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In accordance with the resolution of the Higher Attestation Commission of the Ministry of Education and Science of the Russian Federation, the Problems of Neurosurgery named after N.N. Burdenko was included in the List of Leading Peer-Reviewed Journals and Periodicals issued in the Russian Federation where the main results of Candidate and Doctor Theses are recommended to be published.
Connexin 43 antibodies in intraoperative diagnosis of experimentally poorly differentiated gliomas

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1Department of Fundamental and Applied Neurobiology, Serbsky National Research Center for Social and Forensic Psychiatry; 2Department of Medical Nanobiotechnologies, N.I. Pirogov Russian State Medical University; 3N.N. Burdenko Neurosurgical Institute, Russian Academy of Medical Sciences; 4Prokhorov General Physics Institute, Russian Academy of Sciences, Moscow, Russia

Fluorescent diagnosis was first proposed in the early 20th century and has been used in neurosurgery for about 15 years. The method relies on selective accumulation of strongly fluorescent protoporphyrin IX in tumor cells. Over the past years, the method of intraoperative fluorescence diagnosis has occupied its niche in many neurosurgical clinics around the world and is now used for fast intraoperative diagnosis in brain tumor surgery. However, the efficiency of fluorescent intraoperative diagnosis using 5-aminolevulinic acid is 80—90% and 58.8% for surgery of Grade III—IV and I—II gliomas, respectively. One of the methods to improve the efficiency of fluorescent diagnosis is to use vector systems for delivering fluorescent drugs into the tumor. This paper reports the results of an experimental study of systems for delivering fluorescent agents (protoporphyrin IX, Alexa 488, Alexa 660) using connexin 43 antibodies in rats with transplanted C6 glioma.

Keywords: connexin 43, 5-aminolevulinic acid, glioma, fluorescent diagnosis, laser spectroscopy.

Abbreviations:

5-ALA — 5-aminolevulinic acid;
PP IX — protoporphyrin IX;
Cx43 — Connexin 43;
MAbE2Cx43 — Connexin 43 antibodies;
HGG — high grade gliomas (Grade III—IV);
LGG — low grade gliomas (Grade I—II).

Primary tumors of the central nervous system account for about 2% of all tumors and are in the fourth place in the structure of causes of death in males of 15 to 54 years and females of 15 to 34 years as part of cancer pathology [3]. Treatment of malignant gliomas is currently combined and includes microsurgical resection of the tumor, radio-, and chemotherapy [3, 30]. The main objective of surgical treatment of intracerebral tumors is to remove the tumor tissue (cytoreduction) with minimal damage to the brain and to establish a histological diagnosis. Complete removal of malignant intracerebral tumors is virtually impossible due to the infiltrative nature of growth and damage to functionally important regions of the brain, which may lead to a pronounced neurologic impairment after surgery [18]. Despite this, a maximum possible surgical removal of the tumor remains a crucial factor affecting the length of overall and disease-free survival of patients [25] (Table 1).

Repeated microsurgical interventions and interventions after radiotherapy for recurrent gliomas of the brain are of especial complexity, because the identification of tumor boundaries is complicated in this situation [17].

Currently, the metabolic fluorescent diagnosis using 5-aminolevulinic acid (Alasens) is actively used in the clinical practice [4, 23, 28, 29]. Previous studies have demonstrated that the frequency of complete removal of malignant gliomas using Alasens is increased in 2 times; in this case, the authors have achieved an increase in 6-month disease-free survival [29].

Preferential accumulation of protoporphyrin IX in the tumor tissue compared to the surrounding tissues is observed upon oral administration of Alasens. The fluorescence contrast of the tumor/surrounding normal tissue is developed (from 10:1—15:1 to 20/1—50/1) in 2 h after administration [26]. According to the literature [1, 19], fluorescence is observed in 80—90% of cases in patients with malignant gliomas, when Alasens is used. Non-uniform accumulation of PP IX was demonstrated to be due to the heterogeneity of glioma and to be observed in its anaplastic areas with a high proliferative potential, which allows the identification of the most aggressive glioma parts [13, 19].

However, a part of patients with malignant gliomas lacks detectable fluorescence for several reasons [1, 7]. The question of fluorescent diagnosis in surgery of Grade I—II gliomas remains unresolved. According to some sources [29], the use of the method in this type of patients is not recommended. According to others [1], the efficacy of the FD method in LGGs is significantly lower than in surgery of malignant gliomas and amounts to no more than 58.8%.

This underlies the relevance of the search for new ways to induce detectable fluorescence during surgery.
The first reports about the possibility of using glioma component antibodies labeled with fluorescent dyes for intraoperative demarcation of tumor borders have appeared recently [14, 20, 32], but much uncertainty remains in this issue (Table 2).

Despite the blood-brain barrier defect in tumor microvessels, which causes the enhanced permeability effect, selective accumulation of a diagnostic or therapeutic agent, in particular a photosensitizer, in the tumor is only possible in the presence of a tumor specific vector such as a monoclonal antibody, aptamer, affinity peptide, or other ligand that recognizes selectively a certain target protein on tumor cells.

In the case of highly invasive glial tumors (Grade III—IV), the most relevant area for targeted drug delivery is the tumor periphery and the peritumoral area of the normal nervous tissue. It is this area where active invasion of glioma cells occurs. Application of monoclonal antibodies to the extracellular fragment of Connexin 43 (MAbE2Cx43) is very promising as a vector for fluorescent agent delivery to visualize the perigloma zone (area of active tumor cell invasion). Previously, it has been shown that the intravenous injection of MAbE2Cx43 leads to its accumulation in the peritumoral area [9, 10].

Connexin 43 (Cx43) is a protein of cell homo- or heterologous gap junctions, in particular, formed between astrocytes and glioma cells [33]. Gap junctions between tumor cells and reactive astrocytes play an important role in the invasion of tumor cells into the parenchyma of the normal brain tissue [21]. Application of MAbE2Cx43 in the intraoperative navigation system may increase the extent of resection due to better visualization of the tumor, especially in the absence of detectable fluorescence when using Alasens [2].

**Research aim and objectives**

The aim of research was to evaluate the perspective for the use of Cx43 monoclonal antibodies in visualization of boundaries of the tumor invasion during intraoperative fluorescence diagnosis of gliomas *in vivo*. The objectives of the study included:

1. Studying the selectivity of accumulation of MAbE2Cx43, labeled with Alexa 633 and Alexa 488, in the brain tissues of rats with the C6 glioma by *in vivo* spectroscopy and by an analysis of fluorescence on brain slices.
2. Comparative monitoring of accumulation of PP IX and MAbE2Cx43, covalently linked to a photosensitizer, in the glioma and perigloma zone *in vivo*.

**Material and methods**

All experimental manipulations on rats were conducted in accordance with the GLP requirements and principles of bioethics. Modeling of the experimental C6 glioma was performed on outbred rats with a total weight of 250 g. The animals were implanted with preliminary cultured glioma cells of the C6 line in the amount of 400×10³ into the striatum of the right brain hemisphere.

A LESA-01-BIOSPEC laser electronic spectral setup was used for an intraoperative diagnosis during the experiment that allows one to determine locally the extent of photosensitizer accumulation in a tissue of any organs available for a fiber optic probe. The setup consists of a laser source to excite a photosensitizer and a miniature universal spectrometer to record and analyze a fluorescent signal. The system is supplied with a desktop PC or laptop.

A solid state laser with a wavelength of 532±2 nm (BioSpec) was used as an emission source exciting PP IX fluorescence in the rat brain tissue. A solid state laser with a wavelength of 473±2 nm (BioSpec) was used to detect fluorescence of PP IX and Alexa 488. A laser with a wavelength of 638±2 nm (BioSpec) and a power of up to 50 mW/cm² was employed to measure fluorescence peaks of Alexa 660.

To excite simultaneous fluorescence of PP IX and Alexa 488, laser sources with wavelengths of 405±2 and 473±2 nm (BioSpec) were used. A solid state laser with

**Table 1. Relationship between the tumor resection extent and overall survival median in patients with glioblastomas [25]**

<table>
<thead>
<tr>
<th>Resection extent according to MRI data (removal of the T1 contrast-enhanced tumor portion), %</th>
<th>Overall survival median, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>375*</td>
</tr>
<tr>
<td>80</td>
<td>384*</td>
</tr>
<tr>
<td>90</td>
<td>414*</td>
</tr>
<tr>
<td>100</td>
<td>480*</td>
</tr>
</tbody>
</table>

Footnote. * — p<0.05

**Table 2. Use of antibodies to human glioma components as promising vectors for neuroimaging**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Glioma antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. Ohashi et al. [20]</td>
<td>2004</td>
<td>RFX1</td>
</tr>
<tr>
<td>R. Ueda et al. [31]</td>
<td>2004</td>
<td>SOX6</td>
</tr>
<tr>
<td>Y. Iizuka et al. [14]</td>
<td>2006</td>
<td>GARC-1+T-lymphocyte</td>
</tr>
<tr>
<td>R. Ueda et al. [32]</td>
<td>2007</td>
<td>SOX6</td>
</tr>
</tbody>
</table>
a wavelength of 532±2 nm (BioSpec) was used to excite PP IX fluorescence in the rat brain tissue. A laser with a wavelength 638±2 nm (BioSpec) was used to obtain fluorescence peaks of Alexa 660. When the fluorescence spectra were detected upon simultaneous excitation with short wavelength sources (405 and 473 nm), the laser radiation was attenuated by the KS 18 filter at the spectrometer input. Upon registration of the fluorescence spectra induced by a laser with a wavelength of 532 nm, the spectrometer input was equipped with the OS 13 filter. When fluorescence was excited by a laser source with a wavelength of 638 nm, the laser radiation was attenuated by the KS 18 filter installed at the spectrometer input.

To obtain the diffuse reflectance spectra from the studied tissues, a halogen lamp was used, the radiation of which was delivered to an object through a separate channel of a fiber optic probe. The resultant fluorescence spectra were processed and analyzed with allowance for the backward diffuse reflectance spectra of the broadband radiation, taken at the same point.

To assess accumulation of PP IX, as a fluorescence inductor, in a glioma, a commercial drug Alasens (active ingredient is 5-ALA, the precursor of PP IX) was used. To detect accumulation of antibodies to tumor associated antigens in the glioma and periglioma zone, experimental fluorescent labels, Alexa 660 and Alexa 488, and a Phthalosens derivative modified with lysine amino acid residues were used.

An Alasens physiological saline solution (in an amount of 1.5 g per 70 kg of body weight) was injected into a vein immediately after preparation, 2 h prior to the experiment. Preparations of MAbE2Cx43 at the initial concentration of 2 mg/mL were covalently bound to fluorescent labels by means of succinimide ester, purified from unbound components using gel filtration, and administered to rats in an amount of 1 mg 24 hours before the experiment.

To conjugate MAbE2Cx43 with the Phthalosens derivative, acylation of free amino groups of lysine residues with an acetic acid moiety was performed. Attachment of the Phthalosens derivative to antibodies was performed in dimethyl sulfoxide with addition of a solution of dicyclohexylcarbodiimide and N-hydroxysuccinimide at +4 °C for 24 h. Then the reaction mixture was centrifuged, the pellet was discarded, and the supernatant was loaded onto a Sephadex G-50 column equilibrated with 0.05 M phosphate buffered saline PBS (pH 6.0).

Experimental drugs were administered to rats with a 12-day glioma through the femoral vein under general anesthesia (intraperitoneal injection of ketamine with premedication by seduxen in an amount of 10 mg per 1 kg of body weight).

An approach to the rat brain to detect fluorescent signals was performed by resection craniotomy in the fronto-parietal areas on both sides. Preservation of the venous sinus and prevention of bleeding were a prerequisite to perform the experiment. A spectral analysis of fluorescence using LESA-01-BIOSPEC was performed in two stages.

At the first stage of the experiment, fluorescence was recorded under in vivo conditions. After the introduction of experimental drugs, a rat with a glioma was immersed in narcotic sleep and fixed on a stereotactic table. After removal of a bone flap from the parietal region, the spectral analysis was performed from the cortical surface (from the tumor surface, periglioma zone, and contralateral hemisphere). The spectra of fluorescence activity of PP IX, Alexa 488, and Alexa 660 were alternately studied. Then, the fluorescence activity of each agent upon immersion of a spectrometric probe deep into a glioma was detected (Fig. 1).

![Fig. 1. Approach to the brain surface and spectral analysis of fluorescence from the glioma surface.](image)

Left — an operative wound view after trepanation; right — in vivo measurement of the fluorescence spectrum.
At the second stage, the rat was anesthetized and decapitated; the brain was taken out and rinsed with physiological buffer, and the fluorescence spectrum was analyzed ex vivo (Fig. 2). The measurements, performed in vivo, were repeated in the glioma, periglioma zone, and normal tissue on the front section and then in the sagittal projection.

One rat from each group after the administration of an experimental drug was selected for microscopic studies. The animal was subjected to perfusion with a 4% paraformaldehyde solution to obtain brain glioma samples. Thick brain slices with the thickness of 200 µm were prepared from rat brains using a vibrotome (Microm HM 650 V, Thermo Scientific). The brain accumulation and distribution of Alasens and/or MAbE2Cx43 after their administration to rats were assessed by a fluorescent signal from PP IX, Alexa 488, or the Phthalosens derivative at the excitation by a laser source of a the laser scanning microscope (Nikon, Japan) in the range of 600—700 nm.

**Results**

The obtained results on an objective assessment of application of antibodies in intraoperative diagnosis of the tumor and its borders in the experiment on rats with the C6 glioma were compared to those for application of Alasens.

1. **Results of using the fluorescent agent Alasens**

In the 1st group of rats (4 rats), the accumulation selectivity and spectral characteristics of Alasens were evaluated in 2 h after its intravenous introduction into the femoral vein. Convincing peaks of PP IX accumulation in a glioma that differed in the amplitude from the healthy tissue signal in approximately 3 times were detected using a laser with a wavelength of 532 nm. In this case, significant variations in the intensity of a fluorescent signal from various sites of the tumor tissue and peritumoral region were detected.

**Fig. 3** demonstrates the fluorescence spectrum of Alasens-induced PP IX with the excitation at 532 nm and with the attenuating OS 13 filter in a receiver channel of a spectrometer (fluorescence maxima are at 635 and 710 nm).
2. Results of using the fluorescent agent Alexa 660 conjugated with anti-Cx43

In the 2nd group of rats (4 rats), the brain accumulation and distribution of MAbE2Cx43 Alexa 660 (Cx43 antibodies conjugated with Alexa 660) were evaluated on the 12th day after implantation of the C6 glioma. The spectral analysis of Alexa 660 fluorescence excited by a laser with a wavelength of 638 nm was performed in the glioma, periglioma zone, and intact brain tissue.

The fluorescence intensity was normalized to the signal level from a control object (experimentalist’s hand skin). In this case, the intensity of a tumor fluorescent signal detected transcranially in vivo was 14 RFU (Fig. 4).

The signal intensity of Alexa 660 fluorescence in vivo after removal of a bone flap from the surface of the contralateral hemisphere cortex as well as at a significant distance from the glioma and periglioma zone in the ipsilateral hemisphere was about 2 RFU, and it was 5 RFU from the glioma surface after removal of the extracranial portion. As the sensor is moved laterally from the tumor along the ipsilateral hemisphere surface, a signal is increased to 8 RFU. When moving the sensor to the medial side (towards the ventricles) from the glioma along the cortical surface towards the midline, a signal is increased to 20 RFU (see Fig. 4).

Studying a glioma ex vivo demonstrates that a glioma grows through the brain in the medial direction, which explains a decrease or increase in an Alexa 660 fluorescence signal upon detection from the cortical surface in vivo. On the front section and sagittal section of the isolated rat brain, a signal from the glioma center (necrotic zone) reaches 2 RFU, a signal is increased to 4 RFU along the glioma periphery to the perifocal area (zone of active migration of tumor cells), and a signal is in the range of 12 RFU and above in the periglioma zone.

3. Results of using the fluorescent agent Alexa 488 conjugated with anti-Cx43 and supplemented by Alasens administration (“double” metabolic navigation)

To colocalize accumulation of Alasens and antibodies in the glioma and periglioma zone, MAbE2Cx43 was bound to another fluorescent label, Alexa 488. As in the previous groups, Alasens and MAbE2Cx43 Alexa 488 were injected into the vein 2 and 24 h, respectively, before the examination.

Upon the simultaneous use of laser sources with wavelengths of 405 nm and 473 nm, the Alexa 488 fluorescence peaks in the range of 500—550 nm and the protoporphyrin IX peak in the range of 600—650 nm were detected. The ratio of peak intensity of PP IX fluorescence (635±10 nm) to peak intensity of Alexa 488 fluorescence (520±10 nm) was calculated and the distribution of the intensity ratio in various portions of the tumor was obtained. Table 3 and Fig. 5 provide the values of this ratio normalized to the value for the tumor center.

Table 3. The fluorescence peak amplitude ratio of PP IX to Alexa 488 at different sites of C6 glioma

<table>
<thead>
<tr>
<th>Localization</th>
<th>Fluorescence intensity ratio of PP IX to Alexa 488 normalized to the tumor center value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor center</td>
<td>1,0</td>
</tr>
<tr>
<td>Tumor margin</td>
<td>1,4</td>
</tr>
<tr>
<td>Perifocal area</td>
<td>0,7</td>
</tr>
<tr>
<td>Perifocal area at a long distance from the center</td>
<td>0,2</td>
</tr>
</tbody>
</table>

Fig. 4. Spectral analysis of Alexa 660 fluorescence in the glioma, periglioma zone, and intact brain.
Left — a transcranial measurement; right — a measurement in various sites of the tumor.
antibodies labeled with Alexa 488. Moreover, the further the fiber optic probe is moved from the tumor center, the greater a decrease in the PP IX fluorescence signal is, while the Alexa 488 signal level is actually the same.

4. Results of optical visualization of experimental rat C6 glioma using Alasens and antibodies labeled with a fluorescent dye

A camera equipped with a light filter was used to detect an observable emission. A semiconductor laser with a wavelength of 473 nm and a bandpass interference filter isolating the band of 500–600 nm were used. Fig. 6 reveals intense fluorescence of the C6 glioma due to accumulation of MAbE2Cx43 Alexa 488 in it that was introduced into the systemic circulation a day prior to the examination.

5. Results of confocal microscopy of experimental rat C6 glioma using Alasens and antibodies labeled with the fluorescent label Alexa

Upon intravenous administration of Alasens to rats with the C6 glioma, the selectivity of PP IX accumulation in the tumor tissue and perigloma zone was evaluated by confocal microscopy. C6 glioma cells were labeled by a membrane tracer (dye), DilC18, (fluorescence at 546 nm) prior to implantation. Therefore, accumulation of protoporphyrin in glioma cells was monitored by two fluorescent signals (546 nm laser is for DilC18, and 630 nm laser is for induction of a signal from PP IX).

According to the results of microscopy performed, the tumor tissue actively absorbed Alasens, and PP IX fluorescence was not observed in single glioma cells migrated from the main tumor nidus (Fig. 7). This was probably related to the peculiarities of the tumor cell metabolism.

The fluorescence analysis of rat brain slices 24 h after intravenous administration of MAbE2Cx43 preparations to rats with experimental glioma revealed their accumulation in cells of the peritumoral rim area by means of the Alexa 488 fluorescent probe (Fig. 8). To precisely localize accumulation of MAbE2Cx43, the preparation was further supplemented histochemically by immunovisualization of GFAP-positive reactive astrocytes.

Upon administration of a photo-immunoconjugate (MAbE2Cx43 with the Phthalosens derivative) in an amount of 400 µg/kg of the protein, a fluorescent signal from the photosensitizer was obtained in the perigloma zone (Fig. 9).

Discussion

Guidelines for conducting operations for brain gliomas include the maximum tumor resection with a minimal risk of functional complications with the mandatory use of microsurgical technique and intraoperative optics. When indicated, neuronavigation systems and
neurophysiological monitoring may be used during operative intervention [4].

The maximum radicalness of surgical intervention with allowance for physiological permissiveness leads to one-stage elimination of a large number of viable tumor cells, including those resistant to therapy, reduces intracranial hypertension, and may facilitate the improvement of disturbed neurological functions [5]. Reliable information about the tumor resection extent may be obtained by intraoperative visualization. A solution of this problem is mainly implemented by using intraoperative computed tomography, magnetic resonance imaging, ultrasound scanning and three-dimensional ultrasound frameless neuronavigation, neuronavigation systems, and various combinations of these methods [4, 6, 11].

One of the new methods of intraoperative navigation is the use of 5-ALA in surgery of various brain tumors. This method is based on selective accumulation of PP IX by tumor cells and is used in surgery of gliomas [1, 7, 29], meningiomas [12, 15], metastases [16], and other brain tumors. However, in some cases, fluorescence-negative tumors occur upon using 5-ALA. For example, the sensitivity of optical diagnosis with using an optical microscope with a fluorescence module in surgery of glial tumors amounts to 58.8% for Grade I—II gliomas and 89.7% for Grade III—IV gliomas [1]. This gives rise to the necessity to search for ways to improve the efficacy of intraoperative fluorescent diagnosis. One of the possible ways to do this is the use of vectorized photosensitizers.

Connexin 43 involved in the formation of intercellular gap junctions allows cells to carry out adhesion and to exchange by various intracellular messengers. Furthermore, the protein plays a certain role in active migration of Cx43 positive glioma cells to the peritumoral area [33]. Besides that Cx43 positive C6 glioma cells possess a higher ability to migrate compared to Cx43 negative ones, they are more resistant to oxidative stress and various other damaging factors [33]. Thus, Cx43 seem to be a promising target for inhibition of the tumor invasion and targeted delivery of photosensitizers to the peritumoral area.

In the experiment, upon administration of non-vector photosensitizers, in particular Alasens, to rats with...
Fig. 8. According to the results of microscopic study, the tumor tissue actively absorbed Alasens, and PP IX fluorescence was not observed in single glioma cells that migrated from the main tumor nidus.

Fig. 9. Confocal microscopy of slices of the rat brain with C6 glioma.
a — aligned image; b — cell nuclei counterstained with DAPI; c — fluorescence of a photo immunoconjugate; d — differential interference contrast; e — enlarged image of the perigloma zone.
glioma, we demonstrated their accumulation in tumor cells, but boundaries of the glioma invasion occurred to be Alsens negative. Non-specificity of accumulation of free photosensitizers in the tumor gave rise to experimental vector based studies.

In the spectral analysis of the glioma and periglione zone in rats, when MAbeE2Cx43s with fluorescent labels were administrated to rats, a high fluorescent signal in the area of Cx43 expression (along the glioma periphery, where actively dividing tumor cells are localized, and periglione zone, which is a reactive rim of Cx43 and GFAP positive astrocytes) was detected. Upon administration of MAbeE2Cx43s covalently linked to the Phthaolens derivative photosensitizer, the presence of fluorescence in the peritumoral area was demonstrated using confocal microscopy.

Therefore, the possibility to conduct intraoperative diagnosis of glioma cells migrating from the main tumor nidus was demonstrated using vectorized photosensitizers that may be used for intraoperative metabolic navigation. The pilot experimental study using Connexin43 antibodies conjugated with various fluorescent labels demonstrated effective accumulation of a fluorophore in tumor cells under *in vivo* conditions. This suggests that MAbeE2Cx43 may be an effective target for delivery of fluorescent agents to the glioma tissue, which provides the prospect to develop a method in surgery of non-fluorescent (when using Alsens) gliomas in the future.

REFERENCES

Treatment of patients with glial brain tumors is an important issue in modern neurosurgery. One of the main aspects of surgical treatment of these patients is the intraoperative demarcation of tumor boundaries due to infiltrative nature of the process. The maximum possible surgical removal of the tumor remains a crucial factor affecting the duration of overall and disease-free survival of patients. Various methods of intraoperative multimodal neuronavigation, including the fluorescent diagnosis with 5-aminolevulinic acid, are used for this purpose. However, unfortunately, the method sensitivity, according to the literature, amounts to about 80—90% in surgery of malignant gliomas. The fluorescent effect is more rarely observed in patients with benign glial tumors. Application of laser spectroscopy allows increasing the sensitivity and specificity of fluorescent diagnosis during operative intervention. Another way to increase the efficacy of the method is the use of various tumorotropic antibodies for vector delivery of fluorescent agents to the tumor.

As a vector to deliver fluorescent agents to the area of active tumor cell invasion, the use of monoclonal antibodies to an antigen that is actively accumulated in the perigloma zone, in particular to Connexin 43, is very promising. In the experimental work, the authors demonstrated the possibility to use fluorescent agents Alexa 488 and Alexa 660 coupled to the given antibodies, also in comparison with the use of protoporphyrin IX (Alasens). In this case, the spectral analysis of the distribution of two fluorescent agents (protoporphyrin IX and Alexa 488) administered simultaneously (the method of “double” metabolic navigation) demonstrated their different accumulation in the tumor center and in the perifocal area. For example, the central part of the tumor accumulates primarily protoporphyrin IX, whereas the peripheral zone accumulates the conjugate of Alexa 488 with Connexin 43 antibodies. The use of an additional light source and a camera with filters allowed conducting optical fluorescence visualization of a glioma on brain slices. The work is illustrated with confocal microscopy images confirming accumulation of fluorescent agents in the tumor tissue. This work demonstrated the possibility to perform the intraoperative fluorescence diagnosis using vectorized photosensitizers to visualize glioma cells migrated from the main tumor nidus, which may be used to increase the method efficacy.

Of the paper drawbacks, the lack of data on the use of vector delivery of fluorescent agents in the case of benign brain gliomas can be noted, which may be related to the difficulty to model this pathology in the experiment. In addition, the authors did not indicate, whether the development of possible side effects occurs when using antibodies to Connexin 43. Also, it would be interesting to clarify the possibilities of using antibodies to other tumor antigens with the aim of delivering vector fluorescent agents to glial brain tumors.

Thus, the developed method of vector delivery of fluorescent drugs may be used to improve the efficacy of fluorescent diagnosis during further experimental studies with the prospect of possible application and implementation in the clinical practice in the future after a full cycle of clinical trials in brain gliomas.

O. N. Dreval’ (Moscow, Russia)
Results of using spine assist mazor in surgical treatment of spine disorders

O.N. DREVAL, I.P. RYNKOV, K.A. KASPAROVA, A. BRUSKIN, V. ALEKSANDROVSKI, V. ZIL'BERNESTEIN

In this paper, we describe the possibility of using a bone-mounted miniature robot based on the experience of different surgeries performed in 77 patients divided into four groups according to the general pathology (degenerative stenosis of the vertebral canal, fractures of vertebral bodies, spondylolisthesis, hemangiomas, and tumors). All the patients underwent surgical intervention using SpineAssist, such as stabilization using the GO-LIF system, transpedicular systems, vertebroplasty, and vertebral body biopsy. The new method and the technology of stabilizing surgeries using Spine Assist Mazor allow one to perform preoperative virtual planning based on CT images and to identify an ideal and safe trajectory of placing screws or needles for vertebroplasty or biopsy.

Keywords: robot, robotic assistance, SpineAssist, transpedicular fusion, GO-LIF.

Robots have been gradually introduced into medical practice in the recent years in order to improve the quality of various procedures and surgical interventions, to shorten their duration, and to assist the surgeon during surgery. Robots are produced not only to perform surgical manipulations but also to provide assistance to the patient during the recuperation period. According to the Robotic Institute of America, a robot is re-programmable multi-functional manipulator designed to move materials, parts, tools, or specialized devices through variable programmed motions for the performance of a variety of tasks.

Medical robots are divided into four groups [5, 9, 11, 12, 21—23, 25—27].
1. Remote manipulators (telesurgery);
2. Passive robots: surgical manipulations are guided by the surgeon;
3. Semi-active robots: certain stages of an operation are performed by the surgeon, some manipulations are performed by a robot;
4. Active robots that perform complete surgical intervention under control of a computer.

The majority of neurosurgical operations are performed using a microscope and require a high accuracy and utmost care with respect to the nervous structures. Various studies have been carried out recently on the use of robots in specific branches of neurosurgery such as epilepsy surgery, stereotactic surgery, and stabilizing interventions (transpedicular fixations) [5, 6, 9, 22].

In 2004, a SpineAssist spinal robot was developed and clinically tested in Israel (Mazor Surgical Technologies, Caesarea, Israel). This is the first robotic system approved by the Food and Drug Administration for the use in spine surgery [18]. SpineAssist belongs to the group of passive robots [16, 22, 24]. One of the main objectives taken into account during design of the robot was the necessity for a robot to be firmly fixed within the operative field during surgery. The robot is fixed by mounting a platform (bridge) to a bony (spinous) process within the operative field using a special clip in the case of open intervention or a Kirschner's wire in the case of transcutaneous interventions; in addition, the platform (bridge) is externally fixed by metal rods to the patient's iliac spines. This approach is particularly relevant when placing transpedicular systems. The robotic assistance system consists of a Windows-based workstation, used for preoperative planning on the basis on CT scans (in the DICOM format), and the robot itself. Movements of the robot are also controlled by the workstation. The robotic arm has six degrees of freedom. The arm itself is a rigid steel stem. During surgery, the surgeon and the robot work together; the robot indicates the trajectory for introduction of an instrument, while the surgeon drills the holes for placing implants. The course of surgery is controlled by stage-by-stage X-ray, but in general, operative intervention occurs under the ultimate surgeon’s control [2, 3, 20].

The use of the SpineAssist robot has demonstrated a high accuracy of transcutaneous operative interventions using hardware [10, 19, 22]. Also, open surgeries have been performed using SpineAssist for placing transpedicular systems [2, 3, 7]; under the control of SpineAssist, the placement accuracy is almost perfect both in transcutaneous and in open modes [14]. SpineAssist is used for vertebroplasty of vertebral bodies in the case of their fractures or hemangiomas. The main advantage of SpineAssist is its high accuracy. The main disadvantage of the system is the high cost of the robot [13, 15].

The use of the SpineAssist robot

Between 2011 and 2013, 77 surgeries (39 males and 38 females, the mean age of 56.1±2 years) were performed using the SpineAssist robot.
Table 1. Summary of patients’ groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Pathology</th>
<th>Type of surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. n=36 (46.75%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males — 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females — 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal stenosis, grade I—III spondylolisthesis, one affected level</td>
<td>GO-LIF fusion with or without bilateral decompression</td>
<td></td>
</tr>
<tr>
<td>2. n=14 (18.18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males — 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females — 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal stenosis, vertebral body fracture, two or more affected levels</td>
<td>Fusion with classical transpedicular systems (Viper, Romeo)</td>
<td></td>
</tr>
<tr>
<td>3. n=16 (20.78%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males — 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females — 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiomas, vertebral body fractures</td>
<td>Vertebroplasty of vertebral bodies</td>
<td></td>
</tr>
<tr>
<td>4. n=11 (14.29%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males — 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females — 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral body mass lesions</td>
<td>Biopsy</td>
<td></td>
</tr>
</tbody>
</table>

All the patients were divided into four groups based on the pathology and the type of surgery (Table 1):

— Group 1 included patients with degenerative spinal stenosis and spondylolisthesis at the level of one spinal segment who underwent fusion using the GO-LIF procedure with or without bilateral decompression of the vertebral canal;

— Group 2 included patients with degenerative stenosis, spondylolisthesis, and lesion of two or more spinal segments who underwent fusion using classical transcutaneous transpedicular systems (Viper, Romeo);

— Group 3 included patients with the vertebral body changes of various origin (hemangioma, fractures, and deformities) who underwent vertebroplasty;

— Group 4 included patients who underwent biopsy of the altered vertebral body tissue.

Group 1 consisted of 36 patients who, if necessary, underwent bilateral decompression with or without interbody fusion followed by transcutaneous transpedicular interdisc fusion using the GO-LIF (Guided Oblique Lumbar Interbody Fusion) procedure as the main stage (Fig. 1). Interbody fusion was performed using different type cages, including cages filled with bone autograft.

Good results were obtained in 35 (97.22%) patients (Fig. 2), and an unsatisfactory result was obtained in 1 (2.78%) patient, which is associated with pathoanatomical features at the operative level (exostoses and facet joint hypertrophy) and vertebral body osteoporosis, which hampered the proper screw fixation. The system was removed, and bilateral decompression was performed.

Both the pain syndrome and neurological symptoms regressed during the postoperative period.

The L5—S1 level was most commonly affected (Fig. 3).

The advantage of the GO-LIF procedure is its minimal invasiveness; the lack of injury to the facet joints and minimal injury to soft tissues; a possibility to fuse a spinal segment with two screws, which are placed through the pedicle of a subjacent vertebra to the body of a superja-

Fig. 1. The SpineAssist robot in the operative field with an attached “arm” and guides for the subsequent placement of screws.

Fig. 2. CT control. The GO-LIF procedure is performed at the L4—L3 level.
Application of the GO-LIF fusion procedure requires the use of the SpineAssist robotic-guided system.

Indications for fusion using the GO-LIF procedure include:
- grade I—III spondylolisthesis;
- spinal stenosis;
- degenerative spine diseases.

Contraindications to fusion with the GO-LIF procedure include:
- lumbar hyperlordosis;
- abnormal sacral development;
- infectious spine diseases (osteomyelitis);
- osteoporosis (T-score less than 2.5);
- obesity (body mass index over 40).

Group 2 consisted of 14 patients with multilevel spinal lesions (Fig. 4) such as spinal stenosis and vertebral body compression fractures.

All the patients underwent robotic-assisted implantation of the Viper or Romeo transpedicular fixation system: for vertebral body fracture (3 cases) and for degenerative multilevel stenosis (11). In all cases, fusion was performed transcutaneously (Fig. 5). All the patients also underwent bilateral decompression.
Regression in the pain syndrome and neurological symptoms were observed in the early postoperative period. Patients were activated on the 2nd day after the operation.

The results of interventions were positive in all cases. Group 3 consisted of 16 patients with various changes in the vertebral bodies (hemangiomas, fractures); in one case, multiple vertebral body hemangiomas in the lumbar spine were present (Fig. 6). Robotic-assisted vertebroplasty was performed in all cases.

According to the clinical presentation, all hemangiomas were symptomatic and non-aggressive [1, 4, 8, 17]. The use of the SpineAssist robotic system allowed introduction of the filling material directly into the affected vertebral bodies with good results in all patients (Fig. 7).

Group 4 consisted of 11 patients in whom the SpineAssist robot was used to perform biopsy of vertebral body mass lesions (Table 2).

The use of robotic assistance allowed safe, reliable, and qualitative biopsy sampling from hard-to-reach regions of the vertebral bodies (Fig. 8).

Clinical case

A 30-year-old male N, diagnosed with a “L4 body defect of unknown origin” was hospitalized with complaints of gnawing pain in the lumbar spine that was

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Table 2. Distribution in the pathology and affected level

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Males</th>
<th>Females</th>
<th>Affected level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloma</td>
<td>0</td>
<td>2</td>
<td>T3, T6</td>
<td>2</td>
</tr>
<tr>
<td>Metastases of prostate cancer</td>
<td>2</td>
<td>0</td>
<td>L1, L4</td>
<td>2</td>
</tr>
<tr>
<td>Metastases of breast cancer</td>
<td>0</td>
<td>2</td>
<td>T5, T6</td>
<td>2</td>
</tr>
<tr>
<td>Metastases of lung cancer</td>
<td>1</td>
<td>0</td>
<td>T4, T5, T7</td>
<td>2</td>
</tr>
<tr>
<td>Osteoma</td>
<td>0</td>
<td>2</td>
<td>T11, T12</td>
<td>2</td>
</tr>
<tr>
<td>Chondrosarcoma</td>
<td>0</td>
<td>1</td>
<td>T11</td>
<td>1</td>
</tr>
<tr>
<td>Vertebral body defect</td>
<td>1</td>
<td>0</td>
<td>L1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

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Fig. 7. CT control after vertebroplasty (T7 body fracture). Filling material is located only in the vertebral body.

Fig. 8. Preoperative CT (a): a mass lesion is clearly seen (highlighted by arrows). Preoperative planning of the biopsy needle trajectory based on CT scans (b, c).

Fig. 9. Preoperative CT (a and b). Arrows indicate the L4 vertebral body cavity (a) and endplate defect (b).
increased upon moving (Fig. 9). Biopsy was performed to determine nature of the process in the vertebral body cavity. The material was taken for the histological examination.

No pathological tissue was detected (probably, the cavity was due to previous spondylodiscitis). At the second stage, vertebroplasty of a vertebral body defect was performed (Fig. 10).

**Conclusion**

The use of the SpineAssist robotic system enables minimally invasive, transcutaneous transpedicular interventions with safety and a high accuracy of screw placement. The GO-LIF fusion procedure can be combined with microdiscectomy and decompression of the spinal canal. Fusion of spinal segments using the GO-LIF procedure is impossible without the SpineAssist robotic system. Vertebroplasty using the SpineAssist robotic system allows introduction of the filling material directly into the hemangioma cavity and in the vertebral body fracture region. Biopsy from hard-to-reach regions of the vertebral bodies by means of robotic assistance allows sampling the histological examination material using the optimal and safe trajectory.

Therefore, the use of the spinal robotic assistance system makes it possible to perform high-technology surgical interventions with high accuracy, safety, and efficiency.

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This article describes current issues in spine and spinal cord surgery such as improving the efficiency and accuracy of operations through the use of modern intraoperative navigation systems. The SpineAssist system is a combination of the navigation system and manipulation robot that defines the trajectory for insertion and determines the location of spinal fusion implants or biopsy and vertebroplasty needles. The paper presents the data on the application of SpineAssist in 77 patients. All the patients were divided into four groups based on the type of intervention.

Group 1 consisted of patients who underwent fusion of the affected segment with the GO-LIF procedure; Group 2 included patients who underwent fusion of the affected segments using a transpedicular system; Group 3 included patients who underwent vertebroplasty of the vertebral bodies; Group 4 consisted of patients who underwent biopsy of the vertebral bodies. The article describes the use of the SpineAssist system in degenerative pathologies, trauma, and tumors of the spine. The groups the patients were divided cannot be compared to each other. To our opinion, the article title should be expanded to “Results of using the SpineAssist robot in surgical treatment of spine disorders”. When describing the surgical stages in Group 1, the authors did not indicate that the trajectory in the subjacent vertebra is drilled first, and then decompression of the spinal canal and placement of an autologous bone or Bi-CalPhos interbody implant are performed. The next step is drilling the screw trajectory in the superjacent vertebra. The operation is then completed with fusion of a segment by placement of the GO-LIF screw.

In general, the article is modern scientific work devoted to the state-of-art technologies in spine surgery. Promotion of the robotic assistance method will expand the knowledge of spine surgeons. Undoubtedly, the technologies, such as navigation and robotic assistance, will be available in all specialized clinics in the near future, so it is necessary to learn how to use them right now.

A.N. Konovalov (Moscow, Russia)
Experience of using an intraoperative cone beam computed tomography scanner “o-arm” and the modern navigation system in surgical treatment of spine and spinal cord disorders


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Spine disorders are a highly relevant problem in neurosurgical pathology. The development of diagnostic imaging methods makes it possible to perform intraoperative computed tomography. A special intraoperative cone beam computed tomography scanner “O-arm” has been designed; it combines a function of a C-arm machine and computer tomography scanner. The O-arm system can be used along with navigation system and robotic assistance device. Availability of these devices in an operating room allowed us to study the effectiveness and features of intraoperative CT imaging, Objective. To evaluate the intraoperative use a cone beam computed tomography scanner «O-arm» and the navigation system in surgical treatment of spine disorders. Material and methods. In August—November 2013, 43 patients with degenerative spine disorders, spine and spinal canal tumors underwent surgeries at the N.N. Burdenko Neurosurgical Institute using an intraoperative computed tomography scanner «O-arm» and the navigation system. Results. It is reasonable to use intraoperative CT «O-arm» device with the navigation system when surgical treatment is performed under complex anatomical conditions (a thin root of the vertebral arch, scoliotic or post-traumatic spinal deformity) and the surgery zone cannot be visualized using 2D imaging methods. Intraoperative CT control and navigation system can be employed by neurosurgeons in clinics where the standard stabilizing surgeries and percutaneous methods either are employed rarely or have just started to be used. Conclusions. The use of an intraoperative CT device «O-arm» with the modern navigation system for surgical treatment of spine and spinal cord disorders allows one to perform surgical interventions under complex anatomical conditions, reduces the absorbed radiation dose, and is safe for patients.

Keywords: intraoperative CT scanner «O-arm», navigation system, safety, quality of surgical treatment, spine and spinal cord disorders.

Spine disorders are a significant problem in the neurosurgical pathology. Rapid development of surgical techniques for treatment of spine disorders is associated with several factors. Undoubtedly, achievements in the development and implementation of both diagnostic and therapeutic technologies are of significant importance. An increase in the mean life span in developed countries and the wide spread use of neuroimaging methods (MRI, spiral CT, etc.) have led to improved detection of spine and spinal cord disorders and, as a consequence, to an increased number of patients who need surgical treatment. It is nowadays impossible to conduct spinal neurosurgical intervention without using intraoperative imaging systems. The repertoire of available devices is fairly large. An image converter (IC) is the most commonly used device in everyday practice. The image converter produces two-dimensional images, and it has routinely been used during the last decade. Numerous types of surgical treatment of spine disorders are associated with the need for implant placement. The correctness of the implant position must be controlled intraoperatively. Using IC does not allow making axial images and is a source of radiation exposure to the patient and the doctor, which is a drawback of the system. The correct implant placement minimizes risks of intraoperative complications and improves the quality of surgical treatment.

The development of imaging technologies nowadays makes it possible to conduct intraoperative computed tomography (CT). The O-arm cone beam intraoperative

### Distribution of patients according to the type of surgical treatment

<table>
<thead>
<tr>
<th>Surgery</th>
<th>Number of patients</th>
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<tbody>
<tr>
<td>Decompression and stabilization of one or more segments of the cervical spine</td>
<td>8</td>
</tr>
<tr>
<td>Decompression and stabilization of one or more segments of the lumbaroscal spine</td>
<td>27</td>
</tr>
<tr>
<td>Percutaneous stabilization of one or more segments of the lumbaroscal spine</td>
<td>2</td>
</tr>
<tr>
<td>Removal of spine and spinal cord tumors using intraoperative CT control</td>
<td>2</td>
</tr>
<tr>
<td>Vertebroplasty</td>
<td>3</td>
</tr>
<tr>
<td>Transcutaneous biopsy of spine and spinal cord tumors</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
</tr>
</tbody>
</table>
computed tomography (ICT) scanner was developed specially for this purpose; it combines functions of a computer tomography scanner and IC.

The O-arm system can be used along with a navigation system or a robotic assistance device to improve patient’s safety. Availability of this equipment in an operating room allows studying its effectiveness and features of intraoperative CT imaging.

The objective of this study was to evaluate the O-arm cone beam ICT and navigation system in surgical treatment of spine disorders.

Material and methods

The O-arm cone beam CT scanner has been used for intraoperative imaging at the Burdenko Neurosurgical Institute of the Russian Academy of Medical Sciences since August 2013. The O-arm system can be used both alone and in a combination with a navigation system. Between August and November 2013, 43 patients with degenerative spine disorders and spine and spinal canal tumors underwent surgeries using the O-arm ICT scanner and a navigation system at the N.N. Burdenko Neurosurgical Institute. The distribution of patients according to the type of surgery is shown in the Table.

The navigation system and O-arm ICT consist of five components (Fig. 1): an intraoperative imaging platform with a movable scanning module (gantry), a monitor, a wireless mouse for remote control, a navigation station with a camera, and a monitor to display navigation.

There are certain requirements to an operating room for installation of the O-arm ICT: the operating room area should be not less than 35 m²; slab floor should withstand the load of at least 440 kg per m²; the door height should be not less than 1.95 m, and the door width should be not less than 90 cm.

Availability of an X-ray transparent operating table is an important requirement for using the O-arm system. It

![Fig. 1. O-arm ICT and navigation system.](image)

a — an intraoperative visualization platform with a mobile scanning unit (gantry); b — a monitor; c — a wireless mouse for remote control; d — a navigation station with a camera; e — a monitor to display navigation.
should be noted that in this case the operating table was additionally equipped with carbon fiber side-tables.

During implementation of the O-arm ICT and navigation system, we tried various equipment arrangements in the operating room; the most appropriate of them are shown in Figs. 2 and 3.

During thoracic and lumbosacral spine surgery, the navigation station and its monitor are located at the leg rest end of the operating table. The optimal distance from the navigation camera to the surgical wound is ~ 1.7 m. The patient lies on the operating table with his hands maximally outstretched forward and upward. The scan and park positions should be saved before running the O-arm system. Movements of the movable scanning module are visually controlled to prevent its collisions with the operating table. Upon transition from the scan position to the park position, the scanning unit moves towards the head rest end of the operating table.

During cervical spine surgery, the navigation station and its monitor are located at the head rest end of the
The optimal distance from the navigation camera to the surgical wound is ~ 1.5—1.7 m. The patient lies on the operating table with his arms at sides. The scan and park positions should be saved and checked before running the O-arm system. The scanning unit moves towards the leg rest end of the operating table upon transition from the scan position to the park position.

To master the O-arm ICT and navigation system operating algorithm, all spine and spinal cord surgeries were performed in the presence of the manufacturing company specialist during the first month (Chart 1).

In the operating room, before surgery under endotracheal anesthesia conditions, an intraoperative CT examination in the 2D and/or 3D scanning modes using the O-arm ICT is carried out for precise identification of the surgical intervention area. After completing the surgical approach, the navigation frame is positioned, and a CT examination in the 2D and 3D scanning modes is performed using the O-arm ICT enclosed in a sterile bag. Next, CT imaging data are transferred to the navigation station, and the main stage of surgery is carried out.

It should be noted that the main stage is carried out using special navigation tools. No additional scanning is performed at this stage. After completing the main stage...
Fig. 7. Intraoperative CT examination of the cervicothoracic spine in the 3D scanning mode.
a — at the stage of positioning the needle prior to injecting cement; b — control scanning after vertebroplasty.

Fig. 8. Presurgical MRI of the cervical spine.
a — sagittal projection; b — axial projection. CT of the cervical spine: axial projection of C3 (c).

Fig. 9. Intraoperative control of the removed tumor amount using a CT examinations in the 3D scanning mode.
a — prior to removal of a tumor of the C2 and C3 vertebral arches; b — after removal of the tumor.
**Fig. 10.** MRI of the lumbosacral spine.

a — sagittal projection; b — axial projection.

**Fig. 11.** Intraoperative step-by-step control of placing the interbody implant (a) and transpedicle screws (b) at the L4—L5 level using the navigation system.

**Fig. 12.** Intraoperative CT monitoring of the lumbosacral spine after the placement of the interbody implant and transpedicle screws at the L4—L5 level.

a — 3D scanning mode; b — 3D reconstruction mode.
of surgery, a control CT examination with 3D reconstruction is carried out.

The algorithm of using the O-arm ICT and navigation system is slightly different in the case of transcutaneous interventions (Chart 2).

In the operating room, before surgery, the navigation frame is positioned at the surgical approach area. It should be noted that the navigation frame was fixed using a sterile self-adhesive film in the case of transcutaneous interventions (Fig. 4).

A CT examination in the 2D and 3D scanning modes is performed at the next stage. CT imaging data are transferred to the navigation station, and percutaneous surgery under local anesthesia is performed using navigation tools without additional scanning. A control CT examination with 3D reconstruction is carried out after the surgery.

We present a clinical case of vertebroplasty using the O-arm ICT in a patient S. with hemangioma of the T1 vertebra (Fig. 5).

After an intraoperative CT examination in the 2D mode, it was obvious that the standard fluoroscope could not be used in this case due to failure to visualize the surgical area (Fig. 6).

Surgical treatment, namely T1 vertebroplasty via the anterior cervical approach, was carried out (Fig. 7).

Let us consider a clinical example of using the O-arm ICT in removing a spinal tumor.

A patient Yu. was diagnosed with a tumor (osteoblastoma) of the C2 and C3 vertebral arches. Continued tumor growth. The patient was operated at the place of residence in January 2013. A removal of the C2 and C3 vertebral arch tumor was carried out. Histological diagnosis: osteoblastoma. The control CT examination revealed continued growth of the tumor (Fig. 8).

Surgical treatment, namely a removal of the C2 and C3 vertebral arch tumor, was carried out (Fig. 9).

As a clinical example, we present a case of the placement of a 4-screw system and an interbody implant using the O-arm ICT and navigation system in a 56-year-old patient A. with degenerative spinal stenosis at the L4—L5 level and instability of the L4—L5 segment (Fig. 10).

Surgical treatment was carried out, including decompression at the L4—L5 level, interbody stabilization, and transpedicular stabilization of this segment using the navigation system (Figs. 11, 12).

Results and discussion

The analysis of the main capabilities and benefits of using the O-arm ICT with the navigation system in surgical treatment of spine and spinal cord disorders was carried out. The surgery duration was monitored during this study. The duration of the first surgeries using the ICT and navigation system was long due to mastering the control and system algorithm. However, a tendency towards a decrease in the surgery duration was observed in the course of training within 1 month.

Patients with spine and spinal cord tumors underwent an intraoperative CT examination in the 2D mode immediately before surgery to map the surgical field. In some cases, when a tumor was localized in the anatomically inaccessible area that could not be visualized on two-dimensional images, 3D scanning was performed, which improved the accuracy of the surgical approach. When an osseous growth was present, an intraoperative CT examination was performed to control the completeness of tumor removal.

During the stabilization stage of surgery that requires the placement of interbody implants and transpedicle screws, including percutaneous techniques, the O-arm ICT and navigation system were used to specify the surgical area, to provide intraoperative control of the implant placement accuracy, and to control the decompression areas. 3D scanning and 3D reconstruction were performed after surgery. We suggest that using the O-arm ICT with the navigation system is especially important, when surgical treatment is carried out under complex anatomical conditions (thin vertebral arch pedicle, scoliotic or traumatic spine deformity) and the use of two-dimensional images does not provide visualization of the surgical area.

It should be noted that our series of observations had no complications associated with application of the equipment. Therefore, the use of the O-arm ICT and navigation system is useful for neurosurgeons in clinics where the standard stabilizing surgeries and percutaneous techniques are rare or just have been introduced.

Conclusions

1. Application of the O-arm ICT and navigation system for surgical treatment of spine and spinal cord disorders results in high-quality treatment and is safe for the patient.
3. The use of the O-arm ICT with the modern navigation system reduces the radiation exposure to both the patient and the surgical team.
The present study focuses on the important problem of improving patient’s safety and surgical treatment quality by means of intraoperative imaging tools. An image converter (IC) is currently the most extensively used device. However, experience has demonstrated that information obtained using IC under anatomically complex conditions may be insufficient. Analysis of the literature shows that the percentage of intraoperative complications in spinal neurosurgery is still quite high, about 16—20%. According to A. Carl and H. Khanuja (1997), neurological complications occur in 1.5—6% of cases, and biomechanically inadequate fixation occurs in 31% of cases. The percentage of complications caused by incorrect screw guiding varies from 4 (McAffe et al. 1991) to 21% (Weinstein et al. 1998). According to Graham et al. (1996), damage to neural elements occurs in 14% of cases.

The O-arm Intraoperative cone beam CT scanner is one of the tools to improve the accuracy of surgical interventions. Improving the treatment quality is, among other things, achieved due to the possibility to use it simultaneously with a navigation system. In this article, the authors demonstrated that capabilities of using the intraoperative CT control and navigation system are not limited just to a high accuracy of the implant placement. The O-arm system can also be used to assess the decompression degree in degenerative diseases, to evaluate the completeness of removal of certain spinal tumors as well as to perform puncture interventions (vertebroplasty, biopsy, etc.). This article contains clear recommendations for implementation of the aforementioned devices and states requirements for an operating room and an operating table. The authors’ experience with the O-arm system and navigation equipment provides the reader with the ready-to-use scheme for optimal arrangement of all components of the system within a standard operating room.

Therefore, the presented article is devoted to the topical subject, has a practical significance, and certainly deserves to be published in the journal.

A.O. Gushcha (Moscow, Russia)
Pericranial sinus. Definition, diagnosis, surgical treatment

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Pericranial sinus (Sinus pericranii — SP) is a rare pathology of the extra-intracranial cerebral venous system. However, SP is not just an additional transosseous canal that connects the extra- and intracranial venous systems. This «emissary vein» connects the intracranial sinus and the variceally extended thin-walled veins localized on the outer surface of the skull where blood flows fun in opposite directions. We present a literature review and two case reports of patients with pericranial sinus who underwent surgical treatment. We discuss the problems related to etiology, clinical signs, diagnosis, and surgical treatment of the anomaly.

**Keywords:** pericranial sinus, developmental venous anomaly, microsurgical removal.

Pericranial sinus (Sinus pericranii — SP) is a rare pathology of the extracranial and intracranial cerebral venous systems. About 170 cases of Sinus pericranii have been described worldwide so far [29]. We present two case reports of patients with SP who underwent microsurgical removal.

**Case 1**

A patient M., 7-year-old, was admitted to the Department of Children’s Neurosurgery of the A.L. Polenov Russian Neurological Research Institute in 2012. According to the past medical history, at the age of 2 years, the patient was detected with a soft-tissue lesion in the right postaural area. Transcranial Doppler sonography suggested an arteriovenous malformation in the right postaural area. Magnetic resonance imaging (MRI) of the brain suspected a dural venous malformation. Complaints at admission: a soft-tissue lesion in the right occipital region that, through a defect of the squama of the frontal bone (Fig. 1b), an extracranial lesion was identified in the right postaural area.

Locally: a mass lesion in the frontal fontanelle region, bluish in color, with a rising above the skin, bluish in color. No trophic changes in the skin flap were observed. Their reduction was observed intraoperatively (Fig. 1f). The skin over the lesion is unchanged and easily movable (Fig. 1e). The skin over the lesion, 3 cm above the mastoid bone, 4.0×4.0 cm in size, soft and elastic, with only a slight reduction in size when pressed down to the skull. No trophic changes in the skin flap were observed.


Brain MRI revealed a lesion in the soft tissues of the head (Fig. 2a) that consisted of two portions of 30×48 and 40×17 mm in size. In spiral CT angiograms (Fig. 2b), an extracranial lesion was identified in the posterior portions of the frontal region and in the medial portions of both parietal regions, consisting of two portions of 30×48 and 40×17 mm in size that caused compression atrophy in both parietal bones (a bone defect of the squama of the frontal bone (Fig. 2c) in the frontal fontanelle region, 128×25 mm in size). The lesion drained from the anterior chamber into the middle third of the superior sagittal sinus. No afferent arterial vessels were detected. The patient underwent two-stage microsurgical intervention. During the postop-

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erative period, no signs of venous hypertension were observed, focal symptoms were not detected, there was no cosmetic defect (Fig. 2e).

Discussion

According to C. Marras et al. [28], SP was first referred to in 1760 and was associated with the name of Percivall Pott who for the first time described a soft-tissue lesion formed over a skull fracture. In 1845, A. Hecker [9] presented this pathology under the name of “varix spurius circumspectus venae diploicae frontalis”. In 1850, G. Stromeyer [45] first used the term Sinus pericranii to refer to “a blood-filled reservoir located on the skull bones that communicates with the dura mater sinuses through the diploic veins”. Several terms are used to refer to this pathological condition: simple varix dilatation (varix simplex), cirsoid varix dilatation (varix racemosus), varicose herniosis (varix herniosis), varicose aneurysm (cirsoid aneurysm), venous angioma, dilated varicose vein (varix cirsoideus), osteovascular fistula (fistule osteovasculaire), venous tumor of the skull bones, skull varices [39].

In 1936, M. Févre and L. Modec [18] defined SP as a pathological communication between the extracranial and intracranial venous systems and divided SP into three types according to hemodynamic features: 1) circulation occurs inside SP as in a closed system;
2) the venous blood enters SP via the peripheral veins and then drains into the sinus; 3) the extracranial vascular anomaly is connected with the sinus. In 1950, J. Volkmann [48] divided SP into two types: true (which increases in size as the intracranial pressure is increasing and completely disappears, when the lesion is compressed) and false (which does not completely disappear upon compression). In 1967, J. Gerlach et al. [17] defined SP as varix dilatation and classified it according to the type of varix dilatation into simple, racemous, and hernioid. T. Newton and D. Potts [34] defined SP as a venous malformation and described this pathology as a communication between the intracranial and extracranial venous systems through a thin-walled tortuous vascular channel.

SP, however, is not merely an additional transosseous venous channel connecting the extracranial and intracranial venous systems. This “emissary vein” connects the intracranial sinus and a network of dilated thin-walled epicranial venous vessels located on the outer surface of the skull where pooling of multidirectional blood flow occurs. Taking this into account, C. Gandolfo et al. [16] divided SP, based on its angioarchitectonics, into two main groups: 1) a dominant

Fig. 2. Case 2 (patient D.).
a — spiral CT angiography; b — spiral CT with 3D reconstruction of the bones; c — MRI; d, e — the appearance of the patient before and after the surgery.
type, in which main drainage of the brain parenchyma occurs through the veins draining into SP; 2) an accessory type, in which only a part of the venous outflow occurs through the extradiploic vessels.

SP is usually located along the midline, adjacent to the dura mater sinuses. SP is most frequently observed in the frontal region (40%), in the parietal region at the level of the posterior and middle third of the superior sagittal sinus (34%), in the occipital region (23%), and in the temporal region (4%). There are several reports on its lateral localization [32, 44, 46, 51].

SP etiology is not fully understood. Most commonly SP is a congenital disorder, but an injury may also contribute to SP development [12, 21, 47, etc.].

SP can be divided into congenital, acquired, and post-traumatic [35].

Congenital SP is usually located over the “midline” in the frontal region; however, there are reported cases of congenital SP located in the parietal region and temporo-occipital region [26].

S. Nomura et al. [31] indicate that during embryogenesis when the embryo reaches 40 mm in size, as the cerebral hemispheres are growing in the caudal direction, incomplete involution of small veins of the interperioosteodural plexus occurs on the background of transformation of plexiform interperioosteodural predecessors of the superior sagittal and transverse sinuses into linear structures caused by disemembragenetic lesions. In the postnatal period, these veins are involved the venous outflow, but they are a developmental venous anomaly (DVA), formerly known as venous angiomas [27] (Fig. 3). S. Nomura et al. [31] believe that DVA can be both intradural and extradural.

Pericranial sinus is extradural DVA [31]. This fact explains the often association of SP with DVA not only in congenital but also in traumatic and spontaneous variants [4, 9, 13, 22, etc]. There are also reported cases of SP in the association with arteriovenous malformations [4] and cavernomas [6, 30].

According to K. Sakai et al. [40], SP develops in the late embryonic period as a result of venous hypertension associated with venous outflow occlusion. Since SP is often associated with craniostenosis, C. Gandolfo et al. [16] believe that congenital SP associated with craniostenosis develops due to formation of additional outflow pathways (in association with of intracranial hypertension and impaired venous outflow), which is in accordance with the data of N. Mitsukawa et al. [29].

Spontaneous SP can develop in the case of a short-term increase in the intracranial pressure (vomiting, sneezing, or coughing) that may result in a rupture of the emissary veins accompanied by establishment of an abnormal communication between the extracranial and intracranial venous systems [16]. However, M. Ar runes et al. [5] suggest that spontaneous SP develops in the association with some skull bone changes resulting in their thinning, while a temporary increase in the intracranial pressure and/or a minimal (unobserved) traumatic brain injury may serve as a trigger in the SP development. Traumatic SP occurs at the place of a previous traumatic brain injury and develops as a result of injury to the dural sinuses or emissary veins at their exit point from the cranial cavity [40]. According to T. Ohta et al. [35], a large number of the emissary veins in the patient are a predisposition to the SP development. Many works [8, 40, 42] emphasize the association of various vascular abnormalities, most commonly DVA, with traumatic SP. The period, during which SP may develop after an injury, varies. A. Shah et al. [41] described a case of SP manifestation in a 60-year-old female who suffered a traumatic brain injury with the formation of a frontal bone defect, when was a child.

Fig. 3. Example of a venous angioma (“intradural” DVA) of the left frontal lobe (12-year-old patient girl).
A conventional criterion for determining the underlying cause of this pathology is a type of the sac lining. The endothelial lining is characteristic of congenital SP, whereas the acquired lesion has the connective tissue lining [39]. C. Wen et al. [50] believe, based on a study by E. Hahn [19], that spontaneous and traumatic types do not differ histologically from each other. The histological findings in the case of the patient D. (case 2, see above) (Fig. 4) indicate the presence of a cavernous angioma (cavernoma). However, the presence of the hemodynamic phenomenon typical of SP suggests that this pathological process is a combination of two pathologies, SP and cavernoma.

SP can occur at any age [7, 22, 38]. However, according to M. Sheu et al. [43], manifestations of the disease (progredient increase in lesion size, neurological deficit) in congenital cases are mainly observed in young children; almost a half of the reported cases are patients under 20 years of age, and patients under 40 years of age amount 88% of the total number of reported cases.

Some authors believe that SP is more common in boys, but T. Ohta et al. [35] argue that the higher prevalence rate of SP in boys is due to the higher rate of post-traumatic cases.

SP has no characteristic clinical presentation, and the most common reason for seeking medical attention is a cosmetic defect. However, the clinical presentation of the disease may include headache, dizziness, nausea, and pain and feeling of pressure in the area of SP localization. Extremely rarely patients complain of bradycardia, ataxia, hearing loss, and vomiting [43]. Gradual growth of the lesion is observed [9], although there are also reported cases [37] of spontaneous thrombosis. The only complaint of the patient M. (case 1, see above) was a cosmetic defect. The patient D. (case 2, see above), in addition to a cosmetic defect, had a complication, which had not been reported before, in the form of bleeding from SP that occurred immediately after birth and was probably caused by a damage to SP during passing the fetus through the birth canal.

Fig. 4. Histological characteristics of soft tissue cavernoma of the head combined with pericranial sinus (Sinus pericranii).

a — cavernous vascular cavities with connective tissue septa of varying thickness are lined with the endothelium; b — a vein in the septum between the cavernous cavities; c — a fragment of hair in the malformation septum. Stained with hematoxylin and eosin; a, c — magnification of 100x; b — magnification of 200x.

It is necessary to differentiate between SP and other subcutaneous lesions of the skull, including subgaleal hematoma, atretic meningocele, growing fracture, dermoid cyst, and tumor of the skull bones. A distinguishing feature of this lesion is the dependence of its size on the body position. The lesion has the largest size in the recumbent position, while it decreases in size in the plantigrade position. However, the lesion size increases in the plantigrade position upon compressing the jugular veins on both sides or performing the Valsalva maneuver [7, 21].

This feature is not mandatory for all SP types, which was reflected in the classification of J. Volkmann (1950). For the patient M. (case 1), the lesion size did not depend on the body position. For the patient D. (case 2), a significant increase in the lesion size was observed in the recumbent position.

Neurosonographic studies of SP demonstrate its anechoic structure [24]. Doppler sonography allows one to perform the differential diagnosis between vascular and non-vascular pathologies and to verify the venous nature of blood flow, the direction of blood flow in the emissary veins, and bidirectional turbulent blood flow in the pathological lesion [52], which was detected in both our cases.

Unenhanced CT shows that SP has a higher density compared to the surrounding brain structures. CT also enables detection of a calvarial bone defect. CT with an intravenous contrast shows that SP has the same density as other venous structures, except for the cases of thrombosed SP [26].

MRI provides information on the sinus content based on the signal parameters and the relationship between the pathological lesion and the underlying sinus [39]. MRI examination of the patient D. indicated the presence of a pathological lesion characterized by a heterogeneous signal intensity. This was due to turbulent blood flow. Magnetic resonance or computed venography is used to determine the relationship between the lesion and the dura mater sinuses [7].
In the case of the patient  \( D \), MRI and spiral computed angiography of the brain were sufficient to establish the diagnosis. In the case of the patient  \( M \), spiral CT inconclusively indicated the lack of arteriovenous shunting.

The aim of selective cerebral angiography in SP is to rule out another vascular pathology characterized by arteriovenous shunting and, if SP is detected, to examine in details its angiarchitectonics and identify all venous collectors that connect the sinus and the extracranial lesion \([1, 25]\). In rare cases, venography by direct puncture of a lesion is performed \([46, 49]\). For the patient  \( M \), selective cerebral angiography allowed verification of SP.

**Surgical treatment**

In 1771, Percivall Pott \([13]\) successfully performed trephination and tamponage of SP. There had been no reports in the literature on successful surgeries before 1902. Despite the reports indicating a possibility of spontaneous thrombosis \([20, 37]\), the active surgical approach is valid for this pathology \([2, 12, 44, 47, 50, \text{etc.}]\).

The main goal of surgical treatment is to prevent massive bleeding and traumatic air embolism as well as to eliminate a cosmetic defect. Currently, both endovascular and microsurgical techniques are used for SP treatment \([4, 12, 16, 31, \text{etc.}]\).

The dominant type SP (according to the classification of C. Gandolfo et al. \([16]\)) is a contraindication to any surgery, as it may result in complications such as severe venous insufficiency and/or brain infarction. In the case of the accessory pattern, surgical treatment tactics depends on the particular features of brain parenchyma drainage. According to the foreign literature \([12]\), up to 30% of the reported cases were of the dominant type and were not operated on.

Microsurgical methods can be divided into two types: 1) radical surgery that includes wide craniotomy, occlusion of the venous collectors connecting the sinus and an extracranial vascular lesion, and cranioplasty \([28, 49]\); 2) a removal of the extracranial component, occlusion of the venous collectors, connecting the sinus and an extracranial vascular lesion, without craniotomy \([29]\).

Potential complications of this type surgery include bleeding and air embolism \([2, 5, 14, 15, 25]\).

According to the classification of C. Gandolfo et al. \((2007)\), our cases (the patient  \( M \) and the patient  \( D \)) belonged to the accessory type.

A peculiar feature of the patient  \( M \) was hypoplasia of the left half of the transverse sinus. According to cerebral angiography, stenoses of the right and left halves of the transverse sinus and jugular vein stenosis were not detected. In the case of the patient  \( D \), MRI, spiral CT angiography, and transcranial Doppler sonography showed no significant drainage of the brain parenchyma into SP. In both cases, the surgical approach was chosen. Given the young age of the patient  \( D \) and a high risk of developing severe post-hemorrhagic anemia due to blood loss, the surgery was divided into two stages.

There are few reports \([10, 23, 36]\) on the application of endovascular methods to treat SP with different outcomes.

**Conclusion**

Therefore, pericranial sinus is a rare vascular disease with a favorable prognosis. The goal of surgical treatment is to eliminate a pathological communication between the extracranial and intracranial venous systems.

In cases where main drainage of the brain occurs through veins draining into SP, surgery is contraindicated. In cases where only part of the venous outflow passes through extrapleurocerebral vessels, surgical treatment is indicated. The method of choice for surgical treatment is a removal of the extracranial component and occlusion of the venous collectors, connecting the sinus and an extracranial vascular lesion, without craniotomy. However, if pericranial sinus is considered as an extracranial DVA, the choice of tactics in favor of surgery should be based on a balance between the intended effect and the probability of postoperative complications, such as venous congestion and/or brain infarction.

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One of them, they likely found a combination of two types of pathologies: congenital sinus pericranii and cavernous malformation. It may be true, but in fact, the micrographs presented in Fig. 4 demonstrate just a small blood vessel (under given magnification of about 100x), it is impossible to conclude whether the vessel is arterial or venous and 4 cavernous cavities not filled with blood. It is unclear how these cavities are incorporated in an anomalous venous network and how they participate in blood flow through it. In any case, the article focuses on the relatively rare and little-known problem and arouses interest.

Sinus pericranii is a rare pathological condition. The article discusses the problems of its etiology, clinical manifestations, and surgical treatment approaches. It analyzes the extensive literature and presents different points of view on the pathogenesis, morphology, classification, and indications for surgery. The authors present two their own case reports of successful surgical treatment of children with this pathology. In one of them, they likely found a combination of two types of pathology: congenital sinus pericranii and cavernous malformation. It may be true, but in fact, the micrographs presented in Fig. 4 demonstrate just a small blood vessel (under given magnification of about 100x), it is impossible to conclude whether the vessel is arterial or venous and 4 cavernous cavities not filled with blood. It is unclear how these cavities are incorporated in an anomalous venous network and how they participate in blood flow through it. In any case, the article focuses on the relatively rare and little-known problem and arouses interest.
Placement of the Ommaya reservoir in narrow and slit-like ventricles using a neuronavigation system. Author’s own experience and literature review

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In modern neuro-oncology and onco-hematology, intraventricular injection of chemotherapeutic agents (most typically, methotrexate) is an inevitable part of many protocols for treating patients with malignant tumors of the CNS, neuroleukemia, CNS lymphomas and some other disorders. A ventricular catheter system (also known as the Ommaya reservoir) is used to provide repeated injection of chemotherapeutic agents to cerebral ventricles. The use of modern neuronavigation systems allows one to place Ommaya reservoir in patients with narrow and slit-like ventricles.

Material and methods. During the period between March 2012 and October 2013, 27 patients underwent stereotactic placement of the Ommaya reservoir using a Cart II optical neuronavigation system (Stryker) at the Dmitry Rogachev Federal Scientific and Clinical Center of Pediatric Hematology, Oncology and Immunology. The patients for stereotactic placement of a ventricular catheter were selected on a subjective basis (small ventricular size was the criterion for selection). In one patient, the surgery for placement of the Ommaya reservoir was combined with stereotactic biopsy.

Results. In all patients, a ventricular catheter was placed in the anterior horn of the lateral ventricle on the first try; no intraoperative complications were observed.

Conclusions. Frameless navigation is an illustrative, mobile, and multifunctional method. The same device can be used to perform brain, transsphenoidal, ENT, and spine surgeries, as well as orthopedic interventions. Today, neuronavigation systems are used in neurosurgical operating rooms and make it possible to avoid using stereotactic frames in most cases.

Keywords: neuronavigation, Ommaya reservoir, stereotactic surgery, brain tumor, chemotherapy.

Historical note

Despite the common belief, the ventricular reservoir inventor, Ayub Khan Ommaya, (Fig. 1) had no con-

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He was born in Pakistan in 1930. His father, Nadir Khan, served in the Indian Cavalry Corps of the British Army during World War I in France, where he met his future wife. Ayub Ommaya finished school and then graduated from medical college in Pakistan. For his academic performance, he was honored with a golden medal and a scholarship to further medical education in Oxford. Aside from his brilliant achievements in medicine, Ayub played sports seriously and even won the national championship in swimming in 1953. In Italy, young Ommaya took singing lessons from a renowned opera tenor, and afterwards he often sang before and after surgeries to the delight of his colleagues, patients, and their relatives. Ayub Ommaya immigrated to the United States in 1961, where he had worked in various clinics for more than 40 years. In addition to the invention of his famous reservoir in the beginning of 1960s, he accomplished a great number of achievements in the field of traumatic brain injury biomechanics, he also became one of the pioneers of spinal angiography, was engaged with endovascular and direct surgery of spinal arteriovenous malformations, developed methods for treatment of nasal liquorrhea, and collaborated with Hounsfield for improving the quality of computed tomography.

In his later years, Ayub Khan Ommaya returned with his family to Pakistan, where he passed away from

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**Fig. 2.** Planning of the approach using computed tomography data.
The trajectories for the placement of a ventricular catheter are colored in pink and blue.

**Fig. 3.** Planning of the ventricular catheter placement using a 3D mode.
Skin markers are well seen on the head.
Alzheimer’s disease at his home in Islamabad on July 11, 2008 [4].

The most important part of the Ommaya reservoir placement is to introduce accurately a ventricular catheter into the lateral ventricle. The procedure can be easily performed by a resident, if the ventricles are wide. However, patients who need the Ommaya reservoir placement have usually the narrow or slit-like ventricles, since hydrocephalus develops rarely in leukemia patients. In these cases, surgery is usually performed using a stereotactic frame, which provides a high accuracy for placing a catheter into the ventricle, but it is also accompanied by a number of difficulties, such as the necessity to transport the patient to CT during surgery and overall bulkiness of the equipment. The rapid development of neuronavigation systems in the last decade makes it possible to avoid using stereotactic frames in most cases and makes the procedure to be more mobile [1—3, 10, 13].

Material and methods

During the period between March 2012 and October 2013, 27 patients underwent 27 surgeries for the stereotactic placement of the Ommaya reservoir using a Cart II optical neuronavigation system (Stryker) at the Dmitry Rogachev Federal Scientific and Clinical Center of Pediatric Hematology, Oncology, and Immunology. The age of patients varied from 6 months to 17 years old. According to nosologies, the patients were distributed in the following groups: acute lymphoblastic leukemia in 9 patients; acute myeloid leukemia in 2; CNS lymphoma in 3; malignant brain tumors (medulloblastoma, pineoblastoma, primitive neuroectodermal tumor) in 13.

The patients for the stereotactic placement of a ventricular catheter were selected subjectively, based on the small ventricular size.

Surgical technique

The day before surgery, skin markers are stucked on the patient’s head and the patient is further subjected to computed tomography (CT) or magnetic resonance imaging (MRI) with the slice thickness of not more than 2 mm and the scanning zone from the vertex to maxillary incisors.

After that, the examination data in the DICOM format are transferred to the navigation system and the catheter placement trajectory is planned. We planned two trajectories for both ventricles at once: the main trajectory was for the wider ventricle, and the other was reserved (Figs. 2—4).

At the beginning of surgery, the patient’s head is fixed either in the Mayfield head frame or in a baby frame with gel pads. Further, a patient tracker (reference frame) that serves as the reference point is rigidly fixed on the frame. The surgery can be performed without the Mayfield frame, in which case the patient tracker should be fixed on the patient’s head with a small screw. The latter approach is displeasing due to its invasiveness, but it is convenient in some cases (e.g. upon ventriculoperitoneostomy), since the frame complicates the placement of other components of the shunt system.

After fixing the patient’s head, a registration is conducted. This is one of the key stages that determines the success of future surgery. The registration is conducted based on the skin markers using either anatomical landmarks or surfaces. The registration using the skin markers provides the highest accuracy [2, 5, 9, 11]. However, this method has several disadvantages: markers need to be purchased, they might detach during the night-time, the patient himself (especially a child) or his relatives might take the markers off before the surgery, the markers should not be stucked on the areas, where the Mayfield frame pins are to be placed, or a skin dislocation might occur due to the stiff fixation, additional CT scanning the day before or on the day of surgery is required, when the skin markers are used [5, 11].

After completing the registration, points for burr holes are marked on the scalp using a navigator; the surgical area is prepared. Then, a small scalp incision is made; a burr hole is made, followed by opening the dura mater.

A catheter is placed into the ventricle using a navigation instrument called a mandrin. The surgeon can easily handle it with one hand or firmly lock it in the fixator. The real trajectory can easily be juxtaposed with the planned one using the screen image; the catheter is advanced to the preliminary measured depth. After that, the mandrin is removed, and drops of the neurolymph indicate that the procedure is successful. Further steps
Results

In all 27 cases, the catheter was placed into the anterior horn of the lateral ventricle on the first try, which was confirmed by successful functioning of the Ommaya reservoir and postoperative CT and MRI (Fig. 5). In one patient, surgery for the Ommaya reservoir placement was combined with stereotactic biopsy (STB).

Discussion

Modern neuronavigation devises are divided into two large groups: navigation based on preoperative images, and devises with interactive intraoperative control (real-time). The first group comprises neuronavigators and stereotactic frame systems. The second group includes ultrasound scanners and intraoperative MRI (MRI scanners) [1, 7].

Each system has its own advantages and disadvantages.

The main disadvantage of navigation using preoperative CT/MRI is that the images are consistent with reality only during the first steps of surgery, because further, in the course of changing in the intracranial relations, they may not always reflect the true picture, which decreases the value of this method. On the other hand, the compactness of neuronavigators, their reasonable (compared to intraoperative MRI scanners) cost and ease of use almost have made them a mandatory attribute of the neurosurgical operating room, like a microscope, an endoscopic stand, and an ultrasound aspirator [2, 7].

In the past years, neuronavigators have almost replaced stereotactic frames, except for a small segment of surgeries for placing deep brain stimulation electrodes, where a high accuracy of traditional stereotaxis works well, despite all its inherent disadvantages. Frameless navigation is an illustrative, mobile and multifunctional method. The same devise can be used to perform brain, transsphenoidal, ENT, and spine surgeries as well as orthopedic interventions [7, 9, 11, 13].

![Fig. 5. Postoperative CT control.](image)

The ventricular catheter tip is located in the lumen of the anterior horn of the lateral ventricle.

### Comparative analysis of neuronavigation devises

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Stereotactic frame</th>
<th>Neuronavigators</th>
<th>US scanners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functionality</td>
<td>Puncture manipulations only (STB, placement of catheters and electrodes)</td>
<td>All kinds of surgeries on brain, spinal cord, and ENT organs, orthopedic interventions</td>
<td>Brain surgeries (except for STB and approach mapping)</td>
</tr>
<tr>
<td>Image quality</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Technical planning of the surgery</td>
<td>Intraoperative CT/MRI and planning</td>
<td>Preoperative CT/MRI and planning</td>
<td>Not required</td>
</tr>
<tr>
<td>Rigid head fixation</td>
<td>Required</td>
<td>Depends on a navigator model and the type of surgery</td>
<td>Not required</td>
</tr>
<tr>
<td>Variety of models available on the Russian market</td>
<td>Wide variety</td>
<td>Wide variety</td>
<td>Limited</td>
</tr>
</tbody>
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of the surgery are conventional and do not require any specific description.
Real-time neuronavigation with intraoperative images seems to be a promising approach. It can be provided by two antipodal devices: an ultrasound (US) scanner and an intraoperative MRI scanner. A US scanner has a great number of advantages: it provides a real interactive picture of what is happening inside the wound, it is cheap, mobile, and easy to use. Its only drawback is a quite low image quality that does not allow differentiating the fine structures of the brain and performing operations on the osseous structures [12, 13]. It should also be noted that there is only one model of a US scanner equipped with a sensor capable of locating through the burr hole (BK-Medical) on the Russian market. The literature [12] also describes a device equipped with a sensor of similar size (Aloka). Sensors of other manufacturers require a larger trepanation hole, which is inappropriate in the case of puncture methods of surgical treatment. Nowadays, hybrids of a US scanner and a navigation system are developed [7]. Time will tell on perspectives of these devices.

An absolute opposite to a US scanner is an intraoperative MRI scanner that provides high quality imaging during surgery, which is its main and the only advantage. The other its properties may be regarded as disadvantages. An intraoperative MRI scanner is very expensive, it usually requires an individually furnished operating room, specially trained personnel, and specific surgical instruments [7]. Its application also requires a lot of time and resources. Intraoperative MRI scanners are practically not used in Russia nowadays. Perhaps, in the future, the situation might change as the technology develops and becomes cheaper.

A comparison of neuronavigation devices (except for intraoperative MRI scanners) is provided in the Table.

Therefore, neuronavigators are currently an essential part of neurosurgical operating rooms and make it possible to avoid using stereotactic frames in most cases [7, 10, 13].

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Commentary

The current paper is devoted to the use of a neuronavigation system for an accurate placement of a ventricular catheter into the cerebral lateral ventricles. This application of neuronavigation is certainly reasonable. Twenty seven patients underwent the surgery. In all cases, the lateral ventricle was catheterized on the first try. The authors do not note any technical problems related to both the functioning of the navigation system and the surgery in general. Increasing the capabilities of modern pediatric neurosurgery and oncology leads to an increase in the number of patients who need intrathecal chemotherapy. The described technique is an example of a reasonable use of modern technologies for the benefit of the patient. We would like especially to thank the authors for an interesting historical note about the Ommaya reservoir inventor.

Yu.V. Kushel’ (Moscow, Russia)
Simultaneous operations in patients with kidney cancer with simultaneous brain tumor lesion


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Three clinical cases of simultaneous operations upon synchronous identification of brain tumor and kidney cancer are described. A metastatic lesion of the brain was detected in two of them, and a combination of a primary CNS tumor (glioblastoma) with kidney cancer was identified in one case.

**Keywords:** simultaneous operations, kidney cancer, brain metastases.

Kidney cancer (renal cell carcinoma, RCC) ranks third in the incidence of malignant tumors of the genitourinary system. More than 200,000 new cases of RCC are detected in the world annually, which is 2–3% of the total cancer incidence. The standardized incidence and mortality rates for malignant kidney tumors are 8.9 and 3.78 per 100,000 population of Russia, respectively [1]. Kidney cancer (along with melanoma) demonstrates the highest propensity to metastasize to the brain, but the relative rarity of these tumors explains a low frequency of their metastases to the brain. Kidney cancer metastases comprise 6—7% of the total number of metastases in the central nervous system. RCC metastases are well vascularized, form usually one nodule, and are characterized by intratumoral hemorrhages, whose frequency varies from 9 to 45% [2].

Simultaneous operations for kidney cancer and simultaneous brain metastases have not been widely covered in the literature. There are single clinical observations that describe single-stage surgeries for metastatic lesions of the skull bones [3].

Following are the observations of 3 patients who underwent simultaneous brain and kidney surgeries.

**Observation 1**

A 50-year-old male underwent inpatient treatment at the Neurosurgical Department of the P.A. Hertzen Moscow Research Institute of Oncology (MROI) between 11.12.12 and 19.12.12. On 09.10.12, the patient felt weakness, dizziness, and headache. Later, on 10.10.12, he also felt weakness and convulsions in his left extremities. In connection with these symptoms, the patient was hospitalized through the Emergency Medical Service to a city hospital according to the place of residence. After an examination at the hospital, the patient was referred to the Lipetsk Regional Oncology Center, where he was diagnosed with grade IV, stage T1NxM1 right kidney cancer and brain and lung metastases. The patient applied to the P.A. Hertzen Moscow Research Institute of Oncology (MROI).

At admission, on the background of clear consciousness, left-hemisphere symptoms in the form of right-sided hemiparesis with a reduced strength up to 4 points, cerebral symptoms such as headache and general weakness, and paroxysmal symptoms in the form of focal seizures in the right extremities were observed. The Karnofsky performance status score (KPSS) was 70.

Several lesions in the left frontal lobe, left temporal lobe, and right parietal lobe were detected using brain magnetic resonance imaging (MRI) (Fig. 1). Complete ultrasound investigation (USI) and computed tomography (CT) of the thoracic and abdominal cavities revealed a right kidney tumor lesion (Fig. 2) and several small lung metastases. Discussing the situation resulted in the conclusion that the patient life expectancy and quality of life were mostly affected by:

1) a tumor deposit in the left frontal lobe, causing pronounced edema and brain dislocation, and
2) a primary kidney tumor manifested by recurrent bleedings (gross hematuria).

Lung surgery was not indicated. Radiosurgical treatment for an intracranial nodule causing peritumorous edema was contraindicated, but it was indicated for other nodules, including those in the lungs. Under these conditions, the simultaneous surgery was conducted on 13.12.12 that included a microsurgical removal of a metastatic tumor of the left frontal lobe using intraoperative fluorescence diagnostics (IOFD), right laparoscopic nephrectomy, resection of the right adrenal gland, and para-aortic lymphadenectomy. At the first stage, with the patient in the dorsal position, a microsurgical removal of the left frontal lobe tumor was performed under the control of intraoperative fluorescence navigation. The surgery proceeded without complications and with a minimum blood loss. The bone flap was placed back and the wound was closed in layers.
Then the patient was turned to his left side and laparoscopic nephroadrenalectomy and para-aortic lymphadenectomy were carried out. Brain MRI conducted on the 1st day after the surgery revealed removal of the left frontal lobe tumor and no signs of ischemic and hemorrhagic complications; the remaining tumors were without changes. Abdominal and retroperitoneal USI performed on the 4th day after the surgery detected no signs of complications. Retroperitoneal drainage was removed on the next day.

X 1430721/op (13.12.12) histological examination results: 1) the right kidney tumor — clear cell RCC G3 with an extensive necrosis, invasion of small and large blood vessels, formation of tumor thrombus in the vein, infiltration of the renal capsule, perinephric fat, renal hilar fat, and calyx, and without invasion of the pyelocalyceal system; no tumor growth at the ureteral resection margin; infiltrative tumor growth in the renal hilar fat; 2) the left frontal lobe tumor — a metastasis of clear cell RCC with a central necrosis.

The neurological status was characterized by regression of cerebral and left-hemispheric symptoms. KPSS was 80. The patient was discharged on the 6th day after the surgery with recommendations for further treatment in accordance with a pre-developed plan (radiosurgical treatment using the CyberKnife system and chemotherapy).

Observation 2

A 55-year-old male underwent inpatient treatment at the Neurosurgical Department of MRIO between 28.01.13 and 15.02.13. Since the beginning of January 2013, the patient felt headache, nausea, and vomiting. For this reason, the patient visited the neurologist at the place of residence who referred him for MRI that revealed a brain tumor (Fig. 3). The patient applied to the MRIO. Neurological status: symptoms of a cerebellar lesion affecting the floor of the IVth ventricle and cerebral symptoms. KPSS at admission was 60 and increased to 70 in the course of treatment with dexamethasone. An additional examination revealed a left kidney tumor (Fig. 4).

Given that both tumors posed hazard to the patient life, while other metastatic lesions were absent, a joint consultation of neurosurgeons and oncurologists decided to perform single-stage surgery 07.02.13, simultaneous surgery was conducted that included a microsurgical removal of a metastatic tumor of the cerebellar vermis and IVth ventricle using IOFD. Left laparoscopic nephrectomy and para-aortic lymphadenectomy were performed. At the first step, with the patient in the sitting position, a removal of the cerebellar vermis tumor using...
fluorescence navigation was performed. The patient was then moved to the right lateral decubitus position. Left laparoscopic nephrectomy was performed. Control brain contrast enhanced MRI (08.02.13): the tumor was removed, no complications were observed. A control ultrasound examination of the liver, abdominal cavity, retroperitoneum, and lower extremity vessels (12.02.13): an ultrasound presentation of diffuse changes in the liver, the left kidney was removed, no abnormalities in the removed kidney bed. The postoperative period was uneventful, the postoperative wounds healed primarily, sutures were removed, and the retroperitoneal drainage was removed 12.02.13.

X 27970-78/op (07.02.13) histological examination results: in the kidney — clear cell RCC G3 with an extensive necrosis, invasion of blood vessels, the tumor spread into the renal capsule without going beyond it, the renal pelvis was intact, no tumor growth at the ureteral resection margin; in the brain — similarly structured clear cell RCC complexes without subiculum.

Neurological status: regression of cerebral and brain stem symptoms was observed, persistent instability in the Romberg’s position and slight cerebellar ataxia, KPSS was 80 at discharge. The patient was discharged from the hospital on the 8th day after the surgery.

Observation 3

A 59-year-old female underwent inpatient treatment at the Neurosurgical Department of MRIO between 04.06.13 and 28.06.13.

The patient developed acute speech disorder and weakness in her right extremities in May 2013. She was hospitalized to a clinic according to the place of residence with suspected acute cerebrovascular accident. MRI revealed a tumor of the left temporal lobe (Fig. 5). The patient applied to MRIO. Neurological status: right-sided hemiparesis (3 points), severe motor aphasia, KPSS was 60.

Based on questioning the patient, episodes of total painless hematuria were revealed in her past medical history, with the first of them occurred 4 months before the admission. A further examination at the institute (ultrasound, CT) detected a left kidney tumor (Fig. 6). Given that both tumors posed hazard to the patient life, a joint consultation of neurosurgeons and oncourologists decided to perform single-stage surgery for removal of the left temporal lobe tumor and left nephrectomy.

13.06.13, simultaneous surgery was performed that included left laparoscopic nephrectomy, a microsurgical removal of a tumor of the pole and medial portions of the left temporal lobe using IOFD. At the first stage, with the patient in the right lateral decubitus position, left laparoscopic nephrectomy was performed; then the patient was turned on her back, and a microsurgical removal of the tumor of the pole and medial portions of the left temporal lobe was performed using IOFD. Control brain contrast enhanced MRI (14.06.13): the tumor was removed, no signs of complications. A control ultrasound examination of the liver, abdominal cavity, retroperitoneum, and lower extremity vessels (12.02.13): the tumor was removed, no abnormalities in the removed kidney bed. The postoperative period was uneventful, the postoperative wounds healed primarily, sutures were removed, and the retroperitoneal drainage was removed 12.02.13.

X 27970-78/op (07.02.13) histological examination results: in the kidney — clear cell RCC G3 with an extensive necrosis, invasion of blood vessels, the tumor spread into the renal capsule without going beyond it, the renal pelvis was intact, no tumor growth at the ureteral resection margin; in the brain — similarly structured clear cell RCC complexes without subiculum.

Neurological status: regression of cerebral and brain stem symptoms was observed, persistent instability in the Romberg’s position and slight cerebellar ataxia, KPSS was 80 at discharge. The patient was discharged from the hospital on the 8th day after the surgery.
extremity vessels (17.06.13): the left kidney was removed, no abnormalities of the removed kidney bed were detected.

X 67316-27/op histological examination results: multiple primary synchronous tumors — clear cell RCC, G-2; left temporal lobe glioblastoma.

The postoperative period was uneventful, the postoperative wounds healed primarily, sutures were removed, and the retroperitoneal drainage was removed on 18.06.13. The neurological status showed regression of cerebral symptoms and right-sided hemiparesis, KPSS was 80 at discharge. The patient was discharged in a satisfactory condition on the 15th day after the operation.

**Discussion**

The concept of “simultaneous operations” was introduced by Reiffezscheid [10] in 1971 and was first mentioned in his article “Simultaneous intervention in the abdominal cavity: surgical aspects”.

The term derives from the English word “simultaneously”. Some authors understand simultaneous operations as a surgery that is simultaneously performed on two or more organs for etiologically unrelated diseases. A number of authors [7,9] consider single-case operations as surgical teams at the same time [7,9]. Other authors [4—6,8] suggest that simultaneous operations involve interventions for primary multiple malignant tumors. Simultaneous operations include the main stage and concomitant one (or ones). The main stage surgery is aimed at eliminating the pathological process that is the most dangerous to the patient life, while the concomitant one is aimed at eliminating a disease detected during a preoperative or intraoperative examination.

The aforementioned clinical cases show that the patients sought medical treatment for a neurological deficit they experienced to a greater or lesser extent. A renal tumor was detected during the preoperative examination.

Obviously, the question of conducting simultaneous operations is relevant, when two pathologic processes requiring surgical treatment are detected simultaneously. Definitely, it requires a careful preoperative preparation and evaluation of various surgery- and anesthesia-related risks, since the patient who undergoes simultaneous operation on the brain and kidney is essentially subjected to two full-scale surgical interventions integrated by a single anesthesia procedure. It is usually accompanied by changing the patient position on the operating table, basically different surgical approaches to the pathological process, a double surgical wound, and an increased risk of both intraoperative and postoperative complications. Therefore, the initial condition of the patient and his/her ability to withstand this treatment should obviously be the fundamental factors for making decision about this surgery.

**Conclusion**

We presented three clinical observations of simultaneous operations in patients with simultaneously detected tumors of the brain and kidneys. In the first two cases, it was a metastatic brain lesion, while in the third case, it was a combination of a primary CNS tumor and RCC. The experience we have gained at this stage shows that the use of simultaneous operations can be quite reasonable and relevant. This approach may potentially reduce the time spent for the surgical stage, which is sometimes of crucial importance for complex therapy of patients.

**REFERENCES**

Commentary

Single-stage (or simultaneous, according to authors’ terminology) resection of disseminated or multiple tumors affecting both the central nervous system and distant organs is very rare in neuro-oncology. A common approach is based on removal of a lesion that foremost affects the patient life expectancy, only then followed by removal of another lesion or lesions. The argument in favor of this approach is a less traumatic intervention and a lower blood loss. However, taking into account two premedications, two tracheal intubations, prolonged (or repeated) catheterization of the central vein, and the total time of artificial lung ventilation, it becomes obvious that the traumatism of two successive interventions will be higher compared to single-stage one. More importantly, the sequential surgical treatment approach leads to an unavoidable delay in the beginning with radiotherapy and chemotherapy, which may be critical in some cases.

However, the decision to perform simultaneous intervention should be made carefully. As the authors truly noted, an intervention should begin with a lesion foremost affecting the patient life expectancy, i.e., with that being of the greatest risk. And only in the case of a “smooth” course of the first intervention, the second, less critical, stage of treatment can be started. To my opinion, performing a single-stage intervention by two teams is inappropriate in neurosurgery.

The publication demonstrating the possibility of a single-stage removal of the primary and secondary lesions or two malignant tumors at once is undoubtedly important. By the way, I think that in the last case, the metastatic nature of the intracranial tumor was suggested prior to surgery. At least, I would think so based on the available data. I would like to congratulate the authors on this success and wish them to continue their research with in-depth analysis of results.

A.V. Kozlov (Moscow, Russia)
Case report of an indirect spine and spinal cord injury

N.N. SAL’KOV, D.V. OVCHARENKO

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We present a case report of spine and spinal cord injury caused by wound to the face by a foreign body followed by its penetration to cervical soft tissues and lateral compression of the spinal canal by the foreign body causing a neurological deficit. A significant regression of neurological disorders was observed after the dislocation of the vertebra had been eliminated.

Keywords: spine and spinal cord injury, foreign body, spinal cord compression.

Spine and spinal cord trauma resulting from a wound by a foreign body belongs to rare forms of injury in peacetime that are dominated by puncture and gunshot wounds of the spinal canal with the development of persistent neurological deficit due to complete or partial rupture of the spinal cord [1—7].

In the literature [1, 3—6], spine and spinal-cord injury, which is prevailed by biomechanics of the flexion-extension and axial mechanism, causing the anterior or posterior deformity of the vertebra, is described in detail, but the description of lateral compression that is more characteristic of a combat trauma is very rare.

In our observation, a case of lateral compression of the spinal canal by a foreign body with the development of neurological deficit is considered. The case is remarkable for that the elimination of the vertebral dislocation resulted in a significant regression of neurological disorders within 24 hours. Surgery was performed in the acute period, 11 hours after the injury. Early decompression prevented the development of secondary changes in the brain matter [6—10].

Case description

Patient K., born in 1969, was treated at the Mechnikov Dnepropetrovsk Regional Clinical Hospital from 20.08.13 to 13.09.13. Clinical diagnosis: concomitant injury. Open paravertebral indirect spine and spinal cord injury. Contusion of the spinal cord at the C3—C4 level, a lateral fracture of C4 right. A fracture of the lower jaw.

At the time of examination: the patient was intubated, supported with an artificial lung, under local medical sedation, but performed basic instructions. Medical history: 20.08.13 at around 5:00, at the mine, a rupture of a metal chain occurred, a fragment of which injured the right cheek and lower jaw area and remained fixed in the wound.

Somatic status: a serious condition. The skin and visible mucous coats were clean and pale. Breathing was independent and adequate. Heart sounds were clear and rhythmic, the blood pressure was 100/60 mm Hg, the heart rate was 56 bpm. The abdomen was available for deep palpation. Urine was drained via a catheter.

Neurological status: the patient was awake, quickly exhausted due to sedation, signs of a neurogenic shock were present (BP was supported with moderate doses of sympathomimetics, the heart rate was 56 beats per minute). The pupils were equal, photoreaction was active. Right-sided hemiplegia. The patient reacted actively by the left limbs to painful stimuli. The level of sensitive disorders was not possible to evaluate due to insufficient contact with the patient. The data of the neurological examination correspond to the Brown-Sequard syndrome.

Local status: a contused, chopped wound up to 4 cm was present in the right cheek area. A dense foreign body was palpated under sutures in the wound area.

Computed tomography (CT) of the head and spondylography of the cervical spine revealed a metal body and a comminuted fracture of the body and appendages of the vertebra.

Fig. 1. Spondylography of the cervical spine. A foreign body in the lower jaw and C4 vertebra projection.

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of the lower jaw right with dislocation in soft tissues of the neck on the right (Figs. 1, 2).

20.08.13, maxillofacial surgeon opinion: a foreign body in the perimandibular area of the neck right and a comminuted fracture of the lower jaw right.

20.08.13, angiography of the main arteries of the head: data about injury of the main arteries were not present.

20.08.13 at 16:00, the patient underwent operation: removal of a foreign body in soft tissues right, decompression of lateral compression of the C4 vertebra by the foreign body. During the operation, in order to control the position of the metal rod in neck tissues, the anterior cervical paravertebral approach was performed right. The foreign body lower edge, which was firmly fixed paravertebrally, was identified by palpation. The foreign metal body was removed through a wound in the right cheek. There was moderate bleeding from the bones of the injured lower jaw (Fig. 3).

After emergence from anesthesia, the patient underwent a neurological examination that revealed restoration of the muscle strength in the upper extremity up to 3 points and in the lower extremity up to 4 points. The
level of sensory disorders corresponded to mild hypesthesia left.

Magnetic resonance imaging (MRI), 22.08.13: a lateral compression fracture of C4 right and contusion of the spinal cord at the C3—C4 level (Figs. 4—6).

At the time of discharge from the hospital, there was a decrease in the muscle strength in the right upper extremity (in the hand up to 3 points, in the muscles of the arm and forearm up to 4) and in the right lower extremity up to 5.

Results and Discussion

Primary injury caused the development of the Brown-Sequard syndrome, but performed decompression resulted in a significant regression of neurological symptoms after the surgery. The CT and MRI data and intraoperative findings explained the clinical picture caused by right-sided extravertebral compression of the vertebra and spinal cord by the metal rod.

Conclusions

1. The cause of spinal cord compression can be a rare form of dislocation — a lateral displacement of a vertebra.
2. Early intervention prevented developing secondary injury of the spinal cord.

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Commentary

This clinical observation is certainly rare due to the rarity of penetrating injuries in peacetime, especially if the description of these cases is provided by civilian specialists. For example, in the USA, penetrating spinal injuries caused by compulsive actions are almost comparable in the incidence to those of road traffic accidents, which is associated with general accounting of the statistics of spinal injuries and the frequency of the use of firearms.

Classification of spinal injuries is, in fact, the means for selecting the optimal treatment modality. The description of this clinical observation does not contain the correct diagnosis of spinal injury. It is not entirely clear what the authors put into the concept of “indirect” spine and spinal cord injury. This injury is an open complicated spine and spinal cord trauma. According to the Denis three column concept, a fracture is an instability of the 2nd degree (instability with neurological disorders). Based on the mechanism of injury, it is important to identify the orthopedic type of fracture (e.g., based on the AO classification), i.e. the point and direction of application of the traumatic force. This fracture caused by the translational motion of the wounding projectile is the type C fracture (split fracture) that accompanies injury of both the anterior and posterior support structures of the spine (interspinous ligament rupture — the posterior column — is visible on MRI scans). The lateral displacement of the C4 vertebra, as the authors claim, should be accompanied by a coupled dislocation and a fracture of the articular processes, which is not observed in an analysis of the postoperative data.

Generally, all standards of the preoperative evaluation of patients with spine injury, particularly with penetrating one, include primarily computed tomography, CT, (this examination is mandatory and is included in the Standards for treatment of spinal injuries). The type of fracture and the degree of dislocation as well as the relationship of a wounding projectile and neurovascular structures could be assessed on the basis of CT data that could help identify the nature and extent of spinal deformity and plan the surgical treatment tactics. Due to its instability (injury of more than one support column), the described injury requires, under normal conditions, a single-stage stabilization of the vertebrae by a plate or transarticular procedure. In these cases, we also perform corporectomy of the injured vertebra with spondylosyndesis. Withholding a single-stage instrumental correction of the instability may be due to the penetrating (open) nature of injury, which is primary infected. In this case, the patient needs observation due to the high risk of developing the instability. The fact of the development of unilateral neurological disorder (the use of the term “Brown-Sequard syndrome” in the article is not quite correct, because there is no evidence of sensory disorders on the homolateral side) does not confirm the lateral displacement of the vertebrae, because the latter is often observed in traumatic compression of the anterior spinal artery.

In general, the analysis of this case generates more questions than answers. Based on the famous aphorism on “thanks to all patients allowing us (doctors) to acquire the necessary experience during the treatment process”, these rare cases should be explored thoroughly during the clinical study to avoid repeating the mistakes in similar cases. This observation can be published as a rare case of a combined traumatic spinal injury.

A.O. Gushcha (Moscow, Russia)
Clinical and prognostic significance of genetic markers in craniocerebral injury (Part III)

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It is now becoming increasingly clear that the course and outcome of craniocerebral injury (CCI) are determined not only by its biomechanism, severity, patient’s age, presence of premorbid factors, etc., but also by individual features of the genome of each patient, which puts traumatic brain injury among multifactorial diseases. The genome determines the presence or absence of “genetic predilection” to the development of various complications and sequelae of CCI, which generally determines the progression of traumatic brain injury disease. The first part of the review by Potapov et al. [2] was devoted to the role of apolipoprotein E (apoE) gene polymorphism in CCI, the second one [3] — to the role of inflammation and immune response genes in the course and outcome of CCI. The present (third) part will provide a review of modern data on the effect of genes underlying intracellular processes of oxidative stress, apoptosis, regeneration, and synthesis of neurotransmitters and their receptors.

Keywords: gene polymorphism, craniocerebral injury.

Abbreviations: CT — computed tomography; MRI — magnetic resonance imaging; TBI — traumatic brain injury; GOS — Glasgow Outcome Scale; GCS — Glasgow Coma Scale; ACE — angiotensin converting enzyme (ACE) gene; COMT — catecholamine-O-methyltransferase; MTHFR — methylenetetrahydrofolate reductase; Ngb — neuroglobin; PARP-1 — poly (ADP-ribose) polymerase 1.

«We used to think our fate was in the stars. Now we know, in large measure, our fate is in our genes».

Nobel laureate, James Watson

I. Genes involved in the oxidative stress mechanisms

The angiotensin converting enzyme I (ACE) gene

The angiotensin converting enzyme I (ACE) plays a key role in the synthesis of primary vasoactive peptide, angiotensin II, which regulates arterial vessel tone. ACE occurs widely in the brain: in the blood vessel walls, especially in those of the choroid plexus, on the surface of astrocytes of the periventricular areas, both in the brain regions with a high content of angiotensin II receptors, and, paradoxically, in the areas with a low content of these receptors, such as the basal ganglia.

The angiotensin converting enzyme I gene (ACE) is located on the chromosome 17 (17q23). Most of the studies on association of this gene with various diseases have used the I/D polymorphic marker of the ACE gene that is located in a noncoding region of the gene (intron 16) and caused by the presence or absence of Alu mobile element insertion, whose length is 287 base pairs (bp). This polymorphism was demonstrated to correlate with the level of serum ACE. II genotype carriers are characterized by the
lowest ACE activity, while its activity in DD genotype carriers is about 2 times higher. Heterozygous carriers are detected with an intermediate level of this enzyme [52]. A number of studies [42] have demonstrated that the presence of at least one copy of the D allele increases the risk of cerebrovascular diseases and development of cognitive deteriorations and dementia.

The effect of I/D polymorphic marker of the ACE gene on the outcome was studied in 154 patients of Hispanic origin with TBI (according to the Glasgow Coma Scale (GCS) < 12 points) [8]. 73 of the examined patients were also evaluated for neuropsychological functions. The authors demonstrated that in ID and DD genotype carriers the results of tests that detect abnormalities in frontal lobe functions, motor skills, attention, and reactivity were significantly worse than those in II genotype carriers. At the same time, it was not possible to find a significant association of the I/D polymorphic marker of the ACE gene with the delayed physical and mental development of preterm infants [26].

The exact mechanism of the ACE effect on the TBI outcome remains unclear. It is believed to involve such factors as disorder of cerebral circulation and/or blood pressure autoregulation, causing vasospasm and cerebral ischemia [8]. Another possible ACE effect on the TBI outcome may be due to its ability to inhibit amyloid protein aggregation, which exerts a cytotoxic effect [30].

Thus, the association of the ACE gene with the TBI progression is very likely, but more research in significantly larger groups of patients and with the use of a number of other markers of the ACE gene is required to obtain additional experimental data.

The heme oxygenase 1 (HMOX1) gene

The heme oxygenase activity is controlled by two enzymes: inducible heme oxygenase 1 (HMOX1) and constitutive heme oxygenase 2 (HMOX2). Both enzymes perform a key stage in the catabolism of heme to biliverdin IXα, with ferrous ions (Fe^{2+}) and carbon monoxide (CO) being also formed. Then, biliverdin IXα is converted to bilirubin IXα by biliverdin reductase. Bilirubin and Fe^{2+} ions are among the most potent antioxidants that effectively neutralize highly reactive oxygen species, peroxides, and other free radicals.

The HMOX1 gene encoding heme oxygenase 1 (HO-1) in humans is located on the chromosome 22q12. Mutations in this gene are observed extremely rarely, however, in the case mutations in both copies of the HMOX1 gene lead to growth inhibition, persistent hyperthermia, erythematous rash, anemia, hepatomegaly, and asplenia [63].

Model studies in rats demonstrated that the HMOX1 gene expression in brain glial cells is significantly increased in response to ischemia [57] and hypothermia [24]. In addition, an increased HMOX1 gene expression can reduce the injury extent after experimental intracerebral hemorrhage [60]. On the basis of these data, the authors concluded that the ability to rapidly raise the heme oxygenase 1 level in response to external factors may be important for effective neutralization of inflammatory processes accompanied also by oxidative stress [24, 57, 60].

In the promoter region of the human HMOX1 gene at positions —196 to —276 from the transcription initiation site (position of the latter boundary depends on the total repeat length), the polymorphic microsatellite (GT)n was found, whose number of repeated units varies from 12 to 40 [25]. The allele frequency distribution is usually bimodal. The greatest frequencies were found in the alleles 23 and 30, although the absolute frequency values and the total allele number differ significantly in different populations [25].

The HMOX1 gene expression level, depending on the number of repeated units of the microsatellite (GT)n, has been investigated in vitro using luciferase gene constructs [17, 64]. These studies have demonstrated that the HMOX1 gene promoter regions with the number of repeats of less than 25 provide a higher level of the gene expression than regions with the number of repeats of more than 25. This was true both in the case of a basic

<table>
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<th>Gene</th>
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<tr>
<td>Angiotensin converting enzyme 1 (ACE)</td>
<td>e2/e3/e4</td>
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<td>G(–219)T</td>
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<td>A(–491)T</td>
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<td>Poly-ADP-ribose polymerase 1 (ADPRT1)</td>
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<td>Type 1 kinase with the ankyrin repeat (ANKK1)</td>
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<td>Catecholamine-O-methyltransferase (COMT)</td>
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<td>Serotonin transporter (SLC6A4 or 5-HTT)</td>
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<td>Val762Ala</td>
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expression level [64] and after treating cells with hydrogen peroxide to induce the HMOX1 gene [17].

These data were also confirmed by an elegant study involving 12 healthy individuals, 6 of which had the polymorphic microsatellite (GT)n genotypes with the allele number from 16 to 26 (group S), while the other six had the allele number from 33 to 40 (group L) [28]. Peripheral blood lymphocytes of these patients were used to generate cell lines, in which the HMOX1 gene mRNA level and heme oxygenase 1 enzymatic activity were then determined [28]. The average level of the basic HMOX1 gene expression in groups S and L, which was determined both by levels of mRNA synthesis and by the heme oxygenase 1 enzymatic activity, differed slightly. However, after treatment of cells with hydrogen peroxide to induce the HMOX1 gene, it was found that the mRNA synthesis level in the L group was not changed, whereas in the S group it was increased more than 2 times (2.1±0.49).

Similar results were obtained in measurement of the heme oxygenase 1 enzymatic activity. While the activity level in the L group did not actually change, in the S group it increased significantly (2.4±0.6). It should be noted that the apoptotic cell death frequency was also dependent on the polymorphic microsatellite (GT)n genotype [28]. After incubating cells for 24 h in the presence of hydrogen peroxide, it was found that cells from the L group died much more frequently than cells from the S group (21.56% vs. 4.33%). Two single nucleotide polymorphic markers, G(—1135)A and T(—413)A [49], were found in the HMOX1 gene promoter region. They may also be associated with the HMOX1 gene expression level.

A comparison of the distribution of HMOX1 gene polymorphic microsatellite alleles in a relatively small group of 69 patients with aneurysmal subarachnoid bleeding (ASB) to that in the control group (230) revealed that the frequency of alleles with the length ≥36 was significantly higher in patients with ASB (8% vs. 4%) [45].

Interesting data on a role of the HMOX1 gene were obtained in an examination of patients with a familial form of the Alzheimer’s disease [56]. In patients with mutations in the APP gene, which encodes the amyloid precursor protein (APP), the heme oxygenase activity was 45—50% lower than that in healthy individuals. Furthermore, it was shown that under in vitro conditions, APP, like the amyloid-β precursor-like protein (APLP1), binds to heme oxygenases 1 and 2 in the endoplasmic reticulum and inhibits the heme oxygenase activity by 25—35% [56]. Based on the fact that heme oxygenases are antioxidant enzymes, the authors suggested that the increased oxidative neurotoxicity detected in patients with the Alzheimer’s disease was a consequence of inhibition of the heme oxygenase activity caused by binding of heme oxygenases to APP and APLP1.

Thus, despite the lack of data on the association of the HMOX1 gene with TBI, the gene should be considered as one of important potential candidate genes influencing the TBI course.

II. Genes involved in the apoptosis mechanisms

The TR53 gene encoding the p53 protein

In most cases, TBI is accompanied by the development of secondary brain injuries due to an increase in the intracranial pressure, a cerebral perfusion reduction, formation of ischemia foci, secondary (post dislocation) hemorrhages, and induction of apoptosis of neurons and glial cells [47]. Mechanisms that induce apoptosis after TBI are unknown; some authors believe that the cause may be an excessive release of a neurotransmitter, glutamate [47]. Another possible cause is oxidative stress that accompanies inflammatory processes developing in response to brain injury [29].

The p53 protein plays an important role in the transcriptional regulation and maintenance of genomic stability by interacting with many cellular proteins. DNA damage, including that due to oxidative stress, was demonstrated to lead to accumulation of p53 that in turn inhibits many cellular processes until the damage is repaired. If damage repair is not possible, p53 triggers the apoptosis mechanism [33].

The TR53 gene encoding the p53 protein is located on the chromosome 17q13.1. This gene and its flanking regions contain a number of polymorphic regions, including the G/C single nucleotide polymorphism that corresponds to the Pro/Arg amino acid polymorphism at position 72 of the polypeptide chain [1, 7]. Apparently, in Arg/Arg genotype carriers, TR53 induced apoptosis proceeds more efficiently than in Arg/Pro and Pro/Pro genotype carriers [22] that may be used to study the association of the Pro/Arg polymorphic marker of the TR53 gene with a number of diseases, including TBI.

The association of the TR53 gene Pro/Arg polymorphism with the TBI outcome was explored in one study only. The study included 90 patients of Hispanic origin with severe TBI. The outcome assessment was performed at the time of discharge from the intensive care unit and 6 months later the date of injury. In Arg/Arg genotype carriers, the risk of an adverse outcome was 2.9 times higher than that in Arg/Pro and Pro/Pro genotype carriers [41].

The TP53 gene Pro72Arg polymorphism is associated with the development of diabetic polyneuropathy in type 1 diabetes mellitus in the Russian population [4]. An increased risk of diabetic polyneuropathy is also associated with carriage of the Arg allele (OR=1.96) and the Arg/Arg genotype (OR=2.14). The Pro allele, instead, is associated with a lower risk of diabetic polyneuropathy (OR=0.51).

In conclusion, it should be noted that although there are a few data on the positive association of the TP53 gene with TBI, this gene should be considered as one of the most important candidate genes that affect the course and outcome of the disease. The significance of studying
the association of this gene, and especially its polymorphic marker Pro72Arg, with TBI is determined by that the efficiency of apoptosis of different cell types, including neurons, significantly correlates with carriage of certain genotypes of the TP53 gene Pro72Arg polymorphism. And apoptosis, in turn, is one of the key mechanisms underlying neuronal loss, post-traumatic brain atrophy, and poor TBI outcome.

The neuroglobin (Ngb) gene

A recent study conducted in 196 patients with severe TBI demonstrated the relationship between genetic polymorphism of the neuroglobin (Ngb) gene and the TBI outcome. Patients with the TT genotype for rs3783988 (region encoding the oxygen-binding part of the protein) had a better outcome on the Glasgow Outcome Scale (GOS) and NRS-R (Neurobehavioral Rating Scale-Revised) in 3, 6, 12, and 24 months. Ngb is a protein found in neurons, endocrine cells, and the retina that plays an important role in protecting cells from ischemia and hypoxia in the acute TBI period [15]. Involvement of Ngb in the following functions was established: 1) detoxification/trap of reactive oxygen species and free radicals [19]; 2) glycolysis [14]; 3) carrier of oxygen to mitochondria under hypoxic conditions [21]; 4) anti-apoptotic factor [61].

III. Genes involved in reparative processes

The poly-ADP-ribose polymerase 1 (ADPRT1) gene

In inflammatory processes developing as a result of brain injury, the concentration of reactive oxygen species, peroxides, and other free radicals sharply increases, which leads to an increase in the number of DNA damages. To initiate repair of damaged DNA, activation of synthesis of specific enzymes, PARP polymerases, is necessary, whose function is the synthesis of poly-ADP-ribose chains (60—80 residues). These chains are covalently bound to chromatin proteins at DNA single-strand break sites. In this case, the PARP activity may be increased 500 times or more [20]. Poly-ADP-ribose long chains are a signal and probably a molecular basis for the formation of a complex of specific enzymes and proteins, which repair damaged DNA. NAD+ is used to synthesize these chains. There are several types of PARP encoded by different genes. PARP-1 is the most important one, since it is responsible for synthesis of about 90% of poly-ADP-ribose chains in the cell [5]. PARP-1 is found in endothelial [51] and Schwann cells [11]. Fast activation of PARP-1 is also found in neurons in response to damage by free radicals [55]. The number of DNA damages and intensity of the poly-ADP-ribose chain synthesis largely determine the further fate of the cell: successful repair, apoptosis, or necrosis. Significant or excessive PARP activation, increased poly-ADP-ribose chain synthesis, NAD+ depletion and subsequent ATP depletion may lead to apoptotic or necrotic cell death [55].

Experimental models have demonstrated that the excessive activation of PARP-1 and increased synthesis of poly ADP-ribose chains contribute significantly to the pathogenesis of brain injury and neurodegenerative disorders [12]. Experimental TBI models have revealed a significant neuroprotective effect of PARP-1 inhibitors [35]. The ADPRT1 gene, which encodes PARP-1, is located in the chromosomal region 1q41-1q42. This gene contains two single nucleotide polymorphisms: C/G that corresponds to the Leu/Phe amino acid polymorphism at position 54 of the polypeptide chain, and T/C that corresponds to the Val/Ala amino acid polymorphism at position 762 of the polypeptide chain.

There are a few studies on the association of the ADPRT1 gene polymorphisms with various diseases. Nevertheless, the association between genetic variants in the ADPRT1 gene promoter region and predisposition to the Parkinson’s disease was recently discovered. Furthermore, as in the case of TP53 gene, the polymorphic markers Leu54Phe and Val762Ala of the ADPRT1 gene were demonstrated to be associated with the development of diabetic polyneuropathy in type 1 diabetes mellitus [48]. So, the association of the ADPRT1 gene with TBI has not been studied up to now, but the importance of studying this gene in brain injury is determined by the efficiency of PARP-1 inhibitors in experimental TBI models [32, 58] and its association with other diseases of the nervous system.

The brain derived neurotrophic factor (BDNF) gene

The brain-derived neurotrophic factor (BDNF) belongs to a family of neurotrophins. Neurotrophins are the general name of secreted proteins that support the viability of neurons and stimulate their development and activity. BDNF, in particular, reduces the toxic effect of glutamate, and thereby exerts a neuroprotective effect [40]. Experiments in animals have shown that BDNF reduces severity of ischemia and improves recovery after TBI [6, 54]. BDNF is synthesized as a 247 amino acid long precursor. Mature BDNF is formed after cleavage of the N-terminal fragment of 128 amino acids, called the “pro-region”, either in the Golgi complex by the protease furin or in the secretory granules by specific proteases. The second exon of the BDNF gene contains the G/A single nucleotide polymorphism at position 758 that corresponds to the Val/Met amino acid polymorphism at position 66 located in the N-terminal portion of the molecule (“pro-region”).

An in vitro study of rat neuronal cultures transformed with a vector system based on the Sindbis virus and green fluorescent protein gene demonstrated that transport of BDNF into the secretory granules is significantly slowed in the case of a construct with the Met allele. Furthermore, the depolarization-induced BDNF secretion was significantly lower in neurons transformed by a construct with the Met allele than by a construct with the Val allele.
However, the constitutive synthesis of BDNF was the same in both types of constructs [23].

According to data obtained in a number of studies [23], the human Val66Met polymorphic marker is associated with a complex neuronal phenotype that includes memory and subtle differences in the brain morphology. For example, Met allele carriers perform less well the episodic memory tests, they have a less volume of the hippocampus gray matter and the prefrontal cortex [23]. Carriers of the Val allele, on the contrary, have larger volumes of the gray matter in the prefrontal and occipital cortex, precuneus, hook, and superior temporal gyrus [34]. However, another study conducted in 168 Vietnamese soldiers with injury of the frontal lobes of the brain demonstrated that patients carrying Met—performed a test evaluating the management functions similarly to the control group of carriers of this marker. Val/Val allele carriers performed this test worse than the control group with the same alleles [36]. Another study demonstrated no effect of the Val66Met polymorphic marker on emergence from the vegetative state after 3, 6, and 12 months after severe TBI [10].

Other 7 single nucleotide polymorphic markers of this gene were studied in 109 Vietnamese soldiers before, after 10—15, and 30—35 years after a focal penetrating injury [53]. Two of them (rs7124442 and rs1519480) were independent (on the level of intelligence and degree of brain atrophy) predictors of recovery of cognitive functions.

Therefore, the BDNF gene Val66Met polymorphism can certainly be considered as a candidate gene that influences the TBI outcome and, therefore, requires further investigation.

IV. Genes involved in the neurotransmission regulation

Dopamine receptor genes

The dopamine receptor family includes two subtypes: D1- and D2-like receptors, which are seven transmembrane domain G-proteins. The D1-like receptors (D1 and D5) are encoded by two genes, the D2-like receptors (D2, D3, and D4) are encoded by three genes. The D2 receptor polymorphism may influence cognitive impairments (mainly frontal) caused by dysfunction of the dopaminergic system of the brain as well as is involved in the regulation of emotional state and is associated with the degree of activation of the amygdala and the prefrontal cortex [13]. The ANKK1 (Ankyrin repeat and protein kinase domain-containing protein 1) gene polymorphism (haplotype of three SNPs: rs11604671, rs4938016, and rs1800497 (TAQ1A)), which regulates the expression of D2 receptor genes, leads to a decrease in the number of these receptors [43]. The D3 and D4 dopamine receptor polymorphisms are very important for pharmacogenomics that is the field of genetics studying the relationships between a genetic polymorphism and an effect of drugs, i.e. individual response of the body to the introduction of xenobiotics. For example, the D3 receptor gene Ser9Gly polymorphism is associated with the development of tardive dyskinesias, which are a complication observed in 50% of patients taking neuroleptics for long time [50]. The polymorphism in the number of repeats of 48 bp in the D4 receptor gene region encoding the third intracytoplasmic loop of the receptor affects the efficiency of the atypical neuroleptic, clozapine (H. Hwu et al., 1998).

Genes encoding the D2 dopamine receptor and ANKK1 kinase

Prevalence of the D2 dopamine receptor gene is highest in the limbic system. A study [44] discusses the possibility of association of the gene encoding the dopamine receptor D2 (DRD2) with the degree of recovery of cognitive functions (memory, attention, and functions of the premotor area of the frontal lobes) 1 month after TBI of moderate severity.

In one study, T. McAllister et al. [44] used the C/T single nucleotide polymorphic marker (rs1800497), which was originally designated as TaqIa according to the name of the restriction enzyme TaqI cleaving only a DNA fragment with the C allele [59]. The T allele is associated with a lower level of the D2 receptor synthesis (by 40%) in striatal cells and, respectively, with its lower density on the cell surface. It should be emphasized that the affinity of the receptor for D2 dopamine is identical in carriers of the C and T alleles. In the group of 39 patients with TBI of moderate severity and in the control group of 29 patients, the T allele of the DRD2 gene was associated with the worst performance of the verbal memory test [44]. At the time of the study, it was believed that the C/T polymorphic marker is located in the DRD2 gene promotor region and the T allele is associated with a lower level of the gene expression.

However, in 2004, M. Neville et al. [46] found that the rs1800497 polymorphism is located in another gene, namely in the ANKK1 gene, which is localized in the 10 thousand bp from the DRD2 gene promoter region and encodes serine/threonine kinase with 11 ankyrin repeats. The C/T (rs1800497) polymorphism was established to be located in the last 8th exon of the ANKK1 gene and to correspond to the Glu/Lys amino acid polymorphism at position 713 in the 11th ankyrin repeat of ANKK1 kinase.

In this case, the association of the rs1800497 polymorphic marker with the DRD2 gene expression level may be explained either by a linkage disequilibrium of rs1800497 with some functionally significant polymorphic marker in the DRD2 gene or by that this is the ANKK1 gene that somehow determines the DRD2 gene expression level.

In another study, T. McAllister et al. [43] made an attempt to clarify this issue. The study used 31 polymorphic markers in three genes located close to each other in this region of the chromosome 11q23: NCAM, ANKK1 and DRD2. Four linkage groups were found in the three
genes. One of the groups included, along with the rs1800497 polymorphism, two more polymorphisms (rs11604671, rs4938016), with all of them being located within the ANKK1 gene. The group of patients with TBI of moderate severity was increased to 54 patients and the control group included 21 patients. As in the previous study, the T allele of the rs1800497 polymorphic marker in both groups was associated with the worst performance of tasks of the California test for verbal memory [43]. Of course, similar results were also obtained in the case of polymorphic markers rs11604671 and rs4938016. However, none of the polymorphic markers located in the ANKK1 gene and used in this study was associated with the worst performance of the verbal memory test.

At present, the ANKK1 gene functions are not well investigated. The ankyrin repeats are known to be involved in the interaction among proteins, and proteins with these repeats are involved in a wide range of intracellular processes, including transcription initiation. Members of the serine/threonine kinases family transmit signals within cells and mediate the cell response to external stimuli. Although the presence of different amino acid residues at position 713, apparently, has no effect on the spatial kinase structure in general, but may affect the specificity and/or efficiency of substrate binding. In this case, the difference in activity of two ANKK1 kinase isoforms may explain a different efficiency of signal transduction and a different level of synthesis of the dopamine receptor D2 in cells of the striatum. Against this assumption is the fact that the ANKK1 gene expression in brain cells has still not been detected [46].

The final solution of this problem requires further research both to search for a functionally important marker of the ANKK1 and DRD2 genes and to identify the ANKK1 gene product functions.

The catecholamine-O-methyltransferase (COMT) gene

The COMT gene can certainly be considered as a candidate gene in studying genetic susceptibility to an adverse outcome in TBI, since an enzyme, catecholamine-O-methyltransferase (COMT), encoded by it is involved in the metabolism of catecholamines (dopamine, norepinephrine, and epinephrine). COMT performs methylation of catecholamines, thereby regulating the level of dopamine and norepinephrine in the synapses.

In the 4th exon of the COMT gene, the G/A single nucleotide polymorphism was discovered that corresponds to the Met/Val amino acid polymorphism at position 158. The Met allele compared to the Val allele is associated with a reduction in the activity of this enzyme in 4 times [62].

Since neurons of the prefrontal cortex lack expression of the gene of dopamine transporter (DAT1 or SLC6A3), which provides the reuptake of dopamine from the synaptic cleft in other parts of the brain, the enzymatic activity of COMT probably determines the efficiency of synaptic transmission in this area of the cortex. Thus, it may be expected that the endogenous dopamine level in the prefrontal cortex of Met/Met genotype carriers will be significantly higher than that in Val/Val genotype carriers, while it will have an intermediate value in Met/Val genotype carriers.

In this regard, the association of the COMT gene Met158Val polymorphism with the efficiency of performing neuropsychological tests was studied in 113 patients 1 year after TBI [37]. Neuropsychological tests evaluating memory, attention, reaction rate, speech function, and behavior were used. A relationship between the nature of injuries, according to MRI, and carriage of certain genotypes was not found.

However, a comparison of groups of patients with low and high enzyme activity revealed significant differences in performing tests evaluating the management functions of the brain frontal lobes. Patients with the Val/Val genotype, a high enzyme activity, and a lower level of endogenous dopamine performed tests worse than patients with the Met/Met genotype, a low enzyme activity and, accordingly, a high level of endogenous dopamine in the prefrontal region. It should also be noted that in patients with the Val/Val genotype and a high enzyme activity, the TBI outcome was significantly worse [39].

Therefore, according to the literature, the association of the COMT gene Met158Val polymorphism with implementation of the management functions of the brain frontal lobes and the TBI unfavorable outcome was revealed, so further studying this marker significance in brain injury is required.

Genes involved in serotonin neurotransmission

The association of depressive states with polymorphic markers of the gene encoding the serotonin transporter (SLC6A4 is the old name of the 5-HTT gene) was studied in an ethnically diverse group of 107 patients with TBI and a control group of 66 individuals. The authors used two polymorphic markers. The S/L marker is the insertion or lack of insertion with a length of 44 bp within the tandem repeat located at a distance of 200 bp from the 1st exon of the SLC6A4 gene. Under in vitro conditions and by using the luciferase gene, it was shown that the S allele compared with the L allele is associated with a reduction in the SLC6A4 gene expression level in 2—3 times [27].

Another polymorphic marker, rs25531, is the A/G single nucleotide polymorphism located at a distance of about 300 bp from the tandem repeat in the direction opposite to the transcriptional initiation site. According to some data [65], the rs25531 polymorphic marker may also be associated with the SLC6A4 gene expression level. Methods used to identify the alleles allowed identification of both haplotypes and genotypes of these two polymorphic markers, but no association with the de-
development of depressive states after TBI was found [16, 65].

Despite the fact that the presence of certain polymorphic markers of genes encoding serotonin transporters had no effect on the course and outcome of TBI, the genes that modulate the activity of the serotonergic system may affect the efficiency of pharmacological treatment of post-traumatic depression. For example, an examination of 90 patients with post-traumatic depression revealed the effect of C-(677)T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene on the efficiency of treatment with citruron and the effect of the promoter region of the gene (SHTTLPR) encoding the serotonin transporter protein on the development side effects (dry mouth, nausea, somnolence, and impairment of sexual behavior). The MTHFR enzyme is involved in demethylation of homocysteine, which yields methionine, and the remaining methyl group is then used for the serotonin synthesis. The C-(677)T polymorphism is associated with a low activity of MTHFR and, hence, with a reduction in the serotonin synthesis in the brain. SHTTLPR has two alleles: short (S) and long (L). The presence of the S allele is associated with a reduction in the transcriptional activity of the 5HTT gene and, therefore, with less response to antidepressants — selective serotonin reuptake inhibitors (SSRIs). Furthermore, the presence of the rs25531 single nucleotide polymorphism in the L allele is also associated with a decrease in the 5HTT gene expression level [38].

Therefore, further investigation of genes affecting serotonin neurotransmission may be more important for the assessment of the efficiency of pharmacological treatment and individual selection of therapy than for prediction of the course and outcome of TBI.

Conclusion

Over the last few years, genetic studies in polyfactorial diseases and traumatic injuries of the nervous system have become of great importance. The results of these studies are often contradictory, but generally indicate the influence of many genes (factors of inflammation, apoptosis, degeneration, oxidative stress, reparative processes, and neuroplasticity) on the course and outcome of TBI. Until now, there is no unequivocal assessment of the risk level for developing adverse outcomes or complications of TBI caused by the presence of a certain polymorphism. Nevertheless, this is what has the most significance for the development of algorithms of diagnosis and strategies for treatment of traumatic brain injury in order to prevent secondary injury and to speed up recovery of these patients with allowance for their individual molecular genetic features.

Polymorphisms of the genes of apoE, interleukins, and p53 are the most studied ones for predicting the course and outcome of TBI. However, not only investigation of polymorphisms of individual genes, but also identification of combinations of polymorphisms of various genes (haplotypes) underlying the most important pathophysiological mechanisms (swelling, vasospasm, ischemia, impaired blood-brain barrier, mitochondrial dysfunction, etc.) of traumatic brain injury is of great interest. A promising trend seems to be pharmacogenomics, which allows one to predict the efficiency for using pharmacological agents based on the patient genotypic features. Therefore, there is certainly the need in further, larger research and more detailed investigation of the polymorphism of all the genes listed above as well as the search for new candidate genes (genes of erythropoietin, brain steroidogenic enzymes, neurosteroid receptors, nuclear transcription factors, growth factors, etc.) for deep understanding of the TBI pathogenesis in each individual patient and for selecting individual treatment modalities.

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PROBLEMS OF NEUROSURGERY NAMED AFTER N.N. BURDENKO 3, 2014

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Trepanations in the population of the Altai Mountains in the Vth—IIIrd centuries B.C.

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The history of trepanations beginning with the Mesolithic (10–12 thousand years ago) is known by means of findings in various parts of the world. The article describes three cases of intravital trepanation of skulls from the Pazyryk Culture graves in the Altai Mountains that had existed from the end of the 6th to the beginning of the 2nd century B.C. In two cases, trepanations were performed so skillfully that the operated patients had survived for a long time after the surgery, which was confirmed not only by microscopy but also by MSCT of the skulls. The article establishes causes of surgeries performed, reconstructs the technique of surgical manipulations, and evaluates them in terms of modern medicine. A comparative analysis of ancient trepanations performed by healers of different archeological cultures is provided. It is concluded that prehistoric cranial surgeries in the Altai Mountains had been performed for curative purposes.

Keywords: neurosurgery, trepanation, skull injury, archaelogy, Pazyryk Culture.

Traces of surgical intervention on the human head have been observed for a long period of human history [12], since at least the Mesolithic or the Early Neolithic (10–12 thousand years ago). Not all ancient manipulations can be considered as neurosurgical ones, i.e. aimed at operative treatment of diseases of the nervous system. In part, they might be related to the rituals, through which the operated person reached the altered states of consciousness required for religious and magical activities, because it is possible that behavior deviations observed after skull injuries were considered by the ancients as a manifestation of “divine madness”. From this point of view, trepanation had to promote the emergence of new properties and qualities in a healthy, but specially chosen for some reason person [4]. Nevertheless, it should also be assumed that the main purpose of intravital trepanation was treatment of injuries and elimination of neurological symptoms in human patients.

The term “trepanation” is commonly understood as the process of removing a piece of the skull of a living person without damage to the skull contents [9, 17, 21, 32].

Interest in prehistoric trepanations arose as early as 1865, when Ephraim George Squier (an archeologist, ethnologist, and the representative of the United States in Central America) brought, from graves of the Incas in Peru, a skull with four incisions performed in the right half of the frontal bone perpendicularly to its surface that formed a rectangular opening of approximately one half inch in area [11]. Distinct signs of healing of the opening edges were observed that indicated the patient’s survival, at least for a few weeks after the surgery. While this fact was confirmed by the famous French physician, anatomist and anthropologist Paul Broca, very few people believed in the possibility of successful trepanation under conditions of primitive ancient medicine [17, 24]. In addition to technical complexities of trepanation, the “surgeon” needed to provide effective anesthesia, control of bleeding from the amply perfused scalp soft tissues and bone as well as to prevent the development of wound infection. For example, even in the middle of the 19th century, survival of patients after trepanation at the best hospitals of Europe rarely exceeded 10%, which was associated with the extremely high risk of infectious complications and implementation of this operation only in very severe patients with traumatic brain injury [13].

We studied cases of intravital operative interventions in the head tissues in three skulls found in graves of the Pazyryk Culture that had existed on the territory of the Altai Mountains about 2.5 thousand years ago (from the end of the VIth to the beginning of the 2nd century B.C.). This chronological period is also called the Skythian Epoch, the Early Nomads Epoch, or the Early Iron Age. The southern boundaries of the Pazyryk Culture area covered the northern regions of East Kazakhstan and Mongolia. The leading sector of economy of the Altai Mountains tribes was nomadic herding.

Trepanations of the two skulls were performed so skillfully that operated people survived for quite a long time after surgeries. The article evaluates the performed operations in terms of modern medicine. The historical aspect of the study lies in the comparative analysis of trepanations performed by Pazyryk surgeons and healers from other archeological cultures, the information of which we obtained from publications. The fact that the Pazyryk Culture lifetime coincides with the flowering of medicine in ancient Greece and with the establishment and development of the
school of the great Greek physician Hippocrates (460—
377 B.C.) is of particular interest.

Material and methods

Three skulls were thoroughly examined. Two skulls
were found in the Kyzyl-Dzhar burial mounds located in

the high-mountain valley of the Kyzyl-Dzhar mountain
area, 7—8 km away from the Beltir village of the Kosh-
Agach district of the Gorny Altai Republic [5]. Both

Fig. 1. The skull of a male from the mound 3 of the Kyzyl-Dzhar-V burial mounds.
a — frontal projection; b — lateral projection (arrow points at the linear fracture);
c — occipital projection.

Fig. 2. MSCT of the skull of a male from the mound 3 of the Kyzyl-Dzhar-V burial mounds.
a — 3D model of the skull (arrow indicates at the linear fracture); b — slice through the trepanation defect in the lateral projection; c — horizontal slice through the surface of the trepanation defect. Clear signs of bone regeneration at the defect edge are present.
skulls have been stored in the anthropological collection of the Tomsk State University.

1. Kyzyl-Dzhar-V (mound 3, #KA TGU — 2012): a buried male died at the age of 40—45 years. 2. Kyzyl-Dzhar-V (mound 2, #KA TSU — 2009): a buried female died at the age of about 30 years. The third skull (male, age-related changes at the level of 50—60 years) was found in the mound 3 of the Bike-III burial located in the Bike Mountain area, investigated by V.D. Kubarev [2], in the middle reach valley of the Katun River. This skull has been stored in the anthropological collection of the Institute of Archeology and Ethnography of the Siberian Branch of the Russian Academy of Sciences. According to the authors of the excavation, it may be concluded that all three individuals were living in the 5th—3rd centuries B.C.; descended from the social environment of ordinary commoners and belonged to different ethnic and tribal groups of the Pazyryk archeological culture.

Reconstruction of the treatment process was carried out on the basis of a careful macroscopic examination of the skulls combined with standard osteometric measurements of the trepanation area using surgical optics and multislice computed tomography (MSCT). The latter was performed on a Toshiba Aquilion ONE (320-row 0.5 mm detector, with implementation of VTR and MRR); magnetic resonance imaging was carried out on a GE Signa Infinity (1.5T) device placed on the skull base. CT is known to detect much more clearly the changes in the bone tissue compared to craniography in this kind of studies [8].

Results and discussion

The skull from the Kyzyl-Dzhar-V (mound 3, #KA — TSU 2012), which belonged to a 40—45-year-old male, has a linear fracture (result of injury??) the left temporal and parietal bones that goes to the sagittal suture (Fig. 1). The fracture-sagittal suture intersection is 80 mm away from the coronal suture and 90 mm away from the occipital protuberance. The fracture resulted, with a high probability, from a blow to the left parietal-temporal area by a right handed opponent. Investigators of ancient trepanations have found in most cases openings performed in the left parietal bone. It is assumed that this resulted from interpersonal conflicts with right handed opponents [22, 30].

In the left parietal bone, 12 mm away from the middle line, a rounded skull defect with an internal size of 40×41 mm is identified. The defect edge is bevelled due to dislocation of part of the outer table. Thus, the overall size of the bone defect is 63×64 mm. The bone thickness at the defect is 3 mm. Signs of bone growth are visually defined both at the defect edge and along the fracture line. Healing of a linear skull fracture is known to take many years [3]. MSCT demonstrated good regeneration and healing of the bone (Fig. 2). By the nature of the fracture that goes to the sagittal suture, under which the sagittal sinus is located, it is very likely to suspect the development of an epidural hematoma due to the traumatic rupture of the sinus edge. With allowance for the proximity of the injury to the central motor area, it may be assumed that the affected person had not only cerebral symptoms such as headache, nausea, vomiting, and disturbances of consciousness but also movement disorders in his right leg, and to a lesser extent, in the hand. The clinical picture might also manifest with the development of partial (tonicclonic spasms in the right extremities) or generalized epileptic seizure.

It is curious that the trepanation made by the Pazyryk surgeon was carried out in strict accordance with the recommendations of Hippocrates who believed that bone injuries near the sutures require trepanation in most cases. In this case, Hippocrates warned to avoid trepanning over a suture. “The sutures should be avoided of trepanning, the operation should be performed at some distance in the nearest bone” [1, p. 590; 23]. Hippocrates does not mention sinuses or any vascular structures located under sutures [21]. Sutures were regarded as the weakest bone areas dangerous for injury to the intracranial contents with a trephine of Hippocratic times.

One of the strange Hippocrates recommendations concerned the trepanation technique. He always advised to leave a thin bone layer covering the dura mater without making cephalotomy to the end: “... when the bone became mobile, leave this operation to let the bone part separate spontaneously.... what is left is already sufficiently fine” [1 p. 597]. Later, this statement roused a suspicion that the author was not familiar with the trepanation technique [20]. However, it really is not surprising. Hippocrates nowhere indicated that trepanation is performed for evacuation of hematomas and abscesses or for other intracranial interventions. Hippocrates was also against the use of trepanation to treat depressed fractures. He used trepanation to treat linear injuries of the skull bones. This is confirmed by another recommendation of Hippocrates. Claiming that the thinnest and weakest bone is the crown, and another weak area is the temple [1, p. 582], he was strongly against incisions in the temple because of the risk for injury of the “vein” located there and the development of convulsions in the operated person [1, p. 591]. Today, we know that more than 70% of epidural hematomas that need trepanning are located precisely in the temporal region. As judged by the character of the successful trepanation performed by the Pazyryk surgeon, his purpose was the intracranial contents. The opening is of sufficient size and centered on the fracture line, all layers of the parietal bone are removed. The trepanation window is very suitable for evacuation of an intracranial hematoma!

According to the literature, prehistoric trepanations had a high survival rate for trepanned individuals without signs of post-operative complications. These
figures reached 50—90% [7, 14]. Successful completion of ancient skull operations is associated with a small diameter of the most openings and making them in safe areas to avoid injuries to the sutures and the dura mater [33]. In the described case, upon making the opening, the Scythian surgeon quite carefully backed out of the suture by more than one centimeter, at a distance where the sagittal sinus edge ends, massive bleeding from which requires, even today, special technologies and materials to stop it. However, this is not to say that the Pazyryk healer made a small trepanation window. A modern neurosurgeon in a similar situation would make an opening of a similar diameter.

The second skull from Kyzyl-Dzhar (Kyzyl-Dzhar-IV burial mounds, mound 2, #KA TSU — 2009) belonged to a young female aged about 30 years. Its examination and MSCT data indicate that the female suffered a severe injury in the form of a fracture of the right temporal bone and the middle cranial fossa base. By the nature of the injury, it may be assumed that the injury resulted from falling from a height. A circular opening with the outer size of 39×36 mm and the inner size of 23×16 mm was made in the posterior portions of the crown strictly along the midline over the sagittal suture. The opening is perforated with removal of the outer bone layer, cancellous bone, and inner (vitreous) table (Fig. 3). Signs of...
bone growth are not present. This conclusion is confirmed by MSCT, which did not detect reorganization of the bone trabeculae (Fig. 4). These findings suggest that the injured female died either during surgery or shortly after its completion. Another explanation for these findings is that the operation was performed postmortem. The surgery technique, which will be discussed below, was significantly different from trepanation in the first case.

And finally, the third skull (Bike-III burial, mound 3) belonged to a male of 50—60 years. The visual examination of the skull indicates that it has a congenital deformity in the form of flattening of the right half of the occipital bone due to non-uniform closing of the occipital sutures. No injury traces on the skull were found.

15 mm behind the coronal suture and 50 mm away from the sagittal suture, there is a semi-oval hole with the outer size of 52×45 mm and the internal defect size of 22×34 mm. The bone thickness at the trepanation area is 2 mm (Fig. 5). There are distinct signs of new bone growth, which was confirmed by MSCT (Fig. 6) and indicates long survival of the individual after the trepanation.

This successful result of two of the three presented operations is worthy of a detailed discussion of the technological details of these manipulation.

Five basic methods to perform trepanations have been described [17, 27]. One was already mentioned in the introduction — a procedure to make a rectangular opening in the bone by intersecting incisions. The first skull of this type was found in Peru. The opening was performed with a knife made of silicon or volcanic glass.

The second method is based on bone scraping. Paul Broca, with a piece of glass, performed this trepanation on the skull of an adult, which took him 50 min [28].

The third method involves cutting a circular groove followed by lifting off the bone disc. This method was widely used until recently in Kenya [13].

The fourth method to form a circular trepanation window requires the use of a crown saw. This method was described by Hippocrates, further improved by Roman physicians, and used in modern medicine until recently.

The fifth method is to drill a circle of closely spaced holes the bone and then to cut or chisel the bone between the holes. This technology was recommended by the Romans, adopted by the Arabs, and used in the Middle Ages. It was developed to the method that, in certain cases, is used to this day, despite the availability of high-speed electric and pneumatic drills equipped by craniotomes with routers from high-alloy steel. The method is based on that the bone between burr holes is cut using the Gigli saw, which was carried out under the bone over the dura mater by means of a guide.

Pazyryk surgeons might use iron, copper, and bronze to perform trepanations. High field MRI of the trepanned skulls did not reveal the presence of a ferromagnetic (iron) in the bone cutting area. Copper is rather soft metal to cut a bone. Most likely, Pazyryk healers used firm and suitable bronze tools.

Upon excavation of the Pazyryk cultural artefacts, archeologists did not find the specialized tools that could be attributed to medical ones, which might be used for craniotomy. However, in almost all the graves of Pazyryk people, regardless of their social status, bronze knives...
have been found (Fig. 7). This is a vital tool in everyday life of a cattlemen.

A mass spectral analysis and an X-ray fluorescence analysis of bone samples taken from the place of the ancient trepanations, which we carried out under the RFBR grant, demonstrated that the most likely tool to be used by the Scythian surgeons was a bronze knife [6].

It is noted that the scraping method provided the highest survival percentage of ancient trepanations [15]. A comparison of the probability for bone healing and, consequently, the survival of patients, based on a large set of data from Anatolia, revealed that the best result was obtained for the scraping method compared to sawing and drilling [9].

The ancient Celts, whose settlements stretched from France to the Danube and to the Black Sea shores, continued to use widely the bone scraping method for trepanations [20], and the drilling method, which was already known by that time, was used only in a few places.

Hippocrates described four tools for trepanation: three devices for bone perforation (a trepan, a serrated trepan, and a probe to determine the depth of cutting the bone and the mobility of the bone fragments) and a rasp for scraping the bone tissue and isolation it from the soft tissues of the head [1, p. 579—600]. A Scythian surgeon was enough to use a universal bronze knife.

We used a trasological analysis of the operation traces [6] to simulate the process of removing the bone fragments upon constructing the trepanation skull defect. The operation was obviously carried out in two stages. First, the most firm cortical layer of the bone was cut off using rotational motions without perforating the skull. The bone cutting plane is tangential to the spherical surface of the skull. Only then, at the second stage of operation, directing probably the same tool under a steeper angle, the spongy part of the bone was removed, and then the vitreous table adjacent to the dura mater was carefully lifted off and removed. If instrument motions at the first stage have a relatively long “working stroke”, then at the second stage they are traces of relatively more frequent, short movements of the surgeon hand. A comparison of the operation techniques for the three presented skulls reveals that trepanation in the female with the opening in the sagittal suture area was performed with more rough, sticking movements [6].

Since the Pazyryk Culture has left no written evidence of medical activity, the problem of reconstruction of motivation for Scythian surgeons to conduct cranioto-

Fig. 5. The skull of a male from the mound 3 of the Bike-III burial mound. a — frontal projection; b — projection of the rotation to the right by 3/4; c — lateral projection; d — vertical projection.
my is a challenge. However, it should be noted that if in the case of Neolithic trepanations, it is at all difficult to understand why and who did it [16, 18], with a few exceptions [34], the manipulations on the head in the Pazyryk Culture were obviously of medical nature and performed by specialists with certain knowledge about the organization of the human body and its diseases.

In the first presented case of skull trepanation, operation was aimed at treatment of the patient with severe traumatic brain injury. According to the opening location in the center of the fracture indented from the sagittal suture, the formed defect size as well as precision of manipulations on the bone, the Scythian healer was a perfect diagnostician and trained surgeon with good knowledge of anatomy. The operation clearly was of medical nature and was very likely aimed at removing an intracranial hematoma.

In the second case, this also was an attempt to treat head injury. The adverse outcome of the operation might be associated with many circumstances, of which severity of primary injury should be put first. Eventually, even today, having the state-of-the-art neurosurgical and intensive care technologies, we lose patients after trepanation due to severe primary injury to the brain or other organs in concomitant injury. It is possible that a certain role was played by an unfortunate and inexplicable choice of the place for trepanning on the skull just over the largest venous collector of the brain with a risk for fatal bleeding. It can not be ruled out that a more rough operation technique determined the adverse outcome of treatment. Probably, a human factor, the choice of a doctor, was of crucial importance in those ancient times.

In the third case, where there are no signs of skull injury, the trepanation was performed over the motor cortex and the trepanned person died at the extreme, at that time, age, survived for a long time after the surgery. The causes for the operation remain unclear. It is quite possible that this is the case of treatment of head injury that did not leave marks on the skull. However, other causes can not be excluded, e.g. meningiomas [29] or a parasitic brain lesion. In the modern Siberia and Altai, cases of brain cysticercosis and echinococcosis requiring surgical treatment are rather common. Animals, which were bred by the Altai cattlemen 2,500 years ago, are the main or intermediate hosts in the life cycle of helminthes and could be a source of infection. A high level of diagnosis and successful implementation of intracranial interventions by ancient surgeons do not exclude the possibility of removing helminthes from the brain of the individual from Bike-III.

**Conclusion**

According to the obtained data, the level of diagnosis and implementation of surgical manipulations on the head was high enough in the Pazyryk culture surgeons. They had serious knowledge of the anatomy of the skull and intracranial venous collectors.

One of the operations was carried out as if the Pazyryk surgeon was guided by the recommendations of the great Greek physician Hippocrates. It makes sense to assume either the convergent development of the
linear fracture treatment technology or a connection between the early nomads of Siberia and medical centers of the ancient world.

It is unlikely that the trepanations were performed for ritual reasons, since the cause for two cases was a brain injury. Written sources of the beginning of our era indicate that doctors who performed trepanations realized that it is rather risky surgery. Aretaeus of Cappadocia branded trepanation, calling it “bold remedy” [31]. Galen, describing the risks of brain injury during trepanation, stressed the need in repeated practice in this operation to become a master [25, p. 75, 76, 183]. The analysis of 40 cases of trepanation in Anatolia from the Neolithic to the late Ottoman period revealed that the causes for these operations were mainly skull injuries and, in some cases, tumors and training of doctors [9]. Other authors, analyzing the skull of ancient times from Italy, also conclude on training in performing trepanations [10].

REFERENCES

Commentary

The study of Prof. A.L. Krivosheapkin and authors is, unfortunately, the first in the last 122 years of the Russian history. The previous and only description of the skull with traces of trepanation found on the territory of Russia (now not Russia already) was published in 1892 [2]. Since the primary source is difficult of access, it is worth reminding its content.

During the excavation of the ancient city on the Knyazh’ya mountain (Kiev province), a skull with traces of trepanation dated by the 13th century A.D. was found. According to the decision of archeologists and doctors [1, 2], the skull belonged to a young male who defended a besieged fortification and suffered an open, penetrating traumatic brain injury (probably arrow wound). He was immediately provided with specialized care. The wound was incised, resective trepanation was performed by expanding the edges of the bone defect to 2.75–2.5 cm. The injured male died soon after the operation and was buried in the city. Analyzing this observation, it is difficult to suggest that unique surgical interventions were performed in a small fortified settlement (“city”). It is obvious that craniotomy in Russia, like in other countries, was rather routine operation.

However, the fate of this skull is unknown. I am almost convinced that domestic archeological museums and repositories have other similar skulls, examination of which with the use of modern facilities could provide us with a lot of new information. But as far as I know, the only Russian team carrying out this research is the authors of this article. Much wider studies in this field are conducted in other countries.

There are known the results of excavation of the Peruvian necropolis, where approximately 10% of skulls have trepanation traces, with the nature of bone changes indicating that about 60% of patients underwent the operation and survived after it for at least several months [4].

About 1/3rd trepanations were performed for traumatic brain injury, with their outcome being primarily determined by the severity and localization of injury. In the overwhelming majority of cases of injury to the parasagittal region, the outcome was adverse (as revealed by the lack of reactive changes in the trepanation defect edges).

Indications for craniotomy in the remaining 2/3rd cases remain unclear. Presumably, these might be osteomyelitis of the skull bones, headache, epilepsy, and mental disorders; it is very likely that skull trepanation was performed for ritual purposes. Interestingly that the ancient Incas did not resect the bone over the superior sagittal sinus when performed bilateral trepanation.

In addition to trepanation, the Incas performed also plastics of bone defects with gold or silver plates. Infectious complications (osteomyelitis) were observed only in 15% of cases [3].

In Europe, the remains were not preserved so good, and the earliest ones with traces of trepanation belong to the Middle Ages. For example, in graves found in the territory of Germany and dated by the 6th–8th century A.D., traces of trepanation are present only on 8 of 384 skulls (about 2%). Interestingly that death from osteomyelitis on the 4th week after the operation occurred only in one case, consequently, the mortality rate, which is equal to the infectious complication rate, was 12.5% [4]. It is also interesting that one of the patients of this “series” who had successfully undergone trepanation in the frontal region performed with pliers died much later from a penetrating wound in the parietal-parasagittal region, caused by a blow of the sword.

It is encouraging that interest in the history of Russian medicine has arisen again after a long period. I would like to thank the authors for the pleasure of reading the article and to wish them continued success.

A.V. Kozlov (Moscow, Russia)

REFERENCES


PROBLEMS OF NEUROSURGERY NAMED AFTER N. N. BURDENKO 3, 2014
Topics to be covered in our next issue:

- Reconstruction of complex cranial defects
- Optic nerve decompression in patients with tumors
- Intravascular hypothermia in the treatment of severe head injury