Protocol for optical percutaneous needle biopsy of the liver

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Method Article

Abstract

Liver cancer remains one of the most widespread cancer types worldwide. The time spent on the diagnosis is one of crucial factors ensuring the effectiveness of treatment. As percutaneous needle biopsy remains the gold standard for liver diagnosis, it is necessary to develop new methods for improving this procedure. One of the most promising directions is multimodal optical biopsy. The proposed protocol describes the methodology of real-time *in vivo* optical measurements of liver lesions during the percutaneous needle biopsy of the liver. The multimodal approach combines the methods of fluorescence and diffuse reflectance spectroscopy and conventional histological analysis.

Introduction

In recent decades the incidence of primary and metastatic liver cancer worldwide remains high[1]. One of the causes of this situation is the difficulty of early diagnosis and rapid growth of liver tumors. Therefore, the possibility of earlier diagnosis is crucial for improving the cure rate.

Currently, the cancer diagnostic requires histological and cytological analysis to plan further treatment. Tissue samples for such analysis are commonly obtained by percutaneous needle biopsy performed under ultrasound, CT or MRI control. This technique is the gold standard for liver diagnosis as it provides diagnostic information on oncology disorders with minimal injury to liver tissue and a low risk of complications. However, percutaneous needle biopsy has the probability of false negative results up to 10%[2], which often requires performing additional procedures and increases the time for determining the correct diagnosis.

Optical technologies have exceptional potential to be applied as additional methods for assisting surgeons during conventional biopsy procedures. We present a protocol for simultaneous application of two optical methods for biopsy needle guidance and real-time monitoring of tissues status[3−6]. The methods used include fluorescence spectroscopy (cell and tissue metabolism depending on the intensity of spectral composition and fluorescence of tissues[7,8]) and diffuse reflectance spectroscopy (morphological structure and content of blood, water and other absorbers[9,10]), although the proposed protocol can be supplemented with other spectroscopic methods, which can be introduced into standard biopsy tools.

Reagents

Solutions used during the biopsy procedure:

70% ethanol;

2% lidocaine hydrochloride solution – 10-20 ml;

0.9% sodium chloride.

Solutions for tissue samples fixing:

10% neutral-buffered formalin;

Paraffin.

Disinfectant solutions for biopsy needles and optical probe:

Teflex 8% (Soft Protektor, Russia);

Multidez 3% (Soft Protektor, Russia).

Equipment

Biopsy sampling equipment:

10-20 ml syringes;

17.5G Chiba-type needle;

Sterile cotton pads;

Medical patch;

Glass slides;

Ultrasound scanner.

Optical biopsy device:

365 nm LED;

450 nm laser diode;

HL-2000-FHSA tungsten halogen lamp (Ocean Insight, USA);

Spectralon diffuse reflectance standard (Ocean Insight, USA);

Flame spectrometer (Ocean Insight, USA);

FGL400 and FGL495 filters (Thorlabs, Inc., USA);

Collimator and filter holder;

Optical fiber probe compatible with the 17.5G Chiba-type biopsy needle;

Personal computer.

Procedure

Prior to biopsy:

1. Disinfect biopsy needles and the optical probe with Teflex 8% for 30 min and Multidez 3% for 30 min and carefully rinse with water.

2. Calibrate the experimental setup by acquiring a calibration spectrum of Spectralon, recording a dark signal and controlling the output power of the radiation sources.

During the biopsy:

1. Lay a patient in the supine position.

2. Disinfect the skin with 70% ethanol solution.

3. Detect a tumor using ultrasound guidance and determine the needle path by the shortest safe distance from the skin to the lesion.

4. Mark the puncture site on the skin.

5. Inject a local anesthetic (2% lidocaine hydrochloride solution) at the marked site under ultrasound guidance.

6. Insert a 17.5G Chiba-type needle to the border of the target area of liver tissue under ultrasound guidance.

7. Remove the stylet from the needle.

8. Insert the optical probe in the biopsy needle and then into the liver tissue under ultrasound guidance. The probe should be held still during the measurements described in steps 9-20.

9. Insert FGL400 filter into the filter holder.

10. Turn on 365 nm LED.

11. Perform the measurements of 20 fluorescence spectra.

12. Turn off 365 nm LED.

13. Insert FGL495 filter into the filter holder instead of FGL400.

14. Turn on 450 nm laser diode.

15. Perform the measurements of 20 fluorescence spectra.

16. Turn off 450 nm laser diode.

17. Remove the filter from the filter holder.

18. Turn on the halogen source.

19. Perform the measurements of 100 diffuse reflectance spectra.

20. Turn off the halogen source.

21. Remove the optical probe from the tissue and biopsy needle.

22. Insert the biopsy needle into the tumor under ultrasound guidance.

23. Obtain several samples of tumor tissue from different directions by proper rotatory technique[11].

24. Remove the biopsy needle containing the biological material.

25. Place the sterile aseptic dressing on the puncture site.

After the biopsy:

1. Fixate the samples with 10% neutral-buffered formalin.

2. Dehydrated the samples.

3. Embed the samples in paraffin.

4. Stain approx. 5-μm-thick sections with haematoxylin and eosin according to standard procedures.

5. Perform standard histological examination by light microscopy.

6. Calculate the parameters of obtained fluorescence and diffuse reflectance spectra.

7. Classify the case of tumor by the characteristics of tumor size, cancer type, tumor origin (primary tumor or metastasis) etc., as well as by the optical measurements results (fluorescence intensity, reflectance coefficient, oxygen saturation, etc.).

Troubleshooting

1. Intraoperative complications:

1.1. Ultrasound signs of damage to the vascular structures of the liver.

Actions:

- Stop the procedure;

- Fixation of the biopsy needle in a stationary state with removal 5-7 minutes after the appearance of signs of complication;

- Hemostatic drugs administration.

1.2. There is not enough material to perform an informative morphological study:

Actions: Repeat the biopsy procedure.

2. Postoperative complications:

2.1. Intra-abdominal bleeding and/or hematoma:

Actions:

- Cold on the puncture area for 2 hours;

- Hemostatic drugs administration;

- Percutaneous drainage of the hematoma.

2.2. Pain syndrome:

Actions: Postoperative pain management, administration of analgesic drugs.

Time Taken

DRS spectra recording: 1-2 min per area.

FS spectra recording: 1-2 min per area.

Total optical measurements time including additional manipulations: 5 min per area.

Biopsy sample obtaining: 10 min.

Anticipated Results

The proposed protocol can be used to improve the standard percutaneous needle biopsy procedure and obtain additional diagnostic information in real time to know the state of affected tissues before getting the results of histological examination.

The protocol could be applied not only to liver malignancies diagnostics but also to studying the state of biological tissues of other abdominal organs as well as to determining different pathological states (inflammation, necrosis, tumor, etc.).

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