Current endoscopic techniques used in the diagnosis of preneoplastic lesions of the digestive tract

- Summary -

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**Introduction**

Cancers are one of the leading causes of mortality globally, regardless of the level of development of states. Of all, gastrointestinal cancers rank first in terms of the annual number of deaths. The most affected patients are those in the 6-8 decades of life. Most of the times, the diagnosis is made in advanced stages of the disease when the chemotherapy is no longer effective and the prognosis is reserved. The situation is caused by both the lack of screening programs and their cost, as well as exposure to more and more toxic substances, malnutrition, lifestyle etc. One of the main objectives in gastroenterological and oncological research is the development of new methods for the early diagnosis of premalignant and malignant lesions and their therapeutic sanctioning with much greater benefits for the patient, but also in terms of costs. Currently, there are few countries that have implemented solid screening programs to prevent digestive tract cancers. In Romania, screening is rather opportunistic and preneoplastic or malignant lesions are diagnosed following the investigation of other pathologies or syndromes. Usually, surveillance by upper or lower digestive endoscopy in white light is recommended depending on age, previously diagnosed lesions or diseases, heredo-collateral antecedents, eating habits etc. Conventional endoscopy cannot distinguish lesions according to histological substrate. For this reason, the histopathological examination remains the one that dictates the management of patients. The process is often difficult. Not infrequently, the results of biopsies arrive even after 4 weeks of waiting, precious time especially in the management of patients with malignant lesions. Also, the biopsies taken can be inconclusive if they are not taken on purpose and many lesions can be mismanaged (unnecessary resections, unnecessary surveillance, burdening anatomopathology services with unnecessary biopsies). Through this study we wanted to evaluate the effectiveness of two methods mentioned above, endoscopy in NBI mode and laser confocal endomicroscopy, both in terms of establishing the diagnosis of preneoplastic lesions and in terms of early detection of malignancies. The availability of the two methods is totally different. If virtual chromoendoscopy is available on most new endoscopes at the push of a button and at no extra cost, endomicroscopy is still inaccessible to many centers due to the high price of equipment and consumables. We will also highlight the importance of experience in using each method and the learning curve to obtain optimal results. In the future we will try to implement an instant histological diagnostic algorithm based on the computerized analysis of the obtained images.
Materials and methods

Patients

The present study was a prospective one and took place over 24 months (01.10.2017 - 01.10.2019), within the Gastroenterology and Hepatology Clinic of UMF Craiova. The study included 46 known patients with preneoplastic lesions of the upper and lower digestive tract, suspicious patients with typical digestive symptoms and patients diagnosed with digestive tract lesions following routine checks or prevention. The study was performed on a single group of patients with preneoplastic or malignant degenerative lesions, from the upper and lower digestive tract, which were analyzed by endoscopy in the NBI mode and by laser confocal endomicroscopy. Some of the study participants had been previously diagnosed with preneoplastic lesions of the digestive tract and presented to the clinic for endoscopic reassessment, biopsy and the establishment of appropriate therapeutic conduct. The other patients included in the group were those presented in the Gastroenterology Clinic with digestive symptoms such as: dysphagia, odynophagia, heartburn, abdominal pain (localized, diffuse, intermittent, permanent), involuntary weight loss, vomiting, hematemesis, emission of meleenic stools or hematochezia, rectorage, transit disorders (constipation, diarrhea, alternating diarrhea - constipation). The exclusion criteria from the study were the following: patients who refused to sign informed consent to perform endoscopic procedures; patients allergic to the contrast substance (fluorescein); patients who refuse sedation with Propofol or who have conditions that are incompatible with it; patients undergoing cancer treatment; hemodynamic instability.

Equipment and procedure

Upper digestive endoscopy (EDS): was performed in patients with symptoms typical of the upper digestive tract, after fasting for at least 6 hours for both fluids and solids. The explorations were performed with the GIF-H185 and GIF-HQ190 gastrosopes connected to an Olympus EVIS Exera III system. Endoscopies were performed according to the quality standards established by the American Society of Gastroenterology (ASGE). The identified lesions were examined in white light and subsequently in NBI mode. For each lesion there were made 5-10 captures, from different angles and distances. Lower Digestive Endoscopy (EDI): was performed in patients with symptoms typical of the lower digestive tract and in patients who presented for CRC screening. The scans were performed with the CF-H185
colonoscope connected to an Olympus EVIS Exera III system. All colonoscopies were performed completely, until the visualization of the ileo-cecal valve or until the highlighting of some stenoses or tumor that no longer allowed the advancement. According to ASGE standards, the right colon was intubated twice and the withdrawals lasted at least 6 minutes. In many cases, ENDOCUFF Olympus accessories were also used, which allowed a better visualization of the colon by exposing the mucosal folds. Colon preparation was performed the day before the colonoscopy. This involved ingesting 4 liters of Macrogol 4000 solution for bowel lavage. Oral feeding was also stopped from the beginning of the preparation until the colonoscopy was performed. The identified lesions were examined in white light and subsequently in NBI mode. 5-10 captures were made for each lesion, from different angles and distances. The endomicroscopic examination was performed in vivo and the preparation for was similar to the preparation for EDS / EDI of patients (fasting for at least 6 hours for EDS and ingestion of osmotic solutions for intestinal lavage in case of EDI). Probe-based endomicroscopy (pCLE) was performed with an endomicroscopy system based on mini samples from **Cellvizio (Mauna Kea Technology, Paris, France)** located in the Research Center for Gastroenterology and Hepatology Craiova. The movements (agitated patient, vomiting reflex, peristalsis of the digestive tract, respiratory movements, heartbeat) are the most incriminated in the formation of artifacts. For this reason, all patients with lesions in the upper digestive tract and a few with lesions in the lower tract who could hardly tolerate the procedure were sedated with Propofol 10 mg / ml. The sedation was performed by a anesthezist doctor. As a contrast agent, 10% sodium fluorescein was used in a 5 ml dose (one ampoule) for each patient. Examination of pCLE began approximately 1 minute after injection. Because the mini-samples for pCLE are inserted on the working channel of the endoscope, it was done by using plastic caps fixed to the distal end of the endoscope and by sucking the mucosa. Thus, the lesions of interest were more stable and the frequency of movement artifacts was lower. In order to avoid bleeding and implicitly the artefacts, the biopsies were taken targeted after the completion of the pCLE examination. 4 to 8 biopsy fragments were sampled, which were fixed in formalin and embedded in paraffin. Sections were interpreted in hematoxylin-eosin staining. For each patient, approximately 20 images were recorded, taken at 1-2 second intervals, by pressing a pedal at the examiner's foot. The images were taken both from the normal mucosa and from the lesion of interest.

These were subsequently analyzed by observers with different degrees of training in Gastroenterology and in the interpretation of pCLE images: 1 experienced nurse, 1 resident physician, 1 primary physician. The 3 evaluators were not
present at the real-time examination and the presumptive diagnosis for each patient was not known. Prior to the start of the study, all examiners mastered the Miami classification for pCLE, the NICE, JNET, ME-NBI classifications, and the most recent work on our research was reviewed.

**Histopathological examination as a diagnostic standard**

All biopsies or resections were analyzed by a pathologist specializing in gastrointestinal lesions.

**Statistical analysis**

The data were statistically processed and plotted. The Microsoft Excel package (Microsoft Corporation) with the predefined functions as well as the Data Analysis module was used. The correlation between the results of endoscopic techniques and the result of the histopathological examination with statistical value when $p < 0.05$ was calculated. The sensitivity of NBI and pCLE for the diagnosis of each lesion was also calculated. For the detection of malignancy, the specificity, the positive predictive value and the negative predictive value were also determined.

**Results**

**Group characteristics**

The group of patients used for the study had an average age of 57.87 years. The minimum age was 39 years and the maximum was 78 years. The distribution by sex shows a predominance of men for lower digestive tract lesions (75% of the total) and a slight predominance of women for upper tract lesions (54% of the total). The distribution of the studied lesions is in accordance with the graphs in figures 5 and 6. There were no allergic or adverse reactions to fluorescein during the study. We exclude transient yellowing of the skin and urine.

**NBI correlation, pCLE - histopathological examination for upper digestive tract lesions**

On examination of the NBI we find that the experienced doctor obtains a very close correlation with HP (0.93, $p = 0.05$). The resident doctor and the experienced nurse obtain
poorer results, but they are still within the correlation limits (0.73 and 0.62, respectively). The malignancy / benignity correlation - histopathological examination is maximal for all examiners. Sensitivity, positive predictive value and negative predictive value are also maximal. On pCLE examination, the experienced examiner again shows an almost maximum correlation (0.93). The resident physician achieves a performance similar to that of the NBI examination (0.71) while the experienced nurse does not fall within the correlation limits (0.32, p> 0.05). The experienced doctor and the resident doctor, obtained a maximum correlation in terms of detection of malignant lesions. The experienced assistant gets a correlation of only 0.45. Sensitivity, positive predictive value and negative predictive value were 100% for the first 3. The nurse gets 50%, 50% and 92% respectively.

**NBI correlation, pCLE - histopathological examination for lower digestive tract lesions**

The correlation between the results of the HP exam and those of the endoscopy in NBI mode indicates a high accuracy for the primary physician (0.96) as well as for the nurse (0.93). The performance of the latter emphasizes the importance of an additional experienced observer, especially when endoscopic examinations are performed by resident doctors or even young specialists. The resident doctor participating in the study does not stand out with similar performances but is within the limits of the correlation (0.56). Regarding the detection of malignant lesions, the experienced doctor obtains the best correlation (0.89) and the resident doctor and the experienced nurse obtain similar good correlations (0.761). Sensitivity and positive predictive value were 100% for the experienced endoscopist and 83% for the other two evaluators. The negative predictive value was 92% for all evaluators. At the pCLE examination, the ranking of the 3 evaluators changes. The experienced doctor obtains the best correlation (0.96) followed closely by the resident doctor (0.93). The nurse obtains a modest correlation (0.42). Regarding the correlation malignancy / benignity - histopathological examination, all examiners obtain approximately equal performances (experienced doctor 0.89, experienced nurse 0.89, resident doctor 0.80). Sensitivity and positive predictive value were 100% for all examiners. The negative predictive value was 92% for the experienced endoscopist and the nurse respectively 85% for the resident doctor.
**Discussions**

Upper and lower digestive tract cancers are ideal candidates for inclusion in screening programs especially due to the existence of endoscopic diagnostic and treatment techniques that are constantly evolving. Endoscopy plays a crucial role in diagnosing preneoplastic lesions of the digestive tract and more importantly, offers the chance of detecting cancer in curative stages. The risks associated with diagnostic endoscopy are minimal and the population's access to procedures is increasing. For comparison, Japan has about 30,000 endoscopists serving 127 million citizens and the United Kingdom, with perhaps the best medical system in Europe, has only 3,500 endoscopists for a population of about 55 million.

In Europe, there are currently no clear examination protocols for the detection of preneoplastic lesions of the upper digestive tract or quality standards for upper digestive endoscopy such as those for colonoscopy. Endoscopists are trained more for the detection of major and common pathologies such as esophagitis, peptic ulcer or advanced cancers and not for the detection of minimal changes in the digestive mucosa. In this sense, a protocol for endoscopic analysis of the stomach was implemented, which provides for the taking of 8 photographs during the procedure for a new post-procedural analysis. In contrast, the Japanese protocol considers 20 captures are necessary to analyze the entire gastric mucosa, 3-4 for each area of interest. It is necessary to pay extra attention to the visualization of areas predisposed to the development of neoplasms or difficult to examine such as the upper esophagus, right hemisphere of the lower esophagus, gastroesophageal junction or atrophic gastric mucosa. The sedation of patients is important because low tolerance for endoscopic procedures can negatively influence the outcome of the scan. Any endoscopy performed for diagnostic purposes must last a minimum of 8 minutes: 2 minutes until advancement into the duodenum II, 4 minutes for examination of the gastric mucosa and 2 minutes for examination of the esophagus. Regarding the examination of the lower digestive tract the protocols are much clearer and mastered by many more gastroenterologists. Although the learning curve of colonoscopy is incomparably greater compared to upper digestive endoscopy, the recognition of lesions at risk and their management seems to be assimilated more quickly by clinicians. This fact is proved by our research where a resident doctor and a nurse, additional observer to many procedures, had similar results with the experienced doctor. Whether we are talking about the examination of the upper or lower digestive tract, the first step in detecting preneoplastic lesions is to visualize them in white light: discolored areas of mucosa, excavated or elevated areas of mucosa. New endoscopy systems that provide an HD image
make this much easier. For the second time that requires the characterization of the lesions and implicitly the establishment of the diagnosis and prognosis, the virtual chromoendoscopy techniques were developed. In the upper digestive tract, in terms of detection of preneoplastic lesions, the results are impossible to ignore. In order to highlight high-grade dysplasia in patients with Barrett's esophagus, the use of chromoendoscopic techniques and targeted biopsy collection proved superior to the Seattle protocol, both in terms of histopathological results and in terms of overloading pathological anatomy services. In the case of gastric mucosa examination, classical white light endoscopy does not appear to be sufficient to characterize subtle mucosal changes and the use of the NBI mode may be helpful. It should be noted that the normal gastric mucosa has different aspects when examining the NBI, depending on the area examined. The mucosa of the gastric body is characterized by small round areas surrounded by a capillary network with the appearance of honey figures. The mucosa of the antrum has regular, oval glands with central vascularization. Endoscopy in NBI mode has also been shown to be effective in the diagnosis of gastric intestinal metaplasia. In a multicenter study of approximately 580 patients, intestinal metaplasia was described in 7% of patients when endoscopic examination classic, in white light, respectively 17% when examined in NBI mode. Areas of white-opaque mucosa on examination in NBI mode are suspicious for neoplasia and require attention. In the colon, in NBI mode, the normal colonic mucosa is characterized by a circular and regular pattern of the glands and vessels. The inflamed mucosa has the same characteristics but with thicker and denser vessels. Studies have shown that NBI colonoscopic examination significantly improves the detection rate of adenomas compared to evaluation in white or HD light. The efficiency is comparable to endoscopy with classical chromoendoscopy, in the detection of adenomas and the identification of other lesions. Total NBI colonoscopy is especially recommended in the examination of patients at high risk of developing CRC, Lynch syndrome and polyposis syndromes.

However, there are also studies that refute the usefulness of the method in the detection of neoplastic polyps or dysplastic lesions.

The results in differentiating neoplastic and non-neoplastic colonic polyps were obtained by describing the vascular pattern. It is predictive of the histological stage and even of the level of invasion of cancers in the submucosa. It showed a sensitivity in differentiating malignant lesions from benign ones of 97.5%, equal to that of ME-NBI which associates NBI examination with magnification. However, current classifications do not include serrated polyps which are often difficult to differentiate from hyperplastic or adenomatous
ones. Consequently, the WASP (Workgroup Serrated Polyps and Polyposis) classification was developed that improved the endoscopic diagnosis of serous polyp but more studies are still needed to prove its effectiveness. The use of standardized scales improves diagnostic accuracy and significantly reduces interobserver variability.

Virtual chroendoendoscopy is much easier to use compared to conventional dye-based chroendoendoscopy, but is not used by all clinicians who have it. The situation is caused in most cases by their perception of the training required to use the technique and the additional time required for the examination. We are currently trying to implement a new approach in the management of polyps <5 mm based on the so-called optical biopsies obtained by using high-performance endoscopic techniques, without causing the multiplication of interval cancers. Studies have shown that endoscopy in NBI mode can support the strategies of "diagnose and leave" and "resect and discard" but only in the hands of an experienced endoscopist, as evidenced by the present study.

For our study we used endoscopy in NBI mode available on the new Olympus endoscopy systems. The results obtained for the evaluation of the upper digestive tract complied with the level of training of the evaluators. The correlation of the NBI analysis with the histopathological examination had values increasing from 0.62 for the experienced nurse to 0.73 for the resident doctor and 0.93 for the experienced gastroenterologist. Probably the most important aspect is the fact that no evaluator, regardless of the degree of training, did not underdiagnose a malignant lesion and did not negatively influence the subsequent management. Although the correlation with HP examination, sensitivity, positive predictive value and negative predictive value had maximum values, it is necessary to extend the study to validate the results as the number of patients with malignant degenerative lesions was very small. The appearance is also valid for the sensitivity of NBI endoscopy in the diagnosis of lesions with high degree dysplasia, intestinal metaplasia and gastric mucosal atrophy. Exceptions are hyperplastic lesions and those with low-grade dysplasia, where the values obtained may already have statistical significance. Regarding the lower digestive tract, the correlation of the histopathological examination with the NBI analysis did not respect the level of training of the evaluators. Consequently, the nurse obtains a correlation almost identical to that of the experienced doctor (0.93 / 0.96) and the resident doctor is within the limits of the correlation, but with a value at its lower limit. The explanation may be the nurse's experience and last but not least the lesser variability of the lesions commonly encountered in daily practice at colonoscopic evaluations. From the point of view of the detection of malignant lesions, the performances were not similar to the analysis of upper
digestive tract lesions. The difference was made by a single patient with a benign lesion that was classified as malignant by the experienced doctor. Again, we emphasize that no lesions analyzed by NBI were underdiagnosed, the correlation being very high anyway.

To differentiate the types of polyps, the experienced doctor obtains a sensitivity of 100%, but, as in the case of lesions of the upper digestive tract, additional samples are needed. The present study will be extended to a larger number of patients and the efficiency of the method, interobserver variability and the role of an additional evaluator will be much more strongly supported.

Unlike the NBI technique, available at the touch of a button, the other method of endoscopic analysis, pCLE, requires complex imaging equipment, image processing and still very expensive consumables to be used in daily medical practice. It is an important leap in the development of endoscopy, and the technique of live biopsies has so far proved its effectiveness in several studies, but the success of the examination is not always guaranteed. Experience in the use of equipment and in the interpretation of images proves to be important especially at the first examinations.

In terms of the learning curve, a 2011 study found that new users quickly gain the ability to interpret images obtained with similar efficiency to experienced users. The hypothesis is also supported by the results of our research where the resident doctor, without experience in using the technique and interpreting the images obtained results almost similar to the experienced doctor.

The first study that compared the results obtained at the histopathological examination with the results of the examination with the endomicroscope, was performed by Kiesslich and collaborators when the first classification of the observed changes was established. The study analyzed 13020 images from the examination of 390 lesions. Compared to the classical histopathological examination, an accuracy of 99.2% was obtained, with a sensitivity and specificity of 97.4% and 99.4%, respectively.

Interpretation of images resulting from endomicroscopic analysis is an impediment to the use of this technique due to the limited knowledge of clinicians in the interpretation of histological images. The study aimed in addition to evaluating the effectiveness in the correct diagnosis of preneoplastic lesions and highlighting the importance of clinical experience. Although the number of patients included in the study is not very high, as in the case of endoscopy in NBI mode, we aim to continue the study on a larger group to validate the results obtained. In addition, we want the images obtained to be analyzed with a computer analysis software, with the storage of parameters that provide an automatic diagnosis in the
future. This eliminates the subjective factor and inter-observer variability. For upper gastrointestinal lesions, endomicroscopy has so far shown, according to several studies, a specificity and sensitivity of 56% and 100% in the diagnosis of Barrett’s esophagus, 88% and 90.9% in the case of gastric intestinal metaplasia and an accuracy of 97% in diagnosis of gastric adenocarcinomas in early forms.

The results of our study differ significantly depending on the location of the lesions.

For the upper digestive tract, the correlation of the histopathological examination result with the result of the pCLE examination is almost maximum (0.925) for the experienced gastroenterologist, with training in endomicroscopy. Also, the resident doctor obtains a good correlation (0.717) if we refer to the minimum experience in endomicroscopy. The nurse’s results do not fall within the correlation limits (0.321). Minimum knowledge of histology and high variability of the examined lesions are probably the main causes that can be incriminated. The trend is also observed in terms of the detection rate of malignant degenerative lesions. The correlation is in this case maximum for both the experienced doctor and the resident doctor. The nurse does not fall within the limit of high correlation (0.45). The results obtained after the examination of the lower digestive tract show the same significant differences compared to the level of training of the examiners. The resident physician and the experienced examiner approach the maximum correlation, while the nurse does not fall within the limits of high correlation. For the diagnosis of malignant lesions, all examiners obtain satisfactory results, close to the maximum correlation. It should be noted that in this case the nurse obtains a higher correlation value compared to the resident doctor and equal to the experienced doctor.

Endomicroscopy obtained better results in the characterization of colonic polyps and in the differentiation of the neoplastic ones from the non-neoplastic ones compared to the virtual chromoendoscopy (91% and 77% respectively). In our research, the results are comparable in terms of sensitivity in detecting the degree of dysplasia, except for high-grade dysplasia where pCLE proves superior: NBI (100% for malignancy, 66% for high-grade dysplasia, 100% for dysplasia low grade, 100% for hyperplasia), pCLE (100% for malignancy, 83% for high grade dysplasia, 100% for low grade dysplasia, 100% for hyperplasia). Due to these performances, pCLE can be a real help in implementing the “view and leave” or “resect and throw” strategy.
Conclusions

Digestive endoscopy in NBI mode available on state-of-the-art endoscopy systems proves its effectiveness in our study, and promises good results even in the hands of a less experienced endoscopist. The additional observer, in this case an experienced nurse, can positively influence the diagnosis in the case of digestive endoscopy in NBI mode, if the main examiner is less experienced. On the other hand, pCLE is a revolutionary method of diagnosing preneoplastic and neoplastic lesions of the digestive tract, with results almost similar to the HP exam when used by a gastroenterologist trained in the use of the technique. The learning curve of pCLE is not very large and the results of an uninitiated user are close to those of an experienced doctor. However the results of pCLE still have an important variability depending on the experience of the examining physician. It is necessary to implement an image analysis algorithm to reduce interobserver variability.