Rīgas Stradiņa universitāte

Kaspars Auslands

# TWO AND MORE CEREBRAL METASTASES COMPLEX TREATMENT OPTIONS

Summary of Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

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Doctoral Thesis was carried out at the clinical basis of Rīga Stradiņš University Department of Neurology and Neurosurgery – Riga East Clinical University Hospital (RECUH) Clinical Centre "Gaiļezers" (Heads of Department Dr. K. Bicāns, Professor J. Ozoliņš) – in cooperation with Latvian Oncology Centre Clinic of Therapeutic Radiology and Medical Physics (Head of Clinics, Ph. D., Assistant Professor O. Utehina) and Radiation Therapy Department (Head Z. Liepa). Diagnostic radiology and control of therapy was mostly performed at the clinical base of Radiology Department – RECUH Clinical Centre "Gaiļezers" (Head Professor G. Krūmiņa). Genetic analysis of evacuated cerebral metastases was performed in collaboration with Rīga Stradiņš University Institute of Oncology (Head Professor E. Miklaševičs).

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The Doctoral Thesis is available at RSU Library and RSU homepage: www.rsu.lv

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# TABLE OF CONTENTS

# ABBREVIATIONS USED IN THE THESIS

| Abbreviation | Explanation in English language |
|---|---|
| **CH** | Chemotherapy |
| **CT** | Computed Tomography |
| **DS-GPA** | Diagnosis – specific graded prognostic assessment |
| **FSRT** | Fractionated Stereotactic Radiotherapy |
| **GPA** | Graded prognostic assessment |
| **GY** | Gray |
| **IGRS** | Image-guided radiosurgery |
| **KPS** | Karnofsky performance scale |
| **MRI** | Magnetic Resonance Imaging |
| **MTS** | Metastasis |
| **PCR** | Polymerase chain reaction |
| **RPA** | Recursive partitioning analysis |
| **RT** | Radiotherapy |
| **RTOG** | Radiation Therapy Oncology Group |
| **SRS** | Stereotactic radiosurgery |
| **TU** | Tumor |
| **WBRT** | Whole-brain radiation therapy |

# INTRODUCTION

Unfortunately the prognosiss for the patients with this diagnosis is generally poor, both in terms of survival, and the qaulity of life. As of the moment of detection of cerebral metastases and unless treatment is not applied, patients decease within nearest months upon advancement of the primary disease or neurologic symptoms. The prognosis of median length-of-life for patients with multiple cerebral metastases generally is poor (2.3–7.1 months) and the aim of the applied palliative care is to ensure as optimal qulity of life as possible (17). During the cancer diagnosis, brain metastasis is more frequently the cause of morbidity and mortality, as well as the cause of cognitive impairment (1; 5).

For a long time there was a widespread belief that the diagnosis of „cerebral metastases" equals to the terminal stage of cancer and the further task of the doctor is to ensure an adequate palliative care to the patient. Oncologists found it difficult to recommend any therapy, taking into account the fact that the most part of chemotherapeutic agents cannot pass the blood-brain barrier. In such cases neurosurgeons operated only solitary, surgically easily accessible, symptomatic metastases, but the radiation therapists prescribed a palliative whole-brain irradiation, thereby provoking development of new metastases and continuous advancement of disease.

However with more and more newest technologies becoming available, the therapy of cerebral metastases is experiencing a rapid progress. Also in Latvia, upon improvement of neurosurgical instruments, operation planning and performance equipment and upon frameless image-guided stereotactic radiosurgery system becoming available since 2010, there are preconditions existing for provision of modern and complex treatment for the patients with cerebral metastases.

Our study includes a section on 40 patients diagnosed with two or more cerebral metastases who received surgical and complex treatment, as well as the first 16 patients with one or more cerebral metastases who underwent stereotactic radiosurgery in Latvia. It should be pointed out that the results of stereotactic radiosurgery treatment of one metastase are crucially important for patients with two or more metastases, since, by applying radiosurgical treatment, more important is total volume of the treated pathology that the number of metastases (for instance, equal volume ratios may be for two small or one major metastasis) and in cases where it is not possible to surgically evacuate all nodes in a patient with two or multiple metastases, and one or more of the remaining cerebral metastases may be exposed to radiosurgery therapy.

It should be mentioned that the use of the prognostic indices to triage patients with brain metastases into a proper treatment algorithm is paramount. In 1997, the Radiation Therapy Oncology Group (RTOG) published a recursive partitioning analysis (RPA) of patients with cerebral metastases (statisctical method for patient's classifying) and grouping patients in three prognostic groups, and grounding these grouping with such variables as age, KPS indicators and the severity of extracranial disease (6). In 2008 the RPA classification in general oncology is supplemented with multiple factors and created a GPA(Graded Prognostic Assessment) (18) classification. It has RPA classification as grounds, that is supplemented with multiple criteria that allow more precisely to detect the possible diagnosis of the patient. GPA system is equal to RPA system, but it is less subjective and easier to use. In addition to primary criteria here is taken into account also the number of diagnosed cerebral metastases, considering that patients with 1–3 metastases have a longer survival period than the patients with metastatic dissemination to multiple organs. According to GPA, the age of the patient, KPS indicators and the presence of extracranial metastases and a number of metastases (one, two, three

or more than three) are evaluated. Moreover, recently in year 2010 there has been developed a diagnosis-specific GPA classification (DS-GPA) (19), that allows individualizing the life expectancy of the patient. It distributes these factors, additionally taking into account the histologic diagnosis of metastases. For instance, the prognostic factors of small cell lung cancer and non-small cell lung cancer include 4 factors, whereas the only significant factor of the breast cancer and gastrointestinal cancer are KPS indicators. However this system has also deficiencies – as an example can be mentioned a group of patients where the primary tumour cannot be established. It means that the issue of the nearest future shall be the enhancement and improvement of prognostic system and implementaiton in clinical practice in order prior to adoption of the final decision on issue how radical would be the choice of therapy offered to patient, the decision would depend on individual assessment and would be grounded upon a totality of multiple factors.

A small number of literature sources evidence that the information of genetic analysis of histology material of evacuated cerebral metastases may be expedient and useful for prognosis of postoperative life expectancy (4; 11; 12). TP53 ir classified as tumor supression gene and mutations in this gene provoke loss of tumor suppression abilities. Although there have been passed more than 20 years since there were discovered first *TP53* gene mutations, however the estimate of its status does not provide for clear indications of its clinical application. Major difficulties are caused by the large-scale mutations and heterogeneity of methodological approach in evaluation of mutations , there are not available also joint criteria in assessment of status of TP53 gene as regards the diagnosis and prognosis. In the framework of our study it was first time in Latvia when there were evaluation of genetic materials of surgically evacuated cerebral metastasis for establishing of clinically important *TP53* gene mutation, as well analysis of obtained data.

The research carried out by us (included 40 patients) has given a significant contribution in development of treatment methods of two or more cerebral metastases, taking into account the high level of complexity and poor survival prognosis, there is a small number of patients in the cumulative studies of the leading literature sources. Usually these count 30–60 surgically treated patients with two and more cerebral metastases included in the study (1; 5; 8; 9).

A contemporary approach to the treatment of cerebral metastasis is targeted to adequate combination of the available treatment methods (surgical therapy, radiotherapy and chemotherapy) in order to ensure an improvement of the quality of life, maintenance of functionality and life expectancy. During the last twenty years there are many guidelines available in the world literature, as well as protocols for multidisciplinary therapeutic approach for treatment of cerebral metastasis, nevertheless the adoption of decisions in the clinical practice is related to many unresponded issues. Therefore it is necessary to further carrying out of studies and summarising of results, particularly for patients with two or more cerebral metastases.

## Structure of the doctoral theses

The thesis is written in Latvian language. The dissertation includes the following sections: introduction, review of literature, materials and methods, results, discussion, conclusions and the bibliography. The total volume of the dissertation are 121 pages, including 16 tables and 42 figures.

## The aim of the study

The aim of the study was to analyze and to compare surgical and combined treatment outcomes and possibilities for the patients harbouring two and more brain metastasis, as well as to evaluate the first results of the radiosurgery treatment of the brain metastases in Latvia.

## The objectives of the study

1. To gather the tactics and results of the surgical treatment in the patients having one and more brain metastases.
2. To evaluate the impact of various factors to the post-operative length-of-life.
3. To evaluate the differences of survival indicators upon performance of radical or partial evacuation of brain metastases.
4. To analyse the results of treatment of patients who had partial evacuation of brain metastases with applied complex therapy (WBRT or WBRT and chemotherapy) in postoperative period.
5. To evaluate the first results of radiosurgery treatment of the brain metastases in Latvia.
6. To identify clinically significant mutations of *TP53* gene in cerebral metastases and to detect their relation to the postoperative survival.

## The hypotheses of the study

1. The outcomes of treatment of two or more brain metastases correlate with radicality of surgical manipulations.
2. The use of surgical resection in the case of two and more cerebralmetastases setting is not associated with increased risk of postoperative morbidity and mortality.
3. The application of targeted combined treatment in patients harbouring two and more cerebral metastases preserve the quality of life of patient and extends patient's survival also after a partical evacuation of metastases.
4. The application of radiosurgery treatment in the cases of small (up to 3cm in diameter) multiple cerebral metastases has seen a positive impact.

## The topicality, scientific novelty and practical importance of the study

1. Summarized and analysed results of the treatment in patients with two and more cerebral metastases who have undergone surgical and combined treatment of their metastases.
2. Summarized and analyzed results in first 16 patients with cerebral metastases in Latvia that have been treated with radiosurgery.
3. Specified postoperative survival prognostic parameters in patients with two and more cerebral metastases.
4. First time in Latvia in the framework of the study there was performed an assessment of the genetic material of the surgically evacuated cerebral metastases for detection of *TP53* gene mutation, as well as the analysis of obtained data.

5. Developed recommendations for the choice of tactics of therapy applicable to patients diagnosed with two and more cerebral metastases.

## Personal contribution to the study

The author has independently collected the information about the patients involved in the study upon filling in a particularly elaborated quastionnaire, as well as summarized, systemized and analyzed clinical data of patients, upon using medical documentation.

During the surgical treatment stage, the author had performed a surgery himself, or was engaged as an assistant. Author had participated in a complex treatment planning, the symptomatic therapy and a follow-up of the patients.

# 1. MATERIALS AND METHODS

## 1.1. The group of patients after surgical resection of metastases, and combined therapy

### 1.1.1. The study population

A retrospective analysis (years 2005–2011) of the anamnesis of patients with two and more cerebral metastases surgically treated at Riga East Clinical University Hospital Clinics „Gaiļezers".

The criteria of inclusion of the patients in the study were the folowing:

- Diagnosed two and more cerebral metastases;
- Performed surgical evacuation of all the nodules of cerebral metastases or partial evacuation of nodules of cerebral metastases;
- Preoperative clinical condition of the patient according to the Karnofsky Performance Scale – ≥ 60 score;
- Patient's gender – ≥ 18 years;
- A maximum possible examination performed for detection and evaluation of primary tumor;
- Histologically approved diagnosis of cerebral metastases;
- Information available on the postoperative length-of-life;
- Data available on the course of complex postoperative therapy and the results thereof;
- Available histologic agents of evacuated cerebral metastases for carrying out of genetic analysis.

Patient's gender, primary tumor and the obtained histological diagnosis were not of significance for inclusion in the study.

Exclusion criteria for patient's participation in the study were the following:

- Preoperative clinical condition of the patient according to Karnofsky Performance Scale – < 60 score;
- Localization of metastasis in the area of the brain stem;
- Comorbidities that do not allow performing surgical and combined therapy according to the planned protocol;
- Dissemination of meningeal carcinomatosis.

Patients were treated according to the further mentioned protocols:

- ✓ The 1st protocol. Only surgical total evacuation of all diagnosed cerebral metastases;
- ✓ The 2nd protocol. Only surgical, but partial evacuation of symptomatic cerebral metastases;
- ✓ The 3rd protocol. Partial surgical evacuation of symptomatic brain metastases with subsequent whole-brain radiotherapy (WBRT);
- ✓ The 4th protocol. Partial surgical evacuation of symptomatic brain metastases with subsequent whole-brain radiotherapy (WBRT) and chemotherapy.

### 1.1.2. The characteristics of study group patient's questionnaires

Patient's age, gender, type of primary cancer, the time from the first diagnosis of cancer to the diagnosis of brain metastases, the number of metastases at the time of surgery, the presence or absence of systemic disease, the number of craniotomies, postoperative complications, received postoperative radiotherapy or chemotherapy, and postoperative survival rates were entered into a computer database.

Karnofsky Performance Status (KPS) score was evaluated before surgery and at hospital discharge.

All patients were divided into Radiation Therapy Oncology Group recursive partitioning analysis (RPA) classes according to *Gaspar et al*.. Class I consisted of patients with Karnofsky Performance Status scores of at least 70, age under 65 years with controlled primary disease, and no evidence of extracranial metastases; Class II-patients whose clinical condition according to the Karnofsky scale corresponds to at least 70 score, but who are older than 65 years or with uncontrolled primary disease or with extracranial metastases; Class III klase – patients whose clinical condition according to the Karnofsky scale corresponds to at lease 70 score.

During the time period from 2005–2011 all the patients had planned surgical resection and preoperatively received antibiotics, anticonvulsants and steroids. All the operations included the following methods:

1) neuronavigation to ensure a precise access to metastasis and alleviate its intraoperative localization and a cautious and direct access;

2) cavitron ultrasonic surgical aspriator for the reduction of the volume of metastasis that faciliatated the resection of metastasis in „ne block" manner;

3) surgery microscope in cases where the metastases are localized subcortically or paramedian.

Postoperative complications were evaluated and registered. Deaths occurence within 30 days of surgery were considered as a perioperative mortality. Karnofsky Performance Status (KPS) score of the patient was evaluated before the surgery and at hospital discharge.

In order to calculate the postoperative survival rate, data available at the register of inhabitants were used (survival was calculated from the date of surgery to the termination of the study or *exitus letalis* of the patient).

## 1.2. Radiosurgically treated patients group

### 1.2.1. The study population

The records of patients with brain metastases who were treated with image-guided radiosurgery in Riga Eastern Clinical University Hospital Therapeutic Radiology and Medical Physics Clinic between January, 2010 and March, 2012 were retrospectively reviewed.

### 1.2.2. The characteristics of study group patient's questionnaires

Patients age, gender, type of primary cancer, Karnofsky Performance Status (KPS), the number of metastases at the time of radiosurgery, received radiosurgical treatment or fractionated radiotherapy in combination either with or without the whole-brain radiotherapy, radiation dose and volume limits were entered into a computer database.

The treatment isodose volume for each metastasis was calculated using GammaPlan software. The total treatment volume for each patient was the sum of the treatment volumes for all treated metastases

The patients were followed up with the contrast-enhanced MR imaging at 6–8 weeks following SRS treatment, and then every 3 months until the end period of data collection or patient demise.

The results of examinations of MR imaging of the treated cerebral metastases were defined as effectiviness of applied stereotactic radiosurgery, and these were subdivided in the four further mentioned groups:

1) complete response (CR) – a complete resolution of the enhancing lesion;
2) partial response (CR) – > 50% reduction in the size of the lesion;

3) stable disease (SD) – no change in the dimension of the lesion, or < 50 % reduction;

4) progression of the disease (PD) – > 25% increase in the size of the lesion.

In order to calculate the postoperative survival rate after the received radiosurgery treatment, the data available at the register of inhabitants were used (survival was calculated from the date of surgery to the termination of the study or *exitus letalis* of the patient).

### 1.2.3. Radiosurgery technique

During the computed tomography (CT) and treatment procedures, the patients were immobilized using the BrainLAB non-invasive stereotactic immobilization mask system.

Magnetic resonance imaging (MRI) scan was available for each patient to help to define the target volume. The tumour was delineated using MRI images, and after that co-registration between CT and MRI images was done in order to transfer the target volume to the CT images that are used for dose calculations. The clinical target volume (CTV) was defined as the union of CTVs delineated on MRI images as well as on CT scans. No margin was added for subclinical extension. The margin for the planned target volume (PTV) was 1 mm in all directions added to the CTV.

Stereotactic radiosurgery (SRS) was planned with EclipseTM (Varian Medical Systems INC, USA) treatment planning system (TPS) using volumetric intensity modulated dose delivery by RapidArcTM (Varian Medical Systems INC, USA) or intensity modulated radiation therapy (IMRT) with 7–9 intensity modulated treatment fields (Figure 1). The treatment plan was normalized to 80% isodose line, and normalized 100% isodose line

encompassed the PTV. Linear accelerator NovalisTxTM equipped with a high-definition multileaf collimator (MLC 120HD) was used for SRS delivery. All plans were delivered using photon energy of 6 MV and dose rate of 1000 monitor units (MU) per minute. To correct the patient position ExacTrac® 6D (3 transversal directions and 3 rotations) IGRT (Image-Guided Radiotherapy) System (BrainLAB GMBH, Munich, Germany) was used.

### 1.2.4. Ensuring the quality of performance of the radiosurgery

All treatment plans were verified from dosimetric point of view via complex verification procedure, which included dose plane measurements and point dose measurements in phantom and Winston-Lutz test. Dose plane measurements were performed using Gafchromic EBT 2 films and evaluated performing gamma index method. Generally results were considered acceptable if more than 90% of evaluated points passed gamma criteria 1 mm/5%. Point dose measurements were performed using pinpoint 3D (PTW, Freiburg, Germany) ionization chamber. The tolerance level for the point dose measurements was set to 3%. The treatment unit was considered to be appropriate for treatment delivery if isocentre sphere, as measured via Winston-Lutz test, did not exceed 1 mm.

## 1.3. Genetic analysis of histologic material

### 1.3.1. Isolation of DNA from formalin-fixed paraffin embedded (FFPE) tissues

For isolation of DNA from formalin-fixed paraffin embedded tissues were used DNA Isolation Kit (QIAamp DNA FFPE Tissue Kit, Qiagen, Germany) according to the manufacturer's instruction.

### 1.3.2. PCR analysis fo detection of *TP53* gene mutations (exons 5–8)

Table 1.3.2.1

**Primers for polymerase chain reaction (PCR)**

| Gene | Primer | Exons | Hybr.temp. °C |
|---|---|---|---|
| TP53 | CAACTCTGTCTCCTTCCTCTTCCTAC | 5A | 58 |
| | AGCCATGGCACGGACGCG | | 58 |
| | CTCCTGCCCGGCACCCGC | 5B | 58 |
| | CTAAGAGCAATCAGTGAGGAATCAGA | | 58 |
| | CAACCACCCTTAACCCCTCCT | 6 | 58 |
| | AGACGACAGGGCTGGTTGC | | 58 |
| | AGGCGCACTGGCCTCATC | 7 | 58 |
| | GAGGCTGGGGCACAGCA | | 58 |
| | GACCTGATTTCCTTACTGCCTCTTG | 8 | 58 |
| | AATCTGAGGCATAACTGCACCCTT | | 58 |

The course of the examination process is described in the sequence of performed activities.

Primers for PCR. There is one pair of primers necessary for one reaction, that amplifies the PCR product containing the *TP53* gene (exon) and a part of intron and place of splicing.

Each sample is prepared in triplet in order to exclude the error of dropping and analysis could become more precise and accurate.

The necessary agents are taken from the freezer and defrosted prior to commencement of procedure. If necessary, the agents are centrifuged, ensuring their optimal use.

The PCR mixture is prepared (contents are indicated for one reaction):

| | |
|---|---|
| 10 × PCR buffer solution | 2 µl; |
| 10 mM dNTP mix | 0,4 µl; |
| 50 mM $MgCl_2$ | 0,4 µl; |
| primer 1 | 0,4 µl; |
| primer 2 | 0,4 µl; |
| Syto 9 | 2 µl; |
| DNA | 10 ng; |
| Taq polymerase Speed Star | 0,5 U; |
| $H_2O$ | up to 20 µl; |

The prepared PCR mixture is diffused into 0.2 ml test tubes or in adjusted discs of the device Rotor-Gene 6000, that are sealed off with thermal sealing.

Disc of the device Rotor-Gene 6000 shall be placed in the disc cartridge and affixed with the disc fixation ring.

The computer is on and the PCR real time detection device Corbett-Research 6000 is on as well.

The software Rotor-gene 6000 Series Software 1.7 is activated

The icon „New run” is switched on in the software:

| | | | |
|---|---|---|---|
| PCR | 95 °C | 10 seconds | |
| | 65 °C | 5 seconds | × 10 |
| | 72 °C | 20 seconds | |
| | 95 °C | 1 second | |
| | 72 °C | 1 minute | |
| AIC | 72–86 °C | 2 seconds on a step | 0,1 °/in a step |

### 1.3.3. Sequencing of *TP53* gene (exons 5–8)

The course of the examination process is described in the sequence of performed activities.

Polymerase chain reactions (PCR) fragment preparation for DNA sequencing.

Primers for PCR reaction are indicated in the table 1.3.2.1. There shall be necessary one pair of primers for one reaction that amplifies the PCR product, which contains one or several exones or part of introns and place of splicing.

PCR mixture is prepared (contents is indicated for one reaction):

| | |
|---|---|
| 10 × PCR buffer solution | 5 µl |
| 10 mM dNTP mix | 0,5 µl |
| primer 1 | 1 µl |
| primer 2 | 1 µl |

| | |
|---|---|
| DNA | 50 ng |
| Taq polymerase | 1 U |
| $H_2O$ | up to 50 µl |

PCR mixture by 50 µl is diffused in PCR plate and sealed with the film. PCR plate is put in cycler and the program is chosen as stated hereinafter:

| | | |
|---|---|---|
| 95 °C | 5 minutes | |
| 95 °C | 30 seconds | × 40 |
| 58 °C * | 45 seconds | |
| 72 °C | 40 seconds | |
| 72 °C | 3 minutess | |
| 40 °C | pause | |

* Hybridization temperatures for various pairs of primers are displayed in the Table 2.1.1.

For purification of PCR fragments there is used MinElute 96UF PCR Purification Kit, complying with the manufacturer's instructions.

After the purification of samples the concentration is measured with NanoDrop 1000 spectrophotometer. The concentrations of samples after purification shall be 5 ng/µl. For one sequencing reaction there shall be necessary 7.5 ng of PCR product. If the concentration exceeds the one necessary, the purified PCR product may be diluted with a distilled water until the necessary concentration is obtained, whereas in case of concentration less than necessaary the sequencing reaction shall be added a major PCR product volume, respectively reducing the water content to one reaction.

For DNA sequencing reaction there shall be used one primer from the pair of primers that was used for obtaining the PCR product (Table 1.3.2.1).

The sequencing reaction mixture is prepared (content is indicated for one reaction):

| | |
|---|---|
| Sequencing buffersolution | 2 µl |
| Big Dye v3.1. | 0,15 µl |
| primer | 1 µl |
| PCR product (concentration 5 ng/ul) | 3 µl |
| $H_2O$ | 3,85 µl |

Sequencing reaction mixture is diffused by 10 µl in PCR plate. PCR plate is put in the cycler and a respective program shall be chosen:

| | | |
|---|---|---|
| 95 °C | 1 minute | |
| 94 °C | 25 seconds | |
| 58 °C | 20 seconds | × 30 |
| 60 °C | 30 seconds | |
| 72 °C | 40 seconds | |
| 40 °C | 5 minutes | |

Sequencing reaction product purification:

For the prepared product there shall be added 1 µl NaOAc and 20 µl 96% of ethanol in every fossete.

Plate is put into the Eppendorf centrifuge and cetrifuged for 40 minutes at a temperature of 4 °C and to 3200 rpm spins.

The ethanol/NaOAc mixture is poured off and the plate is put on the filter paper in centrifuge upside down and centrifuged for 2–3 seconds.

In every fosette there is added 70 µl of 70% ethanol and centrifuged for 15 minutes at a temperature of 4 °C and to 3200 rpm spins.

The ethanol is poured off and put for 1 minute at a temperature of 95 °C to let the spare ethanol to evaporate.

Afterwards 10 µl of formamide is added and sealed with the plate film. PCR plate is put in the cycler and a respective program is chosen :

95 °C 2 minutes

40 °C 5 minutes

For sequencing there shall be used a 36 cm long capillary and polymer POP-7 with Applied Biosystems recommended standard conditions for electrophoresis.

## 1.4. Statistical analysis of the data

Data processing was performed upon using the software IBM SPSS v.21.

Quantitative variables were described with the central tendency ratios – mode (Mo), arithmetic mean (M) and Standard deviation (SD). In case where the distribution of data drastically differs from the normal distribution, the median was calculated (Me), first (Q1) and third (Q1) quartile interquartile dispersion amplitude (ΔQ).

For assessment of statistic effect of quantitative signs, upon application of Cohen's „d" value, the following division of effect was used:

- 0,1–0,20 (small)
- 0,20–0,50 (average)
- > 0,50 (major).

For verification of compliance of normality a Shapiro-Wilk test was applied.

Alpha level of 0.05 was chosen as a level of significance; thereby p-value of statistical text that is less than 0.05 shall be an evidence of statistical probability. If the obtained p-value is greater than the chosen alpha level, then the null hypothesis cannot be rejected. If the obtained p-value is less than chosen alpha level then the null hypothesis is rejected and an alternative hypothesis accepted. As a result of statistical test a precie p-value was indicated and 95% of credibility interval (CI).

Categorical or quality variables were characterized as quantity and percentage proportion.

Categorical or quality independent signs were compared with Pearson chi square test or Fisher's exact test according to their terms of use.

For analysis of categorical data for assessment of statistical effect a Cramer's V value was used with the following breakdown:

- 0.1–0.3 (small)
- 0.3–0.5 (average)
- > 0.5 (major).

Analysis of survival probability was estimated with the Kaplan-Meier method log-rank test and Cox proportional hazards regression model.

# 2. RESULTS AND THEIR ANALYSIS

## 2.1. Treatment results and their analysis for the patients after surgical resection of metastases and combined therapy

### 2.1.1. Patient group characteristics

During a time period from 2005–2011 in patients with two or more cerebral metastasis there was performed one or multiple craniotomies during one surgery. The mean age of patients was 58.13 years (standard deviation – 10.56). Minimum age is 35 years, but the maximum – 82 years, age range is 47 years. The modal or most common age was 51 years, the mean age – 56 years. First age quartiel is 51 years, third age quartile – 67 years, interquartile range of age is 16 years meaning that 50% of patients are aged from 51 to 67 years. From 40 patients included in the study, 20 patients (50 %) were female and 20 patients (50%) – male patients. The mean age of female patients (SD) is 54.15 (8.81), but the mean age of male patients (SD) – 62.10 (10.87) years.

The most frequent histologic forms of primary tumours were melanoma (n = 15) and breast cancer (n = 9).

For the major part – 23 patients (57.5%) – data were available on controlled primary tumour, whereas for 17 patients (42.5%) there has been established progression of primary disease.

Meanwhile 24 patients (60%) presented metachronous development of metastasis, whereas 11 patients (27%) with cerebral metastases had synchronous development, but in 5 cases (13%) the primary tumour was not diagnosed.

Prior to planned surgical treatment all patients underwent assessment of their neurological status and the following results were obtained: 23 patients

(57.5%) prior to operations presented with neurological deficits, meanwhile 17 patients were without any neurological deficits, but diagnosis of cerebral metastasis was possible due to symptoms of increased intracranial pressure (n = 10), that exposed as headache, nausea and vomitting. On its turn to5 of 17 patients the disease manifested with seizures (generalized or partial), but for 2 patients the cerebral metastases were detected upon performance of extended work-up regarding their primary malignancy The preoperative clinical condition of 18 patients (45%) was of at least $\geq 70$ scores according to Karnofsky-Performance Status.

Upon dividing patients in RPA classes, the data of 10 patients corresponded to Class I, data of 8 patients – to the Class II and data of 22 patients to Class III respectively.

According to the results of the magnetic resonance contract imaging 24 patients had their cerebral metastases localized in one of the brain hemispheres, but for 3 patients the metastases were localised in any of anatomically deep and clinically significant localization sites (in basal ganglions, *thalamus*, brain stem, *corpus callosum*, cerebellar hemispheres). Meanwhile for 13 patients metastases were localised in both, one and in other of previoulsy mentioned localization sites.

Sizes of metastases according to the data of magnetic resonance contrast imaging were ranging from 1.2 to 4.6 cm, mean size – M = 2.44, SD = 0.80 cm.

Number of diagnosed metastases for surgically treated patients: two metastases – in 20 cases, three metastases – in 11 cases, four metastases – in 6 cases, five and more metastases – in 3 cases.

### 2.1.2. Surgical approach and volume of evacuated metastases

Depending on the number of cerebral metastases and the site of localization there were applied various types of surgical approach: a) in 32 cases – one osteoplastic trepanation; b) in 7 cases – two osteoplastic trepanations; c) in 1 case – three osteoplastic trepanations. All trepanations were performed during one surgery.

In 31 cases there was applied surgical treatment to patients who have been diagnosed with 2–3 cerebral metastases, but in 9 cases – patients with 4–6 cerebral metastases.

For 17 patients all the diagnosed cerebral metastases have been evacuated. In 12 cases these were patients with two metastases, but in 5 cases there have been surgically evacuated all three of the diagnosed cerebral metastases. For 23 patients the metastases were evacuated partly, i. e., thoses metastases were removed that were located in sympotamic and surgically accessible localization sites. In 8 cases these were patients with two cerebral metastases, in 6 cases – patients with three metastasis, in 5 cases – patients with four cerebral metastasis and in 4 cases – patients with five and six cerebral metastasis.

Radical evacuation of metastases was possible in patients with two or three cerebral metastases. In patients with two metastasis one metastase was evacuated in 8 cases (40%), but all metastases – in 12 cases (60%); meanwhile in case of three metastases one metastase has been evacuated in 2 cases (18%), two metastases – in 4 cases (36%) and all – in 5 cases (46%). Whereas in patients with four to six metastases the volume of ecavuation was only partial.

### 2.1.3. Perioperative complications

Perioperative complications were registered in 30 day's time after surgical treatment and and were splitted in two groups mentioned hereinafter:

1) local complications that are related to the site of surgical access;

2) systemic complications related to extracranial metastases and decompensation of comorbidities.

In postoperative period there were observed three local complications (one postoperative meningitis related to a penetrating wound and local haemorrhage in surgical site), that the wound revision and evacuation of haematoma was not necessary to.

30 days postoperative mortality was observed in 4 cases (preoperative mortalitality 10%). The cause of death in the given cases were a rapid progression of extracranial tumour (3 cases, patients with preoperative KPS < 70 scores corresponded to RPA Class III) and acute cardiopulmonary failure (1 case).

### 2.1.4. Evaluation of postoperative clinical conditions of patients according to Karnofsky scale

After the surgery, upon discharge from hospital, many patients has their clinical condition assessment according to Karnofsky scale. The assessment in 37.5% cases (15 patients) indicated to improvement of the early clinically functional condition for 10 scores according to Karnofsky scale. In 60% of cases (for 24 patients) the clinical condition remained unchanged and was assessed as stable, but in one case – decreased for 10 scores.

Upon performance of comparison of KPS ratio in postoperative period for patient groups with 2–3 or 4–6 cerebral metastases it was clarified that

better ratios (a stable clinical condition or improvement of KPS ratio) were observed in patient group with 2–3 cerebral metastases.

### 2.1.5. Postoperative survival ratios and the analysis of infuencing factors

The postoperative survival ratios of patients were anlyzed by using the Kaplan-Meier method, according to which the median survival to group of patients that were surgically treated and who received a complex therapy was 4.86 months (95% CI: 3.24–6.47). The Kaplan-Meier survival core for the group of 40 patients is displayed in the figure 2.1.5.1.

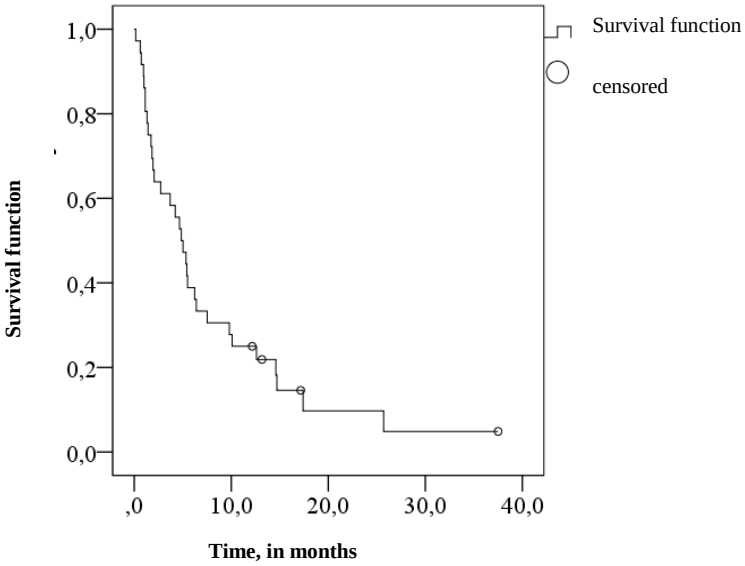


Figure 2.1.5.1. **Postoperative survival curve of a group of 40 patients**

From than group of 40 patients who were treated by applying total or partial surgical evacuation of metastasis and combined therapy, the

postoperative survival ratios for 30 patients (75%) did not reach one year, whereas for 8 patients (20 %) postoperative survival exceeded 1 year, but did not exceed 2 years. More than a 2 years postoperative survival was reached by 2 patients (5 %), please see the Figure 2.1.5.2.

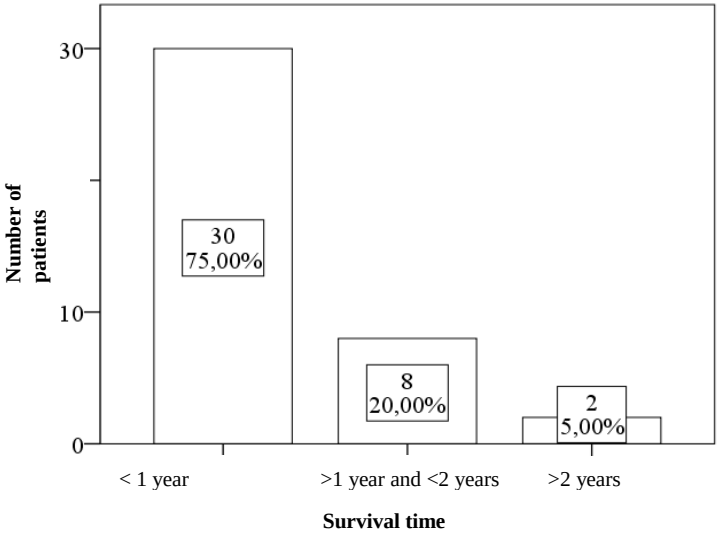


Figure 2.1.5.2. **1 and 2 years postoperative survival ratios**

Taking into account the data of results of the postoperative survival that in majority of patients (75%) death occured during the first postoperative period, additionally in this time period there was carried outanalysis of distributio of survival data. The median survival during the first postoperative year is 3.16 months, but the most frequect or modal mortality is 1.1 month and the ratio of asymmetry of distribution – 0.76 (0.42).In the first postperative year during the time interval of 1.3 to 5.5. month deach occured to 50% of patients from this group of patients.

Upon evaluation of survival rates regarding the results of histological analysis of evacuated cerebral metastases, the poorest prognosis was in case of ovarian and cervix uteri cancer (3.41 months), meawhile the longest survival was observed in kidney cancer patients (14.7 months). Nevertheless the obtained data did not provided for carrying out of statistical analysis taking into account the small number of patients in respective groups. For the most frequent forms of primary malignanices the survival ratios had practically no difference – respectively 7.8 months in case of melanoma and 7.9 months in case of breast cancer.

### 2.1.5.1. Analysis of invariants of factors influencing the postoperative survival

There was carried out the Mann-Whitney U test for the following postoperative prognostic factors (please see the Table 2.1.5.1.1):

Table 2.1.5.1.1

**Indicators characterizing 40 patient group and survival (in months)**

| Variable | N | Median survival (95% CI), months | p-value |
|---|---|---|---|
| Gender | | | |
| Male | 20 | 5.33 (4.45–6.20) | 0.59 |
| Female | 20 | 2.06 (0.30–3.81) | |
| Age (in years) | | | 0.12 |
| ≥ 65 | 30 | 5.33 (4.20–6.45) | |
| < 65 | 10 | 3.70 (0.13–7.26) | |
| Presentation | | | |
| Sunchronous | 29 | 7.53 (2.61–23.45) | 0.46 |
| Metachronous | 11 | 3.70 (1.06–6.33) | |
| Primary tumour controlled | | | |
| Yes | 23 | 3.60 (0.83–6.37) | 0.90 |
| No | 17 | 5.50 (3.65–7.34) | |
| No. of brain metastases | | | |
| 2–3 | 29 | 5.5 (3.09–7.90) | 0.03* |
| 4–6 | 11 | 2.06 (0.1–4.72) | |

Continuation of table 2.1.5.1.1

| | | | |
|---|---|---|---|
| Preoperative KPS | | | |
| ≥70 | 18 | 5.43 (2.58–8.27) | 0.02* |
| < 70 | 22 | 2.06 (0.1–5.73) | |
| RPA | | | |
| I | 10 | 12.07 (2.16–21.97) | < 0.05* |
| II | 8 | 4.86 (3.20–6.52) | |
| III | 22 | 2.06 (0.1–5.73) | |
| Radicalness of Resection | | | |
| All | 17 | 6.23 (3.40–9.05) | < 0.01* |
| Partially | 23 | 2.06 (0.1–5.14) | |
| Postoperative WBRT | | | |
| Yes | 11 | 4.66 (0.1–10.90) | 0.41 |
| No | 29 | 4,86 (2.70–7.02) | |
| Postoperative chemotherapy | | | |
| Yes | 7 | 4,66 (0.1–11.08) | 0.58 |
| No | 33 | 5.03 (3.02–7.03) | |

Statistically credible ($p < 0.05$) influence in survival analysis was established for the following factors: 1) radicalness of resection; 2) number of metastases; 3) RPA Class; 4) KPS preoperative score.

For the group of patients that were performed to the evacuation of all diagnosed cerebral metastases, the length-of-life was significantly longer that in the group of patients that included patient who had been partially evacuated their cerebral metastases, $p < 0.01$. Vin case of evacuatimonths months (95% CI: 3.40–9.05), meanwhile in case of partial evacuation of cerebral metastases the median survival was 2.06 months (95% CI: 0.1–5.14). Grounding upon the *log-rank* test, it was concluded that the radicalness of resection has statistical credibility over the patient's survival ($p < 0.05$) and as it is displayed in the Figure 2.1.5.1.1, resection of all metastases prolongs the length-of-life.

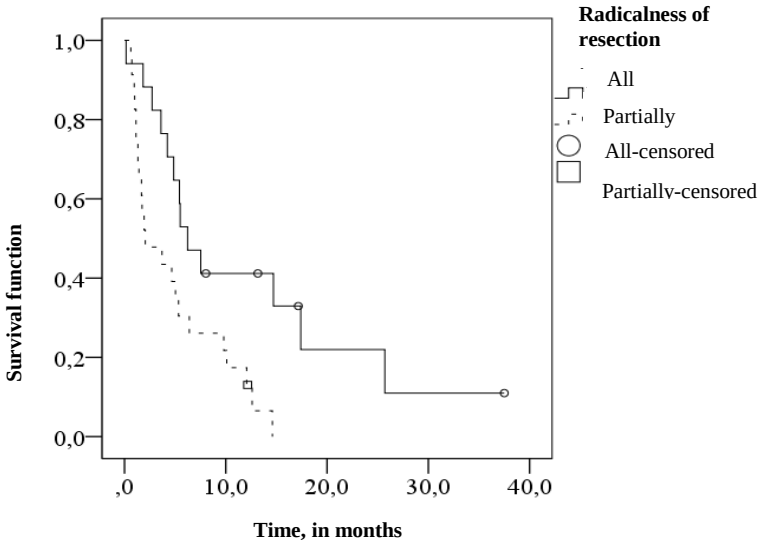


Figure 2.1.5.1.1. **Kaplan-Meyer survival curves depending on volume of resection (all / partially)**

Upon analyzing the number of metastases, in case of 2–3 metastases the median postoperative survival of patients was 6.23 months (95% CI: 3.10–7.10),whereas in case of 4–6 cerebral metastases the median survival was 2.06 months (95% CI: 0,1–4,72). Number of metastases had not only visually observable (Figure 2.1.5.1.2), but also a statistically credible (log-rank test, $p = 0.03$) influence on survival – in the group of patients with 2–3 cerebral metastases the survival was statistically credible longer than for the group of patients with 4–6 cerebral metastases.

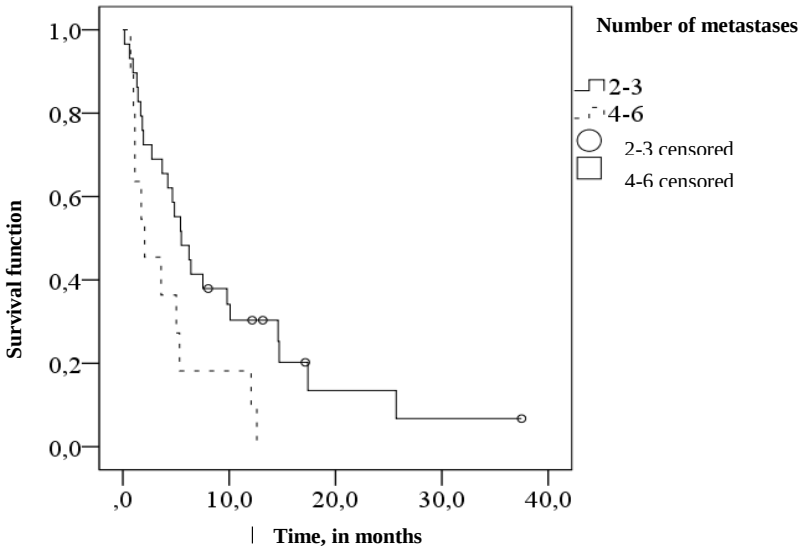


Figure 2.1.5.1.2. **Kaplan-Meier survival curves depending on number of metastases (2–3 / 4–6)**

Upon distribution of patients in RPA Classes I, II and III, the postoperative survival indicators were respectively 12.07 months (95% CI: 2.16–21.97), 4.86 months (95% CI: 3.20–6.52) and 2.06 months (95% CI: 2.06–5.73). Grounding upon the *log-rank* test, it as established that the patient's appurtenance to the RPA Class has a statistically credible ($p < 0.05$) impact on patient's length-of-life and as it can be visually seen from the Figure 2.1.5.1.3 upon increase of RPA Class, the length-of-life decreases respectively.

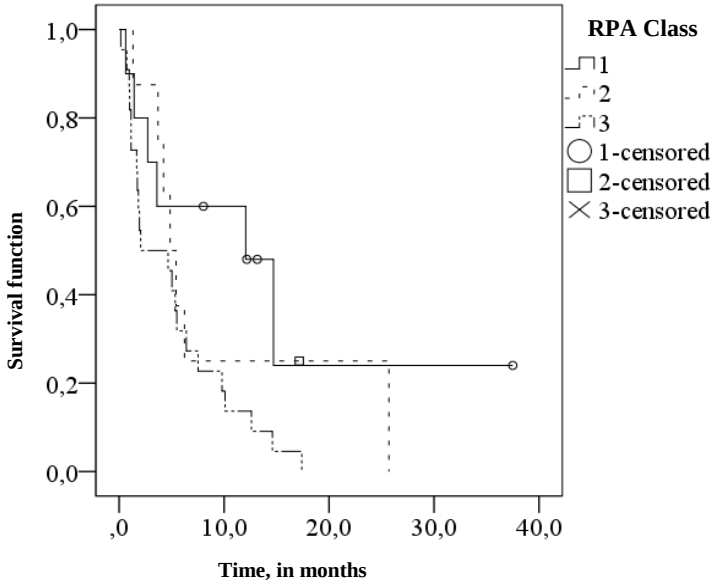


Figure 2.1.5.1.3. **Kaplan-Meier survival curve according to RPA Class I, II and III**

Upon analysing the preoperative KPS ratios, the further mentioned data were obtained displaying that in the group of patients with KPS ratio ≥ 70 scores the survival was statistically credibly (log-rank test, $p < 0.01$) differing from the group of patients that included patients with KPS ratio < 70 score. In patients whose KPS was ≥ 70 score, the median survival was 5.43 months (95% CI: 2.58–8.27), whereas in those patients whose KPS ratio was < 70 score, the median survival was 2.06 months (95% CI: 0.1–5.73). In the patient's group Kaplan-Meier survival curve according to the KPS ≥ 70 / KPS < 70 score is displayed in Figure 2.1.5.1.4.

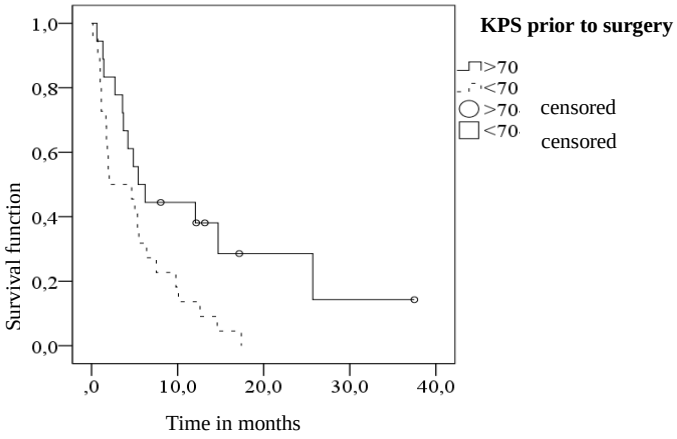


Figure 2.1.5.1.4. **Kaplan-Meier survival curves according to KPS prognostic indicator (≥ 70 / < 70)**

### 2.1.5.2. Analysis of multivariate factors influencing postoperative survival

Those prognostic factors that produced a statistically credible impact ($p < 0.05$) using univariate analysis (radicalness of resection, number of metastases, RPA Class, KPS preoperative indicator), were included in the multivariate analysis, by using Cox's proportional hazards model. Results are summarized in the Table 2.1.5.2.1. Taking into account the number of patients included in the study, none of the signs that was statistically credible in univariate analysis is statistically credible in Cox's proportional harzards regresssion model, nevertheless as it is obvious from the Table 3.1.5.2.1, the hazard ratio (HR) in group whose KPS ≥ 70, is 2.05 times greater than in the group where KPS < 70. Similarly, when analysing the group's number of metastases, we can conclude than in the group with 4–6 metastases the hazard ratio (HR) is 1.58 in comparison to the group with 2–3 metastses.

A slightly higher hazard ratio (HR = 1.95) is in case of resection of all metastases in comparison to the group of patients that the metastases were removed only partially.

Table 2.1.5.2.1.

**Results of multivariate analysis**

| Indicator | p-value | HR (95% CI) |
|---|---|---|
| Preoperative KPS (≥ 70 / < 70) | 0.14 | 2.05 (0.77–5.45) |
| RPA Class (I, II, III) | 0.23 | 1.98 (0.63–6.22) |
| Number of metastases (2–3 / 4–6) | 0.27 | 1.58 (0.69–3.65) |
| Resection volume (resection of all metastases /partial resection) | 0.17 | 1.95 (0.74–5.15) |

## 2.1.6. Treatment results and course of treatment within protocols 1, 2, 3, 4 in the group of patientswho received surgical resection and combined therapy

Out of 40 patients for 17 (42.5%) patients it was possible to perform surgical evacuation of cerebral metastases and taking into account the radicalness of surgical therapy, a further complex therapy has not been applied.

Out of 23 patients for 14 (60.86%) patients after partial surgical evacuation of cerebral metastasis in early postoperative period a complex therapy has been applied – either the whole-brain radiation therapy (WBRT), that was applied to 9 patients, or a whole-brain radiation therapy (WBRT) in combination with chemotherapy (CH) – in 5 patients.

Out of 23 patients for whom not all cerebral metastases were evacuated, for 9 patients (39.13%) a further complex therapy was not applied.

Postoperative median survival indicators for treatment protocol are summarized in Table 2.1.6.1.

Table 2.1.6.1

**Postoperative median survival indicators in various treatment protocol patients**

| Protocol No. | Number of patients | Median survival (95% CI, months) |
|---|---|---|
| 1. Surgery (all) | 17 | 6.23 (3.40–9.05) |
| 2. Surgery (partial) | 9 | 1.73 (0.85–2.60) |
| 3. Surgery (partial) +WBRT | 9 | 5.33 (0.1–15.26) |
| 4. Surgery (partial)+WBRT+CH | 5 | 4.86 (3.15–6.56) |

Upon analysing the postoperative survival outcomes depending on the applied treatment protocol it was established that the chosen treatment protocol has statistically credible impact on survival according to Kaplan-Meier method (log-rank test, $p = 0.01$).

As we can see from the Table 2.1.6.1, the longest survival rate (6.23 months (95% CI: 3.40–9.05)) was in those patients who undergone resection of all metastases , meanwhile the shortest survival rate (1.73 months (95% CI: 0.85–2.60)) was in patients who had undergone partial evacuation of metastases. Upon performance of partial evacuation of metastases and during further treatment period, upon application of combined radiation therapy or radiation therapy and chemotherapy, the median survival indicators were approximately similar.

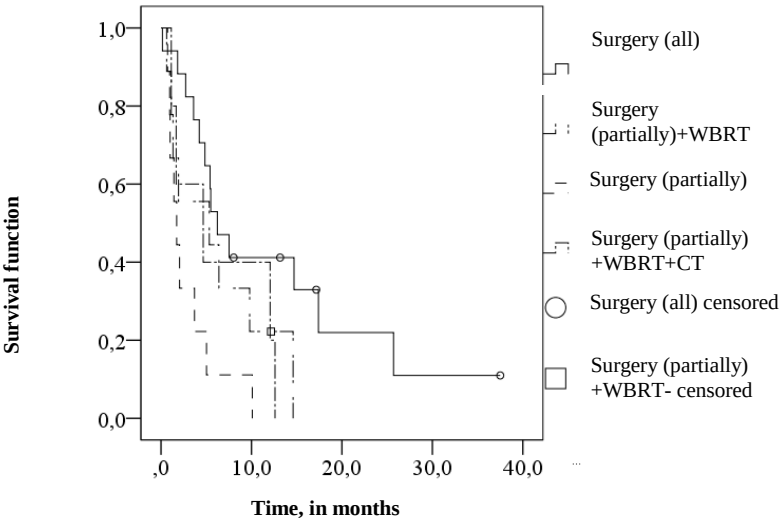


Figure 2.1.6.1. **Kaplan-Meier survival curves according to the applied treatment protocol**

## 2.2. Results in radiosurgically treated patients group

The novelty of radiosurgical therapy in Latvia was grounds for the fact that not only the patients with two or more cerebral metastases were included in the study, but also patients with one cerebral metastase with a purpose to analyse the outcomes of application of absolutely new method in Latvia.

It shall be accentuated that also the outcomes of radiosurgery treatment in patients with one metastasis is very important for patients with two and more metastasis, since upon choosing to apply radiosurgery treatment, more important than the number of metastasis is the total volume of the treated target pathology (for instance, equal volume rations may be for two small and one major metastasis), and in cases where the patient with two or more metastases it

is not possible to evacuate all nodules, one or more of the remaining cerebral metastases may be subjected to further radiosurgical treatment.

During a two years period (2010–2011) 16 patients harbouring one or more cerebral metastases underwent radiosurgical or fractioned stereotactic radiosurgery. The mean age of patients – M = 59.88 (SD = 8.77), minumum age – 45 years, but maximum – 75 years, range of age – 30 years, mean age – 61 years. Histogram of ageis dispalyed in the Figure 3.2.1. 11 of these patients were female patients (M = 59.72; SD = 8.37) and 5 male patients (M = 60.20; SD = 10.63). Independent selection T test indicated that the mean age of female patients and male patients did not have statistically credible differences (p = 0.1).

8 patients prior to stereotactic radiosurgery (SRS) treatment had received the whole-brain radiation therapy (WBRT) with the total dose of 30 Gy (3 Gy in 10 fractions).

In the group of patients that were trated with SRS the most part of them (n = 8) were female patients with breast cancer metastases in brain. Three patients had small cell lunger cancer metastases, two – melanoma metastases, two – ovarian cancer metastasis and one patient with non-Hodgkin's lymphoma brain metastases.

Table 2.2.1

**Distribution of patients according to localization of primary malignancy**

| Primary tumour | Number of patients |
|---|---|
| Breast cancer | 8 |
| Melanoma | 2 |
| Lung cancer (small cell, adenocarcinoma) | 3 |
| Ovarian or cervix cancer | 2 |
| Non-Hodgkin's lymphoma | 1 |

Patients were applied radiosurgery treatment since their clinical condition according to the Karnofsky scale was ≥ 70.

Three patients prior to radiosrugery treatment were diagnosed with neurological deficits. 11 patients were without neurological deficit in preoperative period, but a possibility to diagnoee cerebral metastases allowed symptoms of increased intracranial pressure that manifested as a headache, nausea and vomitting. For two patients the disease manifested with convulsions (generalized or partial).

In 11 patients after the examination with magnetic resonance contrast agent it was established that the metastases were located in one of the brain hemishperes and in three patients - metastases were located anatomically deep and clinically signicant localization sites (within basal ganglia, *thalamus*, *corpus callosum* or cerebellar hemispheres). In two patients metastases were located in both of the previously mentioned location sites.

In 12 patients out of 16 there was observed metachronous development of metastases, whereas in 4 cases cerebral metastases were diagnosed with sychronical development of primary tumour.

In none of the 15 patients who were treated with SRS upon initation of the therapy the metastases in other system organs were diagnosed.

The number of diagnosed metasetases for patients who were treated with radiosurgery, one metastase was establisged in 11 cases, two metastases – in 3 cases, five metastases in 1 case, six metastases – in 1 case and six metastases – in 1 case, please refer to the Figure 3.2.3.

12 patients out of 16 underwent radiosurgery treatment in a manner on one fraction, whereas in 4 cases there has been applied fractioned radiotherapy in 3–5 fractions. In all cases the maximum target diameter (of metastasis) was established by the magnetic resonance T1 contrast agent examination data and was smaller by four centimeters. Seven patients (43.75%) prior to performance pf stereotactic radiosurgery had received a whole-brain radiation therapy (WBRT) with total dose of 30 Gy (3 Gy in 10 fractions), and received therapy

due to progression of already existing metastases or due to new metastases that occured. The other eight patients during the time period of carrying out of study did not undergo the whole-brain radiation therapy (WBRT). The summary of target treatment volume for every patient was a number which was formed by the amount of volume of metastase which was exposed to the radiation therapy. The mean treatment volume of all patients group was 16.63 $cm^3$ (within the range of 1.85–47.03 $cm^3$).

Table 2.2.2

**Applied radiosurgery treatment**

| Treatment modality | Number of patients | Target volume (volume range), $cm^3$ | Marginal dose (range), Gy |
|---|---|---|---|
| SRS | 9 | 25.12 (2.03–47.03) | 18 (15–24) |
| WBRT + SRS | 3 | 8.15 (1.85–15.79) | 18 (18–20) |
| WBRT + FSRT | 4 | 22.36 (6.80–39.47) | in 3–5 fractions |

Taking into account that the median overall survival in the 16 patients group was not possible to calculate, the average calculated survival; was was 16.02 months (95% CI: 10.73–21.32 months).

Survival data are indicated depending on the further mentioned applied modality of radiation therapy:

1) SRS or FSRT, combining together with WBRT (in 7 patients) – the median survival rate was 18.04 months (95% CI: 10.19–25.89);

2) SRS (in 9 patients) – the median survival was 10.75 months (95% CI: 6,25–15,25).

Five patients after radiosurgery treatment were diagnosed with new metastases under magnetic resonance imaging.

Upon summarizing the MR imaging data for all patients 2 months after radiosurgery treatment (Table 3.2.3), all three metastases (11.11%) that after the radiosurgial tratment in MR imaging were established tumor resorption,

were breast cancer genesis. A partial resorption was observed in eight cases (29.62%), and it was observed in five cases of breast cancer and in one – ovarian, one – lung cancer case and one – non-Hodgkin's lymphoma metastase case. Controlled tumor was observed in 13 cases (48.14%) with five breast cancer metastases, with five lung cancer metastases, one melanoma metastase, one ovarian cancer metastase and one cervical cancer metastase. Progression of metastase (MP) was observed in three cases (11.11%) – in two patients with melanoma and one patient with lung cancer metastases.

Table 2.2.3

**Results of evaluation of MR brain metastases after 2 months of radiosurgical treatment**

| | **Malignancy resorption** | **Partial resorption** | **Controlled disease** | **Progression of metastases** |
|---|---|---|---|---|
| Breast cancer | 3 | 5 | 5 | – |
| Melanoma | – | – | 1 | 2 |
| Lung cancer | – | 1 | 5 | 1 |
| Cervical and ovariant cancer | – | 1 | 2 | – |
| Non-Hodgkin's lymphoma | – | 1 | – | – |

At the moment of data processing 9 patients out of 16 who received radiosurgical treatment, were alive, but 7-deceased.

Upon performance of folow-up MR imaging for one patient who had received radiosurgical treatment in one case with melanoma metastase, there were suspected possible complications of the applied therapy – radionecrosis (currently the SPECT or PET examinations for a more precise diagnostics are not available in Latvia). Diagnosis was histologically approved postoperatively. A decision on a surgical therapy was adopted grounding upon the progression

of the malignancy with secondary mass effect and development of general neurologic symptoms.

## 2.3. Results of analysis of *TP53* gene of cerebral metastases

There was analyzed the genetic material obtained from *TP53* gene of histological samples of those 40 patients that had been surgically treated for two or more cerebral metastases. Clinically significant *TP53* gene mutations in histological samples of cerebral metastases were diagnosed in 11 patients (27,5%) (please refer to the Table 2.3.1).

Most frequently (in 3 histological materials out of 9) the *TP53* gene mutations were detected in breast cancer patients.

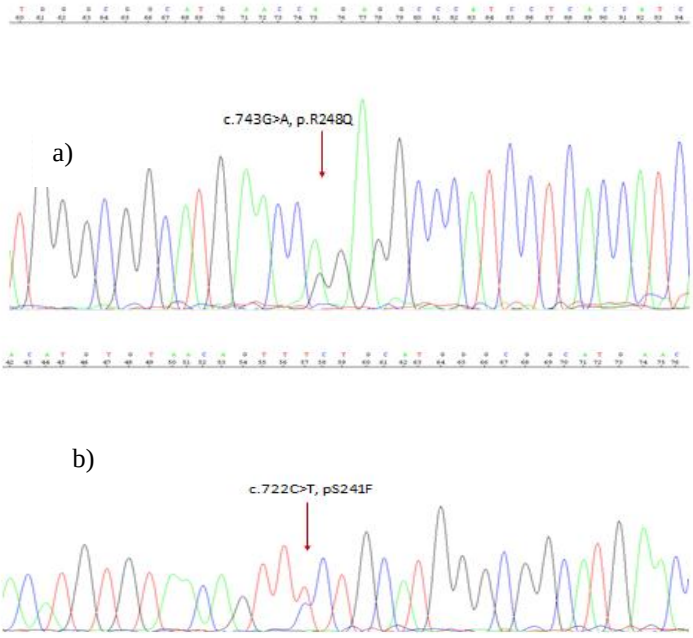


Figure 2.3.1. **Example with clinically significant TP53 gene mutations in a) melanoma and b) breast and cervix cancer metastase materials**

Table 2.3.1

**Clinically significant *TP53* gene mutations**

| Sample | Changes in DNA | Changes in protein | Histologic form |
|---|---|---|---|
| D3b | c.517G > A | p.V173M (*Substitution – Missense*) | Primary source cannot be established |
| | c.637C > T | p.R213* (*Substitution – Nonsense*) | |
| B1-1b | c.738G > C | p.M246I (*Substitution – Missense*) | Primary source cannot |
| B1-2a | c.637C > T | p.R213* (*Substitution – Nonsense*) | Breast cancer |
| B3-5a | IV7+2insG | Nav | Breast cancer |
| G2-3a | c.565delG | p.189fs246* (*Frameshift*) | Gastrointestinal cancer |
| U4a | c.743G > A | p.R248Q (*Substitution – Missense*) | Ovarian and cervix cancer |
| L4-2b | c.743G > A | p.R248Q (*Substitution – Missense*) | Lung cancer |
| R1a | IV7+2insG | Nav | Kidny cancer |
| M1-7b | c.740insC | p.247fs263* (*Frameshift*) | Melanoma |
| M1-1b | c.722_723CC > TT | p.S241F (*Substitution – Missense*) | Melanoma |
| B3-3a | c.542G > C | p.R181P (*Substitution – Missense*) | Breast cancer |

Upon comparing the groups with and without established clinically significant *TP53* gene mutations, the following mentioned data were obtained (please refer to the Table 2.3.2).

Table 2. 3.2

**Comparative indicators of groups *TP53* (+) and *TP53* (-)**

| | TP53 (+) mutations | TP53(-) mutations | p-value | d value |
|---|---|---|---|---|
| Number of patients | 11 | 29 | | – |
| Mean age | M = 61.45; SD = 13.10 | M = 56.86; SD = 9.40 | 0.24 | 0.40 |
| Male patients | 4 | 16 | 0.28 | – |
| Female patients | 7 | 13 | | |
| Mean KPS value | M = 64.55; SD = 9.34 | M = 62.07; SD = 9.77 | 0.47 | 0.25 |
| Mean RPA | M = 2.09; SD = 0.94 | M = 2.38; SD = 0.82 | 0.34 | 0.32 |
| Mean survival | 3.7 | 5.33 | 0.47 | – |

Mann-Whitney U test indicated than in case of *TP53* gene mutations the mean age had no statisticall credibility difference (p = 0.14) from the age of those patients who had not been diagnosed with *TP53* gene mutations. In Pearson's chi-squareed test analysis it was established that the existence of *TP53* gene mutation has no statistically credible impact on patient's age group (p = 0.06). Pearson's chi-squareed test statistical analysis indicated that the existence of *TP53* gene mutations does not have a statistical credible impact on breast cancer (p = 0.19).

In the 11 patients of the group of patients to whom in their evacuated cerebral metastases there were detected *TP53* gene mutations, but the mean survival ratewas 3.7 months (95% CI: 0.93–6.46), whereas to 29 patients who were not diagnosed with clinically significant *TP53* gene mutations, the mean survival rate was respectively 5.33 months (95% CI: 4.32–6.33).

Grounding upon the log-rank test it was established that *TP53* gene mutations do not have a statistically credible influence on postoperative survival (p = 0.47).

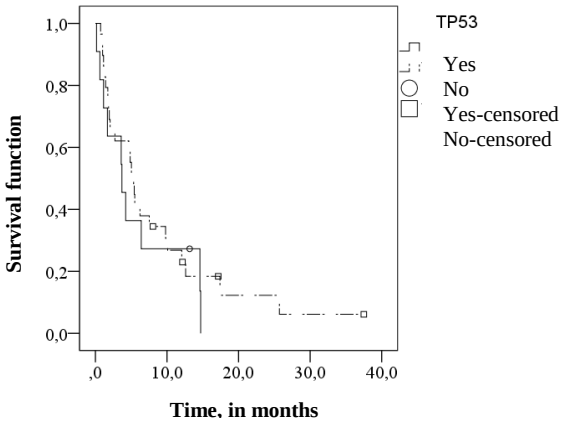


Figure 2.3.2. **Kaplan-Meier survival curve in patient groups with/without established clinically significant *TP53* gene mutations**

# CONCLUSIONS

1. Surgical evacuation of two and more cerebral metastases allows to prolong survival and to preserve the functionality of patients for a particular time interval after the surgery.
2. Better results of surgical treatment of two and more cerebral metastases are obtained in the group of patients with 2–3 metastases after a radical or total evacuation of all diagnosed nodules.
3. Statistically significant impact to a better postoperative survival prognoses have the following factors number of metastases, radical evacuation of metastases, preoperative KPS and RPA Class indicators.
4. Survival prognosis in patients with oligometastases (median survival – 5.5 months) is 2.66 fold greater than in patients with multiple cerebral metastases (median survival – 2.06 months) ($p < 0.05$).
5. In application of complex therapy (WBRT or WBRT and chemotherapy) in case of partial evacuation of brain metastasis gives a small prolongation of survival in comparison to partial, only surgical evacuation of metastases.
6. Assessment of those 16 patients in Latvia who were first radiosurgically treated and that had small (up to 3 cm in diameter) cerebral metastases, produced positive outcomes that require further studies and analyzing.
7. In genetic analysis of histological material (11 observations out of 40) there were established clinically significant *TP53* gene mutations. A statistically significant impact between the postoperative survival and the *TP53* gene mutations in the study was not established.

# PRACTICAL RECOMMENDATIONS

1. In patients with two up to three cerebral metastases the most recommended would be directly surgical, as radical as possible, evacuation of all diagnosed metastase nodules within the range of anatomical and functional possbilities.
2. In cases where the evacuation of cerebral metastases has been only partial, for the further therapy there are recommendations for WBRT and in individual – clinically favourable cases – also an individually adjusted chemotherapy.
3. In the case of one, two or more small (up to 3cm in diameter) cerebral metastases, radiosurgery shall be recommended as a method of choise.

# PUBLICATIONS

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