MEDICAL UNIVERSITY
„PROF. DR. PARASKEV STOYANOV“- VARNA

DEPARTMENT OF „NEUROSURGERY AND ENT“

Martin Nikolaev Moynov, M.D.

Neuronavigated needle biopsy in cranial neurosurgery

AUTOREFERAT

Of the dissertation
For obtaining an educational and scientific “PhD” degree
Scientific specialty: neurosurgery

Supervisor:

Prof. Yavor Enchev, MD, PhD, DrMedSci, MHA, FRCS (Eng)

Varna, 2022
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The dissertation contains 173 pages and is illustrated with 135 figures, 71 tables and 1 equation. The bibliography includes 260 literature sources. There are five publication and scientific communications connected to this work.

The author works as a specialist in Neurosurgery clinic of University hospital „St. Marina“- Varna and as an assistant in the Department of Neurosurgery and ENT of Faculty of Medicine at the Medical University „Prof. Dr. Paraskev Stoyanov“- Varna.

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The defense of the dissertation will take place online on the platform Webex on June 27, 2022 from 15:00 on an open meeting of the scientific council.

The materials on the defense are readily available in the library of the Medical University „Prof. Dr. Paraskev Stoyanov- Varna: http://www.mu-varna.bg

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I would like to thank Prof. Dr. Yavor Enchev and the entire team at the Neurosurgery clinic of University hospital "St. Marina”, Varna without whose assistance the implementation of this study would be impossible.

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INTRODUCTION

Conducting modern oncological treatment of patients with malignant neoplasm of the brain requires accurate histopathological diagnosis (McGirt et al. 2005). Open surgery applied for histological verification of supratentorial intraaxial lesions are often associated with high postoperative morbidity (Hervey-Jumper et al. 2016). There is a risk of affecting functionally significant areas, especially in patients in whom histological verification is combined with radical surgery (Aker et al. 2005). Modern neurosurgical clinical practice is focused on the possibilities of minimally invasive surgery (Ganslandt et al. 2021).

Neuronavigated (frameless) biopsy is a type of high-tech minimally invasive surgery that allows the planning and selection of one or several work trajectories, avoiding functionally significant areas in the brain, locating and tracking the needle biopsy window in real time, controlling the depth and direction while taking biological material for histological examination (Aker et al. 2005, Enchev 2009).

The main advantages of frameless surgery include: real - time visualization of operational objectives, imaging studies with high quality of the converted 3D images, lack of need for a rigid frame allowing for high degree of flexibility of this technique, in terms of safety, time and cost compared to the classic frame based stereotactic surgery (Price et Dorward 2003, Dhawan et al. 2019).

The main disadvantage of neuronavigated surgery is the fact that it is based mainly on preoperative imaging information. This fact in combination with the invasive nature of surgical interventions in general leads to the problem called brain displacement or “brain shift”. There are practical measures that when used lead to the reduction of this problem. Factors to reduce brain displacement are optimal positioning of the patient, maintaining the integrity of the ventricular system, short duration of the operative intervention and intraoperative updating of the preoperative image information with the help of intraoperative ultrasound, MRI or CT (Enchev 2006).

There are a limited number of studies in the literature related to the evaluation of the efficiency and accuracy of this neuronavigated technique as well as lack of a standardized biopsy protocol allowing for objective comparative analysis of different operational results (Dhawan et al. 2019.)

AIM AND TASKS

1. Aim

Analysis and summary of the experience gained with a neuronavigated needle biopsy in patients with supratentorial intraaxial lesions, with view of optimizing the application of this minimally invasive neuronavigated technique for diagnosis and treatment. Considering the operative results to formulate and introduce a surgical algorithm for the application of neuronavigated needle biopsy and to evaluate the effect of its application in the routine practice at the Neurosurgery Clinic of the University hospital "St. Marina", Varna.

2. Tasks

1. To track and summarize the results of neuronavigated needle biopsy interventions in patients with supratentorial intraparenchymal lesions.
2. To make a critical comparative analysis of the operative results with those of other authors.
3. On the basis of a thorough literature review of the existing specialized literature, to formulate indications for the application of neuronavigated needle biopsy in patients with supratentorial intracranial lesions.
4. To analyze the complications and technical difficulties of the neuronavigated frameless surgical technique and to identify guidelines to reduce them.
5. To assess the effectiveness of the procedure and the risks associated with it.
6. To develop a neuronavigated needle biopsy protocol.
7. To carry out training of the medical neurosurgical team at the Clinic of Neurosurgery of the University Hospital - "St. Marina", on the nature, objectives and correct use of a neuronavigated needle biopsy protocol in patients with intracranial lesions.

MATERIALS AND METHODS

1. Study design

The nature of the study is retrospective: Analysis of the medical records of patients operated on in the Clinic of Neurosurgery of the University Hospital "St. Marina" who presented with supratentorial intraaxial lesions.

The following inclusion criteria have been identified:

- Interventions under general anesthesia
- Surgical technique with neuronavigated frameless biopsy
- Operated on in the period between January 2019 - December 2021

Included are fifteen cases of isolated biopsy of supratentorial intraaxial lesions and twenty five cases with biopsy with subsequent open excision of the biopsied supratentorial intraaxial lesions. A total of forty patients with fifty diagnosed intracranial lesions.

Procedures considered to be “successful” are the biopsy procedures that led to a definitive intraoperative histological diagnosis. Interventions considered to be “unsuccessful” are those that led to intraoperative histological diagnosis that include gliosis, encephalitis or normal brain tissue.

2. Clinical population

A total of 40 patients were included in the study: 20 women and 20 men (Fig.1).

The patients were divided into two groups: 15 patients with isolated biopsy and 25 patients with biopsy and subsequent excision (Table 1).

Tab. 1.* *Distribution of all patients in two groups: a group with isolated biopsy and group with biopsy combined with excision.*

Fisher's exact test=2.67, p=0.102		**Surgical technique**		**Total**
Sex		**Biopsy**	**Biopsy and excision**	
Men	N	5	15	20
	%	33.3%	60.0%	50.0%
Women	N	10	10	20
	%	66.7%	40.0%	50.0%
Total	N	15	25	40
	%	100.0%	100.0%	100.0%

Mean age of the patients group where isolated biopsy was performed was 59.1 years at a standard deviation of 16.4 and for the group where biopsy and excision was performed the mean age was 64.2 years with standard deviation 13.7 (Table 2, Fig. 1).

Tab. 2.* *Age distribution of the patients for the two study groups.*

Technique	**Number**	**Average**	**Median**	**Standard deviation**	**Standard Error**
Biopsy	15	59.1	67.0	16.4	4.23
Biopsy and excision	25	64.2	67.0	13.7	2.74

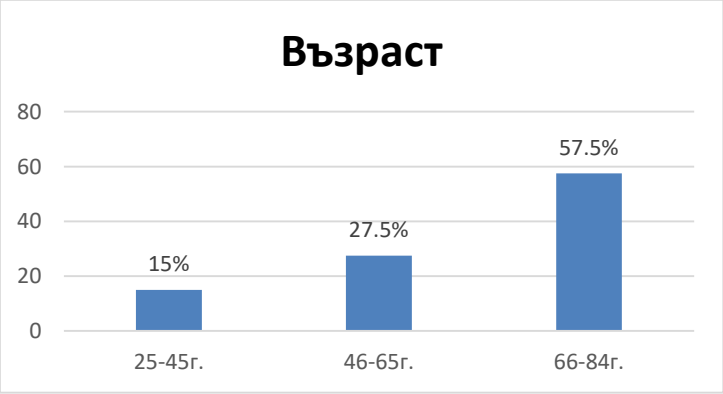

Fig. 1.* *Percentage distribution of patients by age limit of 45 and 65 years.*

3. Methods

3.1. Equipment

Planning is used for all interventions in the study a planning station located in the hospital outside the operating room was used allowing for careful consideration of the available imaging studies and for careful selection and planning of the biopsy targets and the surgical access. (Fig. 2)

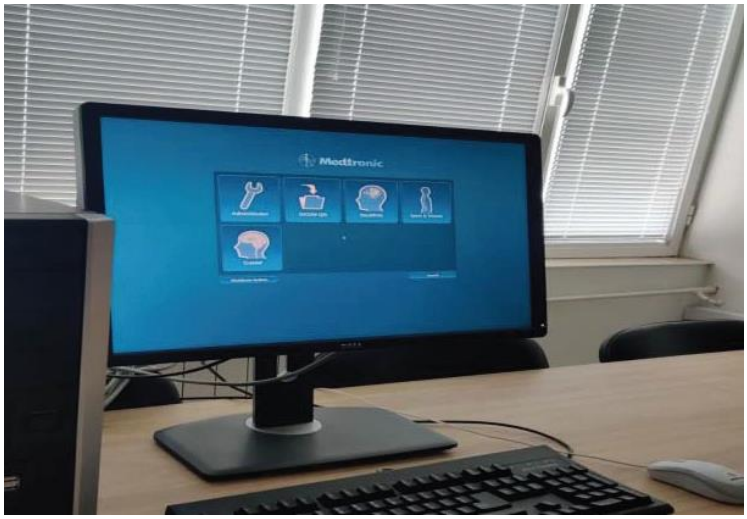

Fig. 2.* *Medtronic ® Planning Stealthstation S7.*

The Medtronic® Stealthstation S7 neuronavigation system is used intraoperatively for all interventions with current software version 3.1.2. (Fig. 3)

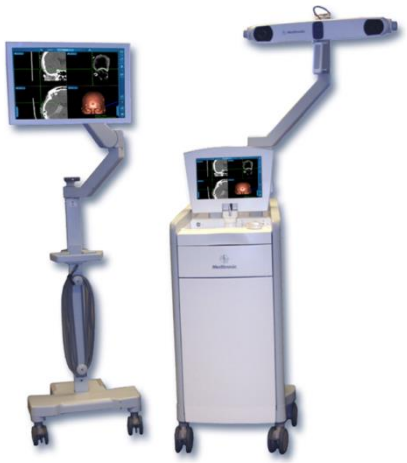

Fig. 3.** *Neuronavigation device Medtronic ® Stealthstation S7 using an infrared camera (localizer) and a reference frame with passive reflectors for navigation (BVM StealthStation 2021).*

The standard navigation set of tools for working with the Medtronic ® Stealthstation S7 navigation device includes a reference frame (Fig. 4), articulation arm (Fig. 5) and passive probe (Fig. 6).

Fig. 4.* *Reference frame with four passive spheres.*

Fig. 5.* *Articulating arm (non-sterile)*

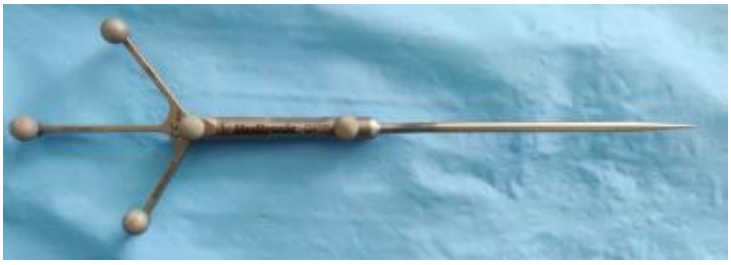

Fig. 6.* *Passive blunt probe*

The biopsy procedure is performed using Cranial Vertek® Passive Biopsy tray (Fig. 7) containing dual starburst for attachment to the Mayfield clamp (Fig. 8), second (sterile) articulating arm (Fig. 9), precision aiming device (Fig. 10), Vertek pointer (Fig. 11), reducing tubes (Fig. 12).

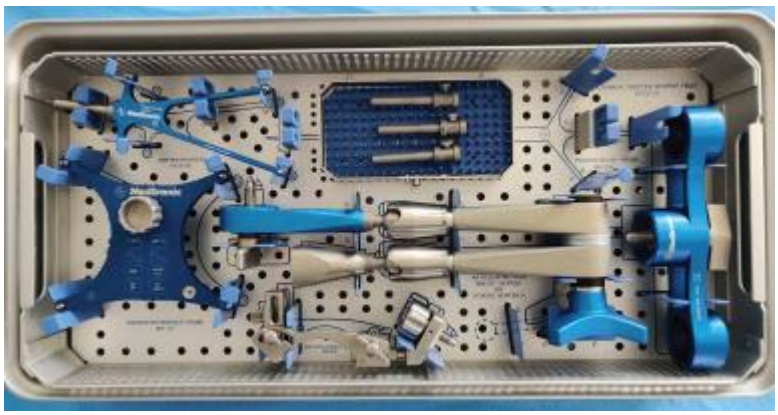

Fig. 7.* *Cranial Vertek® Passive Biopsy tray.*

Fig. 8.* *Dual starburst for attachment to the Mayfield clamp.*

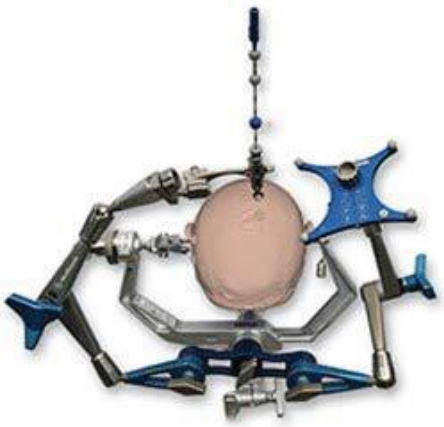

Fig. 9.** *Second (sterile) articulating arm.*

Fig. 10.* *Precision aiming device.*

Fig. 11.* *Vertek pointer*

Fig. 12.* *Reducing tubes with dimensions 1.9 mm, 2.2 mm and 2.6 mm. In the present study the reducing tube of 2.2 mm in diameter was used for all the interventions.*

Biopsy procedures were performed using the Medtronic passive biopsy needle- a cannulated Nashold type of biopsy needle with a diameter of 2.2 mm, rounded tip, laterally situated 9.5 x 1.2 mm biopsy window located at 2 mm from the tip of the needle. The needle is designed to separate a tissue core with preserved cytoarchitectonics for more accurate pathological examination (Fig. 13). The two cannulas of the biopsy needle are marked allowing for reliable determination of the correct position of the biopsy window. By rotating the marked internal cannula the biopsy window is opened (Fig. 14).

Fig. 13.* *Medtronic passive biopsy needle*

Fig. 14.* *Marked inner and outer cannula (black arrow), passive spheres (blue arrows), stopper for regulating the exact length of the working trajectory (red arrow) and a demonstration of the exact size of the biopsy window of the Medtronic passive biopsy needle.*

3.2. Operative technique

Preoperative preparation

Preoperative preparation of hospitalized patients for conducting biopsy intervention involves performing the standard paraclinical tests, ECG and consultation with a cardiologist and anesthesiologist; reviewing the available preoperative imaging studies and confirming their quality and relevance; suspending the osmotic dehydrating therapy to reduce brain displacement; completing a conventional preoperative checklist for cranial intervention.

Planning

Preoperative imaging studies performed using contrast medium are loaded into the neuronavigation planning system via CD, DICOM USB transfer, or a direct DICOM transfer from internal or external PACS server.

Target point planning

The optimal surgical target is the midpoint of the intraparenchymal lesion (Fig. 15). Opportunity to procure biopsy material from the lesion 360 degrees in the axial plane of the lesion by rotating the needle is planned (Fig. 16) as well as within 1-1.5 biopsy windows in the sagittal plane of the lesion by following the same trajectory (Fig. 17).

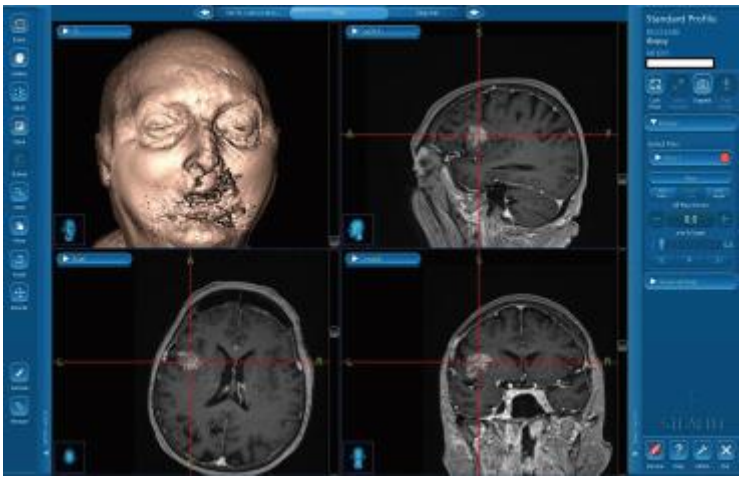

Fig. 15.* *Target selection in the midpoint of the intraparenchymal lesion in the three imaging planes.*

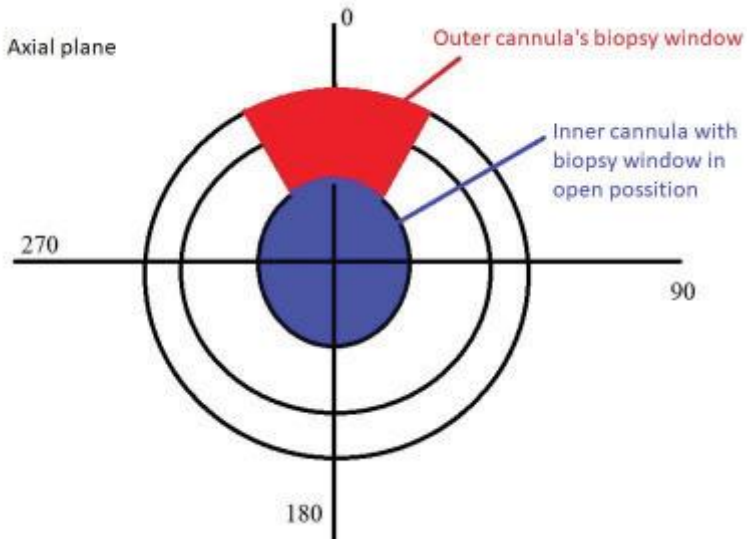

Fig. 16.* *Schematic representation of the cannulated biopsy needle in axial plane with inner and outer cannula in open biopsy window position. Rotation of the outer cannula directs the biopsy window in position at 0, 90, 180 and 270 degrees in the axial plane. Rotation of the internal a cannula allows the biopsy window to be opened and closed.*

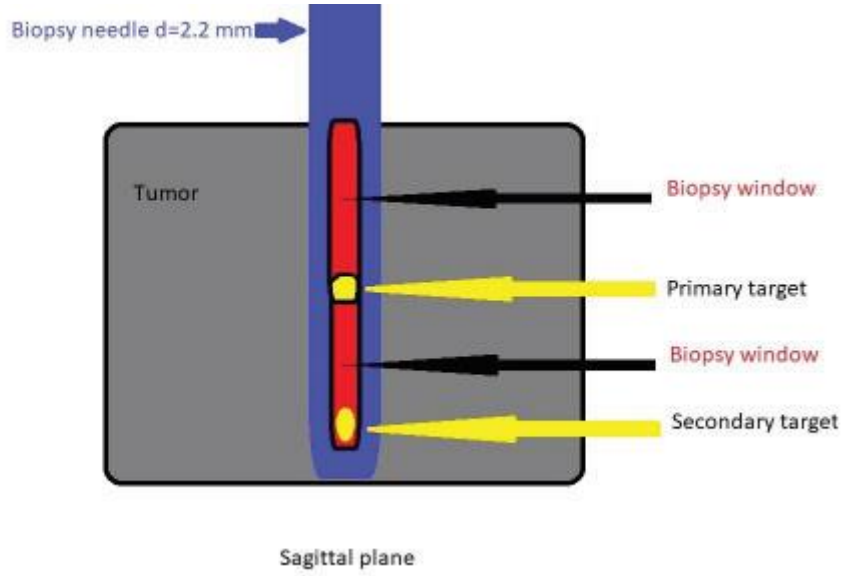

Fig. 17.* *Schematic representation of a cannulated biopsy needle in sagittal plane. Midpoint proximal target is selected and according to the contrast matter distribution and the size of the lesion, a distal target is planned in the same trajectory, near the boundaries of the formation, within 1-1.5 biopsy windows distance apart.*

In case of more than one intraparenchymal lesion, the longest axis of the tumor formation is taken into account. Targets are planned so that if possible they can be reached by a single working trajectory.

Planning of the access point on the cranial convexity

The optimal access point is the nearest safe distance to the intraparenchymal lesion. Care is taken not to affect eloquent areas of the brain, parenchymal vascular structures, sulci (affecting vascular structures located in them), ventricles (increased risk of brain displacement). Assessment of the regional circulation and mass effect of the intraparenchymal lesion is performed.

At least three trajectories are planned (Fig. 18). One optimal trajectory is selected after discussion by the medical team.

The created operational plan is saved, transferred and loaded into the neuronavigation system (Fig. 19).

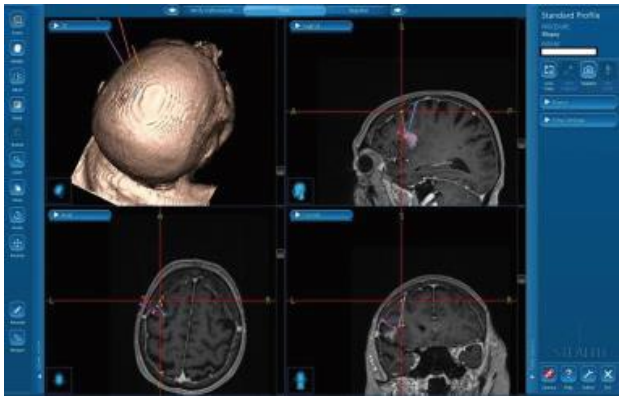

Fig. 18.* *Planning of three working trajectories to be discussed during clinical conference by the medical team.*

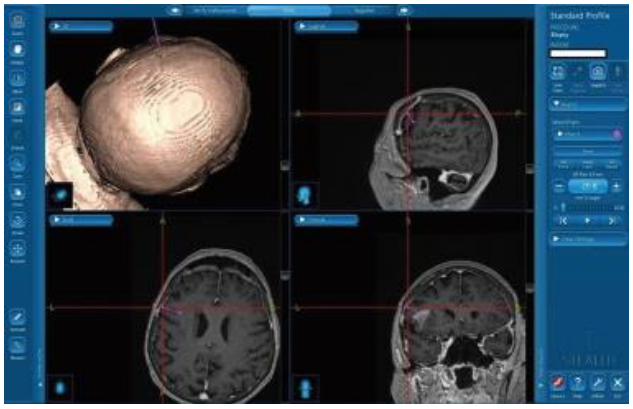

Fig. 19.* *After selection of an optimal working trajectory the surgical plan is transferred and loaded into the neuronavigation system.*

3.3. Surgery

Patient’s positioning

The patient is brought under general anesthesia and positioned on the operating table with the head fixed in a Mayfield clamp (Fig. 20). The dual starburst from the Vertek tray is attached to the clamp. A free corridor between the patient's face, the neuronavigation reference frame and the optical camera (localizer) is insured (Fig. 21 and Fig. 22). The position must allow for the planned access point to occupy the highest point of the patient’s head (Fig. 23). Proper positioning provides conditions for minimal cerebrospinal fluid leakage during operational approach,

minimal displacement of the brain during the intervention and minimal risk of navigation error.

Fig. 20.* *Mayfield clamp with three-point head fixation of the patient's head provides the stability necessary when working with the neuronavigation system. The dual starburst attachment from the Vertek tray is placed on the clamp in non-sterile conditions and allows following attachment of the two articulating arms.*

Fig. 21.* *Positioning the patient by providing free corridor between the patient's face, the neuronavigation reference frame and the optical localizer. The passive probe is verified by placing its tip on the reference frame.*

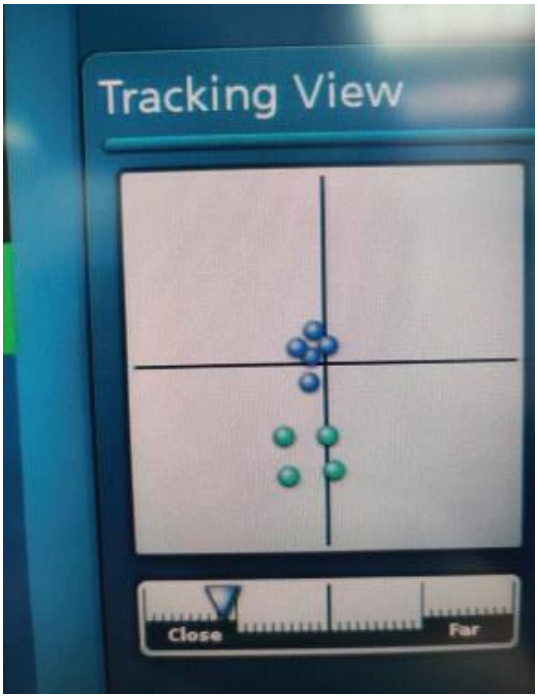

Fig. 22.* *The optical camera of the neuronavigation system tracks in real time the position of the passive probe and the reference frame.*

Fig. 23.* *The patient's position allows the planned access point to occupy the highest point on the patient's head.*

Registration

The registration process allows for the merging of patient's physical space and the virtual imaging space of the navigation system.

Different registration methods are described: Tracer® registration, Touch-n-Go registration, PointMerge® registration and automatic registration methods such as StealthAiR® registration and O-arm® registration (BVM StealthStation 2021). In the present study Tracer® registration was used by a combination of both manual selection of static points (Fig. 24) and by surface tracing of more than 100 points on the superficial anatomy of the patient (Fig. 25), which leads to the merging of the physical space of the patient and the virtual 3D space of the neuronavigation.

Fig. 24.* *The Tracer registration process begins by specifying static points on the patient's superficial anatomy.*

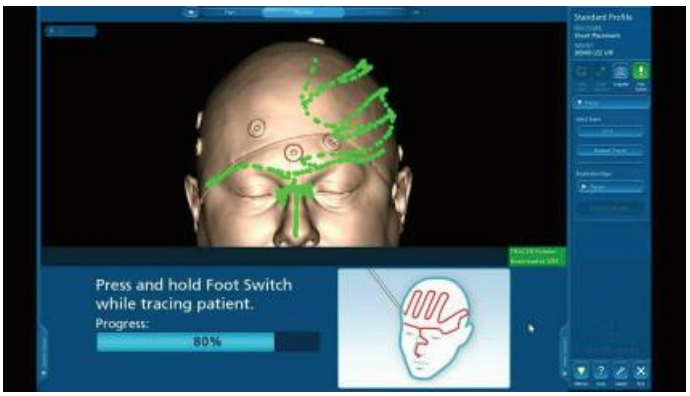

Fig. 25.* *Surface tracing of more than 100 points on the superficial anatomy of the patient leads to fusion of the patient's physical space and virtual 3D space of the neuronavigation system.*

Verification

Accuracy and precision are verified by anatomical landmarks such as lateral (Fig. 26) and medial (Fig. 27) canthus, midline (Fig. 28), mastoid process, external auditory canal, as well as superficial landmarks of the individual anatomy of the patient.

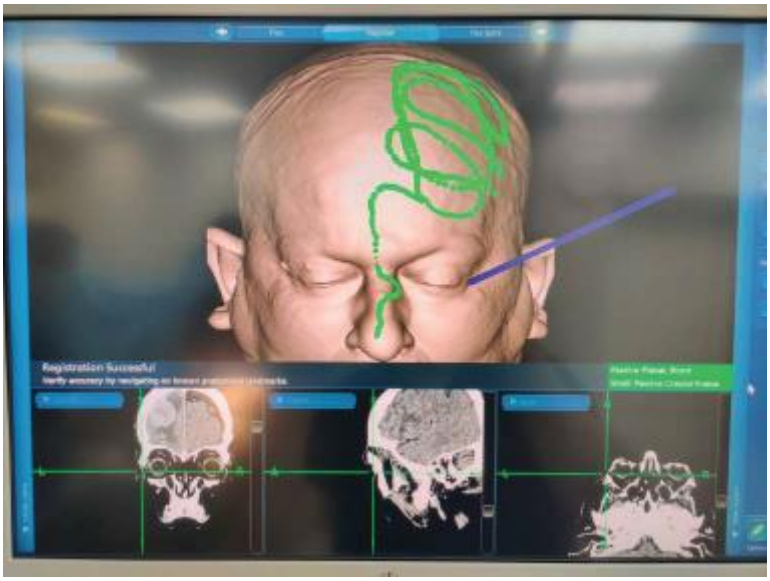

Fig. 26.* *Verification by positioning the passive probe in the area of the lateral canthus*.

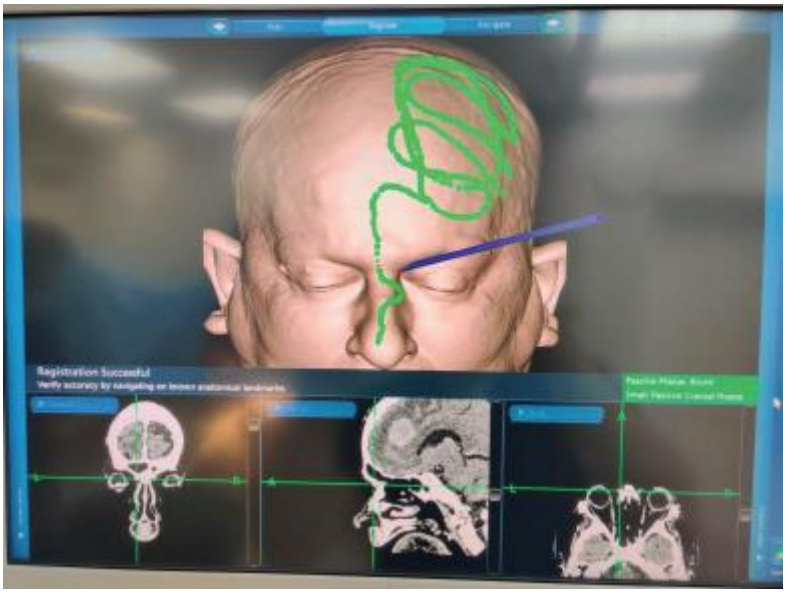

Fig. 27.* *Verification by positioning the passive probe in the area of the medial canthus.*

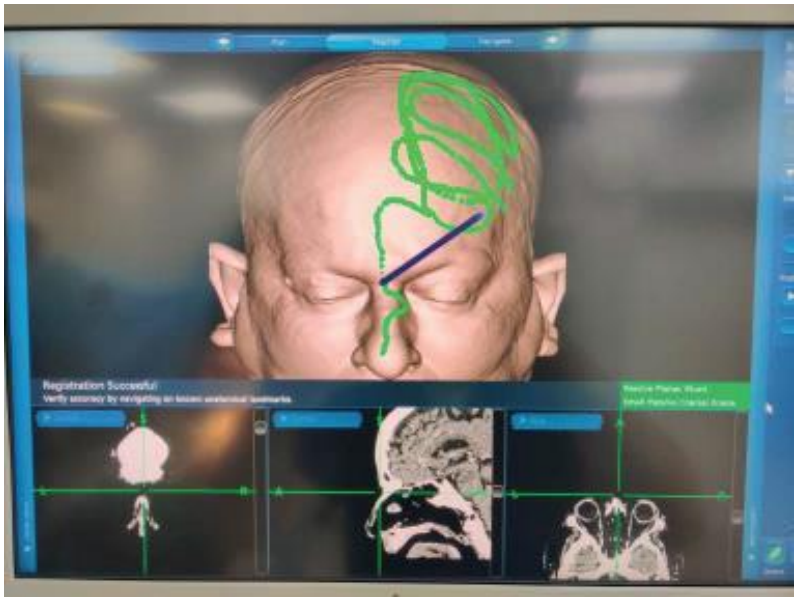

Fig. 28.* *Verification by positioning the passive probe and locating the midline.*

Marking the access point and the surgical incision

The planned access point is marked with the help of the passive probe (Fig. 29). The incision is planned so that it can allow the formation of a skin flap in case a need arise to perform an extensive craniotomy for excision of the lesion or for better control of observed intracerebral hemorrhage.

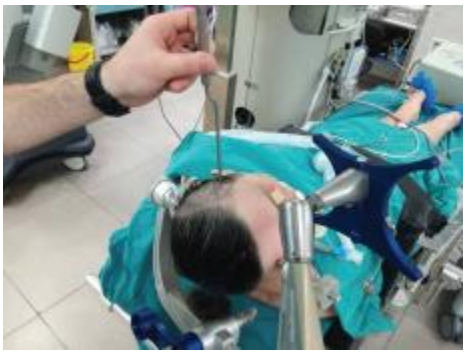

Fig. 29.* *Mark the planned access point. Fixed to the clamp the dual starburst allows the attachment of a non-sterile articulating arm for fixing the reference frame and attaching a second sterile articulating arm to perform the biopsy intervention.*

Biopsy

The non-sterile reference frame is removed from the non-sterile articulating arm. A sterile operative field is obtained. Carefully a sterile reference frame is attached to the non-sterile articulating arm. A sterile passive probe is used for re-verification of the operational access point. Straight incision of the soft tissues is made. A punctiform craniotomy is performed. The sterile articulating arm and the precision guidance device from the Vertek biopsy tray are attached to the dual starburst (Fig. 30). Vertek pointer is registered, placed in the precision aiming device and is approximated over the performed craniotomy. By following the guidance indicators which are automatically provided from the neuronavigation system when the Vertek pointer is placed in the precision aiming device, the trajectory to the target is verified providing accuracy of less than 2 mm, which allows definitive fixation of the precision guidance device in a position ensuring maximum precision (Fig. 31).

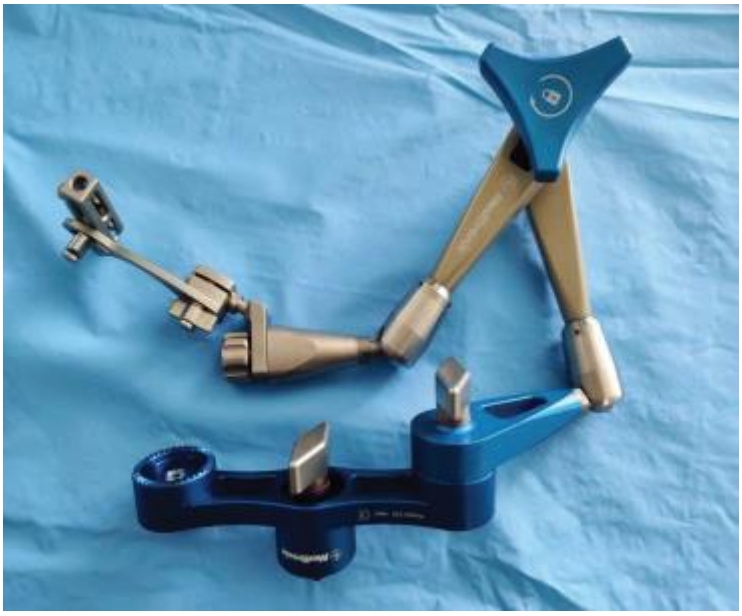

Fig. 30.* *Assembling the sterile articulation arm and the precision aiming device attached to the dual starburst.*

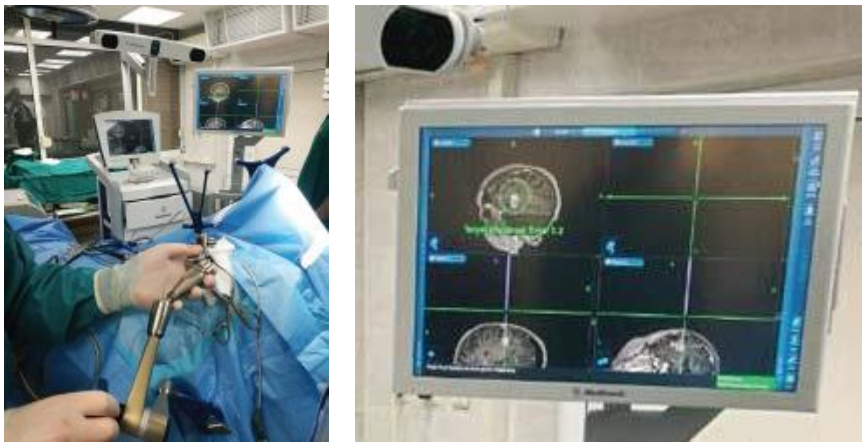

Fig. 31.* *Using the Vertek pointer the accuracy of the trajectory to the target point is verified using the guidance indicators automatically provided by the neuronavigation system allowing for definitive fixation of the precision guidance device providing target alignment error of less than 2 mm.*

After fixing the articulating arm and the precision aiming device with the Vertek pointer attached, the pointer is fixed in space aligned with the planned trajectory providing that target alignment error is less than 2 mm, following a lock of the working trajectory by timely pressing the neuronavigation pedal. (Fig. 32) Once the trajectory is locked, the neuronavigation system automatically calculates and provides the exact length at which the biopsy needle reaches the planned target point (tip stop point). (Fig. 33)

Fig. 32.* *Working trajectory is locked by pressing the navigation pedal.*

Fig. 33.* *Locked working trajectory with deviation from planed target of 0.9 mm and marked biopsy needle length of 108.9 mm, at which the tip of the needle reaches the planned target point.*

The length of the biopsy needle provided automatically by the neuronavigation system after locking of the working trajectory is marked and fixed on the biopsy needle by the needle stopper (Fig. 34).

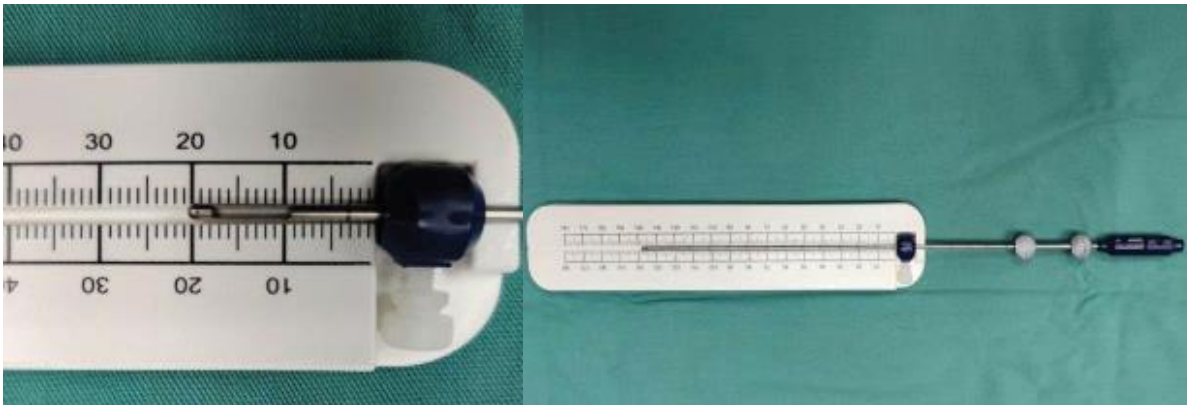

Fig. 34.* *Needle stopper for setting the exact biopsy length needed reach the planned target provided by the navigation system after the lock of the working trajectory.*

The Vertek pointer is removed from the precision aiming device (Fig. 35). Durotomy and coagulation of the arachnoid and pia are performed (Fig. 36). A 2.2 mm reducing tube is positioned in the precision aiming device (Fig. 37).

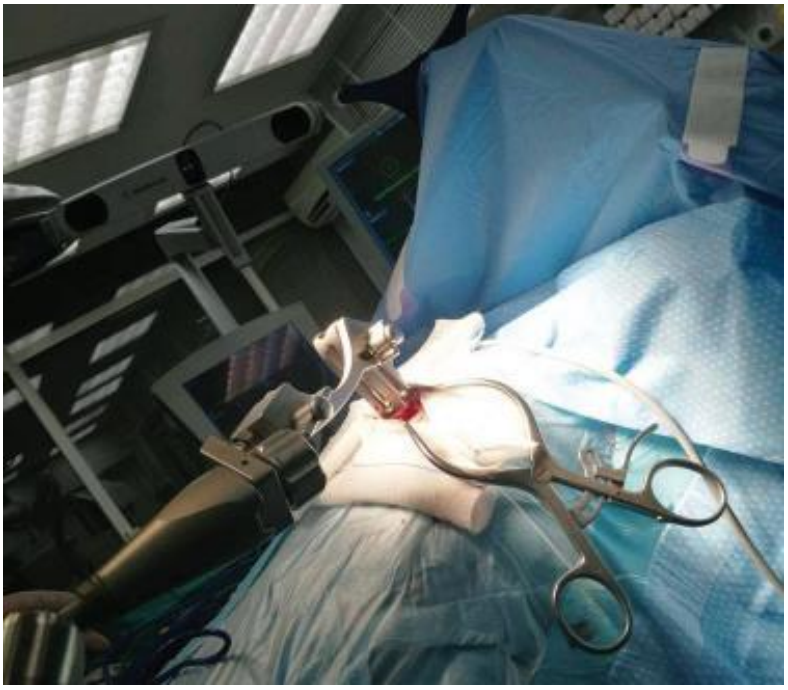

Fig. 35.* *The Vertek pointer is removed from the precision aiming device fixed over the performed craniotomy.*

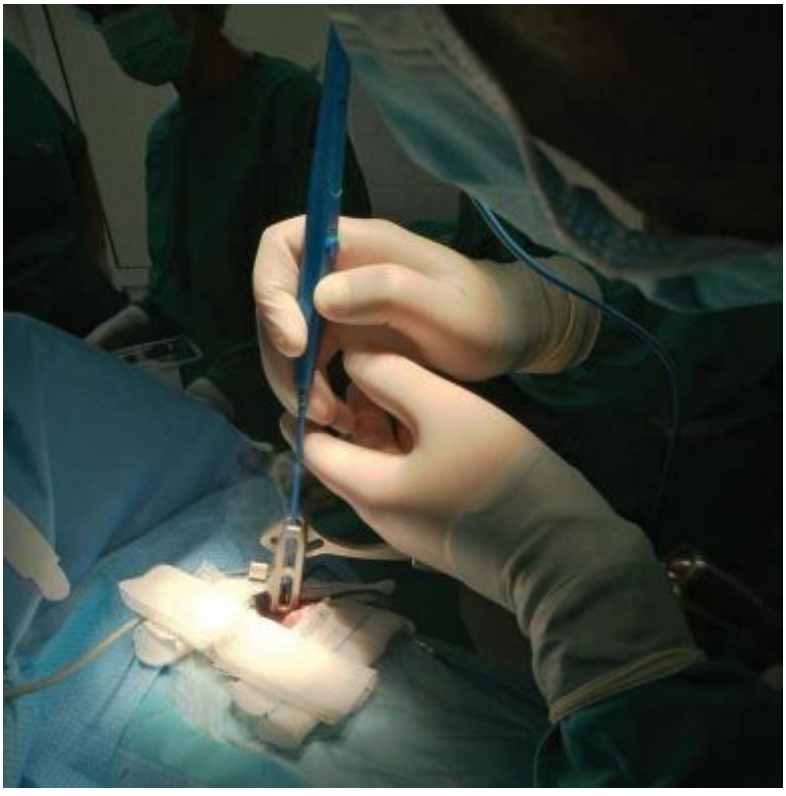

Fig. 36.* *Durotomy and coagulation of arachnoid and pia mater is performed with the help of monopolar and bipolar cauterization.*

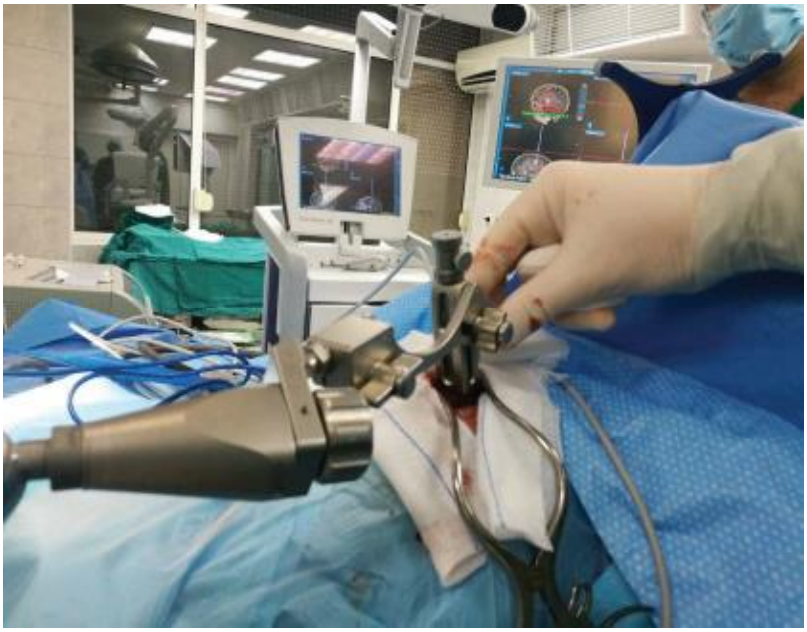

Fig. 37.* *Positioning of 2.2 mm reduction tube.*

The placed reduction tube allows mounting of the biopsy needle in a closed biopsy window configuration (Fig. 38). The tip of the needle and the position and direction of the biopsy window are tracked and controlled by the surgeon in real time (Fig. 39 and Fig. 40).

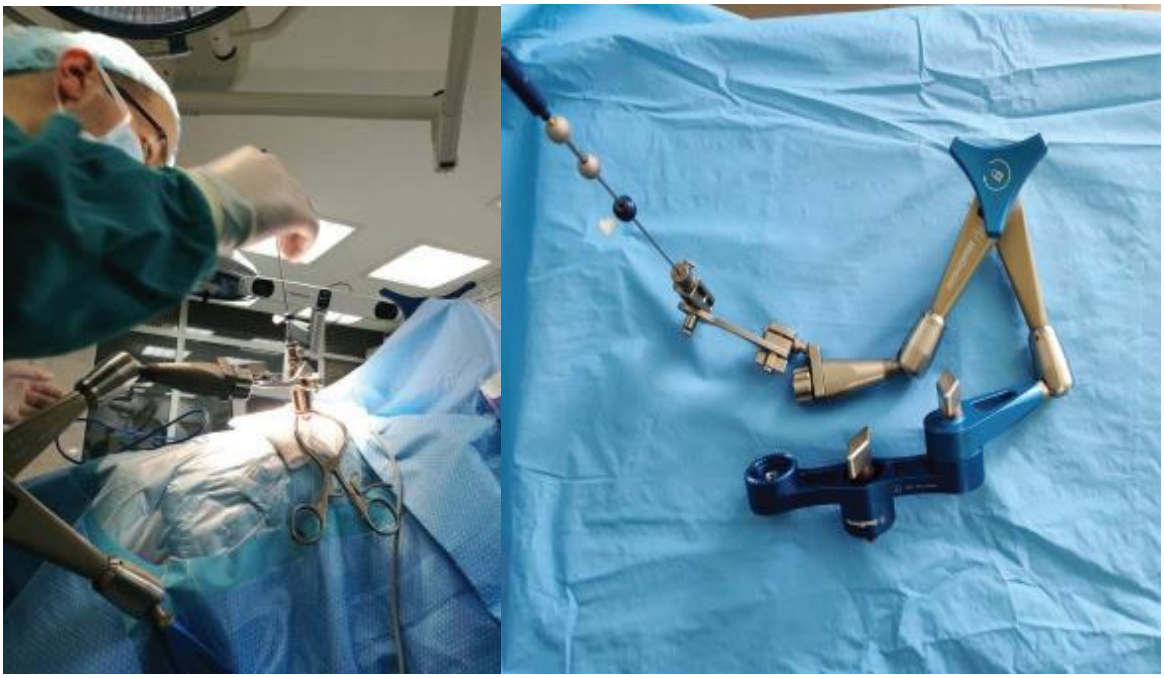

Fig. 38.* *Introduction of the biopsy needle in a closed biopsy window configuration.*

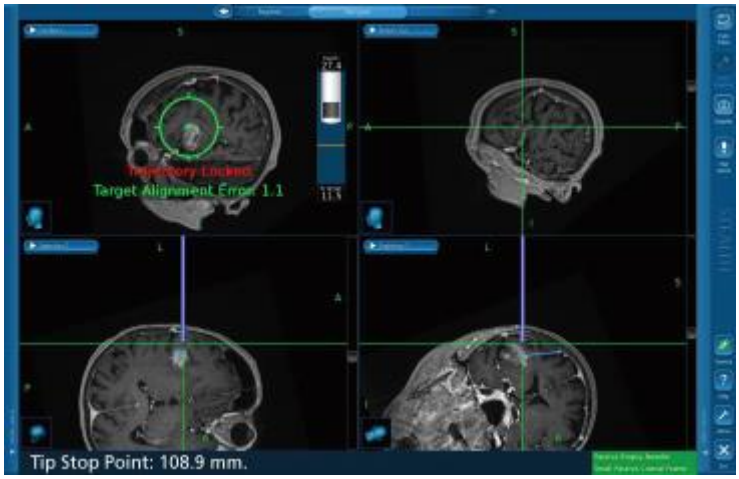

Fig. 39.* *Tracking the depth of biopsy needle's tip and the location of the biopsy window relative to the lesion in real time. The planes of real time neuronavigation tracking are chosen depending on the surgeon's comfort.*

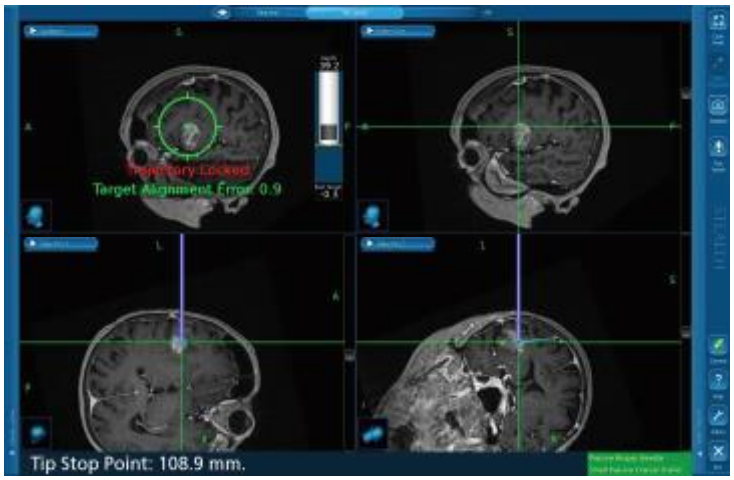

Fig. 40.* *Tracking the position of the biopsy needle's tip at 0.3 mm past the planed surgical target.*

After inserting the biopsy needle follows opening of the biopsy window by rotation of the inner cannula to a position of 180^{o} from the starting 0^{0} position (initial closed biopsy window configuration). In order to secure entering of optimal amount of tissue into the biopsy window a negative pressure is exerted by 5 ml of aspiration using a 10 ml syringe attached to the biopsy needle (Fig. 41). Closing the biopsy window is done by rotating the inner cannula of the biopsy needle up to 360^{o} from the starting position (initial closed biopsy window configuration).

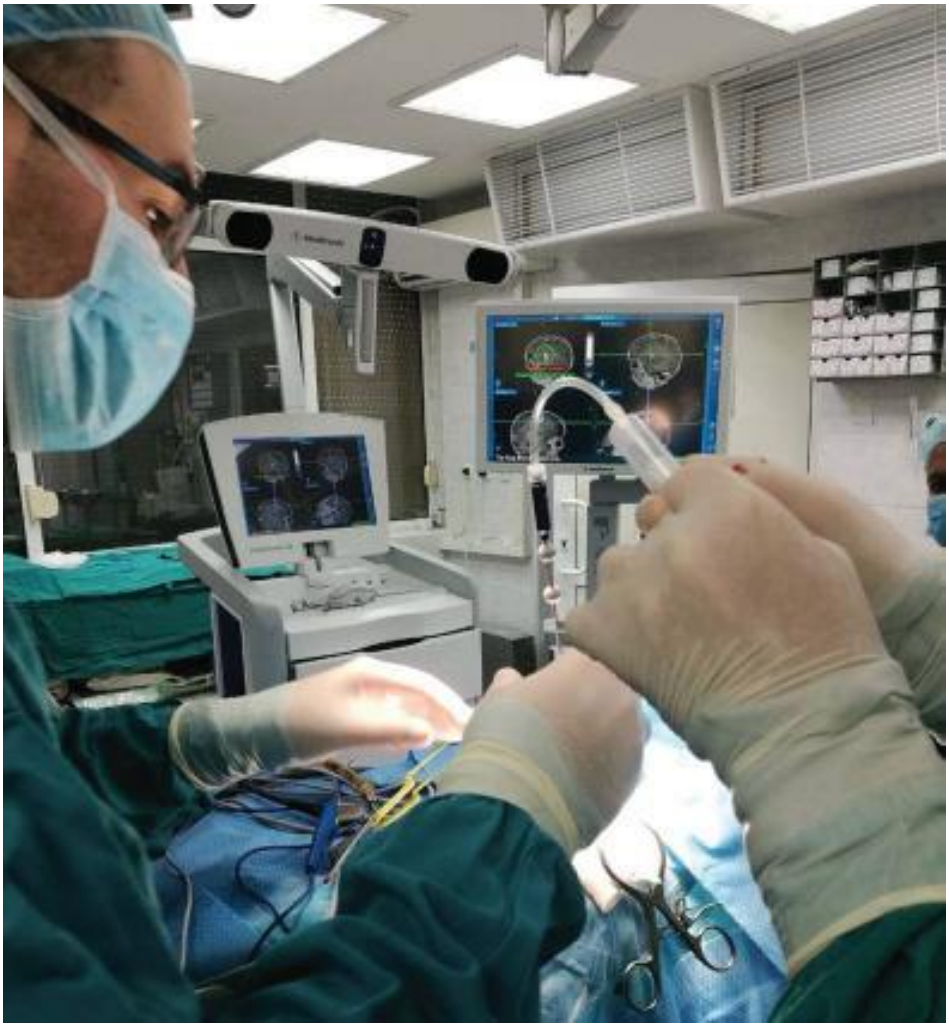

Fig. 41.* *Exerting negative pressure by aspirating 5ml of air using a 10ml syringe ensures that optimal amount of tissue has entered into the biopsy window.*

Closing the biopsy window separates biopsy material which is obtained by removing the inner cannula (Fig. 42). The biopsy material is extracted by flushing the cannula with saline. The inner cannula is reintroduced into the outer cannula accompanied by gentle aspiration to prevent injection of air into the intracranial tissue.

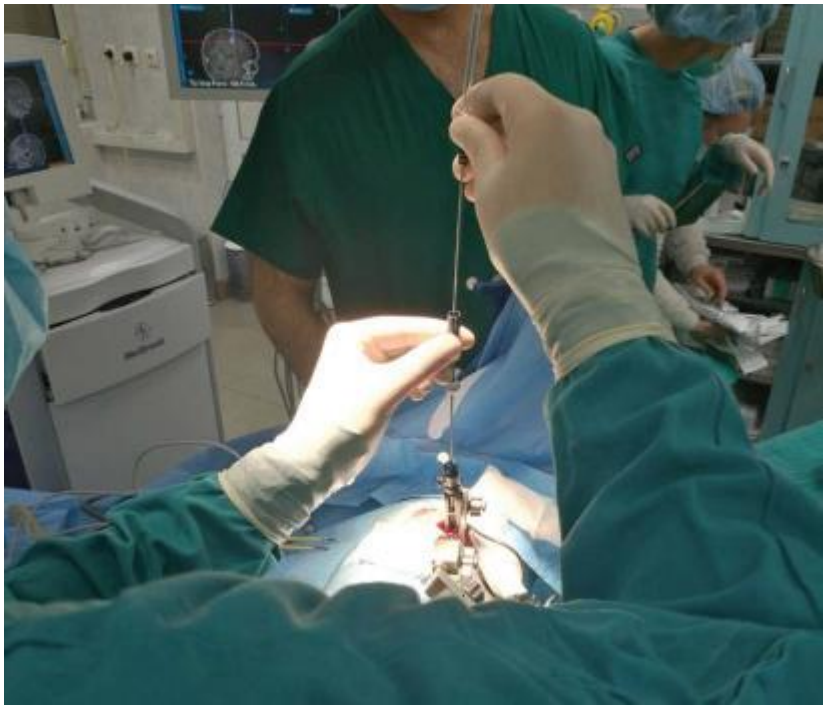

Fig. 42.* *Removal of the biopsy needle's inner cannula in closed biopsy window configuration which extracts tissue for patholohystological examination.*

The procedure is repeated by gradually rotating the outer cannula at 90^{o}, 180^{o}, 270^{o} from the starting position (Fig. 16) on axial plane until extraction of four biopsy materials from the planned axial levels on sagittal plane (Fig. 17). The obtained biopsy materials are sent for intraoperative pathological examination.

After a positive diagnosis on the intraoperative histopathological examination the biopsy needle is removed in closed biopsy window configuration.

According to the intraoperative histological diagnosis the surgical team continues with craniotomy and resection of the intraparenchymal lesion, or with duroplasty and closure of the soft tissues.

3.4. Postoperative period

After extubation the patient is admitted to an intensive care unit for 24 hours observation. After 24 hours, a postoperative head CT scan is performed. If new focal neurological symptoms are registered in that 24 hour period the imaging study is carried out as a matter of urgency.

4. Method for creating a protocol for conducting neuronavigated needle biopsy

Protocol for neuronavigated needle biopsy in patients with supratentorial lesions in the region of the brain is developed on the basis of both accumulated clinical experience and thorough review of the specialized literature. The methodology for creating a protocol, its routine introduction into clinical practice and its gradual modification involves a number of steps (Fig. 43) (Enchev 2015, Kondev 2020).

A surgical protocol standardizes an operative procedure, ensures repeatability and comparability of operational results and reduces the likelihood of technical error in execution of the procedure. Each neuronavigated needle biopsy includes four stages: preoperative preparation stage, planning stage, stage of surgical intervention and postoperative stage. A good preoperative preparation significantly reduces the preventable errors and complications in neurosurgery (Enchev 2015). Careful planning of the surgical target and safe surgical approach to it greatly affects the diagnostic yield and postoperative complications (Taweesomboonyat et al. 2019). Correct positioning of the patient, the method of registration and verification, careful planning of the incision, the technical implementation of the needle biopsy taking into account the heterogeneous nature of the primary brain lesions and the stereotactic nature of the manipulation associated with tracking both the tip of the biopsy needle and the real-time biopsy window are the essential components of this minimally invasive intervention, as inaccuracies in each of them can lead to severe consequences. Lack of standards regarding the postoperative follow up also plays a role in the difficulty comparing the postoperative results for different surgical centers and for different authors.

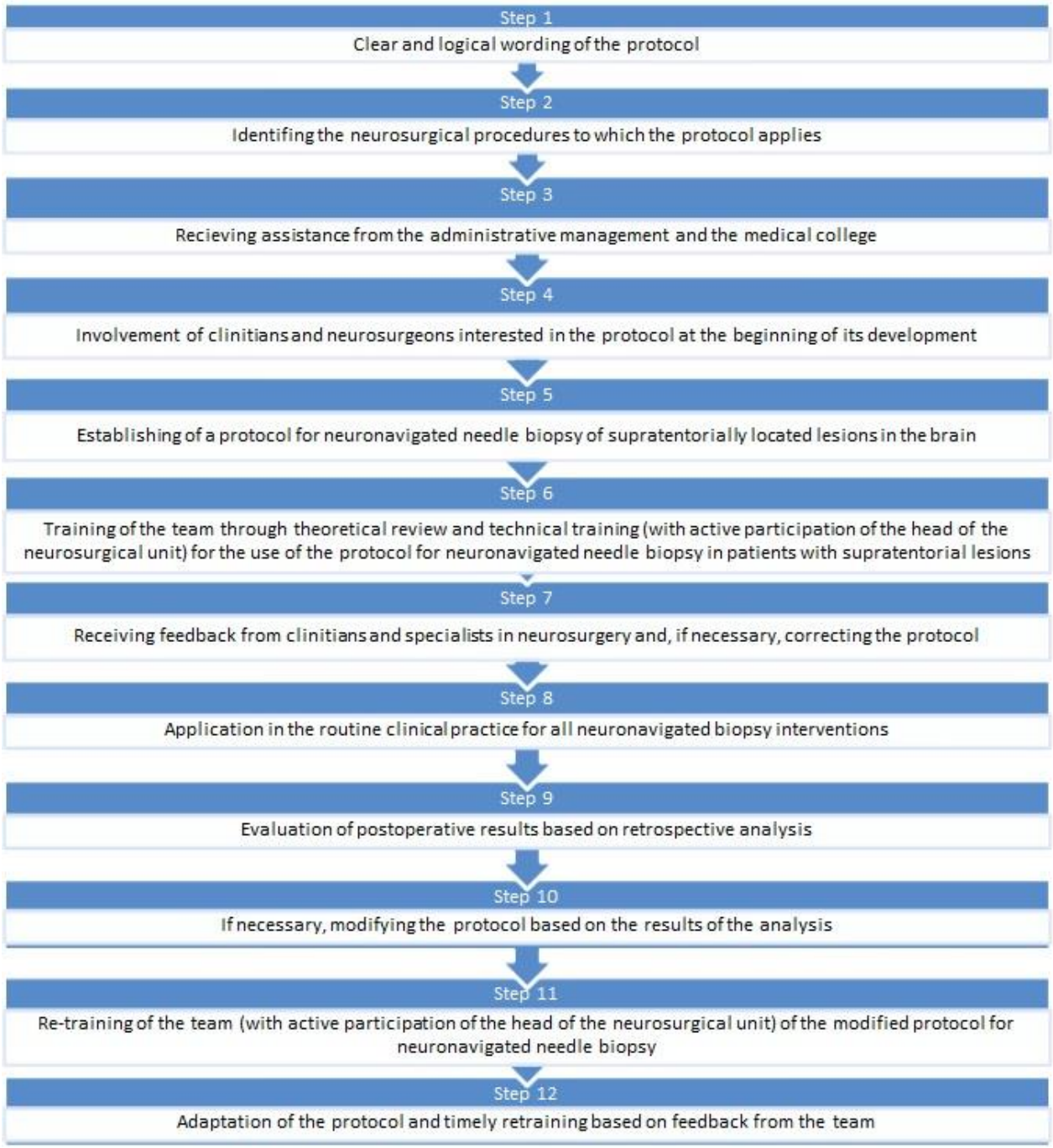

Fig. 43.* *Algorithm including the steps required for construction of a protocol for conducting a neuronavigated needle biopsy of supratentorially located lesions, the introduction of the protocol in routine clinical practice and its modification on the basis of the operative results achieved and the feedback from the surgical team.*

5. Method for training the team in conducting neuronavigated needle biopsy in patients with supratentorial lesions

Prior to the introduction of neuronavigated needle biopsy in patients with supratentorial lesions in the routine clinical practice in January 2019 a thorough study of the scientific literature was conducted as well as training of the medical staff of the Clinic of neurosurgery of the University Hospital - "St. Marina ” in order to properly apply it.

The training was conducted with the active participation of the head of the surgical unit and included: a thorough literature review about the use of neuronavigated surgical methods and introduction of the current data and current trends to the surgical staff; familiarization with the scope and ways of application of the neuronavigation system and the biopsy tray; discussion including questions and answers between the head of the unit, the researcher and members of the surgical team; training on the technical knowhow needed for proper use of the Cranial Vertek passive biopsy tray.

Periodical training of the newly appointed clinicians was conducted including during the period before and after completion of the retrospective analysis.

6. Method for introduction of neuronavigated needle biopsy in clinical practice

The method of introducing neuronavigated needle biopsy in clinical practice includes four stages:

Stage one covers the period of accumulation of knowledge about neuronavigated needle biopsy used in surgically diagnose and treat patients with supratentorial lesions, the training process for its proper technical implementation, training and assessment of acquired knowledge.

Stage two includes the period when attitude is formed. The period includes personal example from the head of the surgical unit when using the neuronavigation apparatus and the biopsy tray. The aim is to be mainly advocated as a biopsy method in the routine clinical practice.

Stage three includes the actual attitude with respect to the use of neuronavigated needle biopsy technique. Accurate use of the neuronavigation system and the biopsy tray is included in this stage in compliance with the established guidelines, avoiding neglect or non-compliance with the individual steps of the neuronavigated needle biopsy surgical technique.

Stage four includes the actual technical implementation during the interventions included in the retrospective analysis of the present study. It includes preoperative preparation, conducting preoperative imaging studies, planning of the surgical target and the point of access on the cranial convexity, positioning the patient on the operating table, registration, verification, planning of the skin incision, stereotactic biopsy of supratentorial lesion according to the following factors: longest axis of the lesion, trajectory allowing biopsy of multiple lesions if such a case is present, the gradual gathering of biopsy material from four orthogonal directions in axial plane on each targeting point in sagittal plane depending on the size of the lesion relative to the biopsy window and taking in account the heterodense structure of most intraparenchymal lesions, as well as case by case assessment if an excision is needed of the lesion given the intraoperative histological outcome.

The four stages interconnect in a repeating cycle, they mutually determine, potentiate and depend on each other. The lack of brake in the cycle is explained by the need for changes due to shortcomings when using the neuronavigated needle biopsy protocol, or changes in the conditions of its implementation (Enchev 2015, Kondev 2020).

7. Statistical methods

7.1 Descriptive analysis

Descriptive analysis is used to describe the basic characteristics of the sample and the indicators included in the study.

The basis for the analysis uses gauges of central trends such as arithmetic mean and nonparametric tests such as Fisher exact test in search of significant differences in frequency presentation of categorical values for small samples.

Regarding the statistical significance of non-parametric tests the assumed value is $p \leq 0.05$.

7.2. Analytical analysis

An independent T-test is used to compare the mean values of operative time as well as dimensions x and y of lesions within the group patients with biopsy followed by excision and patients with biopsy alone. Statistically significant differences are accepted those between groups at $p \leq 0.05$.

Correlation analysis is used to study the dependencies between different clinical indicators to establish the strength of their mutual influence. Assessment of the strength of the relationship between the variables is based on the results of the Pearson coefficient (r) and Spearman (rho). The correlation is measured from -1 to 1, as strong correlation has a value above 0.5, at a level of statistical significance $p = 0.05$. The degree of association between the variables is defined as: significant at $r> 0.5 <r = 0.7$; large at $r> 0.7 <r = 0.9$ and exceptionally large at $r> 0.9$ at $p\leq 0.05$.

Logistic regression is used to establish the Diagnostic yield in the patient group.

Statistical data processing was performed with the computer program SPSS v23 and Jamovi 2.2.0. The graphical representation of the data is presented using Jamovi 2.2.0 and Microsoft Excel, Windows 10.

RESULTS

1. Development of a protocol for neuronavigated needle biopsy in patients with supratentorial lesions

A protocol for neuronavigated needle biopsy in patients with supratentorially located intraparenchymal lesions has been developed. The protocol was created on the basis of a literature review and surgical technique used in all patients in the Clinic of neurosurgery of the University Hospital - "St. Marina" in Varna, where a neuronavigated biopsy intervention was performed.

A surgical protocol standardizes the operative procedure, ensures repeatability and comparability of operational results and reduces the likelihood of technical error in the implementation of the procedure. The protocol includes 15 points:

1. Preoperative preparation.
2. Surgical target point planning.
3. Surgical access point planning.
4. Patient positioning.
5. Registration.
6. Verification of the registration process.
7. Marking the access point and planning the operational section.
8. Verification of the trajectory with target alignment error of less than 2 mm.
9. Marking and adjustment of the length of the working trajectory (the tip stop point) on the biopsy needle.
10. Opening the biopsy window of the inserted biopsy needle and applying negative pressure by aspirating 5 ml using a 10 ml syringe.
11. Stereotactic collection of biopsy material by gradual rotation of the outer cannula at 0^o, 90^o, 180^o, 270^o axially and at distance of 1-1.5 biopsy windows proximal and distal in the sagittal plane depending on the size of the lesion.
12. Intraoperative pathological examination.
13. Depending on the pathological result assessment on the need for open excision of the lesion.
14. Postoperative intensive care for 24 hours.
15. Head CT after 24 hours (earlier if focal deficit is present).

2. Indications and contraindications for conducting neuronavigated needle biopsy under general anesthesia in patients with supratentorial lesions

Indications for neuronavigated needle biopsy include patients diagnosed with a lesion suspected of being primary or a secondary brain tumor, subject to histological verification; immunocompromised patients with lesions in the brain with probable infectious etiology; patients in whom the location of the diagnosed lesion is deep as well as in functional significant regions of the brain, which makes it an inappropriate target for open radical surgery; older or cancer patients with poor performance status - patients for which histological verification is needed so treatment can continue, but radical surgery has too high of a risk, is inadvisable or inappropriate.

Absolute contraindications for biopsy include patients with lesions considered too small to be reached accurately and safely; patients with forms of coagulopathy.

Patients with extensive cranial defects in which the placement of the Mayfield clamp or direct attachment of the reference frame to the skull is impossible do pose a significant challenge. However these circumstances do not disqualify these patients from conducting a navigated biopsy. In these cases, the introduction of electromagnetic field generator and localizer fixed to the soft tissues of the patient could compensate for the lack of opportunity for rigid fixation of the patient's head.

3. Training of the operational team for application of neuronavigated needle biopsy in patients with supratentorial lesions

Routine use of neuronavigated needle biopsy in patients with supratentorial lesions is preceded by theoretical training and technical training of the staff. The neurosurgical team at the Clinic of neurosurgery of the University Hospital "St. Marina" had a training lecture on the use of neuronavigation system and the biopsy tray. The doctors are familiar with the neuronavigated needle biopsy protocol. A discussion was held before and after the conducted retrospective analysis on the operated patients in the Clinic of Neurosurgery of the University Hospital - "St. Marina" included in this study.

4. Routine use of neuronavigated needle biopsy in patients with supratentorial lesions in the Clinic of Neurosurgery of University hospital "St. Marina", Varna

Medtronic Stealthstation 7 neuronavigation system and cranial Vertek biopsy tray have been introduced as a routine tool in surgical treatment of patients with supratentorial lesions subject to histological verification, in the Clinic of Neurosurgery of the University Hospital - "St. Marina", Varna from January 2019 until the present date of issue of this study.

Neuronavigated needle biopsy was introduced after detailed instruction of the surgical team of specialists and residents in neurosurgery about the specifics of this minimally invasive operative technique. Its application is performed by a specialist or resident following the established surgical protocol.

5. Results in the studied patients

For the period January 2019 - December 2021 at the Neurosurgery clinic of University Hospital - "St. Marina" 40 patients with a total of 50 supratentorially located preoperative lesions are biopsied. 41 lesions are diagnosed intraoperatively. In all interventions, the collection of biopsy material is performed via single punctiform craniotomy and via one working trajectory.

Total number of tissue samples taken and sent for hystological study for all patients are 312 materials.

The registered diagnostic yield which demonstrates obtaining a permanent pathological diagnosis is 95% which represents the exact percentage the biopsy is considered successful.

In two of the cases (5%) the biopsy is considered unsuccessful. One case (2.5%) with intraoperative histological diagnosis for Encephalitis in which immunohistochemical postoperative examination diagnoses Lymphoma and one case (2.5%) with intraoperative histological diagnosis for Gliosis, where after an excision is performed, an arteriovenous malformation is diagnosed.

At the postoperative head CT scan of four cases (10%)- two from the cases with isolated biopsy and two from the cases with combination of biopsy and subsequent excision, a discrete hemorrhage is registered which remains without clinical manifestation. No clinically significant postoperative complications are reported nor death associated with surgery. Postoperatively, some patients are directed to conduct oncological treatment, some patients undergo second stage open surgery, and in the case of the microbiological diagnosis- antibiotic treatment is prescribed with good clinical outcome.

5.1. Descriptive data about the present group of patients

The data from the studied population is illustrated and presented in tables and graphs in the original dissertation. There are a total of 40 patients divided into two groups: of 15 cases where it was performed a biopsy procedure and 25 cases who underwent biopsy followed by an excision of the lesion.

5.2. Analytical analysis about the present group of patients

Independent T-test

The test is used to check for statistical differences between the groups of isolated biopsy and that of biopsy followed by an excision with respect to the surgical duration. An expected statistically significant difference in the duration of the operative intervention is registered, given subsequent excision of the biopsied lesion in the group of 25 patients. Mean operative time in patients with isolated biopsy is 109.33 ± 25.56 minutes, while in patients with subsequent excision the mean operating time is 206.4 ± 38.2 minutes (Table 3). An expected longer duration of the performed intervention is observed in the group where an excision is performed.

Table 3.* *Independent t-test is used to compare the average arithmetic surgical duration of patients with isolated biopsy and patients with biopsy followed by an excision. The results demonstrate statistically significant difference in duration for the two groups (t = -8.73, p = 0.01). The mean operative time for patients with excision was 206 ± 38.1 minutes, and for patients with biopsy alone- 109 ± 25.6 minutes.*

Fisher's exact test=2.67, p=0.102		**Excision**		**Total**
Sex		**Without**	**With**	
Male	N	5	15	20
	%	33.3%	60%	
Female	N	10	10	20
	%	66.7%	40%	50%
Total	N	15	25	40
	%	100%	100%	100%

Independent t-test is used to compare the size of the lesions in the groups of patients with biopsy and with biopsy followed by an excision. In terms of x size, the differences between the groups are statistically significant (t = -3.07, p = 0.004), the mean lesion size in patients without excision is 27.4 ± 13.6, and with excision 42.4 ± 15.8. In terms of y size, the differences between groups are statistically significant as well (t = -2.99, p = 0.005), as the mean size of the lesion in patients without excision is 25 ± 13, and with excision 38.9 ± 14.9. (Table 4, Table 5)

Table 4.* *Comparison of the size of the lesions in the two groups of patients with and without excision with respect to X and Y coordinates in the performed preoperative imaging studies for the most representative slice on the axial plane.*

	Group	**N**	**Average**	**Median**	**Standard Deviation**	**Standard Error**
X	Without Excision	15	27.4	29.0	13.6	3.50
	With Excision	25	42.4	43.0	15.8	3.16
Y	Without Excision	15	25.0	27.0	13.0	3.37
	With Excision	25	38.9	39.0	14.9	2.98

Table 5.* *Independent t-test is used to compare the size of lesions in groups of patients with biopsy and excision. With respect to x size, the differences between the groups are statistically significant (t = -3.07, p = 0.004).*

		Statistic	**df**	**p**
X	Student's t	-3.07	38.0	0.004
Y	Student's t	-2.99	38.0	0.005

5.3. Correlation dependence analysis

Correlation analysis is used to study the dependencies between different clinical indicators to establish the strength of their mutual influence. Assessment of the strength of the relationship between the variables is based on the results of the Pearson coefficient (r) and Spearman (rho). The correlation is measured from -1 to 1, as strong correlation has a value above 0.5, at a level of statistical significance p = 0.05. The degree of association between the variables is defined as: significant at r> 0.5 <r = 0.7; large at r> 0.7 <r = 0.9 and exceptionally large at r> 0.9 at p≤ 0.05.

Correlation analysis using the Spearman (rho) method is performed, which tests for a statistically significant relationship between variables: SWI sequence, postoperative hemorrhage and death. The analysis did not confirm a direct relationship. The correlation is measured from -1 to 1, as a strong correlation has a value above 0.5, at the statistical level significance p = 0.05. (Table 6)

Table 6.* *Correlation analysis for direct dependence between SWI sequence and postoperative complications.*

		SWI sequence	Discrete hemorrhage On postop CT	Death
SWI	Spearman's rho	-		
	p-value	-		
Discrete hemorrhage On postop CT	Spearman's rho	-0.2331	-	
	p-value	0.151	-	
Death	Spearman's rho	-0.111	0.480	-
	p-value	0.495	0.002	-

Correlation analysis using the method of Pierson (r) which is applicable to numerically measured data. For both groups of patients the sizes of lesion X and Y, the number of collected materials were examined as well as the surgical duration. The correlation is measured from -1 to 1, as strong correlation has a value above 0.5, at a level of statistical significance p = 0.05. Statistically significant values are marked with an "*". (Table 7, Table 8 and Fig. 44, Fig. 45)

Table 7.* *Correlation analysis is performed using Pierson (r) method for the group of patients who had an isolated biopsy. The analysis demonstrates a strongly positive association (relationship) between dimensions x and y (r = 0.944, p = 0.001) indicating their parallel increase, i.e. as x increases, y increases and vice versa. An expected positive correlation between the obtained number of collected materials (16 pcs.) and the duration of the operation (r = 0.505, p = 0.05) is found showing the directly proportional trend of increase in operating time by increasing the number of collected materials.*

		x	***y***	***4 materials***	***8 materials***	***12 materials***	***16 materials***	***Duration (min)***
X	*Pearson's r*	-						
	p-value	-						
Y	*Pearson's r*	*0.944****	-					
	p-value	*<.0.001*	-					
4materials	*Pearson's r*	*-0.401*	*-0.327*	-				
	p-value	*0.138*	*0.234*	-				
8materials	*Pearson's r*	*0.062*	*-0.060*	*-0.237*	-			
	p-value	*0.826*	*0.832*	*0.396*	-			
12materials	*Pearson's r*	*0.085*	*0.120*	*-0.237*	*-0.364*	-		
	p-value	*0.763*	*0.671*	*0.396*	*0.183*	-		
16materials	*Pearson's r*	*0.407*	*0.431*	*-0.237*	*-0.364*	*-0.364*	-	
	p-value	*0.132*	*0.109*	*0.396*	*0.183*	*0.183*	-	
Duration (min)	*Pearson's r*	*0.128*	*0.100*	*-0.466*	*-0.075*	*0.047*	*0.505**	-
	p-value	*0.650*	*0.724*	*0.080*	*0.790*	*0.868*	*0.050*	-
*Note * p<.05, **p<.01, ***p<.001*								

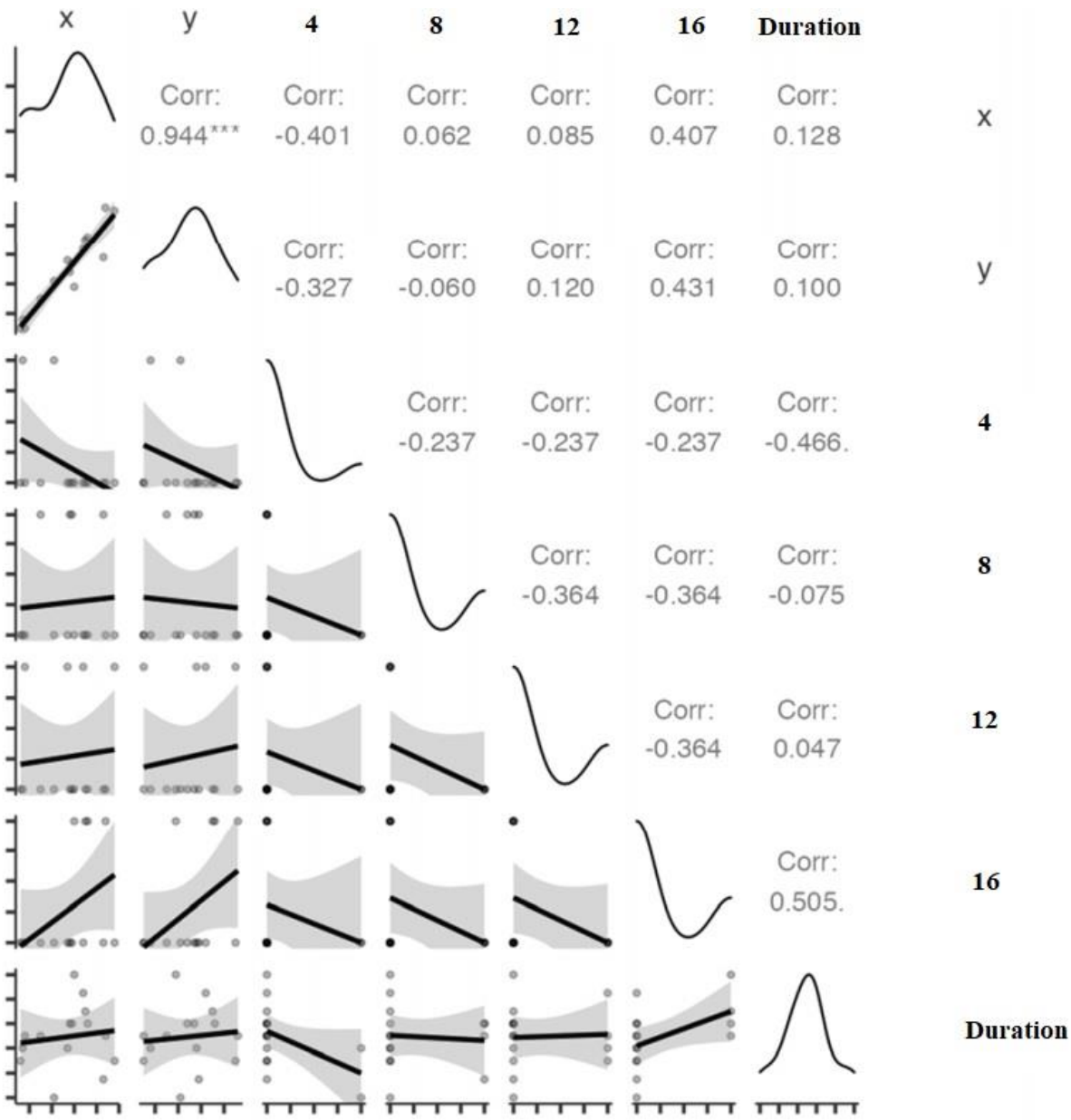

Fig. 44.* *Graphical representation of the correlation analysis using the method of Pierson (r) for the group of patients who had an isolated biopsy.*

Table 8.* *Correlation analysis using the Pierson (r) method for the group of patients who underwent biopsy following and excision. The study demonstrates a strongly positive correlation between the size of the lesions and the duration of the operation showing the expected trend that with increasing lesion size surgical duration is also increasing. An absolute correlation is found for 4 and 8 collected histological materials: data in the tests suggest a direct link between the interventions with excision and smaller number of collected materials for histological examination. One of the reasons for strong correlation dependence may be related to the fact that in the group of patients with excision there is no case of more than eight materials collected.*

		x	***y***	***4 materials***	***8 materials***	***12 materials***	***16 materials***	***Duration (min)***
X	*Pearson's r*	-						
	p-value	-						
Y	*Pearson's r*	*0.767*	-					
	p-value	*<.0.001****	-					
4materials	*Pearson's r*	*-0.157*	*0.197*	-				
	p-value	*0.454*	*0.344*	-				
8materials	*Pearson's r*	*-0.157*	*-0.197*	*-1.000*	-			
	p-value	*0.454*	*0.344*	*<.001****	-			
12materials	*Pearson's r*	*NaN*	*NaN*	*NaN*	*NaN*	-		
	p-value	*NaN*	*NaN*	*NaN*	*NaN*	-		
16materials	*Pearson's r*	*NaN*	*NaN*	*NaN*	*NaN*	*NaN*	-	
	p-value	*NaN*	*NaN*	*NaN*	*NaN*	*NaN*	-	
Duration (min)	*Pearson's r*	*0.663*	*0.522*	*-0.071*	*0.071*	*NaN*	*NaN*	-
	p-value	*<.001****	*0.007****	*0.736*	*0.736*	*NaN*	*NaN*	-
*Note * p<.05, **p<.01, ***p<.001 NaN- not enough data*								

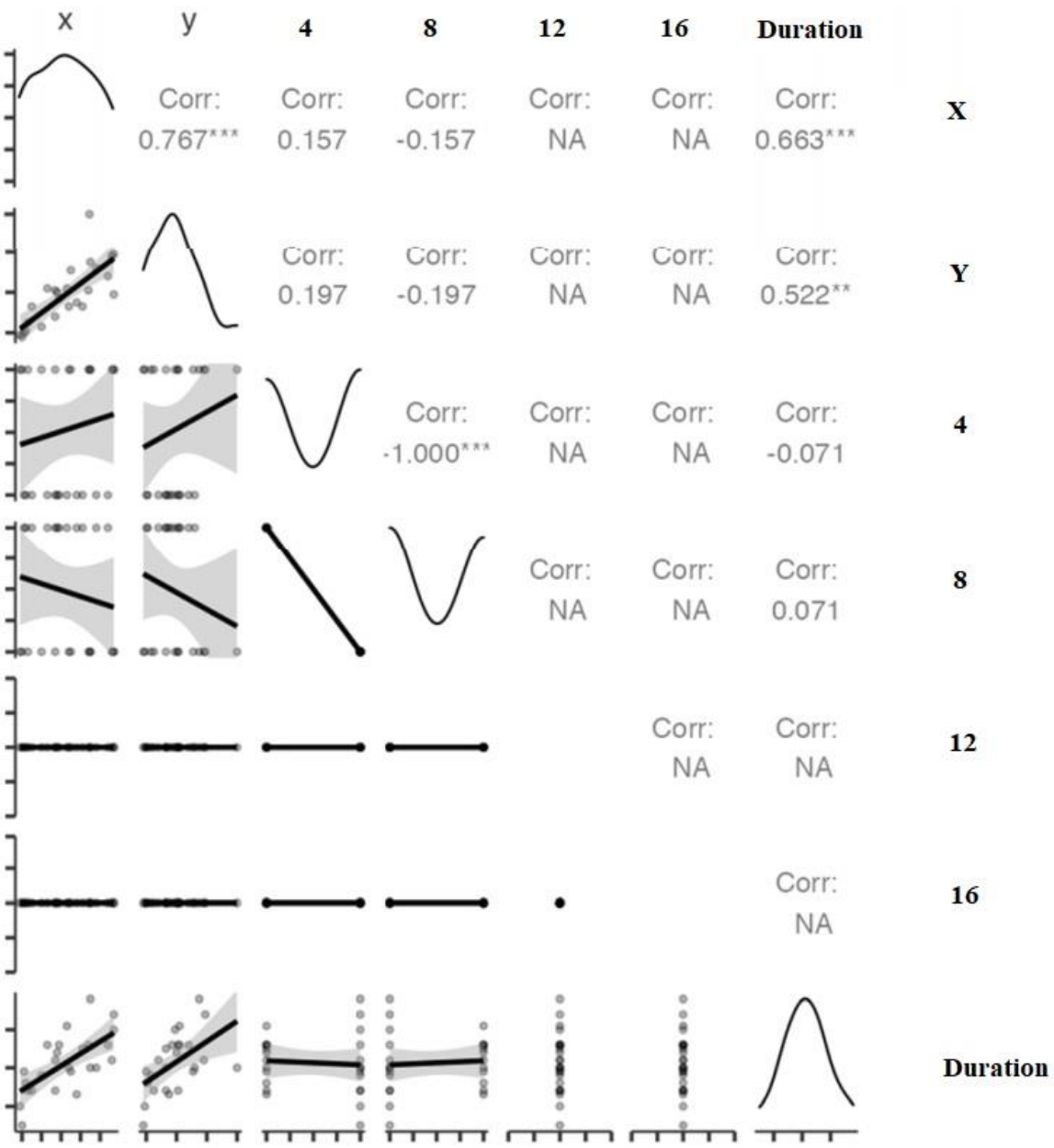

Fig. 45.* *Graphical representation of the correlation analysis by the method of Pierson (r) for the group of patients who underwent biopsy and excision.*

5.4. Diagnostic yield

The total registered diagnostic yield, which demonstrates the production of permanent intraoperative histological diagnosis is 95% representing the exact percentage the biopsy is considered successful. Logistic regression is used to analyze the diagnostic yield in the patient group. It tests whether within the patient sample, factors like age, sex, contrast T1 MRI sequence and anamnestic data for previous radiotherapy/ surgery/ histology, would have some bearing on the diagnostic yield (Tab. 9). The values of the variables studied in the group do not show statistical significance. Regarding the significance of diagnostic output and sex,

within the sample, the data demonstrate a trend for higher diagnostic output in the women's group. The limited amount of data greatly reduces the certainty that when examining another group of patients the results would show the same results.

Table 9.* *Logistic regression is used to analyze the diagnostic yield in the patient group. It tests whether within the sample, factors like age, sex, contrast T1 MRI sequence and anamnestic data for previous radiotherapy / surgery / histology would have some bearing on the diagnostic yield. N.A- not enough data*

Total	**Diagnostic yield**	**Operative Risk**	**p**
Age		0.99	>0.05
<49	97.5% (39/40)		
>49	97.5% (39/40)		
Sex		3.715	<0.05
Male	90%% (18/20)		
Female	100% (20/20)		
MRI (T1C)		8.42	>0.05
With	95.2% (20/21)		
Without	94.7% (18/19)		
Previous radiotherapy		N.A	
With	100% (2/2)		
Without	94.4% (34/36)		
Previous operation		N.A	
With	100% (3/3)		
Without	94.3% (33/35)		
Previous histology		N.A	
With	100% (4/4)		
Without	94.1% (32/34)		

DISSCUTION

Histological diagnosis is a major factor in the treatment of intracranial lesions. Formulation and hasty implementation of a treatment plan without available histological verification in 25% of cases undergoes a significant change after a biopsy intervention is conducted and accurate diagnosis is reached. (Kim et al. 2003)

So far the frame based stereotaxy occupies the main place in minimally invasive biopsy interventions of intracranial lesions. (Zhang et al. 2013)

With the rapid development of modern computer systems, the modernization of neuronavigation systems and the promising data for efficacy, safety and diagnostic value, trends in modern neurosurgical practices are associated with the use of frameless neuronavigated stereotaxy. (Widmann et al. 2012)

1. Diagnostic yield

The number of literature sources related to the frameless neuronavigated biopsy and in particular data for the success rate in reaching a final intraoperative histological diagnosis is limited. Two current systematic comparative analyzes have been published analyzing the effectiveness of classical frame based biopsy and neuronavigated needle biopsy: the first analysis is by Dhawan et al. 2019 including fifteen studies, the second by Kesserwan et al. 2021, it includes the fifteen studies from the previous analysis and in addition includes five new studies. Systematic analysis of the literature and the accompanying meta-analysis do not demonstrate significant clinical difference between biopsy techniques based on stereotactic framework and neuronavigated biopsy technique. Asymptomatic postoperative hemorrhage remains the only one reported statistically significant difference between the two techniques.

A final intraoperative histological diagnosis in neuronavigated biopsy is achieved in 73% -100% (Dhawan et al. 2019). The present study reports final intraoperative histological diagnosis is reached in 38 of the cases (95%) and a lack of final intraoperative diagnosis in 2 of the cases (5%). Deepali et al. 2006 and Jain et al. 2006 studied 86 operated patients, in 16 (18.6%) cases they reported lack of definitive histological diagnosis on intraoperative pathological examination. In a study by Dammers et al. 2008 involving 164 patients, in 89% of those cases is reached

final intraoperative diagnosis. In a study by Livermore et al. 2014 involving 94 patients, the final intraoperative diagnosis is made in 95.7% of the cases. The results of the present study are in the reference frame in comparison to the surgical results in the literature sources.

With regard to diagnostic yield analysis conducted by Kesserwan et al. confirms that contrary to the classical view, compared to frameless neuronavigated biopsy technique, biopsies performed by frame based stereotaxy do not demonstrate higher diagnostic yield. One of the main reasons for the number of collected materials for intraoperative hystological examination to be included in the present study is the hypothesis of Kesserwan et al. 2021 and Dhawan et al. 2019, that due to idiosyncratic or subjective factors leading to the tendency of surgeons to prefer to biopsy small, deep-seated lesions in the brain with a frame based stereotactic technique, and large more superficial lesions- using frameless neuronavigated technique coupled with lesser number of materials collected, the diagnostic yield would be affected. Although the authors do not include in their analysis the depth, average size of the lesion and the number of collected samples, they report a statistically significant difference in terms of lesion's location, specifically midline location of the biopsied lesions in favor of the group of patients operated on by neuronavigated biopsy. The authors take into account two important factors: a possible impact on reliability of some of the published series connected with the permanent transition during the ongoing studies, from frame based to frameless surgical technique and the accompanying changes a result of the learning curve. A similar period of training would change the outcome of a study in favor of the operated patients with frame based technique. The present study only covers patients operated by neuronavigated needle biopsy. The surgical technique and the established surgical protocol were used without any major changes in the surgical stages to be noticed or registered, including the learning period.

Lu Y et al. 2015 explore possible factors that could affect the diagnostic yield of a needle biopsy, including age, sex, characteristics of the used intraoperative imaging studies and anamnestic data for previous radiotherapy, previous surgery and previous biopsy. The results of their study point to statistically significant ($p < 0.01$) influence on diagnostic yield in terms of age: 75.9% diagnostic output in patients under 40 years and 90.6% in patients over 40 years. Statistically significant ($p < 0.01$) effects on diagnostic yield were reported and in terms of anamnestic data: from 90.2% diagnostic production in patients without previous radiosurgery the percentage of diagnostic yield decreases to 69.7% in patients who had previous radiosurgery done; of 90.9% diagnostic yield for patients without any previous surgery done the percentage of diagnostic yield decreases to 75.9% in patients who have undergone previous intervention; from 90% diagnostic yield in patients without previous biopsy the success rate drops to 55.6% diagnostic yield in patients with a previous biopsy done.

In the present study, a logistic regression was performed for the study of diagnostic yield in the patient group, testing whether within the patient sample, factors like age, sex, contrast T1 MRI sequence and anamnestic data for previous

radiotherapy, surgery, histology, would reflect changes in the diagnostic yield. No statistically significant factors influencing the diagnostic yield with respect to age, characteristics of imaging studies and anamnestic data are registered. Possible reason for the significant differences in the risk factors influencing the diagnostic yield is relative to the small number of patients in the present study. Additional research is needed including a larger sample size of biopsied patients, given the reported risk factors by Lu Y et al. 2015 and significant differences in diagnostic yield in patients with previous radiotherapy, surgery and histology in comparison to the present study.

2. Intraoperative histopathological diagnosis

Regarding the intraoperative histopathological diagnosis, a study performed by Zhang et al. 2013 reported that in a series of 62 patients, the distribution of definitive histological diagnoses is: glial tumor- 50%, Lymphoma- 10%, metastases - 10% and infectious diseases - 6.4%. Taweesomboonyat et al. 2019 documented 85 patients with performed intraoperative histological examination showing: Lymphoma - 43.8%, Glial tumor - 30.4%, Infectious disease - 9%. In the present study, the intraoperative histological examination in biopsies with a positive result diagnoses a glial tumor in 75% of patients in the study group. 41 lesions were biopsied in 40 patients, and in 19 (47.5%) the lesion was diagnosed as Glioblastoma (Fig. 46 and Fig. 47), 10 (25%) - Diffuse astrocytoma WHO grade II (Fig. 48), 1 (2.5%) – Anaplastic astrocyte WHO grade III.

In 4 of the patients in the group (10%) pathological examination diagnoses secondary dissemination in the brain of malignant neoplasm, in 2 (5%) - Lymphoma and in 1 (2.5%) – Embryonic tumor WHO grade IV. One of the interventions with a positive histological result (2.5%) leads to the diagnosis of an abscess and to reaching a microbiological diagnosis (Fig. 49).

In two of the patients (5%) in the study the biopsy result is determined as negative - one patient (2.5%) with Encephalitis, where postoperative immunohistochemical examination diagnoses Lymphoma and one patient (2.5%) with intraoperative histological diagnosis of Gliosis, where after an excision is performed a definitive diagnosis of arteriovenous malformation is reached.

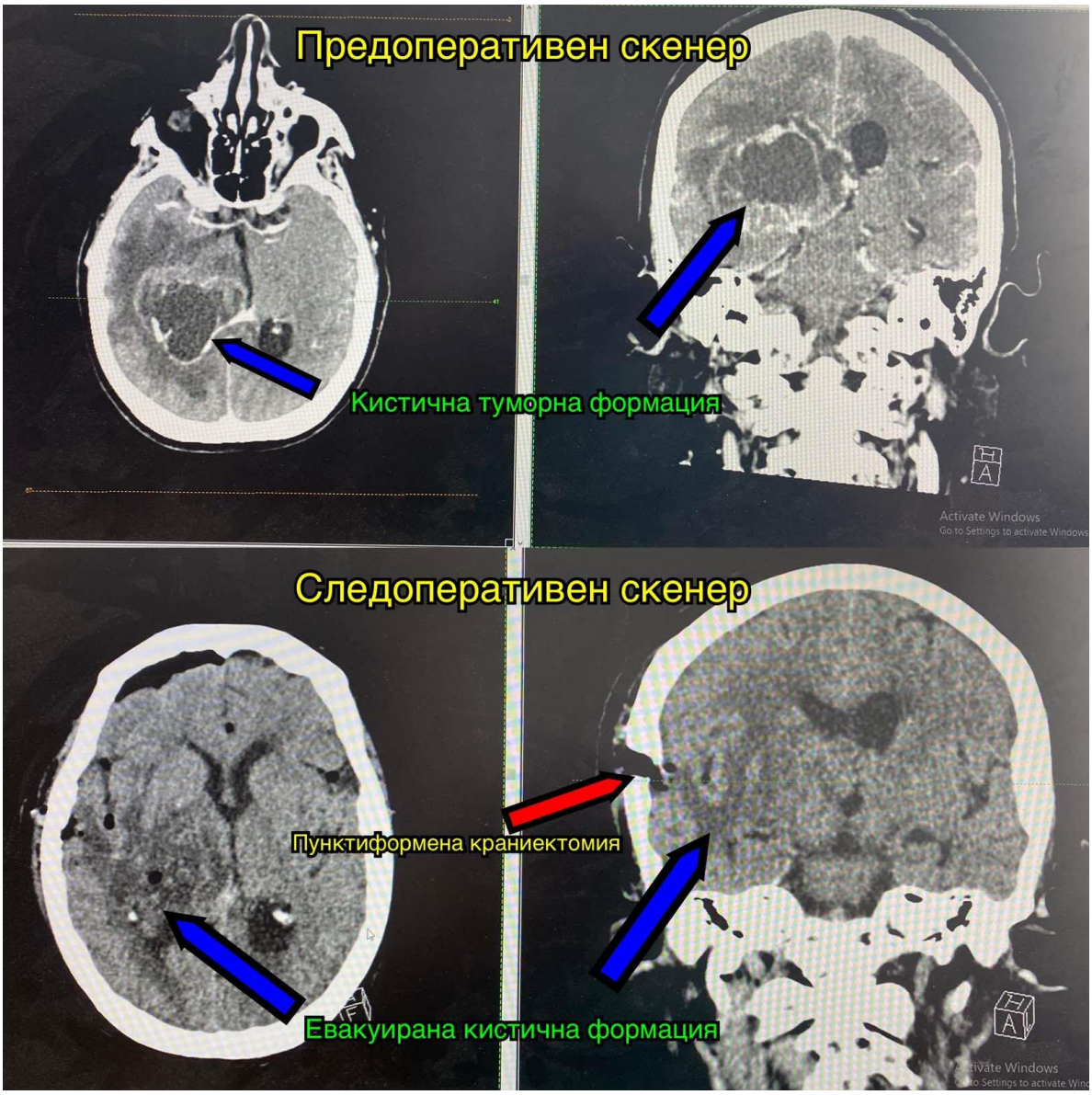

Fig. 46* *Preoperative (top left and right) and postoperative (bottom left and right) head CT with evidence of cystic lesion in the right temporo- arietal area. An intraoperative histological diagnosis is reached- Glioblastoma Multiforme.*

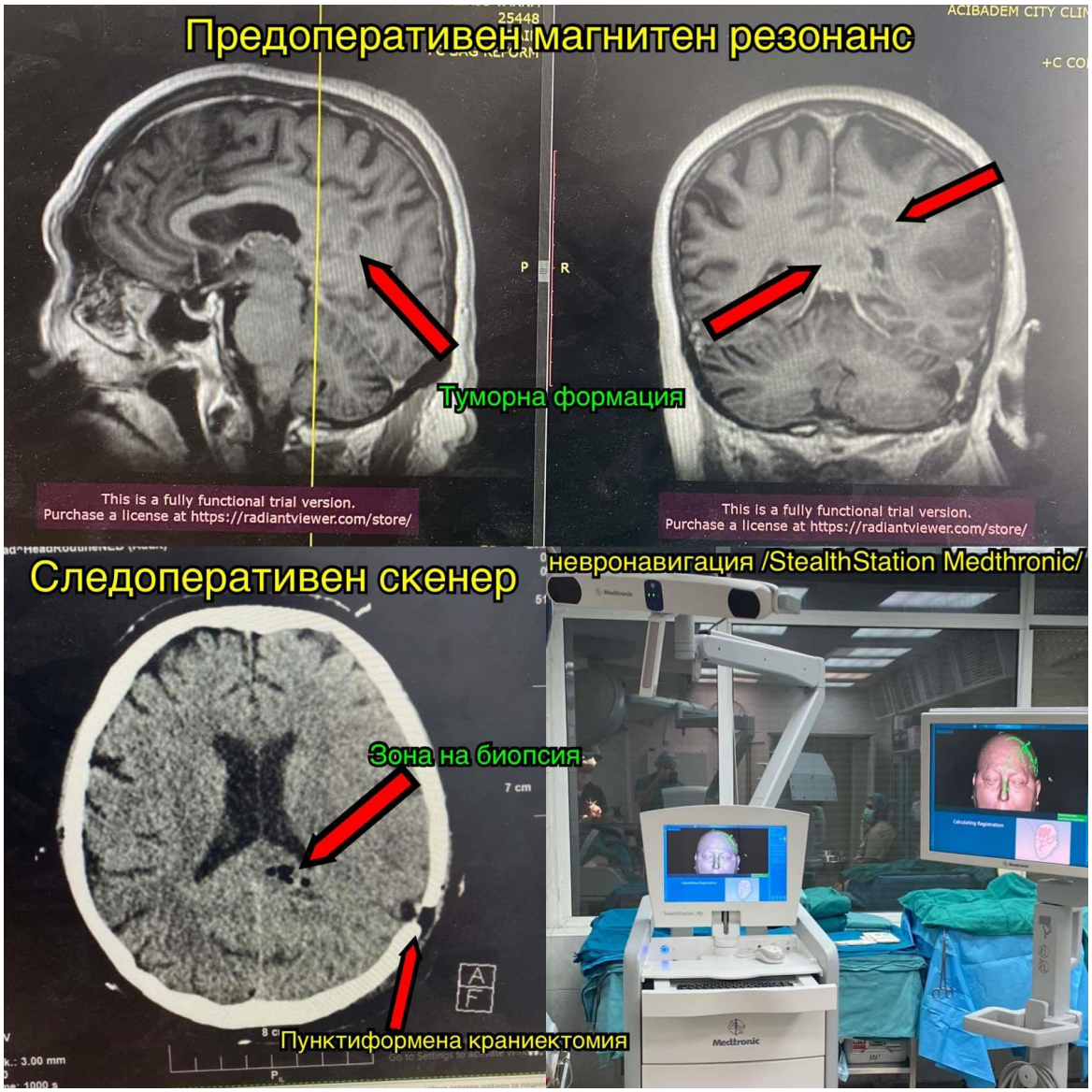

Fig. 47* *Preoperative (top left and right) and postoperative (bottom left) head CT with evidence of tumor formation engaging corpus callosum and left parietal lobe. Intraoperative histological diagnosis is reached- Glioblastoma Multiforme*.

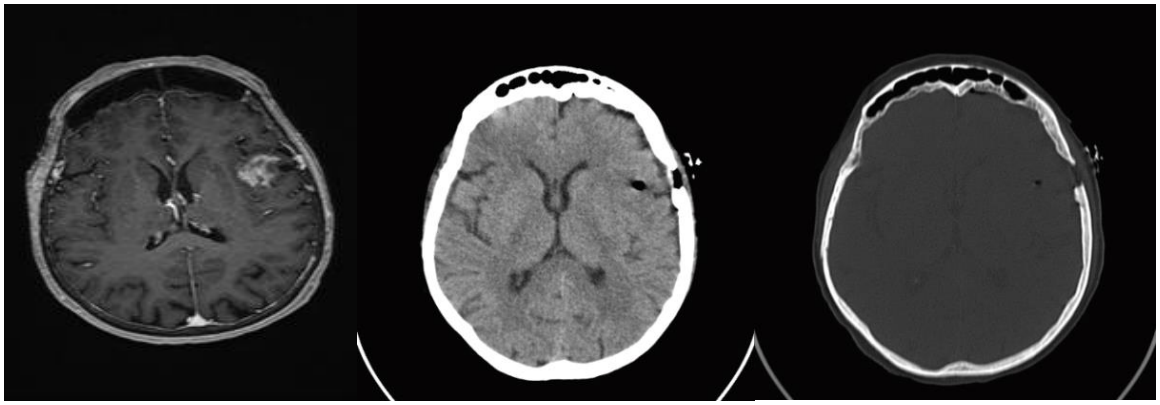

Fig. 48.* *Preoperative MRI (left) and postoperative (center and right) head CT of a 27-year-old man with left fronto-temporal lesion. An intraoperative histological diagnosis was reached- Diffuse astrocytoma gr. II*

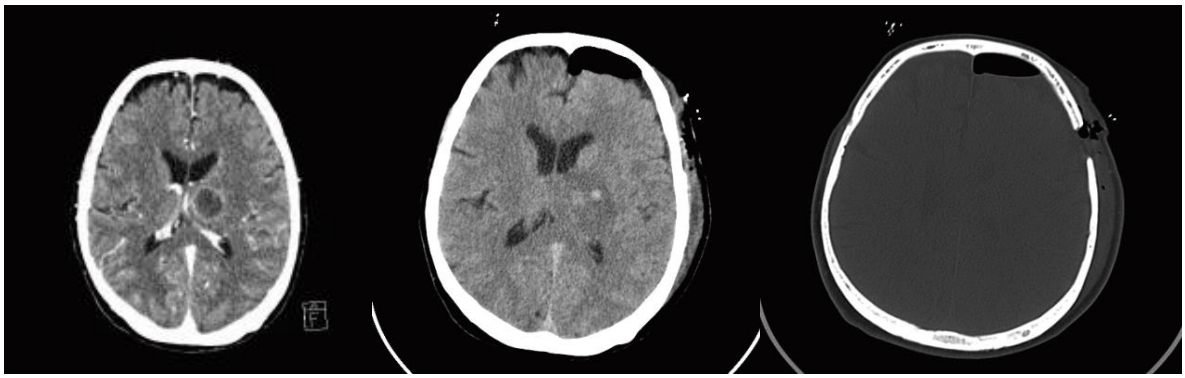

Fig. 49.* Preoperative (left) and postoperative (central and right) head CT of a 67-year-old woman with a left thalamic lesion. Intraoperative histological diagnosis of abscess is reached.

Kesserwan et al. 2021 documented 20 studies conducting a total of 1206 frameless biopsies. Average values with respect to age, sex, anesthesia, lesion location and histology are demonstrated together with the results on the same indicators for the present study (Table 10).

Table 10.** *The results of the present study are presented together with the results for the neuronavigated interventions from the meta - analysis of Kesserwan et al. 2021 which includes 20 studies with a total of 1206 biopsies conducted.*

Indicators		*Meta-analysis*	*Present Study*		
		Medium value	*Isolated biopsy*	*Biopsy and Excision*	*Total*
Age (years)		51.5± 8.6	59.1±16.4	64.2±13.7	62.3±14.8
Sex (%)	N	364	15	25	40
	Women	44.00%	67%	40%	50%
	Men	56.00%	33%	60%	50%
Anesthesia (%)	N	684	15	25	40
	General	97.40%	100%	100%	100%
	Local	2.60%	0	0	0
Location (%)	N	476	24	26	50
	Lobes	56.90%	58%	66%	61%
	Median	39.10%	17%	4%	11%
	Cerebellum	2.10%	0	0	0
	Multiple	1.90%	25%	31%	28%
Histology (%)	N	641	15	25	40
	High grade glioma	53.80%	27%	60%	47.50%
	Low grade glioma	14.70%	47%	16%	27.50%
	Unclassified	2.20%	0	0	0
	Other tumors	2.00%	6.50%	0	2.50%
	Meta	5.00%	0	16%	10%
	Lymphoma	10.30%	6.50%	4%	5%
	Other	12.00%	13%	4%	7.50%

One observes that in the present study patients of old age are found in the group of the biopsies combined with excision. The number of women in the group of isolated biopsies is also significantly higher (67%) compared to the analyzed average percentage (44%). It is possible but unlikely explanation for the statistically significant negative effect of male sex on diagnostic yield in the performed logistic regression for the patients in the group (OR 3.715; $p < 0.05$). The relatively small number of patients included in the study is a more likely explanation for the reduced diagnostic yield in relation to male sex in the current sample.

The main advantage of frame based stereotaxy is that it compensates the need for general anesthesia. In the analysis of Kesserwan et al. 2021 1.2% of stereotactic frame surgeries are performed under general anesthesia, while 97.4% of neuronavigated biopsies require general anesthesia ($P < 0.05$). This fact is of great clinical importance, especially concerning elderly patients, patients with poor performance status, or those patients that need histological verification in order to continue their treatment but cannot tolerate general anesthesia. All surgical interventions in the present study are performed under general anesthesia.

In terms of location, an observation can be made about the higher percentage of multiple localization of the lesions (25% in the group of isolated biopsies, 31% in the group of biopsy and excision, 1.9% of the literature data) for the current sample, at the expense of the lower percentage of lesions with midline localization (17% in the group of isolated biopsies, 4% in the group of biopsy and excision and 39.1% in the analyzed data from the literature sources). Given the deep localization and higher probability of damage to eloquent areas during open surgery, the small percentage of deeply situated lesions that is excised in the study is expected. All lesions that are operated on in the present study have a supratentorially localized. According to the analyzed published data, 2.1% of neuronavigated biopsies are localized in the region of the pontocerebellar angle.

A trend is observed in terms of histological diagnosis: high - grade gliomas tend to be biopsied and excised (60% in the group of biopsy and excision, 27% isolated biopsy, 53.8% in published data), low-grade gliomas tend to undergo isolated biopsy (47% in the group of isolated biopsy against 16% in the group of biopsy and excision and 14.7% according to analyzed published data). Metastatic lesions are overwhelmingly subject of excision (16% in the group of biopsy and excision, 0% in the group of isolated biopsy and 2% of literature data).

It is important to note the fact that some of the analyzed series have a higher percentage of influence on the results of the meta-analysis as a whole. Regarding postoperative asymptomatic hemorrhage, series published by Michaud et al. 2013 contributes 82.8% (p = 0.03) to the reason why the frame based technique is preferred next to the frameless one. Excluding the series in question from the systematic analysis, Kesserwan et al. 2021 demonstrate that after the change, the difference between the two surgical techniques becomes statistically insignificant (p = 0.78).

Dammers et al. 2010 registered yield of tissue with a quality that does not allow histopathological evaluation in 1% of cases in biopsies performed with intraoperative histological examination and up to 11% in biopsies in which the biopsy material is first collected and the histopathological examination is performed at a later stage. In the present study for each intervention after collection of 4 materials the tissue is sent for intraoperative histopathological evaluation.

A study by Nishihara et al. 2014 considered 59 cases biopsied by frame stereotaxy and 38 cases biopsied by neuronavigated technique. The study demonstrates diagnostic yield of 94.9% for the frame based biopsy and 97.4% for the neuronavigated biopsy (p = 0.944), morbidity rate of 5.1% and 0% (p = 0.417) respectively and reduction of the total operative risk by 23.1% for neuronavigated biopsy in lesions with localization in deep regions of the brain. In cases diagnosed with glial tumor in combination with localization of the formation in the basal nuclei, the reduction of the total operational risk reaches 30%. Nishihara et al. 2014 confirm the lack of significant difference between the frame based and neuronavigated biopsy technique in relation to diagnostic yield and morbidity, but for patients with a lesion suspected for glial tumor localized in the area of the basal nuclei, for the purpose of reduction of total operational risk they recommend the use of neuronavigated biopsy technique with the possibility of virtual tracking of the trajectory in the imaging studies of the patient in real-time.

Woodworth et al. 2005 examined a total of 21 patients where 12 frame biopsies and 9 neuronavigated biopsies are conducted with a follow-up craniotomy at a second stage. The results demonstrate similar accuracy at neuronavigated and frame based biopsy - 89% and 66%, respectively (p = 0.21). In 14% of cases, the biopsy material is assessed as adequate for correctly diagnose glioma, and in 5% the glial tumor is incorrectly staged as lower grade than what it actually is. In 5% of cases the intraoperative finding is for necrosis / gliosis. Biopsies of tumors larger than 50 cm^3 are defined as eight times less likely to diagnose correctly the exact stages of a glial tumor compared to lesions below 50 cm3 (OR, 8.8; 95% CI, 0.9-100, p = 0.05). The authors recommend taking a larger number of biopsy materials on a trajectory planned along the long axis of the lesion.

3. Postoperative complications

A study by Taweesomboonyat et al. 2019 that includes 85 patients takes into account the relationship between lesion size, diagnostic accuracy and postoperative complications. Authors demonstrate that volumetric lesions with a diameter of more than 3 cm associate with a higher percentage of positive diagnostic results, and lesions with a diameter of less than 3 cm- with a higher rate of postoperative complications. The mean lesion size in the study range from 33.1 ± 16.9 mm x 30 ± 16.0 mm., no significant difference in the diagnostic accuracy is registered for tumors over 3 cm.

Dhawan et al. 2019 reported that postoperative complications vary in the range 2.4 - 17.8% and largely depend on the criteria one base the evaluation on. Michaud et al.2013 note that a possible reason for the difference with respect to postoperative hemorrhage could be due to the fact that postoperative head CT is performed at different time windows for different techniques.

Owen et al. 2009 noted that the lack of bleeding from the biopsy needle after its introduction is a strong prognostic sign for lack of functionally significant intracerebral hemorrhage, as per their opinion a postoperative head CT can be reserved only for patients for whom one observes intraoperative persistent bleeding from the biopsy needle despite the irrigation performed and for the patients for whom one observes postoperative focal neurological symptoms.

The postoperative head CT is an important point in the established clinical protocol for neuronavigated needle biopsy in the Neurosurgery clinic of the University Hospital "St. Marina”. The control imaging study is planned for 24 hours after the operative intervention. If postoperative focal symptoms are observed the postoperative CT is conducted as a matter of urgency. The lack of the postoperative examination algorithm is one of the reasons for the difficulty in comparing the operational results between the different series. An additional factor is the lack of standards for assessing hemorrhage from a radiological point of view. In studies evaluating postoperative hemorrhage with dimensions over 1 cm as significant, the reported percentage of postoperative complications is significantly lower so that it can reach 0%. In the present study hemorrhages larger than 0.5 mm are assessed as significant, as in four of the cases (10%) the postoperative head CT shows discrete hemorrhage in the biopsy region with dimensions over 0.5 mm. In all patients in the study, the hemorrhage is not clinically manifested. One should consider that postoperative CT control in all patients is performed twenty-four hours after completion of the surgical procedure, as in patients with biopsy and excision the postoperative examination registers changes associated with the wider craniotomy and the invasive nature of the surgical excision of the biopsied lesion. In 100% of those cases the excision covers the region the lesion is biopsied before excision is performed. Although the criteria for the assessment of postoperative hemorrhage did

not differ for the patients in the two groups, the possibility of using direct hemostasis and administration of hemostatic material is a factor that decreases to a greater extent the likelihood of postoperative haemorrhage in this group of patients and remains a factor that cannot be quantified accurately and cannot be compensated for in terms of the present retrospective analysis. Possible way to compensate for such a factor in the case of future research relates to the topic intraoperative imaging (intraoperative CT or MRI). Conducting intraoperative diagnosis before transition to excision would provide reliable and comparable data on the presence of intracerebral hemorrhage after the biopsy material is collected. Inclusion of this intraoperative imaging step in the routine neuronavigated biopsy protocol remains debatable, largely due to the inaccessibility of this intraoperative diagnostic equipment.

In terms of postoperative mortality one can observe similar results considering a series published by Dammers et al. 2008 The reason for reporting a total mortality rate for the frameless biopsy technique of 2.2% and 2% for the framework biopsy, in 64.4% are due to the published by Dammers et al. series. When removing the series from the system analysis Kesserwan et al. 2021 reporte postoperative mortality of 1.35% for frameless technique and 0.93% for frame based stereotactic biopsy. For one patient in the present study an event leading to death is registered without causal connection with the performed biopsy- acute worsening of chronic heart failure on the sixth day after surgery.

Seven studies have been published between 2005 and 2018 that include 61 patients biopsied using the same neuronavigation system used in the present study - Medtronic StealthStation. Published data on the individual study, year of publication, number of patients included in research, diagnostic yield, mortality, morbidity, postoperative hemorrhage, the presence of postoperative neurological deficit and operative duration are demonstrated (Table 50).

Table 50.** Published studies involving biopsy interventions using the same neuronavigation system used in the present study- Medtronic StealthStation.

Study	Year	System	Patients	Diagnostic yield	Death	Morbidity	Haemorrhage	Neurological deficit	Surgical Duration (min)
Georgiopoulos et al.	2018	Stealth Station	28	96.40%	0	21.4%	17.8%	3.5%	79.1 ±22.7
Livermore et al.	2014	Stealth Station	95	95.70%	-	-	-	-	117 ±42
Dammers et al.	2007	Stealth Station	164	89%	3.60%	11.5%	2.4%	1.2%	127 ±33
Jain et al.	2006	Stealth Station	15	86.60%	-	-	-	-	-
Woodworth et al.	2005	Stealth Station	9	88.80%	0	33.3%	11.1%	22.2%	-
McGirt et al.	2005	Stealth Station	110	-	-	18.1%	-	-	-
Smith et al.	2005	Stealth Station, Brain lab, ISG Wand	74	90.50%	1.30%	1.3%	0	1.3%	185 ±6

Regarding the diagnostic yield, the published results are in the range of 72.8 - 95.6%. One can observe that the results published in studies involving a small number of patients, such as that of McGirt et62 al. and Georgiopoulos et al.2018 will tend to emphasize more sharply deviation from the mean values of the meta-analyzes. Given the general number of 40 patients included in the present study, the reported diagnostic yield of 95% and the reported results of the published researches, it is safe to assume that future research including larger representative sample of biopsied patients according to the presented surgical protocol would report diagnostic yield of 72.8 – 95.6%.

The complications related to neuronavigated frameless biopsy, according to the published data, are equal if not less than the complications associated with frame based stereotactic biopsy. The results demonstrate low levels of postoperative permanent neurological deficit (1.4% - 5.5%), intracerebral hemorrhage (1.8% - 9%) and mortality - about 1%. Published data on the risk factors associated with postoperative complications after conducting biopsy procedures include: multiple introduction of the biopsy needle, diabetes mellitus and localization of the biopsy needle lesion in basal nuclei and posterior cranial fossa. The results of a study by Air et al. 2009 emphasize that performing a neuronavigated frameless biopsy of lesions located in eloquent areas is as safe and effective as in a functionally insignificant regions of the brain. A study by Nishihara et al. 2014 demonstrated that compared to the frame based biopsy technique, the use of navigated frameless equipment provides a 23.1% reduction in total risk for lesions located in the basal nuclei and 30% reduction of the overall operative risk in biopsy of glial tumors. In the present study, all biopsy procedures were performed by neuronavigated frameless biopsy with a neuronavigation system Medtronic Stealthstation 7, Cranial Vertek biopsy tray and Medtronic passive biopsy needle, with one trajectory and one insertion of the outer cannula of the biopsy needle. According to McGirt et al. 2005 and Air et al. 2009 the main factor contributing to postoperative neurological deficit and hemorrhage in the biopsied region in patients with intracerebral lesions located in deep regions of the brain (basal nuclei, thalamus, posterior cranial fossa), is the parenchymal trace of the biopsy needle left after its entry. This is precisely the reason for the present study to be used one working trajectory and one entry of the external cannula with the ability to repeatedly collect biopsy material though the internal cannula by progressively increasing the depth by moving the external cannula on the same working trajectory, as the introduction of the external cannula more than once is avoided in order to reduce the likelihood of postoperative complications.

A study by Chen et al. 2009 examines 299 cases where stereotactic biopsy is conducted. The study reports 90.6% diagnostic yield, 7.4% postoperative complications, however almost half of the observed complications lead to permanent morbidity. In 1.34% of the studied group the authors performed a craniotomy to evacuate a hematoma or evacuate a brain abscess. The authors report a mortality rate of 1.34% - four cases from the studied group of patients. Two cases out of five diagnosed with liver cirrhosis prior to the biopsy intervention developed

postoperative hemorrhage. The authors describe a higher probability of developing postoperative hemorrhage in patients diagnosed with liver cirrhosis, especially patients from Taiwan and the East Asian region, where the frequency of liver cirrhosis is higher.

4. Modified universal neurosurgical checklist

The operative protocol for neuronavigated needle biopsy developed based on a literature review and introduced in interventions including patient with supratentorial lesions operated on in the Neurosurgery clinic of University hospital- “St. Marina” starts with a preoperative patient preparation.

In addition to standard paraclinical examinations and preoperative consultations with specialists in cardiology and anesthesiology, the preoperative stage begins with the completion of a modified universal neurosurgical checklist (Modified Universal Neurosurgical Check-List Examiner- MUNCLE) to ensure the safety of all neurosurgical patients by eliminating the preventable medical mistakes (Fig. 51). Such mistakes include all forms of surgery in the wrong place, complications associated with the patient's wrong positioning on the operating table and complications due to device failure necessary for the relevant procedure.

After the excellent results from the application of the safety checklists in intensive care and intensive care, as well as in other surgical areas, checklists are introduced in neurosurgery. The filling of this checklist begins when the patient is hospitalized and continues until the completion of the operative intervention by checking whether postoperative therapy, laboratory tests and imaging studies are appointed in the medical history of the operated patient.

МОДИФИЦИРАН УНИВЕРСАЛЕН НЕВРОХИРУРГИЧЕН ЧЕКЛИСТ(*Modified Universal Neurosurgical Check-List Examiner (MUNCLE)*)

1. *Идентификация на пациента:*
   a. Име
   b. Възраст
   c. ID номер (ЕГН)
   d. Адрес
2. *Диагноза:*
3. *Планирана процедура:*
   a. Краниална
   b. Спинална
   c. Периферен нерв
4. *Очаквана кръвозагуба в мл (.....). Да се поръча еритроцитна маса и/или плазма:*
   Да
   Не
5. *Проверка на компресивни чорапи:*
   Наличие
   Коректно положение
6. *Образни изследвания:*
   a. Име
   b. Възраст
   c. Дата на изследване
7. *Позициониране на пациента:*
   a. По гръб
   b. По корем
   c. Странично
   d. Полуседящо/Седящо
8. *Проверка на положението на процедурата (глава, гръбнак, крайник):*
   Писмена диагноза
   Образни данни
   Съвпадение между Писмена диагноза и Образни данни
9. *Проверка на страната (ляво/дясно):*
   Писмена диагноза (ляво/дясно)
   Образни данни (ляво/дясно)
   Съвпадение между Писмена диагноза и Образни данни (ляво/дясно)
10. *Проверка на нивото:*
    Писмена диагноза
    Образни данни
    Съвпадение между Писмена диагноза и Образни данни
11. *Позициониране на главата:*
    a. Титиера
       i. Проверка за прегъване на подлежащото ухо
       ii. Проверка за компресия на подлежащото око
       iii. Проверка за компресия на шията
    b. Mayfield/Sugita пин-холдер
       i. Позициониране- linea temporalis superior
          1. Проверка за предшестващи фрактури
          2. Проверка за предшестващи краниотомия/краниектомия
       ii. Налягане- 60N
       iii. Заключване
    c. Елевация на гръдния кош- 10-15º
    d. Елевация на главата- над сърцето
    e. Ротация на главата
    f. Накланяне на главата
12. *Позициониране на крайниците:*
    a. Проверка на врата- без екстензивно опъване или компресия
    b. Проверка на Подлежащо рамо и ръка- подлагане/омекотяване- без екстензивно опъване или компресия
    c. Проверка на Надлежащо рамо и ръка- без екстензивно опъване или компресия
    d. Проверка на Краката- подлагане/омекотяване- без екстензивно опъване или компресия
13. *Позициониране на гърдите (при жени) и гениталиите (при мъже):*
    a. Проверка на гениталиите- подлагане/омекотяване- без екстензивно опъване или компресия
    b. Проверка на гърдите- подлагане/омекотяване- без екстензивно опъване или компресия
14. *Очертаване на кожния разрез:*
    a. Позиция
    b. Форма
    c. Размер
15. *Позициониране, проверка и включване на:*
    a. Операционен микроскоп
    b. Невронавигационна система
    c. Невроендоскоп
    d. Интраоперативен ултразвук
    e. CUSA
    f. LASER
    g. С-рамо
    h. Други
16. *Проверка на постоперативните терапия и изследвания:*
    Постоперативна терапия
    Постоперативни лабораторни изследвания
    Постоперативни образни изследвания

Име на проверяващ:

Подпис:

Дата:

Час: preOp (……) / postOp (……)

Fig. 51.** *MUNCLE - Modified Universal Neurosurgical Check-List Examiner*

5. Imaging studies

One of the main points in the safety checklist is verification of preoperative imaging studies. All modern neuronavigation systems rely on imaging studies for creating a virtual image space of the patient. It is necessary to provide an up-to-date preoperative examination of the patient conducted with contrast medium for better visualization of both anatomical structures and surgical targets. In the present study, CT, contrast-enhanced MRI, and given the multimodal capabilities of the neuronavigation system combination of the two.

Currently, the selection of the surgical target is performed on the basis of a contrast enhanced CT or non-quantitative MRI images: contrast enhanced T1W and CET1W sequences and amplification in hyperintensity in T2W or FLAIR sequences. Wang et al. 2016 describe planning biopsy interventions using SWI MRI sequences which significantly improve the visualization of small vessels structures compared to conventional T1W-Gd images, as they document a decline in the postoperative complications. The authors underline that SWI provides higher image contrast especially for mediums with different paramagnetic characteristics such as hemosiderin, ferritin and deoxygenated blood. This MRI sequence clearly visualizes intracranial microvessels, microbleeding and neoangiogenesis in the tumor formation, as it is visualised with a characteristic hypointense signal. The processes of neoangiogenesis and neovascularization in the tumor formation can’t be properly visualized on a CT or other sequences of conventional MRI.

When mentioning quantitative imaging studies such as PET-CT with its radioactive markers, magnetic resonance spectroscopy (MRS) and DSC-MRI, numerous studies confirm the possibility of their use in order to increase the precision in the selection of surgical targets compared to standard MRI or CT examination. (Muragaki Y et al. 2008),(Hermann et al. 2008),(Weber et al. 2010), (Pafundi et al. 2013), (Lopez et al 2015) In the present study no quantitative imaging studies are used in the process of intraoperative navigation, as for the non-quantitative MRI studies used, the conducted correlation analysis of the reflection of the SWI sequences used in thirteen patients does not confirm statistically significant change in the postoperative complications.

6. Tissue samples

The two factors that limit the reliability of the results of one biopsy procedure are: the accuracy of the aiming of the biopsy needle and the quality of the tissue sample collected for histological examination. For targeting accuracy, the main goal is to extract sample from the lesion's core provided the tissue around the lesion is kept intact. As demonstrated, the high quality of modern imaging studies coupled with careful planning and modern stereotactic surgical equipment provide diagnostic yield of 72.8% - 95.6% for neuronavigated needle biopsy procedures.

With regard to the quality of the tissue sample, published data suggests that the volume and the integrity of the biopsy specimen are the two factors of paramount importance. A little amount of tissue collected, as well as its fragmentation constitutes the main limitations leading to difficulty in histological interpretation. Study published by Jackson et al. 2001 demonstrates that the main cause of diagnostic inconsistencies and difficulty in the histopathological interpretation in stereotactic biopsies is less than optimal amount of tissue collected. A study by Torres et al. 2016 documents the average size of needle biopsy materials without diagnostic value of 0.4 cm3 as opposed to an average size of 0.15 cm3 for diagnostic needle biopsies ($p = 0.02$) (Trojanowski et al. 2019).

A noteworthy point is that the targeting accuracy of the biopsy needle and the quality of the tissue sample collected for histopathological examination depend largely on the used surgical technique. A major factor in the technical performance of the biopsy is the negative pressure that needs to be applied for the tissue to shift into the biopsy window. Most of the published studies either do not mention the amount of pressure used at all, or describe it as not quantified, not controlled, with no exact value specified, or define it as a "Light aspiration". A study by Rossmeisl et al. 2015 includes recommendations for conducting needle biopsies with a needle with a lateral position Nashold type biopsy window and technique involving the application of "Slight negative pressure" using a syringe. In research conducted by Flegel et al. 2012 and Wani et al. 2016 is described a technique with direct aspiration through the biopsy needle with 0.5 ml and 5 ml syringe, without specifying the exact value of the used negative pressure.

A study by Kreula et al. 1990 clearly demonstrates the absence of diagnostic biopsy material during a biopsy operation using a technique without applying negative pressure. Simultaneously the authors' study illustrates how the application of aspiration does not lead to direct separation of tissue material but to a shift of the biopsied tissue into the biopsy window. After discontinuation of the applied negative pressure the tissue entering the biopsy window restores its original shape and location.

A study by Haseler et al. 2011 documents the benefits, the shortcomings and implications to patient's safety in the relationship to the used syringe size, vacuum generation and load exerted on the operator's hands during aspiration-related biopsy procedures. The authors demonstrate that despite the progressive increase of the aspiration force of the used syringes, by increasing their size, the

effective difference in the maximum aspiration pressure between the use of 10ml and 20ml syringe is ~ 15% (441 Torr and 517 Torr respectively). Although larger syringes generate more maximum vacuum, the increase of the size increases the load on the operating arm of the surgeon, decreases the control during the performance of the biopsy technique, which leads to unintentional movement of the needle in the direction along the trajectory of introduction, which may be associated with serious postoperative complications. Haseler et al. 2011 describe 4.2 Torr-cm^2 x 10^3 of force required to withdraw the plunger of a 10ml syringe against the 6.2 Torr-cm^2 x 10^3 of power required to pull the plunger on a 20 ml syringe. In the present study a technique is used by carful aspiration of 0.5 ml air (~ 340 Torr) using a 10ml syringe. Additionally as to exclude unintentional movement of the needle collecting the biopsy material, the manipulation is performed by two people – one person who holds the biopsy needle and by applying a rotation of the inner cannula, opens and closes the biopsy window, who by rotating the outer cannula also controls the direction of the biopsy window, and a second person who holds the syringe, pulls the plunger and aspirates while the biopsy window of the biopsy needle is opened.

A study by Trojanowski et al. 2019 examines the quality of the taken biopsy material in a series of ex-vivo needle biopsies using fresh pork brain and a Nashold type needle and a Sedan type needle while the applied aspiration pressure is between 150 Torr and 450 Torr. The authors describe a similar technique of collecting biopsy material as the technique used in the present study, by a rotation of the biopsy needle without describing the exact direction and depth of the biopsy window during the collection of the tissue. The authors demonstrate a 4% statistically significant difference in favor of the Sedan type needle in terms of quality of samples obtained and 4% difference in the quality of the samples in favor of the method by turning the needle, compared to a "classical method" with no rotation. Trojanowski et al. 2019 describe a significant increase in biopsy efficiency from 88.5% to 98% by application of negative pressure, as the lowest measured pressure in the study is 0.02 MPa (~ 150 Torr), while at the same time they describe that additional increase in applied aspiration pressure after 0.06 MPa (~ 450 Torr) has a diminishing return with respect to biopsy efficiency - efficiency is increased by 1% for each 75 Torr of force applied. The authors find no evidence of quality degradation of samples obtained under conditions of high pressure aspiration in compared to tissue samples obtained at low aspiration pressure.

Histopathological artifacts related to the technical method of needle biopsy may also affect the diagnosis (Trojanowski et al. 2019). Studies by Mair et al. 1989 and Chowhan et al. 2014 report higher quality cytoarchitectonics in biopsies performed with aspiration. Kim et al. 2011 underline the mechanical damage of the extracted tissue samples during the intervention as a potential factor leading to inaccurate histopathological diagnosis. The authors describe an artifact that is a semicircular or ribbon-like tissue compression at the periphery of the extracted tissue material. The artifact they describe is linked to the mechanics of the needle with a side cutting biopsy window and can be produced during the rotation of the inner cannula of the biopsy needle, and histologically may be misinterpreted as

hypercellularity, spindle-shaped, increased limitation or pseudopalisade, especially in cases of glial tumor biopsy.

Lu et al. 2015 describe that collection of biopsy material that does not allow proper histopathological assessment can reach 10% of cases. Trojanowski et al. 2019 reported an average of 97% yield of tissue suitable for histopathology assessment, as the percentage rises additionally when collecting material with a higher aspiration pressure.

For non-diagnostic biopsies of deep lesions, McGirt et al. 2005 recommend taking more biopsy samples before performing the biopsy by engaging a second trajectory. In addition, the authors establish a link between the presence of hypoglycemia and the development of postoperative deficit in the population diagnosed with diabetes, emphasizing the advantage of good preoperative control of glucose levels in patients undergoing biopsy intervention.

In terms of number of tissue samples taken during biopsy, Deepali et al. 2006 examine 86 biopsied patients between 6 and 75 years. In 55.8% of cases the lesion is localized in the basal ganglia. The demonstrated diagnostic yield is 81.3% using between one and six tissue samples obtained for histopathological examination. In 68.7% of cases, one or two tissue samples are collected. A change in diagnostic yield is described ranging from 76.5% in the case of one sample to 84% and 88.2% respectively in the case of two and three samples collected up to 100% diagnostic yield for biopsies with five and six tissue samples collected. The authors summarize that in their study there is a tendency for higher diagnostic yield in the cases with a larger number of tissue samples collected. In the present study a total of 41 lesions are biopsied and a total of 312 biopsy materials are send for examination. For 16 lesions that are biopsied the number of tissue samples taken is 4, for 17 lesions biopsied- 8 materials are collected, for the biopsy of 4 lesions- 12 materials are collected. When using a technique by consistently rotating the outer cannula of the biopsy needle and guide the direction of the biopsy window at 0^o, 90^o, 180^o, 270^o stereotactic separation of four biopsy specimens is achieved, aiming to separate material from the central area of the lesion, as well as the border area in sagittal plane on the imaging study, marked by contrast uptake, in order to prevent incorrect histological interpretation especially in primary brain neoplasms. The results in the present study show that greater number of collected tissue samples is obtained in the isolated biopsy group. The results suggest that in this group of patients the longer surgical time is at the expense of the greater number of biopsy samples collected. Conversely, the collected samples for the biopsy interventions followed by excision number four or eight, as the surgical time is being extended at the expense of the subsequent excision. In connection with the study by Deepali et al. 2006 in the present study there is a tendency to achieve high histological production under the condition of a high number of tissue samples in patients who do not undergo excision after the biopsy procedure, in comparison to the patients who undergo biopsy followed by an excision, where intraoperative histological verification is achieved by smaller number of collected tissue samples.

7. Duration of the surgical intervention

A significant difference can be found in reports about the operating time in frame based biopsy interventions in comparison to the neuronavigated biopsies. Smith et al. 2005 examined a group of 213 patients who they operated- 139 by frame based biopsy technique and 74 by navigated frameless biopsy technique. The authors did not find significant difference in the studied groups in terms of demographic data, histopathological diagnosis, proportions in non-diagnostic biopsies and operative complications. 6% of frame based interventions are operated on under general anesthesia. 95% of neuronavigated biopsies are performed under general anesthesia. Average operating time for frame biopsies in the study is 113 ± 3min while reported mean operative time for neuronavigated biopsy interventions is 185 ± 6 min ($p < 0.0001$). The authors comment that the significant difference in operative time between the two biopsy techniques reflects the difference in the intraoperative planning and adjustment of the equipment, as well as the time difference from the incision to closure. The neuronavigated biopsy requires time to set up the navigation system and to perform the registration and verification process before the beginning of the biopsy manipulation. In addition, the whole planning of the intervention in the frame based biopsy takes place outside the operating room. Regarding the neuronavigated biopsy, modern neuronavigation planning system and mobile neuronavigation station allow planning of the procedure outside or near the operation theater. The team of Dorward et al. 2003 confirms that the difference in operative time is in favor of frame based biopsy, even though in their examination the time under general anesthesia includes the placement of the frame and conducting of the intraoperative imaging study.

In a study by Livermore et al. 2014 that includes 351 biopsy interventions: 256 by frame based stereotaxy and 95 by neuronavigation guidance, the authors report that mean operative time including general anesthesia is 122 minutes for frame based biopsies and 103 minutes for neuronavigated biopsies, which they present as an average total operating time of 117 ± 42 minutes.

Dammers et al. 2007 examined 391 biopsy interventions: 227 stereotactic frame based biopsies and 164 neuronavigated biopsies. The study demonstrates average operational time of 149 ± 32 min for biopsy interventions performed with a frame based stereotactic technique and 127 ± 33 min for interventions performed using neuronavigated technique ($p < 0.001$).

Georgiopoulos et al. 2018 examine 28 neuronavigated biopsy interventions with an average operative time of 79.1 ± 22.7 min and 28 frame based biopsies at an average operative time of 113.3 ± 17.2 min. The study demonstrates the average value of the total duration of the preparatory period of the biopsy intervention as a whole of 63.2 ± 13.8 min and average duration of the preparation period in the

operating room for neuronavigated biopsy interventions of 20.4 ± 6.3 (p = 0.01) in comparison to the average duration of the preparatory period in the operating room for frame based biopsies of 29.6 ± 5.9 min (p = 0.001). Despite the statistically significant differences in the preparatory periods of the interventions the study does not demonstrate significant differences in average operating time from incision to closure and additionally no significant differences total time spent in the operating room.

Verploegh et al. 2015 examined 247 consecutive biopsies and note ~ 10 min longer operative time in biopsies performed with BrainLAB Varioguide system (120 ± 28 min) than with biopsy interventions performed with Medtronic Treon Vertek (108 ± 36 min).

The present study reported an average operating time of patients with an isolated biopsy of 109.33 ± 25.56 minutes and mean operative time in patients with biopsy and subsequent excision of 206.4 ± 38.2 minutes. Preoperative preparation of the patients with respect to the planning of the surgical objectives is carried out outside the operational hall through the Medtronic Planning Station S7. The reported operative time does not include the time of general anesthesia but the stretch of time from incision to closure. A tendency to prolong the surgical time is formed in favor of larger number of collected biopsy materials providing correct histological assessment in the group of patients with isolated biopsy. Regarding cases of biopsy and subsequent excision, a trend to compensate for the extended operational time associated with an open excision is formed in favor of a smaller number of extracted tissue materials.

An explanation for the smaller number of materials can be related to the size of the lesions that are biopsied: 84% of patients with lesions larger than 30mm belong to the group of patients with biopsy and excision, only 46.7% belong to the group with isolated biopsy, 53.3% of patients with lesions less than 30 mm in size belong to the group of isolated biopsy and 16% belong to the group of patients with biopsy and excision. Research of Taweesomboonyat et al. 2019 including 85 patients, reported connection between lesion size, diagnostic accuracy, and postoperative complications. Lesions with a diameter of more than 3 cm are associated with higher percentage of a positive diagnostic result, and lesions with a diameter of less than 3 cm are associated with a higher rate of postoperative complications. It is indeed the expected higher diagnostic yield from a biopsy of lesions with diameter over 3 cm and the ability to compensate for the expected prolonged surgical time that can explain the smaller number of biopsy materials collected in the group of patients with performed biopsies with subsequent excision. Regarding isolated biopsies, despite partially prolonging the operative time, by way of using one working trajectory and by collecting larger number of materials provides maximal reduction of possible postoperative complications.

8. Technical features

In November 2021, Medtronic published an emergency safety notice, with reference №FA1204, describing an established anomaly in Stealthstation cranial and Synergy cranial software in biopsy procedures (Depth et al. 2021). The software can entered into a condition in which the biopsy depth gauge is out of sync with the rest of the navigational information on the screen and can display incorrect position of the biopsy needle. In this regard, Medtronic provides the following recommendations when working with the navigation system, which must be taken into account in any intervention carried out with the neuronavigation system:

1. Not to turn off the Guidance view after locking the biopsy path in the StealthStation Cranial and Synergy Cranial software.
2. After locking the navigation trajectory of the biopsy needle the operator must always make sure that the Guidance view remains active in at least one of the quadrants on the screen.
3. Always to provide visual confirmation of the accuracy of navigation as well as confirmation of the match between the graphic of the depth gauge, the values of distance to the target and information for position and distance to the target, provided by the 2D target marker representing the exact position of the tip of the navigated instrument.
4. Always to use the mechanical depth limiter for the biopsy needle

When a discrepancy is found between the depth gauge and other 2D information, Medtronic team recommends the following measures:

To create a disturbance in the visual corridor between the camera and the navigated instrument or reference frame for the purpose of turning the signal of the indicators of the navigation screen red.

To restore the visual corridor between the cameras and the biopsy needle or reference frame and restore their green status.

To Use the Cycle views icon (Fig. 52) to refresh guide view.

To provide visual confirmation of the accuracy by making sure the graphic of the depth gauge matches the distance values to the target represented by the 2D target marking.

Fig. 52.** *Mandatory screen refresh at each intervention in order to prevent the occurrence of an anomaly in the software related to the desynchronization of the depth gauge in relation to the rest of the navigation information (BVM StealthStation 2021)*

The abnormality described may occur in Medtronic StealthStation S7 and i7 systems running Synergy Cranial Ver. 2.2.8. and StealthStation Cranial S7 ver. 3.1.1-3.1.3.

For the period 01-January-2019 to 10-September-2021 Medtronic receives four signals, one of which is confirmed, for the manifestation of the anomaly in question (Depth et al. 2021). In the present study StealthStation Cranial version 3.1.2 is used in all interventions, in accordance to all recommendations from Medtronic, with no manifestation of the described anomaly.

9. Biopsy or excision?

Regarding the intraoperative histopathological diagnosis in 75% of the cases in the present sample the lesion is diagnosed as glial tumor, 10% as metastasis and 4% as lymphoma. The standard treatment of high-grade primary brain tumors include surgery for histological verification, postoperative radiation therapy and concomitant or adjuvant chemotherapy. (Müller et al. 2019) The prognosis of patients with glioblastoma multiforme remains pessimistic - survival rate within one year is described ~ 39%, despite the advanced diagnostic and therapeutic capabilities of modern medicine (Ostrom et al. 2017). The main decision that needs to be made is whether to carry out surgical treatment aimed at achieving surgical radicalism, to perform a biopsy for histological verification and subsequent oncological treatment, or surgical treatment to be postponed in cases of poor performance or other contraindications.

Numerous literature sources point to the association between the degree of resection and life expectancy in patients with high-grade primary brain tumors. (Brown et al. 2016), (Marko et al. 2014), (Chaichana et al. 2014), (Sanai et al. 2011) This association forms the aim of surgical treatment of this pathology to be maximal tumor excision in the condition of minimal functional impairment. This goal determines the results of surgical treatment and is closely related to the active decision-making done by the operator to stop the removal of additional tumor tissue. To stop too early the removal of tumor tissue carries a risk of recurrence of the tumor formation and reduces survival of the operated patient. To cease removal of tumor tissue too late leads to removal of a larger amount of tumor tissue and tumor infiltrated parenchyma, which carries the risk of transient or permanent postoperative functional deficit. The decision in the context of neurosurgical interventions is associated with several factors: general condition, age, concomitant diseases, clinical picture, lesion location, risk /benefit related with the planned intervention and last but not least the patient's motivation.

Müller et al. 2019 indicate the basic techniques for distinguishing tumor tissue from normal brain parenchyma- use of imaging studies for intraoperative navigation such as: MRI, ultrasound, fluorescence, neuromonitoring and brain mapping.

Numerous studies have commented on combinations of factors related to the process of neurooncological decision-making in patients with high-grade primary brain tumor:

Gan et al. 2015 examined 351 patients in 32% of whom it is conducted total resection, in 37% partial resection and biopsy in 24%. Of those patients 56% undergo postoperative radiotherapy, combined or followed by chemotherapy with a reported median survival rate for the group of 14.4 months.

Graus et al. 2013 examined 834 patients in 66% of whom it is conducted surgical resection. The study shows 14% postoperative complications. Of all the operated patients 57% undergo postoperative radiation therapy and chemotherapy,

and 22% do not receive any treatment. The authors emphasize that from the group of patients who undergo surgical treatment, postoperative chemotherapy and radiation therapy that starts before the 42 postoperative day, leads to longer period without progression of the underlying disease.

Scoccianti et al. 2010 examine 1059 patients of which only 11.6% are operated by biopsy technique. Postoperatively, 70.7% undergo course of chemotherapy and radiation therapy. Mean survival period for the group is 9.5 months. Survival until second year the authors observe in 24.8% and 3.9% respectively.

Zouaoui et al. 2014 examined 952 patients, in 541 resection is conducted and 411 biopsies are performed. For 180 cases subsequent treatment is not conducted. For 772, radiotherapy and chemotherapy are given (temozolomide), for 236 patients isolated radiotherapy is conducted, for 157 cases chemotherapy is conducted. The median survival rate for the study is 286 days and the main factors are age, performance status and tumor location.

Chang et al. 2005 examine 788 patients, in 75% of whom it is conducted surgical treatment. Mapping of the cerebral cortex is performed in 19%, intraoperative navigational technique in 29%. Postoperatively 87% receive radiotherapy and 54% receive chemotherapy.

It can be seen that the percentage of operated patients with neuronavigated biopsy varies widely between the studies from 5% to 44%. Distributed in a wide range are also the results of frame based surgeries from 28 to 47%. There are no precise standards for evaluating decisions made by multidisciplinary teams involving specialists in neurosurgery. (Müller et al 2019)

Müller et al. 2019 introduces the use of probability maps for lesion localization, biopsy and resection, based on MRI study conducted before and after the surgery. The maps allow comparison between the choice of a patient and the surgical decision for the different teams. The authors demonstrate a deviation in the reference (type of deviation, which occurs when the individuals included in the study are not representative of the individuals in the general population) of patients with tumors in the left hemisphere and selective removal of the tumor in the region of the right nucleus caudatus in two monitored medical teams. Evaluation of a patient with metastasis in the brain requires in-depth examination and a comprehensive approach to therapy. (PR Ng et al. 2021) Local control of a metastatic lesion can be applied by resection or by radiosurgery, separately or in combination. Modern research shows that the best response to treatment is expected in patients with a single metastatic lesion, good performance status and good control of the underlying disease. In these patients, recommendations include aggressive therapy by resection and adjuvant radiosurgery of the tumor bed. Operational results in treatment with open surgery and radiosurgery are similar, but surgery has an advantage especially in cases of a need of emergency relief of neurological symptoms.(Yaeger et al. 2013)

In the present study, 10.3% of all patients are diagnosed with secondary dissemination of a malignant neoplasm in the region of the brain. For all patients

intraoperatively diagnosed with metastasis is conducted an excision of the lesion. Of all patients with biopsy followed by excision 16.7% have metastasis.

Given the lack of evidence in the modern literature for a prevalence of the results for the use of frame based stereotactic biopsy technique over the results of using frameless neuronavigated biopsy and based on provided evidence of safety and efficacy, a recommendation for the use of a frameless biopsy technique in the routine neurosurgical practice can easily be made.

CONCLUSION

The beginning of any modern neurooncological treatment is related to individual judgment and approach by the medical team in terms of selecting the optimal method of obtaining accurate histological diagnosis. The main ways to ensure histological verification for oncological treatment are biopsy and excision (resection).

When possible, extensive resection is preferred, but often biopsy surgery remains the only alternative option in lesions located in deep regions of the brain, eloquent regions of the brain, diffuse lesions and patients with poor performance status for whom open resection holds high risk.

Modern neurosurgical practice is directed at the potential that minimally invasive neuronavigated biopsy surgery holds.

Based on an extensive literature review and based on the used surgical technique a surgical protocol is formulated in order to: optimize current clinical neuronavigated methods, reduce surgical errors in case of unwilling non-compliance of members of the surgical team, achieve comparable surgical results, as well as to introduce the protocol into routine clinical practice.

The technique applied in the present study demonstrates diagnostic yield of 95%, postoperative complications of 10% of which 0% with clinical manifestation and 0% mortality associated with the surgical intervention.

Neuronavigated frameless biopsy under general anesthesia, used in patients over 18 years of age with supratentorial intraparenchymal lesions is an effective and safe intervention with high diagnostic value.

SUMMURY

Neuronavigated biopsy is a minimally invasive surgical method that provides the neurosurgeon with objective and evidence-based intraoperative information on the histological nature of the intraaxial lesion, which is a major factor in deciding on radical surgery.

Given the lack of evidence in the modern literature for prevalence of the surgical results of the frame based biopsy technique over the surgical results of the frameless neuronavigated biopsy technique and based on growing evidence of safety and efficacy a recommendation for the use of a frameless biopsy technique in the routine neurosurgical practice can easily be made.

The formulated protocol for the use of a neuronavigated needle biopsy in patients with supratentorial intracerebral lesions provides high diagnostic yield, and in terms of postoperative complications and mortality is comparable to the frame based biopsy technique given the extensive data in the published literary sources.

The neuronavigated needle biopsy application protocol in patients with supratentorial lesions permits real-time tracking of the biopsy needle tip and the biopsy window in the working trajectory, providing stereotactic collection of tissue material from different parts of the surgical objective, thus compensating for the heterodence structure of the primary brain tumors witch often is the reason for a difficult histological assessment.

The protocol for the use of neuronavigated needle biopsy in patients with supratentorial intracerebral lesions can provide collection of optimal number of biopsy specimens for histological examination, and can provide intraoperative grading of one or more lesions during conditions like operative time and postoperative complications comparable to the frame based biopsy technique, based on the extensive data in the published literary sources.

Neuronavigated needle biopsy increases the surgeon's confidence when deciding on the need for radical surgery.

CONTRIBUTIONS

1. Introduction of a surgical protocol for neuronavigated needle biopsy and evaluation of the effect of its use in the routine practice in the Neurosurgery clinic of University Hospital "St. Marina", Varna.

2. Training of the medical neurosurgical team at Neurosurgery clinic of University Hospital - "St. Marina", for the nature, the purpose and the use of a neuronavigated needle biopsy protocol in patients with intracranial lesions.

3. Determining the indications and contraindications for use of neuronavigated needle biopsy in patients with intracranial lesions, on the basis of a thorough literature review of the existing specialized literature,

4. Implementing the protocol for working with neuronavigated needle biopsy in the routine practice during surgical interventions of patients with supratentorial intracranial lesions.

5. Evaluation and retrospective analysis of the effectiveness of neuronavigated needle biopsy.

PUBLICATIONS RELATED TO THE DISSERTATION

Moynov M. Monocentric clinical experience with frameless biopsy of supratentorial intraaxial lesions. Int. Bull Otorhinolaryngol. 2022;17(4):4-12. Journal article.

Y. Enchev, **M. Moynov.** Surgical protocol for frameless needle stereotactic biopsy of supratentorial intraparenchymal lesions. XVII World Congr. Neurosurg. WFNS-Bogota, Colomb. 2022-03-13 Conference abstract and oral presentation.

М. Мойнов, Я. Енчев, Б. Илиев, Т. Кондев, Б. Иванов, Е. Мойнова, Ст. Мариянова, Д. Димов. Приложение на невронавигирана иглена биопсия в неврохирургичната практика-начален опит. Периодично издание XXVIII Национална конференция по неврохирургия 06.10.2019. Conference abstract and oral presentation.

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