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Review article: in vivo imaging by endocytoscopy

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Review article:

in vivo imaging by endocytoscopy

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Review on Endocytoscopy ¶

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1 Keywords: Endocytoscopy; Ecy; eCLE; pCLE; iCLE; endomicroscopy; red flag technique; in vivo;
2
3 confocal; magnification; Barrett; IBD; ulcerative colitis; polyp; cancer; celiac disease; Helicobacter
4 pylori; endoscopy; methylene blue; chromoendoscopy; toluidine blue; review; systematic; advanced
5 endoscopic imaging; pEC, iEC.
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10
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15 RA: none

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18 MFN: Pentax research grant, speaker fee, and consultant.
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Abstract

Introduction: Endocytoscopy (EC) enables *in vivo* microscopic imaging at 1400-fold magnification, thereby allowing the analysis of mucosal structures at the cellular level. In contrast to fluorescence imaging with confocal laser endomicroscopy which allows analysis of mucosal structures up to 250 µm in depth, EC is based on the principle of contact light microscopy and only allows visualization of the very superficial mucosal layer.

Aim: To systematically review the feasibility and diagnostic yield of EC for *in vivo* diagnosis of diseases.

Material and Methods: A systematic search of the literature on diagnostic interventions in the gastrointestinal tract using EC was performed by searches in MEDLINE, Current Contents, PubMed, cross references and references from relevant articles using the search terms “endocytoscopy”, “endocytoscope”, “magnification endoscopy”, “endocytoscopic imaging”, “virtual histology”, and “optical biopsy”. Only full manuscripts and case reports published in English were included.

Results: Overall twenty-nine relevant reports were identified. EC was feasible to detect esophageal squamous cell cancer with sensitivity, specificity and accuracy of 95%, 84% and 82%, respectively. Moreover, EC reached excellent sensitivity and specificity for *in vivo* diagnosis of colon polyps (91% and 100%, respectively). Other diagnostic applications of EC included diagnosis of Barrett’s oesophagus, *Helicobacter pylori*, celiac disease, and small cell lung cancer. No serious complications of EC have yet been reported.

Conclusions: EC is a safe and effective new endoscopic imaging technique to obtain *in vivo* histology and guided biopsies with high diagnostic accuracy. Therefore, EC has the potential to facilitate both diagnosis and patient management.

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Deleted: colon polyps

Deleted: EC was able to characterize cellular changes *in vivo* and permitted guided biopsies with high diagnostic yield.

Introduction

„Whatever you can do or dream you can, begin it. Boldness has genius, power, and magic in it.”

Johann Wolfgang von Goethe, German poet.

Recent advances in endoscopic imaging have substantially influenced the diagnostic approach of various gastrointestinal diseases and our understanding of disease pathogenesis. Traditionally, standard white light endoscopy only allows the investigation of mucosal surfaces and surrounding blood vessels at a magnification of about 70-fold. Subsequent histopathological analysis of biopsy specimens remained the gold standard for final diagnosis and corresponding patients management.

Emerging endoscopic imaging modalities including both, vital (e.g. methylene blue, cresyl violet) and virtual chromoendoscopy (Narrow band imaging, NBI; Fujinon intelligent color enhancement, FICE; i-Scan) and magnification endoscopy enabled the endoscopist to visualize and interpret increasing mucosal details [1].

By introducing confocal laser endomicroscopy in 2004 *in vivo* microscopic imaging at 1000-fold magnification became possible, now allowing the endoscopist to obtain real time *in vivo* optical biopsies during ongoing endoscopy [2]. Currently, two endomicroscopy systems are available. One is integrated in the distal tip of a high-resolution standard video endoscope (iCLE; Pentax, Tokyo, Japan) one is probe-based and capable of passage through the accessory channel of a standard endoscope (pCLE; Cellvizio, Mauna Kea Technologies, Paris, France). Various studies have shown the potential of both types of endomicroscopy-systems for *in vivo* diagnosis of different disease entities including esophageal squamous cell

1 carcinoma, Barrett's esophagus, *Helicobacter pylori* infection, celiac disease,
2
3 ulcerative colitis and colon polyps [3].
4

5 Endocytoscopy (Olympus, Tokyo, Japan) was recently introduced and represents
6 another emerging endoscopic imaging technique enabling real time *in vivo* diagnosis
7 of cellular patterns at extremely high magnification. Recent published data indicate
8
9 that endocytoscopy has the potential to change the current paradigm for the
10
11 diagnosis of various diseases (Table 1). Nevertheless, to date no systematic review
12
13 on this emerging endoscopic imaging technique has been performed. The aim of this
14
15 systematic review was therefore to summarize the diagnostic yield and relevance of
16
17 endocytoscopy in the clinical setting.
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Deleted: Besides this fascinating technique, endocytoscopy (Olympus, Tokyo, Japan) allows real time *in vivo* diagnosis

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23 **Methods and Techniques**

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25 A systematic search of the literature on diagnostic interventions in the gastrointestinal
26
27 tract using endocytoscopy was performed by searches of MEDLINE, Current
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29 Contents, PubMed, cross references and references from relevant articles using the
30
31 search terms "endocytoscopy", "endocytoscope", "magnification endoscopy",
32
33 "endocytoscopic imaging", "virtual histology", and "optical biopsy". Only papers and
34
35 case reports published as full text in English were included. Abstracts were not
36
37 considered for this review. The literature search was performed in December 2010.
38
39 Overall, twenty-nine suitable manuscripts on endocytoscopy were finally identified.
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43 **Techniques of endocytoscopy**

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45 Endocytoscopy is based on the principle of contact light microscopy. The system
46
47 consists of a fixed-focus, high-power objective lens that projects magnified images
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49 from the sampling site onto a charge-coupled device [4].
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Mucosal preparation

To enable ultra high-magnification imaging using the endocytoscope adequate bowel preparation and mucosal preparation is necessary. Based on its principle of contact light microscopy and in contrast to endomicroscopy, endocytoscopy only allows visualization of the very superficial mucosal layer. The light focusing depth ranges from 0 to 50 μm . Therefore, mucolysis using *N-acetylcysteine* is recommended before the procedure to remove surface mucus [4]. Additionally, prestaining of the mucosa with an absorptive agent is mandatory. One recent study evaluated the optimal staining regimen for endocytoscopy (pEC; XEC-300) using different dye concentrations of crystal violet, methylene blue and toluidine blue [5]. Optimal conditions for endoscopic mucosal imaging were found after staining with 1% methylene blue in the esophagus and with 0.25% toluidine blue in the stomach and colon. The dyes are usually sprayed onto the mucosal surface using standard spraying catheters. After an appropriate time of exposure (about 60 seconds) to the dye, repeat washing of the mucosa is necessary to remove excess contrast dye before high magnification imaging. While using absorptive contrast agents, for extended visualization repeat staining of the mucosa may be needed [4].

Noteworthy, previous tattooing of suspicious lesions (for example for surveillance examinations) may limit the use of endocytoscopy (pEC; XEC-300) due to decreased image quality because the black pigment is likely to interfere optically with staining of crypts [6].

1 *Procedure*

2
3 Currently, two types of endocytoscopes are available (Table 2) [4]. One is integrated
4
5 in the distal tip of a standard endoscope (iEC) and one is probe-based (pEC, Figure
6
7 1). Endocytoscopy images are displayed on a monitor at 30 frames per second,
8
9 which corresponds to the frame rate during routine high-resolution video endoscopy.
10
11 Integrated endocytoscopy systems use two separated lenses and are integrated into
12
13 an 80-fold magnification endoscope enabling 450-fold magnification (field of view 400
14
15 x 400 μm). Recently, another endocytoscopy system was introduced consisting of
16
17 only one lens that can consecutively increase the magnification up to 380-fold
18
19 magnification using a hand lever (GIF-Y0002; field of view 700x600 μm). For the first
20
21 time, this new endoscope-generation enables continues magnification from standard
22
23 overview to endocytoscopy therefore representing an 'all-in-one' scope. Upper
24
25 endoscopes (XGIF-Q260EC1) are 103 cm and lower endoscopes (XCF-Q260EC1)
26
27 133 cm in length (Table 2).

28
29 Probe-based endocytoscopy consist of handheld miniprobes, which are capable of
30
31 being inserted through the accessory channel of a standard endoscope. Two
32
33 different pEC devices exist, providing either 450-fold (XEC 300F) or 1390-fold (XEC
34
35 120U) magnification images on a 19-inch monitor. The probes are 380 cm in length
36
37 and 3.2 mm in diameter thus requiring an accessory channel of 3.7 mm. By
38
39 introducing the pEC-device through the working channel targeted optical biopsy
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41 under guidance of the mother endoscope becomes possible. Additionally, two
42
43 monitors can be used during the procedure; one is displaying the endoscopic picture,
44
45 one the *in vivo* histologic picture. To avoid motion artefacts during the
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47 endocytoscopic observation of mucosal structures, a soft short (4 mm) plastic cap is
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49 recommended to be affixed at the tip of the endoscope (Figure 2). By using slight
50
51 suctioning the probe is stabilized against the mucosal wall and high-magnification
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1 imaging in focus become possible. The horizontal observation field is given with 300
2
3 x 300 μm (0.09 mm^2) for the 450-fold magnification probe and with 120 x 120 μm for
4
5 the 1390-fold magnification probe.
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10 *Interpretation*

11 Interpretation of optical biopsies is based on several architectural details (e.g.
12
13 epithelial structure), cellular features (e.g. size, arrangement of cells) and vascular
14
15 patterns (e.g. size, leakage, and tortuosity). In addition to these factors,
16
17 endocytoscopy enables the assessment of cytological features, such as the density
18
19 of cells, the size and shape of nuclei, and the nucleus-to-cytoplasm ratio (Figure 3)
20
21 [4]. These are inevitable features for the diagnosis of intraepithelial neoplasia
22
23 (formerly termed dysplasia) and cancer. Additionally, by appraisal of the staining
24
25 pattern differentiation of mucosal inflammatory cells become possible (personal
26
27 unpublished data). Eberl et al. evaluated if endocytoscopy (pEC; XEC-120 and XEC-
28
29 300) could predict histology in neoplastic lesions [7]. Therefore, twenty-five patients
30
31 with esophageal lesions, twenty-eight patients with colonic lesions, and twenty-three
32
33 patients with gastric lesions were examined. The sensitivity and specificity for the
34
35 evaluation of the blinded pathologist was 81% and 100%, respectively, in the
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37 esophagus, 56% and 89% in the stomach, and 79% and 90% in the colon.
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39 Nevertheless, to date no prospective study has validated endocytoscopy-based
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41 image criteria. Importantly, if an endoscopist evaluated the endocytoscopic pictures
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43 in combination with the macroscopic endoscopic images sensitivity and specificity
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45 increased significantly (sensitivity 96%, specificity 95%).
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1 *Comparison of endocytoscopy to magnification endoscopy and endomicroscopy*

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3 Endocytoscopy, magnification endoscopy and endomicroscopy represent completely
4 different endoscopic techniques and all require totally different endoscopes.
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9 As mentioned above, endocytoscopy is based on the principle of contact light
10 microscopy. *In vivo* imaging is achieved by a fixed-focus, high-power objective lens
11 that projects images onto a charge-coupled device [4]. Therefore, the depth of field is
12 dependent on the absorption spectra and light penetration depth of the intestinal
13 tissue and can not be altered manually. Because absorption spectra and light
14 penetration depth are themselves dependent on tissue structure (e.g. squamous
15 epithelium, cylindric epithelium) and composition (e.g. inflammation, haemoglobin)
16 the light focusing depth ranges theoretically from 0 μm up to 50 μm [8]. Of note it is
17 currently not possible to determine the exact level of endocytoscopic imaging.
18
19 Furthermore, in order to realize ultra high-magnification imaging up to 1400-fold,
20
21 prestaining of the mucosa with either methylene blue ([Akorn, Lake Forest, USA](#)) or
22 toluidine blue ([Dr. Franz Köhler Chemie, Bensheim, Germany](#)) is strictly necessary.
23
24 Consequently, endocytoscopy is able to identify cellular (e.g. visibility of nuclei) and
25 architectural features of the surface epithelial layer. In contrast to endomicroscopy,
26 endocytoscopy does not allow the visualization of deeper layers of the epithelium and
27
28 is therefore not suitable to evaluate the depth of invasion in early neoplasia. In
29
30 addition, concerns about the use of methylene blue have risen, which may increase
31
32 DNA damage in the tissue after illumination [9]. As a consequence, methylene blue
33 was recently withdrawn from the German market but is still available in other
34 countries (i.e. USA). Toluidine blue is approved by the Federal Institute for Drugs and
35 Medical Devices for vital staining. To the best of our knowledge, no concerns
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37 regarding the safety of topically applied toluidine blue are known.
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4 In contrast to endocytoscopy, magnification (also called “zoom”) endoscopy uses a
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6 movable lens controlled by the endoscopist to vary degree of magnification, ranging
7
8 from 1.5-fold to 150-fold. Both, high-resolution and magnification features are
9
10 integrated in one endoscope and enable the endoscopist to analyze the pit pattern
11
12 (e.g. villiform or distorted in Barrett’s esophagus) of lesions [10]. Besides optical
13
14 magnification endoscopy, modern video processors enable digital magnification
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16 features. These are based on special software algorithms which enlarges the original
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18 image. Because the resolution of the digital sensor is fixed the software tries to
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20 interpolate additional pixels which markedly reduce the quality of the resulting image.

21
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23 In contrast to endocytoscopy, confocal laser endomicroscopy is based on tissue
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25 illumination after application of fluorescence agents which can either be applied
26
27 systemically (fluorescein) or topically (e.g. acriflavine, cresyl violet). Laser light is
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29 focused to a single point within a defined microscopic field of view. The laser light
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31 stimulates the fluorescence agent and light emanating from this point is focused
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33 through a pinhole to a detector while light emanating from outside the illuminated
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35 spot is excluded from detection. The gray scale image created is an optical section
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37 representing one focal plane within the specimen [11]. The iCLE system offers the
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39 operator to manually adjust the imaging plane depth ranging from 0-250 µm using an
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41 optical slice thickness of 7 µm. Endomicroscopy offers *in vivo* imaging of cellular and
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43 subcellular structures at 1000-fold magnification compared to 1400-fold magnification
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45 of endocytoscopy [3]. While concerns about the use of acriflavine have raised
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47 (potential mutagenic effects) one recent large multicenter study have addressed the
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49 safety of intravenous fluorescein for endomicroscopy in the gastrointestinal tract [12].
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Current applications of endocytoscopy

Esophagus

Endoscopic imaging of esophageal disorders is well established in clinical practice due to the increasing incidence of cancers at this location and their poor prognosis in advanced disease stages. Moreover, the opportunity of gentle curative endoscopic resection methods in early disease stages increases the need for imaging modalities which allow the detection of subtle mucosal changes with a high diagnostic yield (Table 3).

In 2004, Kumagai et al. observed the surface of the squamous epithelium in normal esophageal mucosa and superficial esophageal cancers using endocytoscopy (pEC; XEC-120 and XEC-300) [13]. Normal cells were homogeneously arranged, and the nucleus to cytoplasm ratio was uniform and low. In contrast, in esophageal cancers, the cell distribution and nucleus-to-cytoplasm ratio was irregular and the density of cells was increased. However, no statistic analysis was done in this study.

Additionally, three case reports confirmed the potential of endocytoscopy for *in vivo* diagnosis of early esophageal squamous cell carcinoma [14-16]. Using pEC with 450-fold magnification (pEC; XEC-300), it was possible to visualize typical neoplastic mucosal alterations like irregular and enlarged nuclei during ongoing endoscopy.

Inoue et al. conducted a pilot trial including twenty-nine consecutive patients with esophageal lesions who were evaluated using endocytoscopy (iEC; XGIF-Q260EC1) after prestaining of the esophageal mucosa with 0.5% methylene blue [17]. The positive predictive value for malignancy was 94%, the sensitivity 83.3% and specificity 93.7% with an overall accuracy for differentiating between malignant and non-malignant tissue of 82%.

To investigate whether endocytoscopic images of cancerous and non-cancerous esophageal mucosa correspond with conventional histopathology, a multicenter ex

1 vivo pilot study was conducted [18]. Overall, twenty-seven patients with esophageal
2 squamous cell cancers were included and evaluable pairs of an endocytoscopic
3 image and a histological picture of the same site were obtained at twelve cancerous
4 and fourteen normal areas. The pattern of the cellular arrangement and density and
5 the size and shape of nuclei were morphologically identical between endocytoscopy
6 (pEC; XEC-300) and conventional histopathology. However, the cytoplasm was not
7 identifiable in endocytoscopic images. Mean (\pm SD) total numbers of nuclei per
8 endocytoscopic image were 129 ± 14.8 at normal areas and 550 ± 66.5 at cancerous
9 areas ($P < .0001$).

10 One recent published study aimed at evaluating whether endocytoscopic observation
11 of esophageal squamous cell carcinoma could replace histopathological examination
12 of biopsy specimen [19]. Therefore, fifty-seven iodine-unstained areas in resected
13 specimens of the esophagus from twenty-eight patients were examined. Additionally,
14 seventy-one lesions of esophageal squamous cell carcinoma were analyzed *in vivo*
15 using endocytoscopy (pEC; XEC-120 and XEC-300). The sensitivity and specificity of
16 endocytoscopy for malignant lesions was 94.7% and 84.2%, respectively. In 93.5% of
17 cancerous lesions diagnosed using endocytoscopy further biopsy examinations were
18 considered as unnecessary by the histopathologist.

19 Barrett's esophagus is strongly associated with gastroesophageal reflux disease and
20 well recognized as a precursor lesion of esophageal adenocarcinoma. Early
21 detection of mucosal alterations is important to provide an efficient surveillance and
22 endoscopic therapy in those patients.

23 One study conducted by Pohl and coworkers evaluated the feasibility of
24 endocytoscopy (pEC; XEC-120 and XEC-300) in the surveillance of patients with
25 Barrett's esophagus [20]. Overall 166 biopsy sites from sixteen patients without
26

1 visible lesions, who presented for Barrett surveillance were analyzed using
2
3 endocytoscopy and correlated to histopathology. Adequate assessment of
4
5 endocytoscopy images was possible in 51% with magnification x 450 and in 22% with
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7 magnification x 1125. At most, 23% of images with lower magnification were
8
9 interpretable to identify characteristics of neoplasia, and 41% with higher
10
11 magnification. Interobserver agreement was fair with kappa values from < 0 to 0.45.
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13 Positive and negative predictive values for high-grade intraepithelial neoplasia or
14
15 cancer were 0.29 and 0.87, respectively, for magnification x 450 and 0.44 and 0.83,
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17 respectively, for magnification x 1125.
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23 **Key message:**

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25 Endocytoscopy could detect esophageal squamous cell carcinoma with good
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27 sensitivity and specificity.

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29 In Barrett's esophagus endocytoscopy lacks sufficient image quality to be currently
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31 of assistance in identifying neoplastic areas.
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37 *Stomach:*

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39 *Helicobacter pylori* (*H. pylori*) infection affects about fifty percent of the general
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41 population (10-30% in industrialized countries and 80-90% in developing countries)
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43 and is strongly associated with gastroduodenal ulcer disease, gastric lymphoma and
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45 non-proximal gastric adenocarcinoma.

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47 Kimura et al. cultured *H. pylori ex vivo* from gastric mucus of three patients suffering
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49 from gastric ulcer disease. Subsequently, *H. pylori* microorganisms could be
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1 observed in the supernatant of the culture medium using endocytoscopy with 1100-
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3 fold magnification (pEC; XEC-300) [21].
4

5 In addition, Fasoli and coworkers published one case report highlighting the potential
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7 of endocytoscopy (XEC-300) for *in vivo* evaluation of gastric signet ring cell
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9 carcinoma. Endocytoscopy was reliable to identify disease specific histologic aspects
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11 and there was a strong concordance between *in vivo* and *ex vivo* histology [22].
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17 **Key message:**

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19 Currently, no studies are available concerning endocytoscopy based *in vivo*
20
21 diagnosis of *H. pylori* infection, hyperplastic and precancerous lesions of the
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23 stomach.
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26
27 *Duodenum:*

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29 Celiac disease represents an autoimmune enteropathy in genetically susceptible
30
31 hosts induced by gluten. Histopathological diagnosis is based on villous atrophy,
32
33 hyperplasia of intestinal crypts, and an increased number of intraepithelial
34
35 lymphocytes.

36
37 Up to now, two studies have evaluated the potential of endocytoscopy for *in vivo*
38
39 diagnosis of celiac disease.

40
41 In the first study, a total of 166 endocytoscopy (pEC; XEC-120 and XEC-300)
42
43 recordings from forty patients with established (n= 32) or suspected celiac disease
44
45 were prospectively acquired and corresponded to conventional histopathology [23].
46

47 When adjusted by multivariate analysis four criteria were identified to be significant
48
49 predictors for Marsh III pathology: low number of villi per visual field (<3; odds ratio
50
51 [OR] 9.1; 95% CI, 1.3-62.0), confluence of villi (OR 37.1; 95% CI, 1.3-1021.2),
52
53

1 irregular epithelial lining (OR 10.9; 95% CI, 2.5-46.7), and inability to delineate loop
2 capillaries (OR 14.9; 95% CI, 3.3-67.0). Nevertheless, none of these factors was a
3
4
5 good predictor for Marsh I pathology.

6
7 Sixteen patients with documented celiac disease and seven control patients were
8
9 included in the second trial, evaluating the potential of endocytoscopy (pEC; XEC-
10
11 300) in celiac disease [24]. Endocytoscopy visualized the presence of normal-
12
13 appearing, long, thin villi, lined with clearly distinguishable surface epithelial cells, the
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15 presence of thick, shortened villi, reflecting partial villous atrophy, and the total
16
17 absence of villi and the presence of enlarged crypt orifices, reflecting total villous
18
19 atrophy. Sensitivity and specificity of endocytoscopy for the diagnosis of villous
20
21 atrophy was 88% and 100%, respectively.
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24

25 **Key message:**

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27 In celiac disease endocytoscopy enables accurate determination of severe
28
29 abnormalities like villous atrophy. Nevertheless, mild changes cannot be assessed.
30
31 Therefore, diagnosis and follow-up of patients should still be based on biopsies.
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36
37 *Colon:*

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39 According to most national and international guidelines, colonoscopy is the
40
41 recommended surveillance method of patients aged 50 years and older to exclude
42
43 adenomatous polyps. However, most polyps are diminutive in size (1-5 mm) and
44
45 harbour only a very low prevalence of cancer [25]. Therefore, sending diminutive
46
47 polyps to the histopathologist is generally a profusion of resources. By using real time
48
49 *in vivo* histology, suspicious polyps could be identified for targeted biopsy or
50
51 endoscopic polypectomy (Table 4).
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1 Indeed, Sasajima et al. evaluated in a prospective study the usefulness of
2 endocytoscopy (pEC; XEC-120 and XEC-300) for the differentiation of colorectal
3 lesions in 113 consecutive patients [26]. Endocytoscopy was able to differentiate
4 normal colonic mucosa, hyperplastic polyps, low-grade and high-grade adenomas
5 and invasive cancers. The overall accuracy of endocytoscopy was 93.3% with an
6 excellent kappa value between endocytoscopy and the corresponding
7 histopathological analysis of 0.910. The differential diagnosis between neoplastic and
8 non-neoplastic lesions and between adenoma and invasive cancer was statistically
9 significant ($P < .001$).

10 In addition to this context, one recent published case report highlighted the potential
11 of endocytoscopy (pEC; XEC-120) for *in vivo* diagnosis of very focal high-grade
12 intraepithelial neoplasia in colonic polyps [27]. This is of tremendous importance,
13 because polyps often show only focal lesions which are surrounded by normal
14 colonic mucosa.

15 Meroni et al. addressed this point in another case report [28]. They performed
16 endocytoscopy (pEC; XEC-300) in one surgical specimen obtained after resection of
17 colon cancer and found focal abnormalities of colonic glands highly suggestive of
18 aberrant crypt foci seven cm apart from the primary tumor within macroscopically
19 normal mucosa.

20 Finally, Cipolletta et al. conducted a prospective study including forty-one
21 consecutive patients with colorectal aberrant crypt foci [29]. The mean duration of the
22 endocytoscopy procedure was 44 ± 12 minutes (range 31 - 62 minutes) and it was
23 possible to observe lesions at the cellular level and evaluate both cellular and
24 structural atypia *in vivo*. In normal mucosa, crypts had preserved individuality and
25 round-shaped contours. Nuclei were located at the basal third of the crypt in a single
26 line, and the lumen was circular. In dysplastic aberrant crypt foci, crypt contours were

1 polygonal, cell nuclei were elongated with pseudostratification toward the luminal half
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3 of the crypt and irregularly arranged, and the lumen was linear. Sensitivity and
4
5 specificity of endocytoscopy (pEC; XEC-300) for the detection of intraepithelial
6
7 neoplasia was 91.4% and 100%, respectively. Interobserver agreement between
8
9 endoscopist and pathologist was good (weighted kappa 0.68; 95 % CI 0.59 - 0.78).

10
11 The same group aimed to assess the capability of endocytoscopy (pEC; XEC-300) in
12
13 differentiating neoplastic from non-neoplastic lesions in the colorectum [30]. Overall,
14
15 fifty-two lesions were examined in 49 patients (17 polypoid and 35 non-polypoid).
16
17 Final pathological diagnosis was normal mucosa or hyperplastic polyp in ten cases,
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19 low-grade adenoma in twenty-nine, high-grade adenoma in eleven, and submucosal
20
21 invasive cancer in two cases. Positive predictive value of endocytoscopy was 100%
22
23 for hyperplastic polyps, 93.1% for low-grade intraepithelial neoplasia, 90.1% for high-
24
25 grade intraepithelial neoplasia, and 100% for invasive cancer. Additionally,
26
27 endocytoscopy diagnosis correlated completely with histopathology in the
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29 differentiation between neoplastic and non-neoplastic lesions.

30
31 One recent case report identified endocytoscopy (pEC; XEC-300) as a potential
32
33 method for imaging of a carcinoid tumor which was located in the terminal ileum.
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35 Nests of monomorphous cells separated by poor cellulated strands without glandular
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37 structures were evident on endocytoscopic imaging [31].

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39 Very recently, Hosoe and coworkers prospectively evaluated the feasibility of
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41 endocytoscopy with 450-fold magnification (iEC; CF-Y0001) for the *in vivo* diagnosis
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43 of amebic parasites in five patients. The authors could successfully visualize amebic
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45 trophozoites in all cases and therefore diagnose amoebic colitis *in vivo* during
46
47 ongoing endoscopy [32].

Key message:

Endocytoscopy can predict the presence of preneoplastic and neoplastic lesions in the colon with high accuracy. One drawback is the prolongation up to one hour of the duration of endoscopy.

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Lung:

Very recently, our group addressed the potential of endocytoscopy for *in vivo* diagnosis of small cell lung cancer during ongoing bronchoscopy in four patients [33]. After topical application of methylene blue, endocytoscopy (pEC; XEC-120) was able to reliably identify numerous small blue cells with hyperchromatic nuclei (Figure 4). Corresponding histopathological analysis confirmed *in vivo* diagnosis of small cell lung cancer. This exciting initial experience is now evaluated in a larger ongoing prospective study.

Shibuya and coworkers performed endocytoscopy (pEC; XEC-300) in twenty-two patients (7 squamous cell carcinoma, 11 squamous dysplasia and 4 after PDT therapies). Both abnormal areas of interest and normal bronchial mucosa were stained with 0.5% methylene blue and afterwards examined with endocytoscopy at 570-fold magnification. In this study, endocytoscopy was useful to discriminate between normal bronchial epithelial cells, dysplastic cells and malignant cells during ongoing bronchoscopy [34].

1 *Others:*

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3 Besides the above mentioned areas, endocytoscopy was also proven to be efficient
4
5 in different disease entities.

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7 In a pilot study including five patients, endocytoscopy was used to evaluate bladder
8
9 carcinoma [35]. Using the pEC system with 450-fold magnification (XEC-300), the
10
11 cell structure and nuclear morphology of the tumors were identified and graded
12
13 correctly in 80% of the cases.

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15 In another case report endocytoscopy (not nearly specified which system was used)
16
17 was used to assist intraoperative diagnosis of carcinoma in a patient who was
18
19 suffering from chronic pancreatitis. Diagnosis of cancer was based on the
20
21 visualization of cells with hyperchromatic nuclei and a high nucleus to cytoplasm ratio
22
23 [36].

24
25 While it is often challenging to optimally sample the muscularis propria
26
27 endoscopically for the diagnosis of muscle layer diseases, especially for motility
28
29 disorders resulting from neuroenteric dysfunction, Sumiyama and coworkers
30
31 designed an *ex vivo* and *in vivo* porcine animal study to access the muscular layer
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33 using endocytoscopy (pEC; XEC-120 and XEC-300) [37]. Cellular microstructures
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35 resembling spindle-shaped smooth muscle cells and neuron-like cells were visualized
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37 by endocytoscopy *in vivo*.

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39 Endocytoscopy was used in another report of two patients for intraoperative
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41 diagnosis of disseminated malignancy. During diagnostic laparoscopy,
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43 endocytoscopy (pEC; XEC-300) of peritoneal nodules revealed malignant cells and *in*
44
45 *vivo* diagnosis was confirmed by corresponding histopathology [38].

46
47 Additionally, after methylene blue staining onto normal rectal mucosa, endocytoscopy
48
49 (pEC; XEC-300) revealed red blood cell flow within the microvasculature circulating
50
51 through the arterioles [39].

Key message:

Potential applications of endocytoscopy were shown in different pilot studies including lung, bladder and intraoperative diagnosis of malignancy. Larger trials are now needed to confirm these interesting and promising preliminary results.

Conclusions

Modern endoscopy with advanced endoscopic imaging techniques has revolutionized the diagnosis and management of patients in the endoscopy suite. Besides other emerging endoscopic imaging techniques, including chromoendoscopy, magnification endoscopy and confocal laser endomicroscopy, endocytoscopy has become an inherent part of advanced endoscopic imaging. In different disease entities, endocytoscopy allowed the endoscopist to obtain real time *in vivo* histology during ongoing endoscopy with a high diagnostic yield and to guide subsequent biopsy acquisition for histopathological analysis. Up to now, no serious complications of endocytoscopy have been reported.

Currently, the spectrum of endocytoscopy ranges from esophageal disorders, including esophageal squamous cell carcinoma and Barrett's esophagus to celiac disease and *in vivo* diagnosis of colon polyps and intestinal pathogens like *Helicobacter pylori* and amebic trophozoites. Besides, the value of endocytoscopy was also proven for the *in vivo* diagnosis of small cell lung cancer and to facilitate final histological diagnosis during diagnostic laparoscopy.

Nevertheless, most studies so far only include small sample sizes or are case reports. No multicenter trial evaluating the technique has yet been performed. In addition, as endocytoscopy is a developing technology the performance of different

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1 instruments varies widely. Up to date, no information regarding the learning curve or
2
3 the inter-observer and intra-observer agreement of endocytoscopy are available.

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5 Therefore, endocytoscopy is a promising new development providing advanced
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7 endoscopic imaging. Nevertheless, multicenter studies evaluating the technique in
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9 larger settings are highly warranted.

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11 In the near future, new 'all-in-one' scopes for stable ultra-high magnification imaging
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13 and functional imaging could further improve the diagnostic applicability of
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15 endocytoscopy.
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For Peer Review

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51 **Statement of interest:** There was no financial support for this study.
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Tables:

Table 1: Current potential clinical applications of endocytoscopy.

Esophagus	
	- Esophageal squamous cell carcinoma [8-14] - Barrett's esophagus [15]
Stomach	
	- <i>Helicobacter pylori</i> - infection [16] - Signet ring cell carcinoma [17]
Duodenum	
	- Celiac disease [18,19]
Colon	
	- Polyps [21-26] - Amoebic colitis [27]
Lung	
	- small cell lung cancer [28, 29]
Others	
	- Bladder carcinoma [29] - Intraoperative carcinoma diagnosis [30, 32]

Table 2: Technical characteristics of different endocytoscopy devices.

Type	Probe-based (pEC)		Endoscope-based (iEC)		
	XEC-300	XEC-120	XGIF-Q260E C1	XCF-Q260E C1	GIF-Y0002
Endoscope magnification	N/A		x 85	x 110	x 380 (x 600 digital)
Endoscope working channel (mm)	N/A		2.8	3.2	2.8
Total length (cm)	380		103	133	103
Functional length (cm)	240		103	133	103
EC magnification	x 450 (14' monitor) x 570 (19' monitor)	x 1100 (14' monitor) x 1390 (19' monitor)	x 580		x 380 (x 600 digital)
Field of view (μm)	300 x 300	120 x 120	400 x 400		700 x 600 (440 x 380 digital)
Horizontal resolution	4.2	1.7	4.0		3.7
Outer diameter	3.2 mm		11.6	13.6	10.7
Imaging plane depth	30 μm		5 μm		50 μm

EC =endocytoscope.

Table 3: Summary of studies evaluating endocytoscopy for diagnosing esophageal diseases.

Author and reference	Disease entity	Study design	Patients	Results
Kumagai Y, 2004 [8]	SCC	Descriptive study	12	In cancerous mucosa, cell distribution and nucleus-to-cytoplasm ratio was irregular and density of cells increased.
Dumonceau JM, 2006 [9]	SCC	Case report	1	
Fujishiro M, 2008 [10]	SCC	Case report	1	
Banerjee R, 2008 [11]	SCC	Case series	3	PPV 94%, accuracy 82%
Inoue H, 2006 [12]	SCC	Prospective study	29	
Fujishiro M, 2007 [13]	SCC	Ex vivo pilot study	27	Endocytoscopic images closely correlated with conventional histology
Kumagai Y, 2009 [14]	SCC	Prospective study	21	Sensitivity 94.7%, specificity 84.2%
Pohl H, 2007 [15]	Barrett's esophagus	Prospective study	16	Assessment of EC-images possible in 51% (x450) and 22% (x1125). PPV, NPV 0.29 and 0.87 (x450) and 0.44 and 0.83 (x1125). Interobserver kappa 0-0.45.

EC, endocytoscopy; SCC, squamous cell carcinoma; PPV, positive predictive value; NPV, negative predictive value.

Table 4: Summary of studies evaluating endocytoscopy for diagnosing colon polyps.

Author and reference	Study design	Patients	Results
Sasajima K, 2006 [21]	Prospective study	113	Accuracy 93.3%; kappa between EC and histopathology 0.91
Neumann H, 2010 [22]	Case report	1	EC could detect focal HGIEN
Meroni E, 2007 [23]	Case report	1	EC could detect tissue abnormalities in normal mucosa surrounding colorectal cancer
Cipolletta L, 2009 [24]	Prospective study	41	Sensitivity 91.4%, Specificity 100%, Interobserver agreement 0.68
Rotondano G, 2010 [25]	Prospective study	49	PPV for hyperplastic polyps, LGIEN, HGIEN and invasive cancer was 100%, 93.1%, 90.1% and 100%

EC, endocytoscopy; PPV, positive predictive value; NPV, negative predictive value, LGIEN, low-grade intraepithelial neoplasia; HGIEN, high-grade intraepithelial neoplasia.

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Figure 1: The probe-based endocytoscope is advanced through the working channel of a standard endoscope. Inserted image highlights the magnification probe. Images with kind permission from Olympus, Hamburg, Germany.

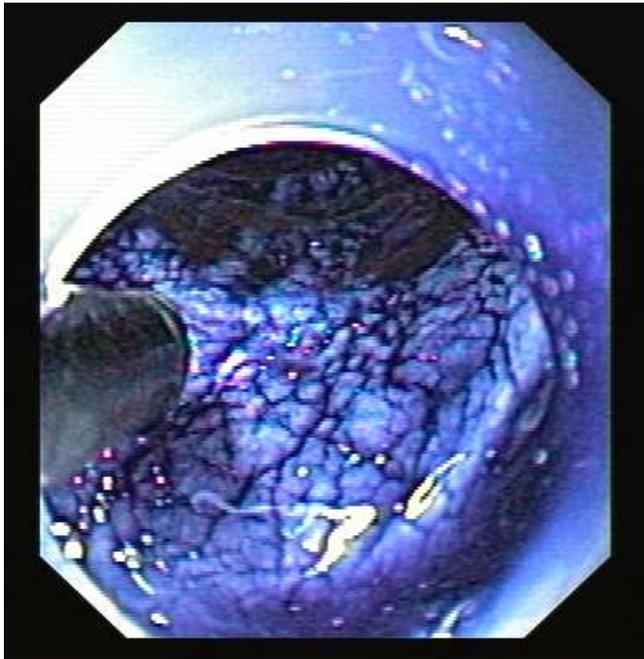


Figure 2: After mucosal staining with methylene blue the probe-based endocytoscope is gently pushed against the mucosal surface to enable high-magnification imaging. Note the plastic cap at the distal end of the endoscope to reduce motion artefacts.

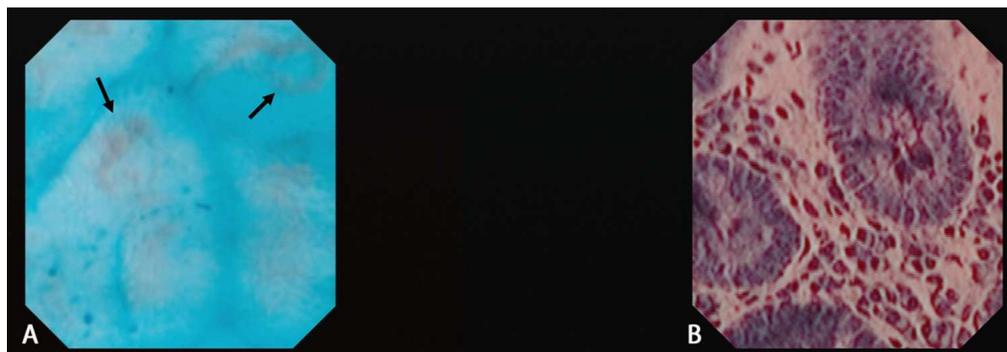


Figure 3: Optical biopsy at 450-fold (Panel A) and 1390-fold (Panel B) magnification using the endocytoscope highlights density of cells, the size and shape of nuclei, and the nucleus-to-cytoplasm ratio. Panel A shows normal gastric epithelium after toluidine blue staining. Microvessels are clearly visible (arrows). Panel B highlights normal colonic epithelium after toluidine blue staining.

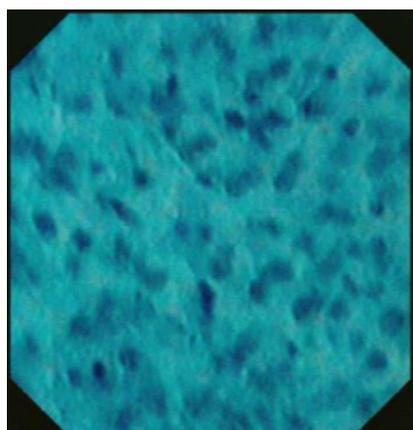


Figure 4: Methylene-blue aided endocytoscopy in a patient with small cell lung cancer visualizes numerous small blue cells with hyperchromatic nuclei.

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