OPINION

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SUBJECT: PROCEDURE

Of the defense of a dissertation on "**Multimodal intraoperative electrophysiological neuromonitoring in neurosurgical operative interventions**" in the doctoral program "Neurosurgery", in the field of higher education 7. "Health and Sport" in professional field 7.1 Medicine, of the PhD student Dr. Milko Dimitrov Milev at the Clinic of Neurosurgery, Acibadem City Clinic University Hospital Tokuda.

The documents submitted by Dr. Milko Dimitrov Milev on the basis of Article 4 of the Law on the Development of the Academic Staff of the Republic of Bulgaria, Article 31 of the Regulations on the Implementation of the Academic Staff Development Act of the Republic of Bulgaria, Article 29 of the Rules for the Development of the Academic Staff of Acibadem City Clinic University Hospital Tokuda, the decision of the Scientific Council (Protocol No 43/29.09.2022) and Order No. 15.03.393#1 from 18.11.2022 provide the necessary legal basis to open a public defense procedure for a dissertation on "**Multimodal intraoperative electrophysiological neuromonitoring in neurosurgical operative interventions"** by Dr. Milko Dimitrov Milev.

1. BIOGRAPHICAL DETAILS OF THE APPLICANT

Dr. Milko Dimitrov Milev acquires Master's degree in Higher Medical Education in Sofia, in 2010. For the period 2011 - 2016 he acquired a specialty in neurosurgery, initially enrolled as a resident-neurosurgeon at UMHATEM "N. I. Pirogov" and subsequently at the Clinic of Neurosurgery at the University Hospital Tokuda. In the course of his medical education he completed training courses in pain management and treatment: "EFIC – European Pain School 2007" in Siena, Italy and "Pain Medicine" at the Medical University of Sofia.

2. SCIENTIFIC AND RESEARCH ACTIVITIES

2.1 QUANTITATIVE CHARACTERISTICS OF SCIENTIFIC PRODUCTION

The presented publications of Dr. Milko Dimitrov Milev are 22 in number, covering the period 2005 -2022, published in Bulgarian and foreign referable journals - Bulgarian Neurosurgery; J Bulg Cancer Soc; Mol Genet Genomics; Surg Neurol Int.; Asian Journal of Neurosurgery; Journal of Biomedical and Clinical Research. There are also 55 participations in scientific forums, both in the country, mainly National Neurosurgical Conferences, International Congress of Medical Sciences,

Sofia, as well as participations in international events - EANS Virtual Congress, Belgrade; International Society of Intraoperative Neurophysiology Meeting, Madrid, Spain; Proceedings of the EANS congress, Brussels, Belgium. For the period from 2006 to 2010 he participated in a series of research projects and, with relevant publications in the field of modulation of neuropathic pain, the effects of nonsteroidal (phyto-) and steroidal estrogens of nociception/hyperalgesia and inflammatory reaction.

Presented are 6 publications related to the dissertation and 5 participations in congresses, with the corresponding presentations.

2.2 CHARACTERISTICS OF THE CANDIDATE'S SCIENTIFIC ACTIVITY

The scientific paper submitted for evaluation on **"Multimodal intraoperative electrophysiological neuromonitoring in neurosurgical operative interventions"** is structured as follows:

- Volumetric - contains 159 pages, 91 figures, 3 chapters, summary and conclusions and a list of cited literature consisting of 189 titles. The dissertation paper was discussed and directed for public defense before a Scientific Jury, consisting of 5 habilitated persons, 2 internal and 3 external members.
- The structure is implemented in the required form – abbreviations, introduction, literature review, aim, tasks, material and methods, results, discussion of results, conclusions and contributions;
- In the first part, concerning the **literature review**, the doctoral student has managed, with the necessary clinical skill, to declare the possibility of reduction of the structural damage during neurosurgical treatment by the application of intraoperative neuromonitoring. The aim of IONM is to prevent the occurrence of persistent or transient neurological deficit in the course of preparation and execution of neurosurgical operative interventions. It is achieved through two main divisions of the methodology – localization of functionally important structures (cortical zones, nuclei or bundles of nerve fibers) and permanent or intermittent evaluation of the functional integrity and proper functioning of certain zones, structures or networks in the nervous system. Intraoperative neuromonitoring is the main method for the determination of the location of functionally important areas of the nervous system in the course of neurosurgical operative procedures.

The literature review **covers 2 functional areas of assessment: motor cortex and cortico-spinal tract and the system of the oculomotor nerves**. It is clear from the presentation that mapping of the primary motor cortex and cortico-spinal tract and monitoring of the somatomotor system can aid in the control of the risk of their damage in the course of the surgical intervention and the likelihood of development of stationary postoperative motor deficit in supratentorial pathological processes. Last but not least is the possibility of functional demarcation of the resection limit in

volume-occupying lesions, allowing its refinement and safe resection volume in case of violation of normal anatomical relationships. The analysis of the characteristics of motor evoked potentials from direct stimulation of the primary motor cortex is distinguished as a test with high sensitivity in terms of identification of postoperative deficits, However, the relatively high rate of false positive results requires that it should be combined with other electrophysiological criteria. Similar are the advantages and disadvantages of risk assessment based on the electrophysiological measurement of distance to the cortico-spinal tract by means of subcortical stimulation, which is also a method with localization characteristics, allowing dynamic assessment of the relationships of the surgical boundary with the motor pathways. In addition, there is an increased accuracy of the prognosis of postoperative deficit and an actual decrease in the frequency of these events **with the combination of two or more electrophysiological tests.**

From the literature review in the second field, it is evident that monitoring of the oculomotor nerves can contribute to **the preservation of their functional integrity** in the conditions of surgical interventions of the cranial base and the brainstem. However, it is also clear that **there is a lack of a unified methodological approach** towards the intraoperative localization and monitoring of the oculomotor nerves, and sometimes the preferences of different authors to one or another method of application differ dramatically. Similarly significant is the lack of reliable electrophysiological criteria for detection of damage to the above-mentioned group of nerves. It is necessary to conduct both extended, in the size of the series, and thorough, in terms of clinical, specific operative and electrophysiological modifiers, studies on the nature and the predictive characteristics of **electrophysiological** criteria for the development of injury to the oculomotor nerves, applicable under specific variations of the clinical case.

- All this, on the basis of what is known, semi-known and unknown about the problem, has allowed the doctoral student to define his **aim and a number of tasks**, namely: **To establish a set of electrophysiological criteria in order to ensure timely and reliable identification of an increased risk of injury to the motor cortex and cortico-spinal pathways and the oculomotor nerves. Based on this aim, 6 tasks are defined: The first 3 include strategies aimed at identifying factors modifying postoperative functional status, modifying electrophysiological criteria, and identifying the relationships between electrophysiological criteria and postoperative functional status. The second three tasks pertain to the generation of multifactorial predictive classification machine learning models, their optimization and, finally, the generation of an oligofactorial machine learning model.**

This review is based on clinical, medical imaging and electrophysiological data from 174 surgical interventions in the period 2016 – 2021, in which intraoperative electrophysiological neuromonitoring was conducted. The clinical cases in the study were divided into two independent groups in accordance with the two directions of the above goals and objectives.

The first group consisted of 130 clinical cases, in which, the spatial relations of the **area of surgical intervention to the primary motor cortex and cortico-spinal pathways were examined electrophysiologically or an electrophysiological assessment of the functional status of the aforementioned structures was performed** during neurosurgical operative interventions.

The second group consisted of 44 operative cases (for the period 2018-2021), in which electrophysiological localization and functional integrity assessment of 61 oculomotor nerves were performed during cranial base and brainstem operations.

- **The following methods (study design) were applied:**

- An assessment of the motor function of the limbs according to the Manual Muscle Testing (MMT) scale with values from 0 to 5 points for the examined muscle group was carried out in the group of operative cases with mapping and monitoring of the primary motor cortex and cortico-spinal tracts.

- In the comportment of this study, 3 apparatuses with different ranges of programmable assignments and available modalities were used – NIM Eclipse E3, NIM Eclipse E4 and NIM Neuro 3.0. The selection of the apparatus for each specific case was dictated by the number and type of modalities the number of recording channels required. As a standard, each apparatus includes a stimulation module and a preamplifier module for signal acquisition.

- In different cases of the series, the following techniques for direct cortical stimulation, localization of the primary motor cortex and monitoring of motor cortex and cortico-spinal tract were applied alone or in combination:

1) Low-frequency bipolar cortical stimulation – Penfield technique using a bipolar stimulator with an electrode spacing of ~5 mm and biphasic pulses with a duration of 0.5–1 ms and a frequency of 50-60 Hz.

2) High frequency monopolar cortical stimulation – described by Taniguchi, anodal monopolar stimulation with pulses in groups 3 to 7 and frequency 300-500 Hz.

3) Cortical motor evoked potentials – generated by intermittent stimulation of a motor cortex by Taniguchi technique through cortical electrodes positioned on the somatomotor cortex.

4) Transcranial motor evoked potentials.

5) Cathodal monopolar subcortical stimulation for the localization of fibers from the cortico-spinal tract – the stimulation parameters are approximately equivalent to those of the Taniguchi technique (a sequence of 5 pulses with a pulse duration of 0.5–1 msec, with a temporal pulse spacing of 4 msec), with stimulation intensities between 2 and 15 mA.

6) Phase reversal of somatosensory evoked potentials — achieved by means of differential recordings from adjacent cortical electrodes located in a row perpendicular to the sulcus of Rolando. Somatosensory potentials were evoked by means of peripheral stimulation per standard

methodology of nn. medianus, ulnaris or tibialis depending on the set objectives. In each of the listed techniques, standard stimulation and recording points, record lengths, filter settings and averaging parameters were used.

In the course of this study, for the purpose of improving postoperative functional outcomes in neurosurgical operative interventions in the cranial base area, a protocol for positioning intraorbital electrodes for intraoperative electrophysiological localization and monitoring of the three ocular nerves was developed. Compound muscle action potentials were recorded from three external ocular muscles: m. rectus inferior (for n. oculomotorius), m. obliquus superior (for n. trochlearis) and m. rectus lateralis (for n. abducens). The recordings were acquired by a bipolar assembly of a pair of electrodes positioned by means of a needle applicator of 25G diameter and 30 mm length. The use of these electrodes has allowed, due to their flexibility after removal of the applicator, safe placement in the orbital tissues and protection of the orbital tissues during positioning of the patient and throughout surgery. Also these electrodes enable selectivity of the recordings of the signals from the target muscle groups due to the bipolar assembly with small distance between the electrodes and the rejection of noise and parasitic signals generated peripherally to the survey area.

In order to validate the results, the necessary statistical methods have also been used.

- **Results and discussion**

The doctoral student with the necessary skill level has analyzed the electrophysiological criteria in the context of assessment of the risk of damage to the primary motor cortex and cortico-spinal tracts in supratentorial surgical interventions by analyzing the functional outcome in terms of primary motor cortex and cortico-spinal tract.

Preoperatively, in 48 cases, preoperative somatomotor deficit (36.9%) was not recorded. In nearly half of the cases (46.9%), the pathological process leading to the intervention resulted in preoperative somatomotor disorders to a mild extent (4 out of 5 points on the manual muscle testing scale, MMT). Significantly less frequent are cases with moderately severe or severe preoperative motor deficit. The same applies to the analysis concerning the electrophysiological criteria for damage to the primary motor cortex and cortico-spinal tract. The analysis of the data series found a significant correlation between the presence and the severity of the preoperative motor deficit and the presence of postoperative damage in the somatomotor system. No significant differences were found in the late postoperative outcome in patients operated on for supratentorial tumor formations (over 90% of cases) when comparing subgroups according to infiltrative characteristics or histological type. The inability to achieve total resection of the defined solid tumor formation is associated with a tendency to a worse late postoperative status than that in cases with complete removal of the solid tumor, which, however, does not reach statistical significance. This result can be explained by the

fact that premature cessation of resection is prompted by intraoperative findings of anatomical or electrophysiological nature for intimate involvement of a primary motor cortex or cortico-spinal tract by the tumor formation.

These circumstances are associated, on the one hand, with the higher risk of damage following from the continuation of resection, leading most often to the decision to leave a tumor residuum, as well as with the known primarily worse prognosis in these patients in terms of postoperative motor status. In support of this interpretation are also the observed statistically significant differences in the group-average value of the minimum intensity of subcortical stimulation with suprathreshold motor response between the cases with total (7.43 mA) and with subtotal (5.39 mA) resection. The latter value is close to the most commonly discussed in the literature values of the indicator of </=5 mA, associated with an increased risk of development of motor deficit, and reflects the respective participation of the indicator in the decision to terminate the resection in 11 of the 27 cases with subtotal resection in the series.

Based on this analysis, the following conclusions are generated: 1) Monofactorial analysis of electrophysiological criteria does not provide a sufficiently reliable assessment of the risk of neurological damage, unlike multifactorial machine learning models, which can successfully classify cases in which damage to the somatomotor system will occur. 2) The multifactorial classification models can be optimized by selection of the variables with actual significance for the classification process, simplifying their application while maintaining or increasing the accuracy of the prognosis. 3) The analysis of the model for the directions for classification of cases in the positive or negative class at different values of electrophysiological criteria, in the context of concomitant modifiers and risk factors, can identify threshold values of electrophysiological indicators in terms of the risk of damage to the somatomotor system. 4) An amplitude of cortical MEPs below 50% of the baseline is associated with the occurrence of neurological deficit (≤ 4/5 MMT), amplitudes between 50 and 100% are at increased risk of deficit, with the end result determined by additional modifying factors: preoperative status, intensity of stimulation, age and inability to execute total resection of the solid tumor. 5) The combination of recording of a suprathreshold motor response to subcortical monopolar stimulation with an intensity of ≤ 5 mA with a drop in the percentage amplitude of transcranial MEP < 30% or cortical MEP < 50% of baseline is associated with a risk of late moderate or severe postoperative motor deficit (≤ 3/5 MMT points).

In turn, the cross-validation of the models allow them to be evaluated neutrally, without the risk of false increase of the reliability indicators of the model. This is possible because in the process of cross-validation, the model does not, at any time, have access to the entire dataset, i.e., it cannot be "accustomed" to give an absolutely accurate, but only for the particular investigated group, prognosis (the phenomenon of overfitting is avoided). The classification models generated in the

process of machine learning allow the creation of individualized to the specific clinical case simulation nomograms for assessment of the risk of motor deficit corresponding to specific values of a particular electrophysiological indicator, as well as the complex risk analysis with combinations of two electrophysiological methods. The classification machine learning models successfully identify cases at risk of development of postoperative damage to the oculomotor nerves, as well as values of electrophysiological criteria associated with an increased risk of deficit.

Of particular significance is the ultrasound navigation of the application needle for the reduction of the risk of incorrect positioning of both of the electrodes in adipose tissue and away from muscle tissue in the comparatively less frequently placed in the series electrodes for monitoring of the third and fourth cranial nerves. With the demonstrated importance of the values lower in the couple electrode pair impedance on the results of the classification machine model for assessment the risk of postoperative deficiency, an indirect effect of ultrasound controlled positioning of electrodes on the functional result can also be expected due to the expected increase in the selectivity of the recordings, the amplitude values of the recordings and the signal to noise ratio, which, however, could not be demonstrated in this study due to the limited number of cases.

The other aspect of ultrasound control over electrode positioning should also be noted, and this is the prevention of periprocedural complications of the type of damage to the orbital contents. Here the doctoral student has again correctly drawn the relevant conclusions and they are: 1) Ultrasound control over the position of intraorbital electrodes supports their accurate positioning and leads to lower impedances in anatomically difficult localizations.2) Control over electrode positioning based on its impedance is associated with higher incidence of responses to direct nerve stimulation.3) The combination of ultrasound and impedance-based control allows the recording of high-quality responses from n. trochlearis/m. obliquus superior. In addition, a high risk of damage to the oculomotor nerves was found in operations on basal meningiomas with exposed cisternal segment of the nerve.

Based on analyses of variables latency and amplitude values, as well as their interactions with other predictors, a risk for new postoperative n. abducens deficit is identified in patients for which the intervention is for extraaxial tumor resection, and the access to the tumor formation includes the exposure of the cisternal part of n. abducens (postoperative deficit is associated with a decrease in the amplitude of the compound muscle potentials to direct stimulation of n. abducens below 100 microvolts). Also the thesis that the latency of the response to direct nerve stimulation is a stable indicator correlating with the stimulation zone could be proposed.

And last but not least, I want to stress upon the doctoral student’s contributions.

A. Theoretical:

1. In a literature review the current concepts and practical results of the electrophysiological intraoperative monitoring of primary motor cortex and cortico-spinal pathways and oculomotor nerves are specified.

2. The methods for location of the primary motor cortex and cortico-spinal tract are analyzed in detail and the electrophysiological criteria for risk assessment allow the proposition of appropriate sets of electrophysiological tests for the prevention of their damage in the course of surgery.

B. Methodological:

1. The established relationships between postoperative status, the characteristics of the clinical case and the electrophysiological findings from intraoperative neuromonitoring for cases with interventions in the supratentorial space with danger to the somatic motor function and near the oculomotor nerves and their nuclei, provide the basis for the conduction of a selective analysis of their complex interrelations.

2. For the first time, a machine learning model based on the gradient boosted decision trees (XGBoost) algorithm has been applied to the fields of neuro-oncology, operative neurosurgery and intraoperative neurophysiology.

3. For the first time, an assessment of the accuracy of the prognosis of the classification models has been implemented on the basis of indicators resistant to omission of the effect of false positive and false negative results, which allows for neutralization of the effect of imbalance in the classification classes.

4. The determined threshold for the examined electrophysiological criteria, as well as the changes of these threshold values with changes in concomitant modifiers, risk factors and other electrophysiological indicators, allow the assessment of the risk of damage to the primary motor cortex and cortico-spinal tract and n. abducens.

C. Practical:

1. The generated oligomodal classification models for assessment of the risk of damage to the primary motor cortex and cortico-spinal tract in operations for supratentorial tumors based on characteristics of the patient, tumor, surgery and electrophysiological criteria are routinely applicable in neurosurgical surgical practice.

2. An algorithm has been developed to generate individualized calibrated curves of the values of electrophysiological criteria versus the risk of damage to the primary motor cortex and cortico-spinal tract for the purpose of dynamic assessment of the findings of intraoperative neuromonitoring.

3. A technique for accurate, ultrasound and impedance-controlled positioning of flexible intraorbital electrodes for the purpose of monitoring oculomotor nerves with generally available equipment has been created and described.

4. For the first time in the literature, a protocol is presented for the ultrasound assisted positioning of intraorbital electrodes for monitoring m. obliquus superior.

After all that has been said so far, I believe that the presented materials show that the applicant meets the requirements of the dissertation procedure on " **Multimodal intraoperative electrophysiological neuromonitoring in neurosurgical operative interventions** " both documentary and thematically, the arrangement of the presented material and the contributions resulting from it.

Following all of the above, I give my positive assessment of the candidate and strongly recommend to the distinguished members of the Scientific Jury to award the PhD student Dr. Milko Dimitrov Milev from the Clinic of Neurosurgery at Acibadem City Clinic University Hospital Tokuda with the academic position "Doctor" in the field of higher education 7. "Health and Sport" in professional field 7.1 Medicine in scientific specialty "Neurosurgery for the needs of Clinic of ″Neurosurgery - Acibadem City Clinic University Hospital "Tokuda″.

ACIBADEM CITY KILINIK TOKUDA HOSPITAL

30.12.2022 Head of CAIC prof. N. Mladenov, DSc

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