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# The role of confocal microscopy in the diagnosis of pancreatic neoplasms

## Rola endomikroskopii konfokalnej w diagnostyce chorób nowotworowych trzustki

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**Słowa kluczowe**
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Summary

Confocal laser endomicroscopy (CLE) is a novel tool allowing for *in vivo* histologic evaluation of human tissue. The method has the potential for differentiating malignant changes from benign at their early stage and has proven its clinical value in many G.I conditions such as Barrett's esophagus or colonic polyps. The data is accumulating on the usefulness of this new technique in pancreatic cysts evaluation. This may allow for the early detection of advanced neoplasia or small cancer, which may increase the survival in those diseases with otherwise very poor prognosis. Instead of offering to the patients with pancreatic cyst of unknown origin the different surveillance programs it would be more valuable to subject them to the test determining their malignant versus benign character. In particular, mucinous adenoma or intrapapillary mucinous neoplasms have high risk of malignancy and their early detection is warranted. This way we could also avoid many unnecessary surgical procedures performed in patients with the cysts of uncertain origin. Confocal endomicroscopy detects pancreatic cystic neoplasms with sensitivity of 59-80% and specificity: 100%. There is also emerging data on the high accuracy of CLE in the differential diagnosis of pancreatic solid masses.

Streszczenie

Laserowa endomikroskopia konfokalna (CLE) jest to nowatorskie narzędzie umożliwiające ocenę histolologiczną *in vivo* tkanek ludzkich. Metoda ta umożliwia różnicowanie zmian złośliwych od łagodnych w ich wczesnym stadium i udowodniła swoją przydatność m.in. w przełyku Barretta lub w polipach jelita grubego. Wzrasta liczba danych na temat użyteczności tej nowej techniki w ocenie torbieli trzustki. Może to pozwolić na wczesne wykrycie zaawansowanej neoplazji lub niewielkiego raka, a w konsekwencji podwyższyć wskaźniki przeżywalności u chorych, których rokowanie jest niekorzystne. W chwili obecnej, chorym z torbielami trzustki niejasnego pochodzenia oferuje się wieloletnie programy nadzoru. Tymczasem CLE umożliwia różnicowanie postaci łagodnych i złośliwych we wczesnym etapie po rozpoznaniu torbieli. W szczególności, gruczolaki śluzowe i gruczolakowaty wewnątrzprzewodowy gruczolak brodawczakowaty mają wysokie ryzyko zezłośliwienia i ich wczesne wykrywanie jest uzasadnione. W ten sposób możemy także uniknąć wielu niepotrzebnych zabiegów operacyjnych wykonywanych u pacjentów z torbielami niepewnego pochodzenia. CLE wykrywa nowotwory torbielowate trzustki z czułością 59-80%, a swoistością 100%. Ostatnio pojawiają się również nowe dane o wysokiej miarodajności CLE w diagnostyce różnicowej guzów litych trzustki.

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## INTRODUCTION

Confocal laser endomicroscopy (CLE) was introduced in 2004. It is a method that enables the endoscopist to obtain real time *in vivo* histology also described as an "optical biopsy". The principle of this method bases on tissue illumination with a low-power laser. The laser beam of 488 nm wavelength (blue light) reflects from the examined epithelial surface and is refocused by the same lens, reaching a filtering "pinhole" and then a detector (photomultiplier or avalanche photodiode). The illumination and detection system are lying in the same plane creating

a confocal system. The field of view ranges from 240 to 600 $\mu$m and images are collected at the rate of 12 frames per second (1, 2).

The CLE technique requires the use of a fluorescent dye which enables the reflection of laser light. The contrast agents can be introduced systemically (fluorescein, tetracycline) or locally (acriflavine, cresyl violet) by spraying them on the examined area. The most commonly used agents are intravenous 10% solution of fluorescein or topically applied acriflavine (0.2%). There are two endoscopic systems of CLE – integrated with an endoscope (eCLE) or a separate probe introduced by the accessory channel of an endoscope (pCLE) (2). The obtained endomicroscopic image allows for examining the mucosa of gastrointestinal tract at cellular level with the possibility to evaluate vascular architecture and connective tissue structure.

The clinical use of CLE is well established in evaluating metaplasia and dysplasia in Barrett's oesophagus allowing for more precise biopsy. Similarly, CLE is a useful method for determining histologic type and grade of dysplasia in colonic polyps. There is an ongoing research in the CLE evaluation of biliary strictures, duodenal neoplasia, gastric neoplasia, dysplasia in inflammatory bowel diseases and also in diagnosing solid and cystic tumours of the pancreas. The recent data on CLE diagnostics in gastroenterological diseases has been summarized and presented as Miami classification (2).

The evaluation of pancreatic masses with CLE requires the use of endoscopic ultrasound fine needle aspiration biopsy (EUS-FNA). EUS allows for diagnosing and staging pancreatic malignancies by establishing precise tumour size, blood vessel or organ infiltration (T status) and lymph node involvement (N status). Additionally, EUS allows for endoscopic biopsy of solid pancreatic lesions with the accuracy of 90% but negative predictive values of 64% (3-5). The sensitivity of cytology in pancreatic cystic lesions is lower – 54%, with specificity of 93%. The biochemical analysis of the cystic fluid (carcinoembryonic antigen – CEA) shows 63% sensitivity and 88% specificity for diagnosing malignant lesions (6).

**The use of CLE improves differentiation of pancreatic lesions. This method requires EUS-FNA of examined mass with a 19-G needle. The CLE probe is introduced through needle directly to tumour tissue (nCLE).** The first studies were conducted in 2010 by Becker et al. on porcine models. Ten pigs were examined under general anaesthesia with the use of EUS guided biopsy with nCLE and with natural orifice transluminal endoscopic surgery (NOTES). Confocal miniprobe was used to evaluate multiple structures (lymph nodes, diaphragm, ovaries, liver, spleen and pancreas) after intravenous fluorescein injection. The results were compared with standard histological evaluation. The obtained images were of acceptable quality and sufficient resolution. The authors concluded that the method is technically feasible (7).

## DIFFERENTIAL DIAGNOSIS OF PANCREATIC CYSTIC LESIONS

The EUS-FNA of cystic lesions of the pancreas frequently does not allow for sufficient cytological assessment, mostly due to small amount of cells present in the fluid. The aim of EUS guided nCLE of pancreatic cysts is the evaluation of the cystic wall epithelium which enables for accurate differentiation of histological type of the neoplasm.

Giovannini et al. published a description of nCLE images of various pancreatic cystic lesions. Serous cystadenoma presented with ultra-thin straight bright grey bands and a vascular network of branch vessels (fig. 1). Mucinous cystadenoma showed a large white band with few vessels (fig. 2). Intraductal papillary mucinous neoplasms (IPMN) in benign stage were characterized by the presence of finger like projections and regular vessels (fig. 3) whereas in malignant IPMN dark clumps with a neovascularization and large vessels > 20 $\mu$m of diameter were observed. In pseudocysts small black floating particles, large dark homogenous floating structures and heterogenous bright particles (macrophages) were present (fig. 4) (8).

Konda et al. preformed a pilot study evaluating the diagnostic potential of nCLE in differentiating pancreatic cyst types and the safety of the method (INSPECT study). The research involved 66 pa-

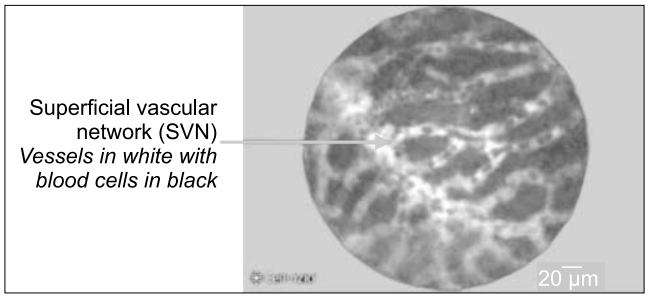


**Fig. 1.** Serous cystadenoma with superficial vascular network (nCLE image by the courtesy of www.celivizio.net educational program administrators).

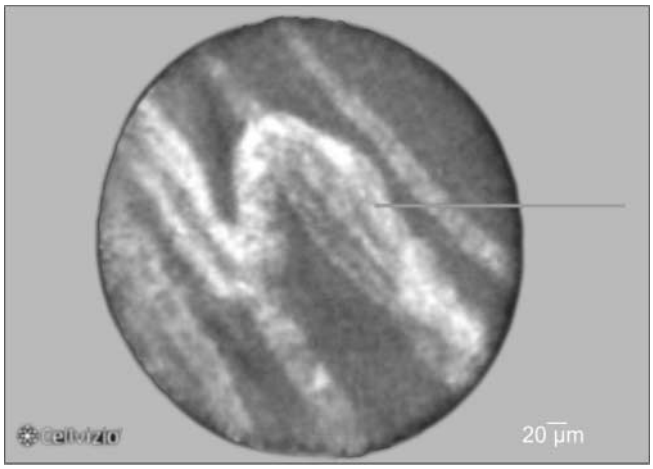


**Fig. 2.** Mucinous cystadenoma with visible numerous large vessels > 15 $\mu$m diameter (nCLE image by the courtesy of www.celivizio.net educational program administrators).

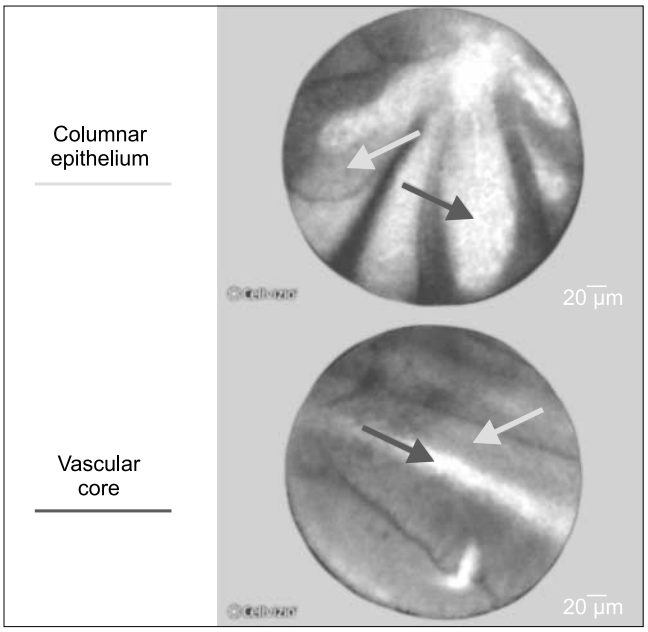


**Fig. 3.** Intraductal papillary mucinous neoplasms (IPMN) with the presence of finger like projections and regular vessel pattern (nCLE image by the courtesy of www.celivizio.net educational program administrators).

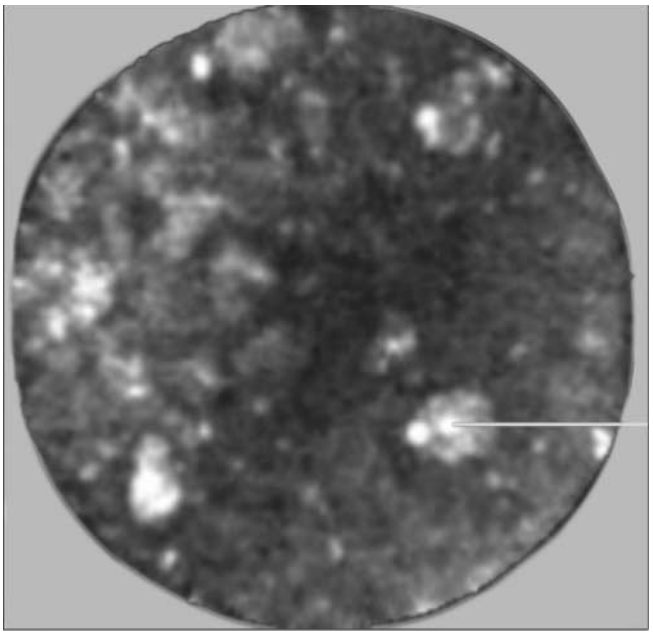

**Fig. 4.** Pseudocyst with visible bright particles – macrophages (nCLE image by the courtesy of www.celivizio.net educational program administrators).

tients in whom nCLE examination was performed under EUS guidance. The data from nCLE was correlated with histology diagnoses. The results provided 59% sensitivity and 100% specificity with positive predictive value of 100% and negative predictive value of 50% in determining the correct diagnosis of pancreatic cyst histological type. The overall complication rate was 9% (mild and moderate pancreatitis – 2 cases, abdominal pain – 1 case and intracystic bleeding not requiring further treatment – 3 cases) (9).

Similar study was conducted by Nakai et al. which assessed the feasibility, safety, and diagnostic yield of the combination of cystoscopy and nCLE in the clinical diagnosis of pancreatic cystic neoplasms (DETECT study). The research involved 18 patients. Specific features associated with mucinous cysts were identified: mucin on cystoscopy, papillary lesions and dark rings on nCLE. The sensitivity of for cystic lesions was 90% and of nCLE was 80%, and the combination was 100%. Complications occurred in 2 patients (7%) as pancreatitis (10).

Napoléon et al. conducted a study on a group of 31 patients with a solitary pancreatic cystic lesions of unknown diagnosis. The established nCLE microscopic criteria allowed for diagnosis of 13 serous cystadenomas, 6 mucinous neoplasms, 5 intraductal papillary mucinous neoplasms and 7 pseudocysts. Diagnosis was confirmed by histologic evaluation of surgical specimen or a committee consensus. The specificity of the method reached 100%, sensitivity 69%, positive predictive value 100% and negative predicitive value of 82% (11).

## DIFFERENTIAL DIAGNOSIS OF PANCREATIC SOLID MASSES

The sensitivity and specificity of nCLE in diagnosis of solid pancreatic masses has not been yet evaluated. However, Karstensen et al. performed a study on 25 patients in whom EUS guided nCLE was used to differentiate pancreatic tumours. The results of examinations in which fluorescein administered intravenously was used as a contrast agent showed low diagnostic value due to inadequate ability to elucidate cell nuclei. The use of topical acriflavine improved the examination sensitivity. The procedure was safe and feasible in all patients (12).

Similarly, there were no large studies on lymph node nCLE evaluation in patients with diagnosed pancreatic masses. However Giovannini et al. described the appearance of inflammatory lymph nodes by the presence diffuse small cells in a homogenous stroma with a normal vascularization whereas malignant lymph nodes show glandular structures with dark cells, neovascularization and leakage of fluorescein (13).

## MOLECULAR STUDIES

It is necessary to mention preliminary studies focusing on molecular evaluation of pancreatic tissue with the use of nCLE. Nakai et al. analysed epidermal growth factor receptor (EGFR) and survivin expression in *in vivo* study on porcine pancreas. The fluorescein isothiocyanate (FITC) labelled anti-EGFR antibodies were injected into pancreas with a 19-G needle under EUS guidance. After 30 minutes nCLE was performed to determine the areas of higher expression of investigated molecules. The results were evaluated by standard histology, confirming the nCLE observations (higher immunoreactivity of EGFR in duct lining

cells and survivin in acinar cells). The study suggested the potential of improving pancreatic masses diagnosis by introducing immunostating techniques in nCLE examinations (14).

Another study on rat model of PanIN and pancreatic cancer involved fluorescent staining of Cathepsin E. Confocal microscopy was used to determine the immunoreactivity of the molecule, showing its increasing expression in association with progressive pathological grades (PanIN I, II, III and pancreatic cancer). The authors suggest that this finding might play a role in monitoring high risk populations of pancreatic adenocarcinoma (15).

## CONCLUSIONS

EUS guided nCLE is a novel method capable of diagnosing pancreatic masses. The procedure is feasible and safe. The most encouraging results were presented in studies focusing on differentiating pancreatic cystic lesions. Further research might improve nCLE accuracy and impact patient management.

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