Evolving endoscopic technologies for the detection of dysplasia in inflammatory bowel diseases

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Patients with long-standing and extensive ulcerative colitis (UC) and colonic Chron's disease (CD) have an increased risk of CRC compared with the general population. Although no large controlled trials have proven that surveillance reduces mortality, cancer prevention in inflammatory bowel disease depends on the detection of dysplasia during scheduled surveillance colonoscopy and is widely recommended by gastroenterological associations. Dysplasia in IBD may occur in flat mucosa or in raised lesions (DALM) which have sometimes endoscopic features similar to adenoma (adenoma-like DALM). Recently, new endoscopic techniques to facilitate the distinction between dysplastic and actively inflamed or normal mucosa have been proposed. Chromoendoscopy significantly increases the sensitivity of detecting subtle dysplastic lesions and has emerged as the new standard of cancer surveillance in patients with IBD. Confocal laser endomicroscopy (CLE) is a novel technique that enables the endoscopist to obtain real time in vivo microscopic images of the gastrointestinal mucosa and can be used for targeting biopsies to relevant areas. CLE in conjunction with chromoendoscopy proved able to increase the diagnostic yield of dysplasia in ulcerative colitis and reduce the number of biopsies needed. The role of digital filtering technologies (virtual chromoendoscopy) and autofluorescence in IBD surveillance will be also discussed.

KEY WORDS: Chromoendoscopy, Confocal laser endomicroscopy, Displasia, IBD.

Patients with inflammatory bowel disease (IBD) are at increased risk for colorectal cancer (CRC) [1]. Although the precise extent of this risk is difficult to quantify due to methodological and selection biases of different studies, in a large meta-analysis involving patients with ulcerative colitis. Eaden et al. have estimated that cumulative risk of CRC is 2% at 10 years, 8% at 20 years, and 18% after 30 years of disease [2]. Recent population-based studies have shown that CRC risk is equivalent among ulcerative colitis (UC) and Crohn's disease (CD) patients and is approximately 2-3 fold greater that general population. Factors contributing to the development of CRC include disease duration, anatomic extent, severity of inflammation [3], association with primary sclerosing cholangitis (PSC) [4] and a family history of sporadic CRC [5,6]. Macroscopically, dysplasia in IBD can be classified as raised or elevated and flat depending on whether it can be detected by endoscopy. Raised dysplasia encompasses endoscopically detectable lesions often referred as dysplasia-associated lesion or mass (DALM). These lesions appear similar to the inflammatory abnormalities often encountered in IBD colon with active inflammation, making them difficult to be recognized by even experienced endoscopists. Since the inconsistency in the literature on the criteria to define DALM, raised dysplasia has been recently categorized into "adenoma-like" and "non-adenoma-like" DALMs. These lesions have a different natural history, risk of malignancy and treatment, making this classification useful both microscopically and clinically, with important implications in the management and raised dysplastic lesions [7]. Although flat dysplasia refers to non visible lesions, the recent use of

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enhancing endoscopic modalities have allowed to described with this term even slightly raised endoscopically detectable lesions.
Although no large controlled trials have proven that surveillance reduces mortality, cancer prevention in inflammatory bowel disease depends on the detection of dysplasia during scheduled surveillance colonoscopy and is widely recommended by gastroenterological associations. However, the detection and diagnosis of dysplasia, remain challenging. Careful inspection and 2 to 4 random biopsies for every 10 cm of the whole colon has been recommended [8]; this approach is time consuming and dysplastic lesions, particularly flat or slightly elevated, can still be missed. Recently, new endoscopic techniques to facilitate the distinction between dysplastic and actively inflamed or normal mucosa have been proposed.

Chromoendoscopy

Indigo carmine and methylene blue and are the most commonly used dyes in UC surveillance. Indigo carmine is an example of contrast dye that coats mucosa enhancing the recognition of mucosal changes or irregularities not perceived by white light endoscopy. Methylene blue is an absorptive dye which stains non-inflamed mucosa but is poorly absorbed by inflamed zones and dysplastic epithelium. Briefly, the procedure consists in the preparation of 0.1% indigo carmine or 0.1% methylene blue (100 mL of either) drawn into 50-mL syringes; a dye-spray catheter is inserted down the bioptic channel of the endoscope and the tip is protruded 2 to 3 cm. During colonoscope withdrawing the dye is applied in the entire colonic mucosa (panchromoendoscopy). The SURFACE guidelines have been introduced for standardize and implement the use of chromoendoscopy in the IBD surveillance programme [9]. Bowel cleansing and thorough examination of the colonic mucosa, like any screening or surveillance colonoscopy is of paramount importance and the need of appropriately trained endoscopists has been also underlined.
Several recent studies on cromoendoscopy have demonstrated a greater diagnostic yield for dysplastic lesions in chronic colitis [11-15]. Kiesslich et al. randomized patients with longstanding UC to conventional endoscopy or methylene blue chromoendoscopy, showing that in the chromoendoscopy group more targeted biopsies were possible and more dysplasia detected [14].
The efficacy of pancolonic chromoendoscopy with indigo carmine dye spraying to recognize intraepithelial neoplasias in patients with longstanding UC has been shown in a "back to back" colonoscopy study by Rutter et al.: during the pre-dye spray colonoscopy only two dysplastic lesions were detected whereas other seven were highlighted by the dye spray [13]. These findings support the concept that pancolonic chromoendoscopy and targeted biopsies are more effective than routine endoscopy with random biopsies.
The use of magnifying endoscope in conjunction of chromoendoscopy allows the detection of flat lesions and a detailed surface mucosa examination. Neoplastic changes can be recognized and the malignant potential of a suspected lesion predicted through the crypt architecture analysis and applying the pit pattern classification. According to the pit pattern classification five major types can be distinguished: pit pattern types I and II are considered non-neoplastic lesions, types III to V are compatible with dysplastic and neoplastic lesions [10]. Recent data seem to suggest that high resolution and high definition endoscopy can also provide sufficient details to characterize the superficial mucosal pattern.
Hurlstone et al in a prospective controlled study on 162 patients with longstanding UC has demonstrated that magnifying chromoendoscopy with indigo carmine significantly increase the diagnostic yield for epithelial neoplasia and flat dysplasia compared to conventional colonoscopy; the overall sensitivity of magnifying chromoendoscopy in predicting neoplasia was 97% with a specificity of 93% [15].
Concern about the duration of the endoscopic procedure when the dye spraying is applied has been raised; however data from Kiesslich et al have shown that the procedure time of chromoendoscopy do not significantly exceed conventional colonoscopy. Moreover, since it has been estimated that the number of random biopsies needed to exclude dysplasia is nearly double than when the dye enhancement is applied, dye spray targeted-biopsies might reduce the pathologist workload.
Chromoendoscopy increased the diagnostic yield of dysplasia 3- to 4.5- fold compared with conventional colonoscopy [16] and its use by trained endoscopists has been endorsed in the Crohn's & Colitis Foundation of America consensus conference recommendations and the British Society of Gastroenterology (BSG) guidelines for surveillance of patients with long-standing IBD [9,8].

Narrow band imaging (NBI)

Angiogenesis plays an important role in IBD pathogenesis and represent a potential therapeutic target. During malignant progression, mucosal pit pattern changes are associated with alterations of the superficial microvasculature network as part of the the so called "tumour neoangiogenesis". Narrow band imaging (NBI) represents a new endoscopic technology that enhances mucosal surface structures and vascular architecture. Since this technique do not imply the use of dye spraying, the term of "digital chromoendoscopy" has been coined when referring to NBI or to other similar filter post-processing modalities such as i-scan (Pentax) and Fuji Intelligent Chromo Endoscopy (Fuji) [17]. NBI uses narrow-bandwidth filters in a red-green-blue (R/G/B) sequential illumination system which has different penetration through

the mucosa depth. Blue band lights, corresponding to 415 nm absorption which is typical for haemoglobin, enhances the images of capillary vessels on surface mucosa whereas thick vessels located in the deep mucosa layer are detected by the red band light (600nm) [18]; when combined, a great contrast between vascular pattern and the surrounding mucosa is achieved, allowing the characterization of the surface epithelium architecture (pit pattern) and the analysis of the vascular network [19]. NBI can detect pit pattern and vascular pattern abnormalities differentiating inflammatory and neoplastic lesions in the mucosa of esophagus, stomach and large bowel. NBI and chromoendoscopy have the same sensitivity and specificity in differentiating neoplastic from non neoplastic lesions and both are superior to conventional white light endoscopy [20]. The role of NBI in CRC surveillance in patients with IBD is still under scrutiny. There is one prospective cross-over study by Dekker et al. analyzing the feasibility of NBI in detecting colitis-associated dysplasia in 42 patients with longstanding UC. Although more suspicious lesions were found during NBI, the overall sensitivity for neoplasia was comparable to conventional endoscopy [21]. Since NBI focuses on capillaries abnormalities, it can be anticipated that detecting and differentiating dysplasia associated angiogenesis from similar changes due to the inflammatory background would be difficult. Based on these data, large-scale clinical trial are required to establish the role of NBI in the surveillance of longstanding IBD.

Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is an imaging technology frequently used in ophthalmology and more recently applied in the gastrointestinal tract. OCT is an optical analogue of ultrasound, based on light waves instead of acoustical waves which provides real time cross-sectional images formed by back-scattered light reflected from different tissue microstructures [22]. OCT can predict malignant infiltration of the submucosa of neoplastic lesions, assisting endoscopists in selecting the appropriate endoscopic approach for malignancies infiltrating only the upper third of the submucosal layer.

In the IBD field, OCT can effectively detect transmural inflammation and distinguish Crohn's colitis from other forms of colitis [23]. The current resolution of OCT is not sufficient for visualize the nuclear features of dysplasia but only the architectural changes of glands and crypts. For this reason the diagnostic potential of OCT in IBD CRC surveillance is probably restricted to raised neoplasia such as adenoma and non adenoma-like DALM.

Fluorescence endoscopy

Fluorescence endoscopy has been used for the early detection of neoplastic lesions in different organs such as oesophagus, bronchi and bladder. In this technique, a sensitiser, which accumulates selectively in malignant and premalignant tissue, fluoresces under blue light excitation and enables targeted biopsies. 5-Aminolaevulinic acid (5-ALA), a prodrug in haeme biosynthesis, is converted within the cells into the sensitising agent protoporphyrin IX (PPIX) which accumulates selectively in malignant tissue. In an animal model of IBD (dextran sulphate sodium induced colitis) 5-ALA induced photosensitisation enabled the detection of dysplastic lesions in a dose dependent manner, with high sensitivity but low specificity due to false positive fluorescence induced by inflammation. It has been (24*)* demonstrated that higher concentrations of 5-ALA are needed in the lower compared with the upper gastrointestinal tract to selectively unmask neoplastic lesions. Messmann et al. have assessed the ability of FE to detect dysplasia in patients with UC by either local application or oral administration of 5-ALA. Local application was superior to systemic sensitisation in detecting dysplasia and appeared to be appropriate even for patients with pancolitis [25]. The higher sensitivity and negative predictive values showed with local sensitisation seem to suggest that in patients with no selective fluorescence after local application with 5-ALA in their colon the presence of dysplasia can almost be excluded.

The combination with fiber endoscope has been shown to alter the value of fluorescence endoscopy. However, a new system combining autofluorescence and video endoscope has been developed (Olympus, Japan). Auflorescence imaging (AFI) relies upon endogenous fluorophores changes during malignant transformation. Differences in mitochondria, lysosomes and submucosal collagen autofluorescence make dysplastic lesions purple coloured whereas normal tissue appear gren.

It can be anticipated that this and other technological improvements will expand the clinical use of fluorescence endoscopy, enabling optical guided biopsies and increasing the detection of dysplasia.

Confocal laser endomicroscopy (CLE)

CLE is a novel technique that enables the endoscopist to obtain real time in vivo microscopic images of the gastrointestinal mucosa. Currently, two different CLE systems are available including an integrated endoscopy system (iCLE, Pentax Endomicroscopy, Japan) and a probe-based system (pCLE, Cellvizio Endomicroscopy, MKT, France). The iCLE consists of a confocal fluorescence microscope integrated in the distal tip of a white light endoscope which can collect images with adjustable depth level of scanning from 0 to 250 µm. Since the integrated lens is slightly prominent to the tip, the mucosal lesion can be targeted under videoendoscopic control and both endoscopic and emdomicroscopic

images can be simultaneously displayed. The working channel can be used to stabilize the contact between the endoscope and the target either applying suction to the mucosal area of interest or by grasping "flapping" lesions (e.g. peduncolated polyps) with conventional biopsy forceps. The pCLE consists of a confocal probe which can be easily advanced through the working channel and employ a single laser power and a fixed imaging plane depth at 60 µm. Confocal images are streamed at a rate of 12 frames per second and real time videos of the mucosa can be acquired. Moreover, increased field of view can be obtained in real time or post-processed via a computer algorithm termed "mosaicing" (Fig. 1). In both CLE systems a non toxic fluorescent agent (fluorescein 10%) is administered intravenously; fluorescein diffuses to the vasculature and reach tissues through capillaries. High contrast images of vessel architecture and extracellular matrix are obtained with cellular and subcellular details. Direct nuclear characterization for defining and grading intraepithelial neoplasia is not possible due to the fact that fluorescein does not stain nuclei. However, changes in cellular, crypt and vessel architecture can reliably predict the histology and distinguish between inflammatory, regenerative and neoplastic tissue. Dedicated training in the endoscope handling and imaging interpretation are needed for both systems and close collaboration with an expert pathologist is also recommended. Since the first introduction in 2004, several studies have addressed the clinical use of CLE in different parts of the gastrointestinal tract. Barrett's esophagus and associated intraepithelial neoplasia can be detected with high accuracy and interobserver agreement [26]. CLE was also able to highly predict squamous cell carcinoma [27] and precancerous gastric lesions [28]. Confocal images can predict histology of colorectal neoplasms during screening colonoscopy with high specificity and sensitivity (97.4% and 99.4% respectively). In IBD endomicroscopy not only can detect mucosal alterations but also assess the degree of inflammation activity. Since it is not possible to examine the whole surface of the colonic mucosa in the endomicroscopy mode, it is essential to combine CLE with a "red flag" technique such as chromoendoscopy. Chromoendoscopy can reveal circumscribed lesions and subsequent confocal analysis can guide targeted biopsies. Kiesslich et al demonstrated that chromoendoscopy and confocal laser endomicroscopy are capable to detect dysplasia in UC patients with longstanding disease with 94.7% sensitivity, 98.3% specificity, and 97.8% accuracy and significantly reduce the number of biopsies needed [29]. In IBD surveillance, CLE can distinguish in real time hyperplastic and inflammatory lesions from adenoma (fig. 3) and non adenoma-like DALM, leading to a correct management of raised lesions. Adenoma like DALM located outside and within area of colitis could be treated conservatively by endoscopic polypectomy and followed up regularly if the lesion has been completely excised and presence of dysplasia has been ruled out from resection margins and the mucosa adjacent or distant to the lesion.

Molecular imaging (Immunoendomicroscopy)

Targeting of cell surface molecules that are up regulated at an early disease stage represents an attractive approach to identify lesions based on the molecular fingerprint rather than morphology or endoscopic appearance. CLE is currently the only device compatible with

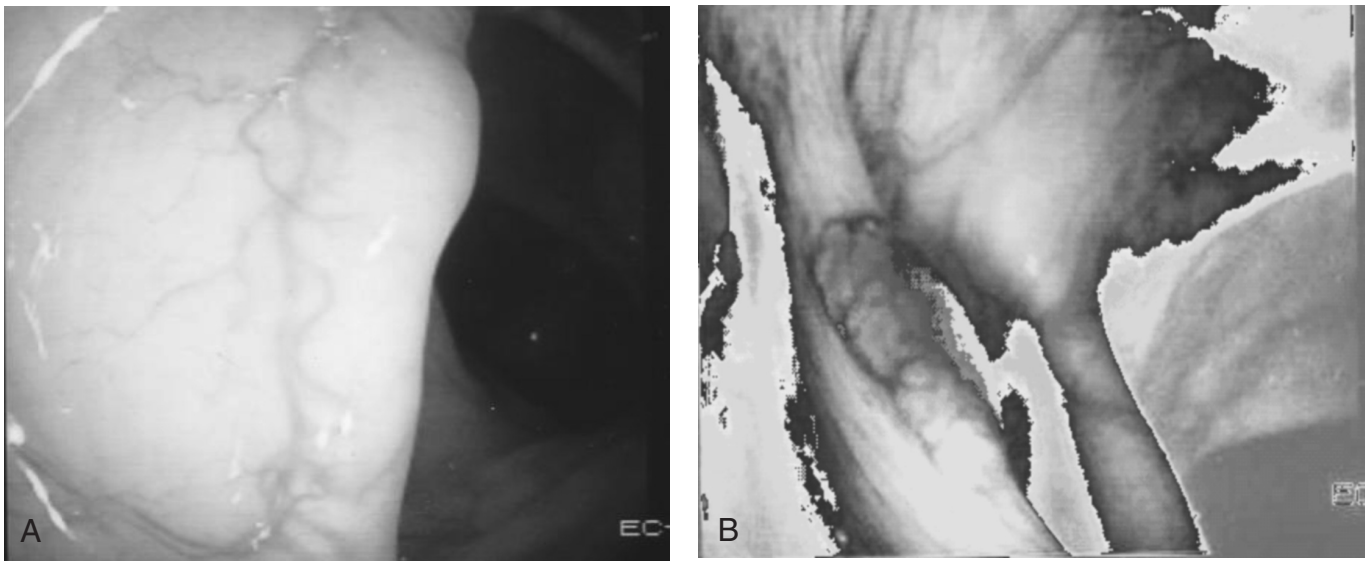

Fig. 1: Slightly elevated lesion (IIa Paris classification) in a 53 year old male with long standing UC in clinical and endoscopic remission. Conventional white light endoscopy revealed a slightly elevated lesion (A); surface architecture and border were better visualized after chromoendoscopy with indigo carmine. Histology demonstrated a sessile serrated polyp.

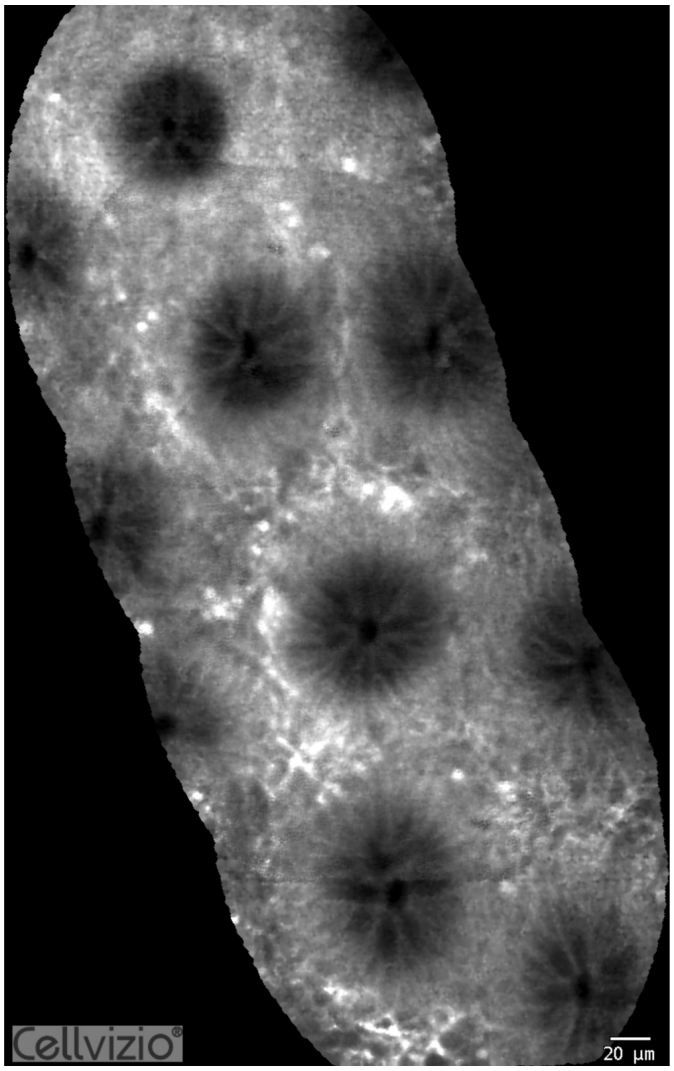

Fig. 2: pCLE images (Cellvizio, MKT, France) of normal colonic mucosa. Mosaic showing typical arrangement of crypts, goblet cells and regular vessel distribution.

conventional endoscopy to efficiently detect and characterize molecular fluorescent probes against marker of neoplasia. Initial in vivo animal studies have now been translated into humans. Using topical application of a labelled peptide with specific binding to colonic neoplasia, endomicroscopy was able to differentiate stained neoplastic crypts from non-neoplastic unstained crypts and detect adenoma during colonoscopy [30]. Confocal endomicroscopy selectively detected fluorescent labelled antibodies against molecules overexpressed in GI cancer such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) in experimental models [31,32].

In IBD molecular and functional imaging could dramatically improve the efficiency of surveillance and the ability to detect flat dysplasia. In vivo molecular characterization of colonic epithelium will allow to distinguish dysplasia in normal appearing mucosa and guide

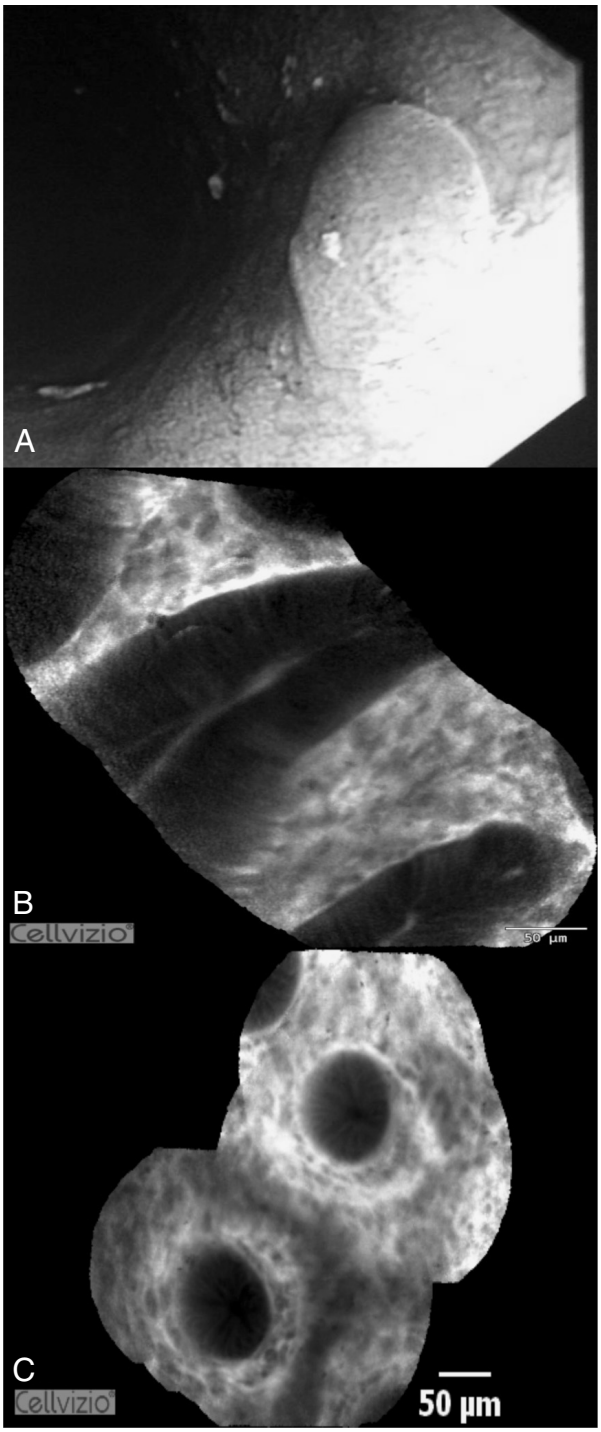

Fig. 3: A) Endoscopic appearance of a slightly raised lesion (IIa Paris classification) in mild UC (Mayo endoscopic score =1); B) Endomicroscopy analysis showing epithelial cells thickening, irregular crypt architecture and depletion of goblet cells. Histology revealed a tubular adenoma with low grade dysplasia with absence of dysplasia in the resection margins and surrounding mucosa C). Endomicroscopy of mild UC surrounding the lesion. Increased intercryptic distance and lamina propria thickening are observed due to the presence of inflammatory infiltrate; although cellular architecture is preserved, crypts' diameters are reduced due to gland regeneration and goblet cells depletion can be identified. Increased number and irregular course of blood vessels in the lamina propria and fluorescein extravasation (bright dye outside the blood vessels) due to increased microvascular permeability can be observed. B) and C) images are obtained by pCLE, Cellvizio, MKT, France).

targeted biopsies reducing sampling errors and biases due to tissue processing during conventional immunohistochemistry.

Conclusion

Longstanding UC and Crohn's colitis are associated to a well established increased risk of CRC. The goal of surveillance is to detect neoplastic changes preceding invasive tumours. Thus, correct detection and interpretation of dysplasia is crucial. High resolution chromoendoscopy in combination with targeted biopsies has emerged as the new standard of CRC surveillance in patients with IBD and incorporated into clinical guidelines [9,8]. New enhanced endoscopy techniques including NBI, I-scan and FICE can highlight subtle irregularities in the colonic mucosa without the application of dyes ("virtual chromoendoscopy") and improve detection and classification of colonic neoplasms; AFI can recognize the changes in the endogenous fluorophores in dysplastic tissue which appear purple compared to the greenish colour of normal mucosa. However the real value of these technologies in IBD surveillance is still to be determined. CLE systems, either endoscope integrated or probe based, allows in vivo real time microscopic examination of intestinal mucosa at subcellular level. Like any other novel endoscopic technique, CLE needs training in the endoscope handling and in the imaging interpretation. In patients with IBD, CLE has been proven to accurately recognize mucosal alterations and assess inflammatory activity. Indeed, chromoendoscopy with CLE resulted in higher diagnostic yield for neoplastic changes and significantly reduced the number of biopsies needed. The ability to differentiate and characterize in real time raised dysplasia could have important implications for the correct endoscopic management of these lesions. Molecular imaging in addition to videoendomicroscopy has the potential to disclose a new era in the endoscopic surveillance and CRC prevention in IBD.

Riassunto

I pazienti affetti da colite ulcerosa (CU) e malattia di Crohn (MC) a localizzazione colica e di lunga durata presentano un aumentato rischio di neoplasia colo-rettale rispetto alla popolazione generale.
Sebbene non ci siano studi controllati che dimostrino che i programmi di sorveglianza endoscopica riducano il rischio di cancro del colo-rettale, la prevenzione del cancro nei pazienti con malattia infiammatoria cronica intestinale (IBD) si basa sul riconoscimento della displasia in corso di colonscopia. Il programma di sorveglianza endoscopica, infatti, è ampiamente raccomandato dalle maggiori società di gastroenterologia. Nei pazienti affetti da IBD la displasia può insorgere su mucosa piatta o come lesione protrudente (DALM). Recentemente sono state proposte nuove tecniche encoscopiche al fine di facilitare la distinzione tra lesioni displastiche, lesioni di tipo infiammatorio e mucosa normale.La cromoendoscopica aumenta significativamente la sensibilità nel diagnosticare minute lesioni displastiche ed è riconosciuta come tecnica standard per la sorveglianza del cancro colorettale per i pazienti con malattia infiammatoria cronica intestinale. L'endomicroscopia confocale è una nuova tecnologia che consente all'endoscopista di ottenere in tempo reale immagini microscopiche *in vivo* della mucosa del tratto gastrointestinale ed effetuare biopsie mirate. È dimostrato come la cromoendoscopia associata ad endomicroscopia confocale incrementi l'accuratezza diagnostica nell'individuare lesioni displastiche nei pazienti affetti da CU riducendo il numero di biopsie necessarie. Verrà discusso inoltre il ruolo di tecnologie che sfruttano la modulazione difitale dei filtri cromatici (cromoendoscopia digitale) e dell'autofluorescenza nell'ambito della sorveglianza di pazienti affetti da IBD.

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