be minimally at 2 cm from the biopsy scar in all directions. We allowed an exception of that rule in thin lesions, and lesions localized in the facial region, while in these cases the borders were at about 1-1.5 cm. When unable to close the wound defects, we applied skin plastics. The mean duration of sentinel biopsy with repeated excision was 65 minutes with variation between 30 and 150 minutes (Fig. 20).

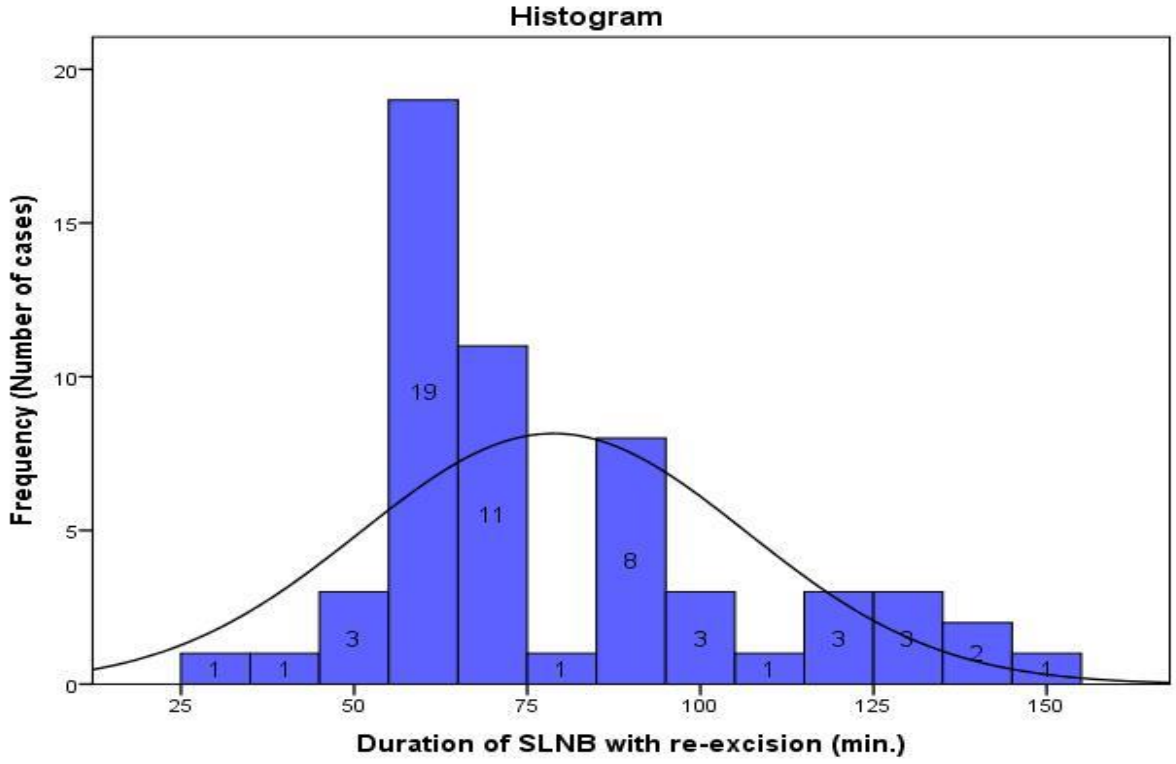

Figure 20. Duration of sentinel biopsy together with repeated excision

We performed repeated excision immediately after ending of sentinel biopsy, and not before it, in order not to disturb the lymph flow from the respective region and not to debase the result from search of the sentinel lymph node. This occurred within the mentioned in the literature 4-6 weeks after making of diagnosis. The main question, that we faced, was at what distance from the scar of previous biopsy of melanoma to be the resectional line of skin. That is why we implemented a very detailed analysis of literature and of the most recent guidelines for management of cutaneous malignant melanoma in leading countries in this regard. We did not find explicit opinions, which required making of choice of operative tactics that was not easy. We began to perform repeated excisions within 2 cm from the biopsy scar in all directions, while we permitted an exception of that rule for thin lesions and lesions localized in the facial region with borders at about 1-1.5 cm. We also evaluated all histologic pathology results of flaps of repeated excision, which showed that there were no residual tumor cells. That helped us to make a decision for continuation of the operative intervention in those borders, moreover - according to the literature - the major part of medical teams had already applied them. The mean duration of sentinel biopsy with repeated excision was 65 minutes, while the extension of time of repeated excisions was related with the need of performing of non-free and free skin plastics in order to be covered the major skin defects as a result of the intervention.

III.3 Lymph node dissection

We applied lymph node dissections only in the cases of positive clinically or positive sentinel lymph node. The mean duration of lymph node dissection was 90 minutes with variation from 30 to 210 minutes (Fig. 21).

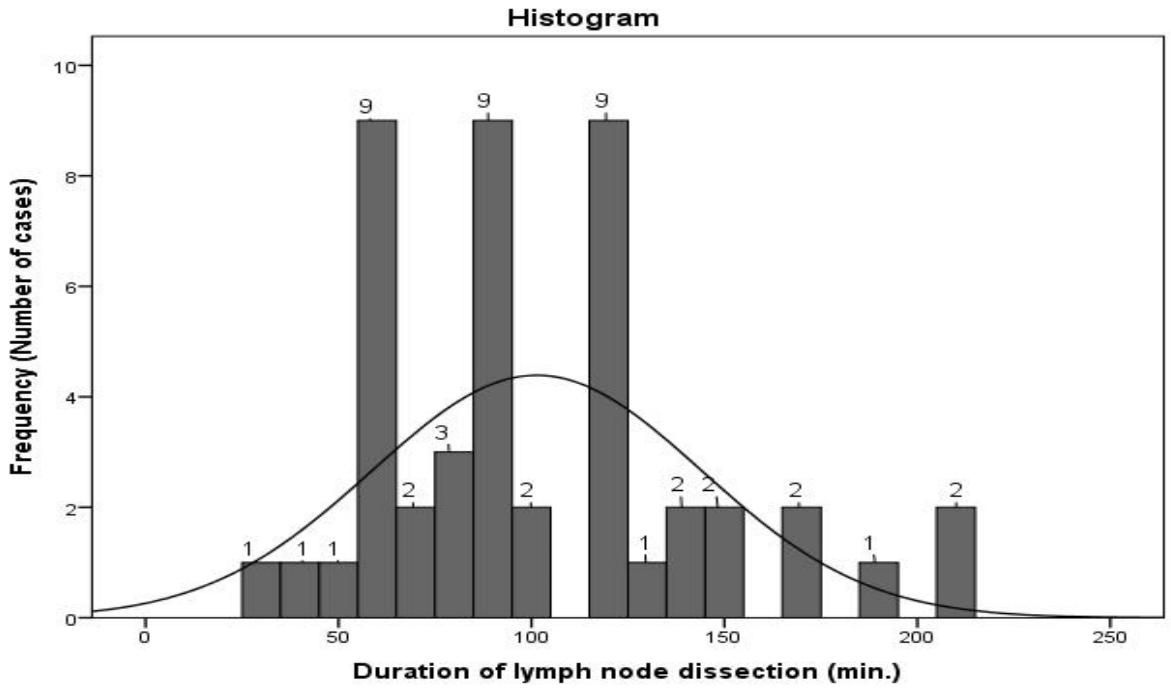

Figure 21. Duration of the lymph node dissection

Our medical team realized only the axillary and inguino-femoral lymph node dissections, while the indications for that were the cited in the literature cases of positive clinically or positive

sentinel lymph node. We used standard operative techniques, while the axillary lymphadenectomies were obligatorily at three levels. The operative incisions, which provided us with better access to the axilla were longitudinal, lying along the middle axillary line, while for the inguinal region they were transverse, leaf-shaped, with sufficient width of the flap. The dissections were performed with strict observation of the rules for lymphostasis and hemostasis, which helped us to avoid intraoperative complications. The areas of operative interventions were obligatorily drained with vacuum systems, which prevented the postoperative lymph retention. The mean duration of dissections was 90 minutes, which was determined by the very careful and complete cleaning of the areas of complex lymphatic and adipose tissue.

IV. To be created an algorithm of the diagnostics, treatment, and follow up of patients with malignant melanoma of skin - adapted to the conditions in Bulgaria - which to be presented in an individual printed form named "Medical Form for a Patient with Malignant Melanoma of Skin"

Until now, all of the medical information with respect to patients with malignant melanoma of skin was collected physically in their oncological files without existence of any certain order. This made very difficult its further processing, which raised in our team the idea for creation of an algorithm adapted to Bulgaria, including diagnostics, treatment, and follow up of patients with malignant melanoma of skin, presented in a printed form with the title "Medical Form for a Patient with Malignant Melanoma of Skin". The algorithm created by us was involved in it together with the information from all diagnostic and therapeutic procedures. The form was divided in three main parts for greater clarity.

1. The first part serves as a general one, and it includes height, weight, body surface, exact diagnosis, TNM classification, determination of stage, concomitant diseases, data from the diagnostic biopsy, repeated excision, biopsy of sentinel lymph nodes, data for performed lymph node dissections after positive sentinel or positive clinically lymph nodes, as well as the data for operatively removed local relapses, metastases, and clinical follow up. One is left with the impression about the introduction of criteria in it - regarding the anatomic pathology response for the primary tumor or the flap of repeated excision - where along with the main among them (Breslow's thickness of melanoma, Clark's level of invasion, and pTNM) were included also new ones (microsatellites, level of mitoses, presence of ulcerations, lympho-vascular invasion, neurotropism, lymphocytic infiltration of tumor, growth phase, and tumor regression), which turned out to be very important for the further therapy, determination of stage, and prognosis of disease. At the end of this part, there is an item of clinical follow up, where patients were set in the respective clinical stage in relation to the TNM classification of melanoma. The subsequent therapeutic tactics depend mainly on the latter setting in a certain stage.

2. The second part includes data from the follow up of patient, while for each particular stage, there is a different algorithm. In this part namely our medical team made greatest efforts, because there is virtually no unified opinion regarding that question in Bulgaria. We did a thorough analysis of the above cited guidelines, while in every single moment we took into account the economic condition and the state of health care system in Bulgaria, and so we

introduced completely feasible, and not so expensive for realization criteria in connection with the separate stages.

- **Follow up / Stage 0 - TisN0M0**

We accepted in this stage as sufficient the performing of annual clinical examination for a period of ten years, which included inspection of the affected site of skin and the regional lymph node basin, because virtually the risk of worsening of the disease was minimal.

- **Follow up / Stage IA - T1aN0M0**

Basic role in this stage as well plays the clinical examination, which is already performed once every month during the first year, once every three months during the second and third year, and once every six months during the fourth and fifth year,and thereafter once every year from the sixth to tenth year, while it must be in the same volume as in Stage 0.

- **Follow up / Stage IB-IIA - T1b,2a,2b,3aN0M0**

The clinical examination in that stage was performed once every month during the first year, once every three months during the second and third year, and once every six months during the fourth and fifth year, and thereafter once every year from the sixth to tenth year in a volume as in the two above- mentioned stages, but besides that examination there were also carried out echography of scar from repeated excision of the primary tumor, of the adjacent skin regions and of the regional lymph nodes once every three months from the first to the third year, and thereafter once every six months for the fourth and fifth year. All of that is performed for the purpose of active searching - as early as possible – of the in-transit and lymph metastases of melanoma, which to result in more effective subsequent therapy. Besides the above-mentioned measures in this stage, examination of 100 protein in serum once every three months from the first to the third year becomes necessary. This protein is produced and released from melanoma cells with abrupt increase, when their number grows rapidly, as is the case with the local relapses and metastases of tumor. Unfortunately, it is not strictly melanoma-specific, and its level increases also in diseases of central nervous system, benign tumors of GIT, infectious diseases, AIDS, etc.

- **Follow up / Stage IIB, C - T3b,4a,4bN0M0**

A clinical examination must be implemented - in this stage - once every month during the first year, once every three months during the second and third year, once every six months during the fourth and fifth year, and thereafter once every year from the sixth to the tenth year, with exploration of the affected site of skin and regional lymph node basin. Ultrasound examination is performed: of the scar from removal of primary tumor, the adjacent skin regions, and the regional lymph nodes once every three months from the first to third year, and thereafter once every six months from the fourth to fifth year for searching of in-transit and lymph metastases. In stage IIC abdominal echography may be performed, chest X-ray, or CAT, MRI or PET/CT once every six months during the fourth and fifth year. This becomes necessary in order to be timely found distant disseminations of melanoma. Examination of CBC and S100 protein once every three months from the first to third year. Examination of CBC becomes necessary in order to be found influence on the blood cell series by the standard immunotherapy with interferon, which is administered in that stage, and correction of its dose or possible discontinuation in case of toxic effect.

- **<u>Follow up / Stage III</u> - T every ≥N1M0**

A clinical examination also is applied in this stage, and it includes inspection of the affected site of skin and regional lymph node basin, as well as ultrasound examination of the scar from removal of the primary tumor, the adjacent skin regions, and the regional lymph nodes according to the plan of the previous two stages. Examination of CBC and S100 protein is implemented once every three months from the first to third year, and once every six months from the fourth to fifth year, as well as examination of BRAF V600 mutation in the tumor or metastatic tissue. Abdominal echography, chest X-ray or CAT, MRI or PET/CT were also performed once every six months from the fourth to fifth year for search of any worsening of disease. The examination of BRAF V600 mutation is a very important aspect in the up-to-date follow up of patients with advanced malignant melanoma of skin, because its expression in cells from local relapses, lymph or distant metastases allows patients to be included in a very modern and effective targeted therapy with BRAF inhibitors.

- **<u>Follow up / Stage IV</u> - T every N every M1**

The clinical and ultrasound examinations in that stage are realized according to the same plan as in the previous three stages. Examination of CBC, S100 protein and LDH once every three months from the first to the third year, and once every six months from the fourth to fifth year. Examination of BRAF V600 mutation in tumor or metastatic tissue. Abdominal echography, chest X-ray or CAT, MRI or PET once every three months from the first to third year, and once every six months from the fourth to fifth year. Examination of LDH is performed due to the fact that its levels are very elevated in cases of mass death of cells, as it is observed in lysis of distant metastases on melanoma. Unfortunately, this marker for worsening of diseases is not a specific one, but it still gives us guideline for the development of disease and effect of the administered neoadjuvant therapy.

3. The third part includes data from different forms of therapy applied to patients with malignant melanoma of skin. In this part the noting of the type of therapy is important according to the method, as well as the use of medications together, with the date of its application and the doses per square meter of body surface. The evaluated therapeutic results are entered in this part, and, finally, the reason for discontinuation of patient's report for the study. The medicinal treatment in printed form is divided into lines, which allows the doctor to be guided along them and to apply exactly the definite groups of medications according to the stage. That on its part will result in reduction of the expenses of incongruous prescribing of chemotherapeutic agents. The analysis of the data in this part will allow determination which particular medication will have the best effect for the specific patient and if none is found to be proceeded to another one. As for the non-medicinal conservative treatment, which has been noted, it also must be strictly consistent with the stage of disease and the patient's benefit from it.

Conclusions

1. MM of skin is more common in men while the average age of our patients was higher compared to the data of studies from foreign countries. In the group with SLNB, women predominated while the average age was significantly lower compared to the group without SLNB.

2. A predominant part of our patients were in stages III and IV, while the local relapses were two times more and had briefer time for occurrence. This is in absolute contrast to the data of world literature. An exception from these tendencies are only patients in the group with sentinel biopsy.

3. The evaluated by us mortality rate in patients with malignant melanoma is higher than the standard one. There is no significant difference by that parameter in the groups without or with SLNB, while it is still a little lower in the second group.

4. The factors which significantly worsen the prognosis in our patients with cutaneous malignant melanoma are: location of the tumor in the region of head and neck; achromatic histological variant; Breslow's thickness of the tumor of more than 4.1 mm; Clark's level of invasion in the subcutaneous tissue; presence of distant metastases; presence of local relapses; as well as higher number of patients in later stage.

5. There are no significant differences in survival rate of our patients in the groups without and with SLNB. Such one is, however, evaluated between the groups with and without lymph node dissection, while it is in favor of the second one.

6. The strict observing of the indications and rules for performing of the repeated excision, sentinel biopsy, and lymph node dissection in patients with malignant melanoma of skin, guarantees the success of procedures and is of huge benefit for patients.

7. The double-detection method for sentinel lymph biopsy used by us is sufficiently effective and economically up to the standard.

8. The algorithm, created by us, for diagnostics, treatment, and follow up of patients with malignant melanoma of skin, which we presented in an individual printed form named "Medical Form for a Patient with Malignant Melanoma of Skin" was consistent with the newest world tendencies and guarantees the best complex medical care to patients.

Contributions

1. We, for the first time in Bulgaria, routinely used double-detection method for implementing of SLNB in patients with MM of skin.

2. An algorithm, for diagnostics, treatment, and follow up of patients with MM of skin, was created for the first time in Bulgaria, consistent with the most up-to-date world tendencies.

3. An individual printed form was created for the first time in Bulgaria, named "Medical Form for a Patient with Malignant Melanoma of Skin" based on an algorithm for diagnostics, treatment, and follow up.

Publications and Scientific Reports Related to the Dissertational Work

1. **Strashilov S**, Kirov V, Yordanov A, Nanev V, Mihailova M, Iliev I. Medical Form for a Patient with Malignant Melanoma of the Skin, Made in Accordance to the Most Recent Guidelines for Diagnosing, Treating and follow up the Disease. Journal of Pakistan Association of Dermatologists 2018; 28 (2), 245-249

2. **Strashilov S**, Kirov V, Yordanov A, Nanev V. Contemporary Tendencies in Surgical Treatment and Biopsy of Sentinel Lymph Nodes in Malignant Melanoma of the Skin. Open Access J Surg. 2018; 6 (2), 001-004

3. **Strashilov S**, Yordanov A. Treatment of Malignant Melanoma of the Skin. It is now being printed for the 2019 issue of Journal of General Medicine.

4. **Strashilov S**, Tonchev P, Yordanov A, Kirov V, Nanev V, Mihailova M. Amelanotic Melanoma of the Skin - Comparative Analysis with Pigmented Melanoma of a 5-Year Period. International Journal of Surgery 2018; 55, S101.Abstract of a Poster Presented at the Annual Surgical Conference of ASiT; April 6-8, 2018, Edinburgh, Scotland.

5. Tacheva D, Naydenova T, **Strashilov S.** Sentinel Lymph Biopsy on Malignant Melanoma of the Skin – Role of Operation Theater Nurse. XVIth National Congress of Surgery with International Participation; October 4-7, 2018, Golden Sands, Varna, Bulgaria.

6. Tacheva D, Naydenova T, **Strashilov S.** The Role of Operation Theater Nurse in Realization of Axillary Lymph Node Dissection in Patients with Malignant Melanoma of the Skin. VIIIth Scientific Conference on the Subject: "Novelties in Oncology" University Specialized Hospital for Active Treatment in Oncology; June 15-17, 2018, Pravets, Bulgaria.

7. Kafadarov G, Goranova Z, Kostov I, Ivanova L, Borisov N, Kirov V, **Strashilov S**, Karaivanov M. Rare Cases of Malignant Melanoma Combined with Adenocarcinoma. XVth International Medical Scientific Conference for Students and Young Doctors, October 9-14, 2017, Pleven, Bulgaria.

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