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RESEARCH ARTICLE

New approaches to diagnostics and treatment of cholangiocellular cancer based on photonics methods

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Abstract Cholangiocellular cancer (CCC) is an oncological disease of the bile ducts characterized by a high mortality rate. To date, the use of standard methods for the diagnosis and treatment of CCC has not been able to reduce mortality from this disease. This work presents the results of fluorescence diagnostics (FD), which consists in using a modified optical fiber and photodynamic therapy (PDT) using a therapeutic laser instead of a low-intensity laser. This technique was tested on 43 patients in a clinical setting. The results obtained indicate a direct correlation between spectroscopic and video FD methods. Furthermore, a direct correlation was found between the photobleaching of a chlorin e6-based photosensitizer, with the commercial names of Photolon Radachlorin and Photoran and stricture regression. Our findings demonstrate the possibility of using a therapeutic laser with a wavelength of 660 nm for both diagnosis and treatment of bile ducts cancer, which results in a significant reduction of the operation time without decreasing its effectiveness.

Keywords cholangiocellular cancer (CCC), fluorescent diagnostics (FD), photodynamic therapy (PDT), chlorin e6, Photolon, spectroscopic method, video fluorescent method, photobleaching, cancer, bile ducts, tumor, intraoperation diagnostics, photosensitizer (PS)

1 Introduction

Cholangiocellular cancer (CCC) is a rare oncological disease of the bile ducts, which is generally characterized by slow growth; however, invasion of regional lymph nodes as well as spread to the surrounding liver parenchyma are possible in the early stages [1,2]. One of the dominant causes of adverse outcomes due to CCC is the late diagnosis of the disease. Other relevant causes are attributed to the low success rate of tumor resectability, as well as the low efficiency of modern treatment methods, which hinder approaching the focus of the disease. In general, the first clinical symptom of CCC is obstructive jaundice, due to which the patient is admitted to hospital [3]. The appearance of obstructive jaundice may indicate a stage of the disease at which radical surgical treatment is no longer possible [4]. The initial care in the treatment of these patients consists of biliary excretion, the variants of which may be antegrade or retrograde endoscopically-guided cholangiostomy [3,5]. Radical surgical intervention is possible in approximately 20%–30% of CCC patients with a low extent of the process and the absence of concomitant diseases that make the risk of surgery extremely high [6–8]. Upon successful surgical treatment, the 5-year survival rate is approximately 20%–40% [2]. With unresectable CCC, life expectancy does not exceed one year, and this duration decreases with the development of obstructive jaundice [2,9,10].

Today, the diagnosis of CCC is often made on the basis of clinical and instrumental data (such as ultrasound, magnetic resonance imaging, multispiral computed tomography, and cholangiography). This may be due to the intrinsic difficulty of sampling biopsy material from a bile duct tumor during endoscopic examination. Performing a percutaneous diagnostic puncture may be complicated by difficulties related to the location of the tumor in the area of the hepatic hilum. In addition, due to the complexity of

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collecting morphological material from the bile ducts, biopsy not infrequently provides little information [10–12].

On the basis of the patient's quality of life, the most preferred option for percutaneous bile removal is bile duct stenting [11,12]. However, upon tumor invasion or spread of tumor infiltration outside the stent, recurrence of obstructive jaundice is possible [8,9,13].

Currently, with the development of radical and palliative treatments of oncological neoplasms of various localizations and natures, modern theranostics methods, such as fluorescence diagnostics (FD) and photodynamic therapy (PDT), are proving to be the most efficacious. The main advantage of FD and PDT is the possibility of combining tumor treatment and diagnosis in one joint procedure [14,15]. In addition, unlike chemotherapy, obstructive jaundice is not a contraindication for FD and PDT [16]. An important advantage of the spectroscopic analysis of tissues consists of the possibility to obtain information regarding the dynamics of various biological processes in real-time. With FD, the degree of photosensitizer (PS) accumulation in the tumor tissue is determined by the intensity of the fluorescent signal. This makes it possible to accurately detect the boundaries of the pathological growth, select the optimal parameters for the PDT session, and evaluate the therapy effectiveness after the session. PS accumulates selectively in tumor cells and is activated by irradiation with light with a wavelength corresponding to the peak absorption of the drug. In this case, a photochemical reaction occurs, causing the formation of singlet oxygen, which destroys tumor cells. This is due to that the fact that singlet oxygen, the main photodynamic process intermediator, possesses a short lifetime in biological systems. Theoretical assessments have indicated that the mean free path of singlet oxygen in biological media is approximately 10–100 nm [17]. Hence, the photodynamic reaction causes molecular and cellular destruction only in close proximity to the PS binding sites. In addition to the direct phototoxic effect on cancer cells, an important role in the destruction mechanism is played by the disruption of blood supply to the tumor due to microthrombosis and damage to the vascular endothelium. These methods have also been widely used in the diagnosis and treatment of bladder cancer and skin cancer, as well as to enable recanalization of a tumor-associated stricture of the esophagus, etc. [18,19]. In previous studies, investigating the damage to the bile ducts caused by the tumor, two lasers with different characteristics were used: a diagnostic laser with a wavelength of 635 nm and a power density of 3–5 mW/cm^2 for video FD and a therapeutic laser with a wavelength of 660 nm and an energy density of 150–200 J/cm^2 for PDT. However, these systems were accompanied by a number of inconveniences, in particular, the need to substitute the optical fiber and laser after FD to proceed with PDT. In this work, the radiation source for FD and PDT was optimized, and a single laser source with a wavelength of 660 nm and an adjustable power up to 2 W was developed.

2 Experimental

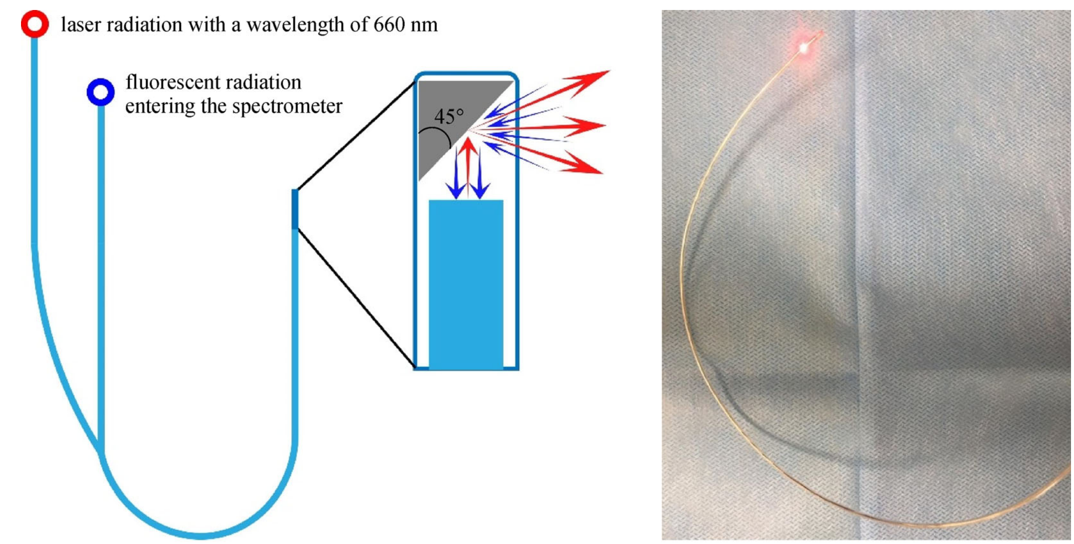
This work presents the results of studies conducted at the First Sechenov State Medical University, Ministry of Health of the Russian Federation (Sechenov University). The treatment group included 43 patients with unresectable CCC complicated by obstructive jaundice. The average age of the patients was 68 years. Fourteen patients were diagnosed as having extrahepatic bile duct cancer (according to the ICD-10: C24), whereas 29 patients were diagnosed with intrahepatic bile duct cancer (according to the ICD-10: C22.1). In all patients, FD and PDT were performed intraoperatively during cholangiography.

To perform FD and PDT, the following intravenous PSs were used: chlorin-based drugs — Radachlorine "Radapharma" (7 patients) or Photolon "Belmedpreparaty" (26 patients) and Photoran "Deko Company" (10 patients) at a dose of 1.0 mg/kg intravenously 3–5 h before surgery. The chlorin e6-based drug is approved for clinical use in Russia, and all PS parameters are regulated. The main benefit of using this PS is the 3 h time it takes to reach maximum accumulation, as well as its ability to be quickly eliminated from the body (48 h), which shortens the need for the patient to follow the light regime. Another advantage of this drug is the possibility of being used for both FD and PDT. Contact FD was performed using a LESA-01-BIOSPEC spectroscopic device and a Y-shaped optical fiber with a side irradiator through a 9-Fr introducer with a 3 mm diameter inserted into the bile duct. The laser excitation wavelength was set to 660 nm for recording chlorin e6 fluorescence in pathological tissue. When working with CCC, a therapeutic laser with a generation wavelength of 660 nm was first used for theranostics. In the future, the use of this type of devices will allow the estimation of real-time PS photobleaching in the tumor to be recorded. Monitoring of the processes occurring in the pathologic region using this device was carried out using a diagnostic optical fiber probe with a side irradiator. Laser radiation was applied to the research sample via an emitting fiber. When passing through the optical path in the biological tissue, photons migrate along a "banana-shaped" trajectory toward the receiving fiber, which is connected to the spectrometer. Subsequently, the signal from the spectrometer is sent to the computer where it is processed using a unique software. The optical fiber was modified from the walls of the bile ducts to increase the accuracy of the diagnostics. A silver rod with a polished surface at 45° was attached to the distal tip of the diagnostic fiber. This resulted in the laser radiation, which is reflected from the rod, to fall onto the investigated area at an angle of 90° with the minimum loss of signal (Scheme 1). The numerical aperture (NA) of the fiber was 0.22 (NA = 0.22). Owing to this modification of the optical fiber, the fluorescence spectra of the investigated area of the bile ducts could be obtained with greater efficiency than when using an optical fiber with an end feed.

Additionally, using a fluorescent video system, images of the bile duct sections before and after PDT were obtained in color and fluorescent light, as well as in the overlay mode using a BIOSPEC UFF-630/675-01 fluorescent endoscopic video system and a BIOSPEC flexible optical endoscope with a diameter of 2.8 mm (Figs. 1 and 2) [20]. The combined overlay mode consists of a color image with fluorescence sections aligned over the top in green pseudo color. Images were obtained at maximum PS accumulation at the site of the malignant neoplasms. The change in fluorescence index was evaluated using a combined image generated by the video system software. The fluorescent video system consists of a white light source, namely, a laser source with a wavelength of 635 nm to excite the chlorin e6-based PS. The video system includes a registration unit comprising a navigation camera for recording color images and a monochrome camera for recording the PS fluorescence signal in the studied area. A light-conducting flexible Y-shaped bundle of optical fiber was used to deliver radiation from two sources to the biological tissue. A flexible endoscope was used to deliver radiation to the test object and to collect the scattered reflected light. The optical resolution of the endoscope is 0.5 mm. Cameras and radiation sources were controlled using a special software. Using this system, fluorescent images of the zone of concern were obtained (Figs. 2 and 3). During diagnostics in overlay mode, an interactive target is present on the monitor screen. When targeting different parts of the studied area, the fluorescence index corresponding to this area is calculated and displayed, which allows to pinpoint pathological areas with greater accuracy [21,22]. During video FD, the laser radiation power density was 5 mW/cm^2, and the laser radiation energy density did not exceed 1 J/cm^2 to exclude the presence of the photodynamic effect. The laser excitation wavelength was 635 nm.

For good visualization, a 7–9 F introducer with a check-flo valve was placed in the bile ducts. The use of an introducer is important, since it allows to navigate the bile ducts during cholangioscopy, as well as to irrigate the area of concern and clear it of incoming bile and blood, which cause false fluorescence. In addition, it is always possible to simultaneously place a safety guide, a therapeutic and/or diagnostic optical diffuser, and a cholangioscope with a diameter of 1.2 mm through an introducer of the indicated size.

PDT was performed after spectroscopic and video FD. For this purpose, a cylindrical optical fiber of diffuser type with a length of 15–20 mm at the distal end was passed through an introducer and placed at the center of the tumor stricture under endovideoscopy and/or X-ray control. The power of the BIOSPEC LFT-675-01 therapeutic laser with an excitation wavelength of 660 nm was 1.5 W, thus permitting to obtain a power density of 150–200 mW/cm^2. The procedure was carried out for 10$\pm$2 min with interruptions; to exclude painful effects, the total radiation dose was 150–200 J/cm^2. All patients followed the light conditions during FD and PDT, in order to avoid the phototoxic effect. To evaluate the effectiveness of the CCC therapy, both visual and spectroscopic fluorescence control were performed before and immediately after PDT.



(a) (b)

Scheme 1 (a) Modified optical fiber. (b) Real image of the modified optical fiber

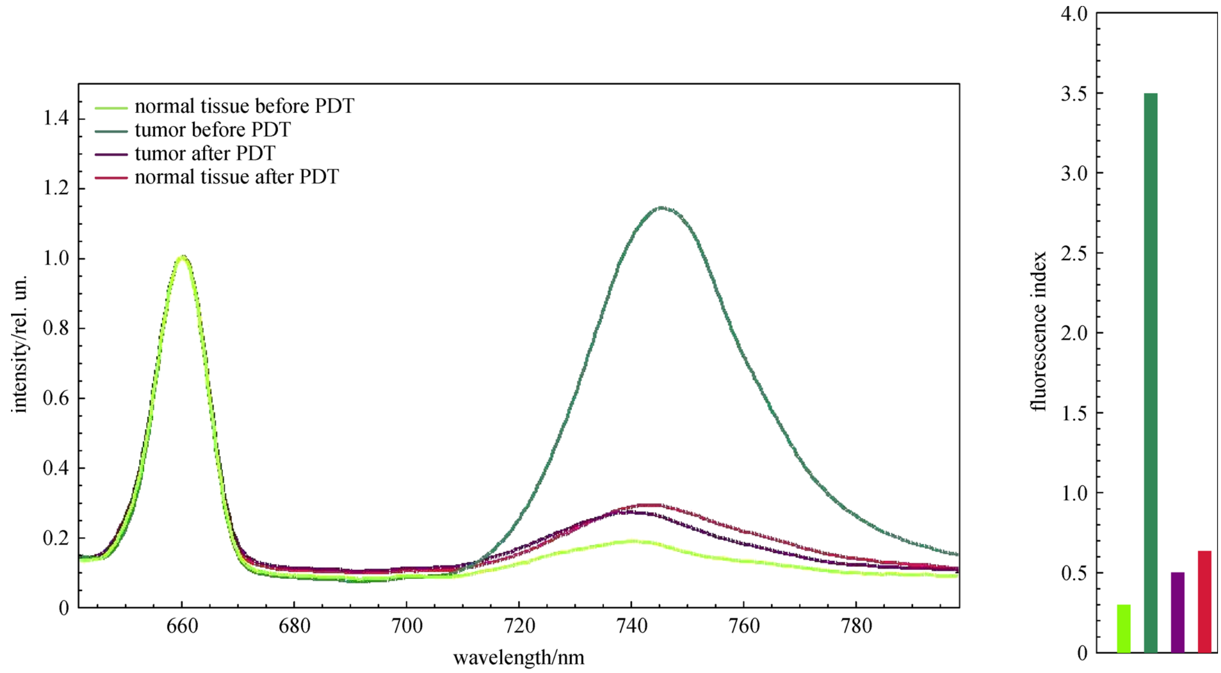


Fig. 1 (a) Fluorescence PS spectra normalized to the laser line (λ_{ex} = 660 nm) at the moment of maximum accumulation in the tumor before and after PDT, compared to normal tissue. (b) Histograms of the fluorescence index expressed as the ratio of the area under the chlorin e6 spectral fluorescence curve to the area under the laser radiation curve

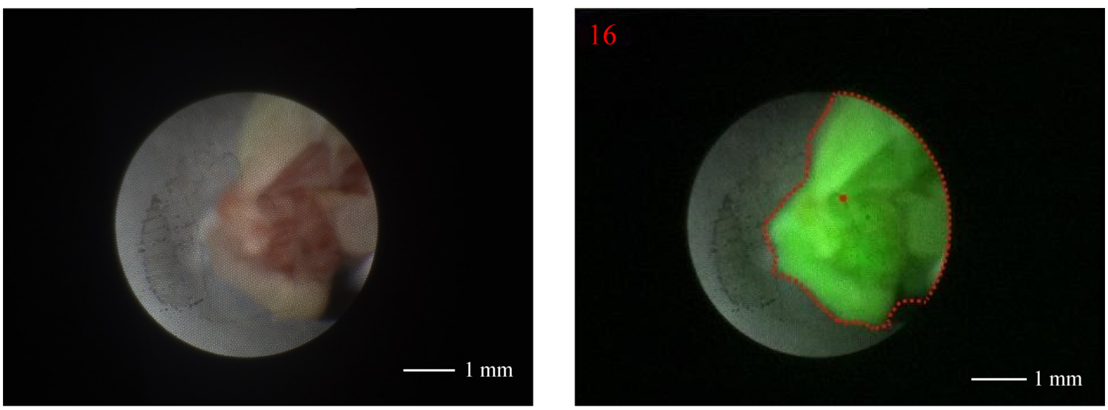


Fig. 2 Image of the bile duct region with the tumor before PDT. (a) Color mode. (b) Combined mode. The dotted line marks the region of the bile duct tumor. The fluorescence index obtained from the interactive target is displayed in the upper left corner of the image. The fluorescence index outside the tumor is less than 10-rel. units, which corresponds to normal tissue

3 Results and discussion

Spectroscopic FD was performed by sensing the fluorescence foci identified by the video fluorescence control. Using a spectral fluorescence device, the drug fluorescence spectra after PS injection were obtained in the pathological and normal tissues studied before and after PDT. Figure 1 shows fluorescence spectra averaged over one studied localization and normalized to a laser line (in the control zone of the study) for one patient before the PDT session (in the zone of maximum PS accumulation) and after the PDT session. Outside the pathologic zone, drug accumulation was not observed, as confirmed by the absence of a markedly pronounced PS fluorescent signal. The left peak in the spectral curve, with a maximum at a wavelength of 660 nm, corresponds to backscattered laser radiation. The peak in the range of 720–780 nm corresponds to the PS fluorescence in the tissue of concern. Based on the fluorescence indices, which are calculated as the ratio of the area under the fluorescence curve to the area under the laser radiation curve (Fig. 1(b)), it is possible to determine the increased accumulation of the drug in the pathological region. After PDT, the signal level decreased to the level of normal tissue, thus indicating the occurrence of PS photobleaching.

To determine the fluorescent signal, a bile duct tumor was selected. The fluorescence index values obtained from an interactive target and correlated with a relative chlorin e6 concentration of more than 10-relative units (rel. un.) ($\leqslant 10$ mark normal tissue) indicate the presence of pathological tissue in the area of concern (Fig. 2). A decrease in the fluorescence index is indicative of PS photobleaching in the irradiated zone (Fig. 3).

A direct correlation between spectroscopic and video FD methods was noted. The effectiveness of a one-treatment session was determined through the decreased intensity of the fluorescent signal in the pathologic region after irradiation. A direct correlation between PS photobleaching and stricture regression was demonstrated.

In this work, the FD and PDT methods for CCC were first applied using only a therapeutic laser with a generation wavelength of 660 nm. Furthermore, an optical modified fiber was used for FD for the first time. A combined FD and PDT session was performed on 43 patients. All patients experienced positive effects in terms of recanalization of tumor stricture and complete regression of jaundice. In all clinical cases, no side effects and complications were detected when PDT was administered.

Spectroscopic and video fluorescence with minimally-invasive analyses following a PDT session showed a decrease in the PS fluorescence signal intensity. Quantitative estimates of the fluorescence signals obtained via spectroscopic and video fluorescence methods showed a correlation between the fluorescence intensity averages in tumor and normal tissue before and after PDT. The observed decrease in PS concentration after a PDT session in the pathologic area is due to the partial destruction of PS molecules targeted by radiation with wavelength matching their absorption peak. A decrease in fluorescence intensity indicates that the radiation has reached its target and that the chlorin e6 present in the cells has produced the photodynamic effect. Thus, a direct correlation exists between photobleaching and the achievement of a therapeutic effect. After only one treatment session, a significant attenuation of the fluorescent signal in the pathologic region was recorded in all patients, indicating good prognosis and treatment effectiveness. To increase the effectiveness of the therapy, the radiation dose can be increased taking into account the individual characteristics of the patients.

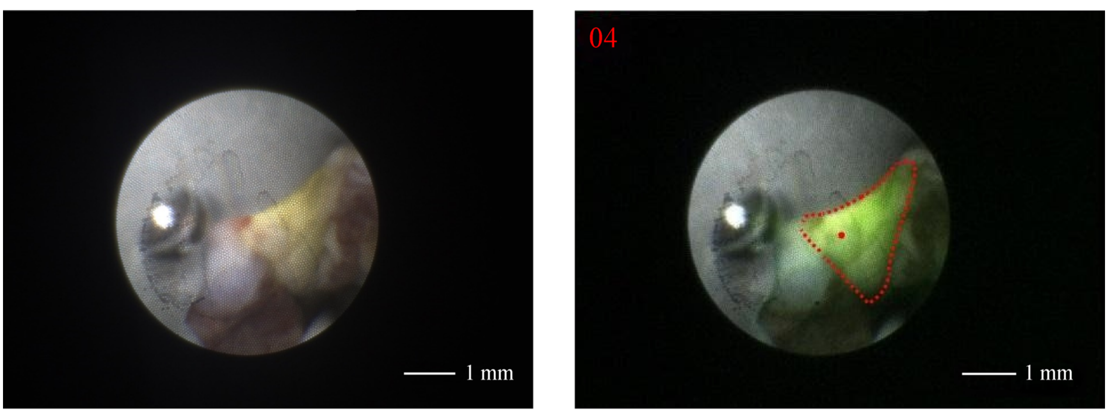
To confirm the positive effects of PDT, patients underwent contrast-enhanced cholangiography before therapy and on the 5th day after therapy (Fig. 4).

4 Statistics

Using the Kolmogorov–Smirnov test, two groups were tested for normality of distribution, showing a negative result. A nonparametric Mann-Whitney statistical test was used to test for differences between the two groups. The *P*-value was found to be 7.22×10^{-16}, thus the Ho hypothesis regarding the equality of the averages was rejected, indicating that differences exist between the fluorescence indices before the therapy and after PDT.

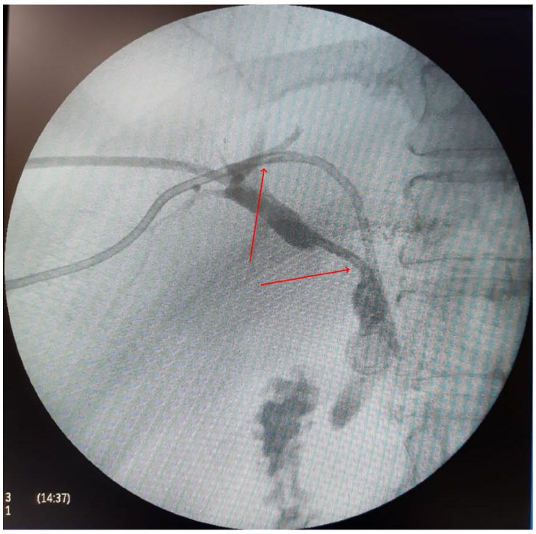
5 Conclusions

The FD methods considered in this article are promising in the field of treatment of oncological diseases of the bile ducts and in the prevention of relapse. The obtained results demonstrate positive effects for the patients' treatment, as well as an enhancement of life expectancy with high quality. The proposed approach is characterized by simplicity of execution, selectivity of action on

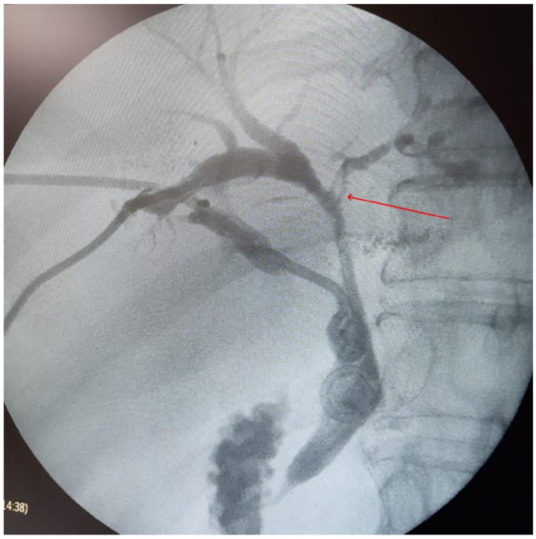


(a) (b)

Fig. 3 Image of the bile duct region with the tumor after PDT. (a) Color mode. (b) Combined mode. The dotted line marks the region of the bile duct tumor. The fluorescence index obtained from the interactive target is displayed in the upper left corner of the image



(a)



(b)

Fig. 4 Cholangiographic picture of the bile ducts. (a) Before PDT, a complete contrasting block is observed in the left lobar duct (the arrows indicate the length of the stricture). (b) After PDT, during cholangiography, the contrasting block in the left lobar duct is observed (the arrow indicates recanalization of the left lobar duct)

pathological tissues, reduction of the procedure time, and absence of adverse reactions and complications. This diagnostic system, equipped with a modified fiber and therapeutic laser rather than a low-intensity laser, allows to identify the PS fluorescence intensity with high efficiency for successful differentiation between normal and tumor tissue, which is necessary for local treatment of the tumor and a prolonged relapse-free period.

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Ethics Approval The trial was conducted in accordance with the Declaration of Helsinki. This study was reviewed and approved by the Integrated Research Ethics Council of First Sechenov State Medical University, Ministry of Health of the Russian Federation (Sechenov University) (approval number 127, 12.09.2018). All registered patients completed an informed consent form.

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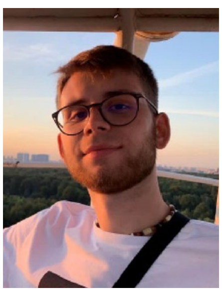

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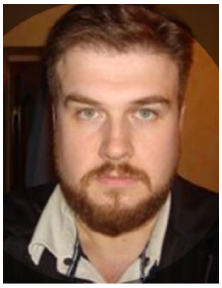

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