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Διπλωματική Εργασία

«Σχεδιασμός πρωτοκόλλου για τυχαιοποιημένη μελέτη σύγκρισης πολυπεκτομής κάτω από το νερό (underwater polypectomy) και συμβατικής πολυπεκτομής (conventional polypectomy) σε άμισχους πολύποδες παχέος εντέρου μεγέθους 5-10χιλιοστών με χρήση ψυχρού βρόχου»

«Study Protocol Design : Randomized clinical trial to compare Underwater Cold Snare Polypectomy (UCSP) to Conventional Cold Snare Polypectomy (CCSP) for non pedunculated colon polyps of size 5-10mm»

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Θεωρητικό Υπόβαθρο

Ο καρκίνος του παχέος εντέρου αποτελεί διεθνώς την τρίτη αιτία θανάτων από κακοήθη νόσο. Η προληπτική κολονοσκόπηση σε ενήλικες >45 ετών αποσκοπεί στην έγκαιρη διάγνωση και θεραπεία προκαρκινωματωδών βλαβών , δηλαδή πολυπόδων παχέος εντέρου. Η ενδοσκοπική αφαίρεση των πολυπόδων γίνεται με διάφορες τεχνικές ανάλογα με το μέγεθος, την μορφολογία, τη θέση του πολύποδα και άλλα. Σύμφωνα με τις επικαιροποιημένες κατευθυντήριες οδηγίες οι άμισχοι πολύποδες μικρού μεγέθους θεραπεύονται με χρήση ψυχρού βρόχου σε εντερικό αυλό διατεταμένο με αέρα (συμβατική πολυπεκτομή). Τα τελευταία χρόνια έχουν γίνει αρκετές μελέτες για τα οφέλη της κολονοσκόπησης με χρήση νερού γενικά, αλλά και της πολυπεκτομής κάτω από το νερό σε μεγάλου μεγέθους πολύποδες. Ωστόσο, δεν υπάρχουν επαρκή δεδομένα για τους μικρούς πολύποδες οι οποίοι είναι συχνότεροι.

Μεθοδολογία

Πρόκειται για μια προοπτική πολυκεντρική τυχαιοποιημένη διπλή-τυφλή κλινική μελέτη ώστε να συγκριθεί η ασφάλεια και αποτελεσματικότητα της συμβατικής πολυπεκτομής σε έντερο διατεταμένο με αέρα και της πολυπεκτομής κάτω από το νερό σε άμισχους πολύποδες μεγέθους 5-10 χιλιοστών. Η μελέτη θα συμπεριλάβει 398 πολύποδες και η τυχαιοποίηση θα γίνει μέσω διαδικτυακού προγράμματος. Πρωτογενής στόχος της μελέτης είναι ο προσδιορισμός του ποσοστού εκτομής της βλεννογονίου μυικής στιβάδας. Λοιποί δευτερογενείς στόχοι είναι το βάθος και το ποσοστό των R0 εκτομών, ο χρόνος , πιθανές επιπλοκές και το ποσοστό υποτροπής.

Συζήτηση

Πρόκειται για την πρώτη τυχαιοποιημένη κλινική μελέτη που συγκρίνει τη συμβατική πολυπεκτομή με την πολυπεκτομή κάτω από το νερό, με χρήση ψυχρού βρόχου, για τους άμισχους μικρούς πολύποδες παχέος εντέρου. Αναμένουμε από την πολυπεκτομή κάτω από το νερό να εξασφαλίσει υψηλότερο ποσοστό εκτομής βλεννογονίου μυικής στιβάδας και προσπαθούμε να εξετάσουμε εάν επιτυγχάνει βαθύτερη εκτομή στο υποβλεννογόνιο χιτώνα. Επιπλέον θα διερευνηθεί αν ο χρόνος πολυπεκτομής διαφέρει μεταξύ των δύο τεχνικών, καθώς και περιπτώσεις όπου η πολυπεκτομή κάτω από το νερό δεν μπορεί να εφαρμοστεί. Αυτά τα αποτελέσματα θα παρέχουν δεδομένα χρήσιμα για την ανάπτυξη οδηγιών στις επιλεγόμενες τεχνικές πολυπεκτομής για άμισχους πολύποδες 5-10χιλιοστά.

A. ABSTRACT

Backround

Colon cancer is internationally the third cause of deaths from a malignant disease. Screening colonoscopy in adults >45 years of age aims at the early diagnosis and treatment colon polyps that are precancerous lesions. Endoscopic polyp removal (polypectomy) can be done with various techniques depending on the size, morphology, location of the polyp etc. According to updated guidelines, non-pedunculated polyps of small size are treated with a cold snare in air dilated intestinal lumen (conventional cold snare polypectomy - CCSP). In recent years, several studies have described the benefits of water aided colonoscopy, as well as safety and efficacy of underwater polypectomy in large colon polyps. However, there is not enough data on small polyps which are the most commonly diagnosed.

Methodology

This is a prospective multicenter randomized double-blind clinical trial to compare the safety and efficacy of CCSP to underwater cold snare polypectomy (UCSP) for non-pedunculated polyps of size of 5-10 mm. A total of 398 polyps will be randomized and randomization will be performed via random numbers method of Microsoft Excel 2016. Primary outcome of this study is to determine muscularis mucosa resection rate. Secondary outcomes are the depth and percentage of R0 excisions, polypectomy time, possible complications and the recurrence rate.

Discussion

This is the first RCT comparing CCSP to UCSP for non-pedunculated small colon polyps. We expect UCSP to ensure a higher muscularis mucosa resection rate and we attempt examine whether it achieves a deeper resection in the submucosal layer. In addition, it will be investigated whether the polypectomy time differs between the two techniques, as well as cases where polypectomy under water cannot be applied. These results will provide useful data for the development of guidelines in polypectomy techniques for non-pedunculated polyps 5-10mm.

PROTOCOL INFORMATION PAGE

Trial Title
Randomized clinical trial to compare Underwater Cold Snare Polypectomy (UCSP) to Conventional Cold Snare Polypectomy (CCSP) for non pedunculated colon polyps of size 5-10mm»

Acronym
COLDWATER STUDY

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Protocol Version Number
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Investigators
A
B
C

TABLE OF CONTENTS

1.BACKGROUND

1.1. OVERVIEW OF UNDERWATER POLYPECTOMY

Polyp removal during colonoscopy is carried out in a dilated colon lumen after air insufflation. However, using water infusion to dilate the enteric lumen instead of standard air insufflations has been associated with higher patient comfort, less dose of intravenous sedation and higher adenoma detection rate [1-5] Underwater Polypectomy (UP) was first described as Underwater Endoscopic Mucosal Resection (UEMR) in 2012. The term "EMR" (Endoscopic Mucosal Resection has been improperly used to describe this new method, due to its unique feature to create a natural separation between mucosal/submucosal and muscular layer of the colon wall. In conventional EMR such separation is achieved by submucosal injection (lifting). During Endoscopic Ultrasound examination of the colon, where water is infused as an acoustic window, Binmoeller noticed that the water driven partial dilation of the colon lumen causes a "floating" effect on the colon wall. This causes the mucosal and submucosal layers to create protrusions towards the lumen (resembling the Gastric rugae) and to move away from the underlying muscular layer. The latter appears to remain circular and retain its normal thickness. [6-7]. Also, water due to its higher refractive index compared to air increases the sensitivity of the endoscopy, causing an optical zoom effect of the mucosal architecture [7-8]. This increases the sensitivity of the endoscopic overview [7-8], improving identification of the lesion margins before polypectomy and of any residual lesion or recurrence after polypectomy [7, 9]. Another advantage of UP is that the colon is not fully dilated, making mucosal lesions more compact with polypoid configuration in comparison with the flatter appearance of the same lesions in a fully dilated colon. This allows the endoscopist to capture larger mucosal surface with the snare increasing the chance of en-block resection even in larger lesions (eg. flat polyps of 20-40mm) [10]. As a result of all the above, UP offers shorter resection time, lower cost due to the reduced use of disposable materials (injection needles, syringes, lifting agents), higher en-block resection rates (89% vs 73%)

in large lesions and higher rates of R0 resection (based on histology results) in comparison with the conventional EMR. [10-17].

1.2.OVERVIEW OF COLD VS HOT SNARE POLYPECTOMY

UP has been applied in studies with colonic lesions >10mm and >20mm in particular. Nevertheless only 5% of the polyps found in everyday clinical practice are of large size with the majority of the polyps being smaller. In regard to small polyps European Guidelines recommend the use of Cold Snare (CS) over Hot Snare (HS) due to improved safety profile. [18] HSP can cause thermal injury of the vessels and of the colonic wall and lead to delayed bleeding (0,04%- 7,8%) [19-23], post polypectomy syndrome, abdominal pain or even perforation with the latter being a rare (1.4%- 1.5%), but clinically serious complication [24-25]. Complication risk after HSP is proportional to the polyp size, histological grade, use of antiplatelet agents and it is higher in Right Colon lesions [26-29]. Incomplete Resection Rate (IRR) in HSP is 6.8% based on CARE study [30]. Complete Resection Rate (CRR) using CSP (R0 resection proved on histology results) ranges from 32.9% to 96.6% in various studies, [31] with the highest rates being demonstrated in the most recent ones. [32-34] One disadvantage of CSP is that the depth of the vertical resection margin is shorter and that found in HSP. This is based on histological examination of the center of the resection specimen, where the muscularis mucosa layer is identified. This finding highlights the incomplete mucosal resection during CSP rendering the method suitable for resecting intraepithelial lesions. [35-36] A metanalysis in 2020 showed that using CSP for polyps up to 9mm IRR was 17.3% vs 14.2% when using HSP, [35] justifying the need for further research regarding the most suitable polypectomy method for this polyp group.

2.STUDY RATIONALE

The aforementioned disadvantage has led many endoscopists to continue to use HS for the removal of small polyps despite the increased risks associated with the electrocautery. [36-37] In order to overcome the relatively short vertical resection margin found in CSP, UCSP has emerged given the theoretical advantage of improved complete polyp resection. Both the safety and efficiency of UCSP has been shown in a study published in Digestive Endoscopy in 2019. This study included 209 adenomatous colon lesions removed by UCSP which were compared with lesions removed previously by conventional CSP where air insufflation had been used to dilate the colon lumen. [38] The vast majority of colon polyps however were microscopic <5mm (157/209, 75.1%) and the rest were 6-8mm in size (52/209, 24.9%). The study showed that both techniques were equally safe, but R0 resection rate was 80.2% for UCSP vs 32.7% for CCSP respectively ($p<0.001$). The percentage of mucosal layer per polyp removed was 50% vs 35.3% ($p= 0.015$), in UCSP and CCSP respectively, while the presence of submucosa in the removed polyps was found to be 20.8% in UCSP and 12.9% in CCSP. The rate of R0 resections in this study was calculated similar to the rate of R0 resections with HSP as described in other studies. [38]

2.1. STUDY HYPOTHESIS

Underwater cold snare polypectomy ensures a higher muscularis mucosa resection rate and deeper resection than conventional cold snare polypectomy

3.STUDY OBJECTIVES

The aim of this study is to compare the safety and efficacy between the two endoscopic polypectomy techniques (UCSP vs CCSP) regarding resection of non-pedunculated colon polyps with size 5mm to 10mm

3.1. PRIMARY OBJECTIVE

Primary outcome of the study is the investigation of the difference in the rate of area containing muscularis mucosa (%) between the two polypectomy techniques.

3.2. SECONDARY OBJECTIVES

Investigation of the difference at the following points between UCSP and CCSP group

- Presence or absence of submucosal in the specimen and its depth (if present)
- R0 Resection Rate (R0RR)
- Incomplete Resection Rate (IRR)
- Procedure time
- Complication rate
- Residual lesion rate during repeat colonoscopy in 12months

4. METHODS AND DESIGN

4.1. STUDY DESIGN

This is a prospective multicenter randomized, double-blind, controlled study to compare underwater cold snare polypectomy (UCSP) to conventional cold snare polypectomy (CCSP) for non-pedunculated colon polyps of size 5-10mm.

Prior to this study, the safety and effectiveness of the UCSP method was confirmed in the literature and by the experience of two qualified endoscopists of the General Hospital Sismanoglio in 35 patients who agreed to undergo UCSP. The resection of polyps with this method was videotaped and will be used to explain the procedure to the endoscopists of the other centers that will participate in the study.

4.2 STUDY SITES

The Gastroenterology Departments of the following hospitals will participate in this trial : General Hospital of Attica "Sismanogleio-Amalia Fleming", General Hospital of Athens "Ippokratio", University General Hospital of Heraklion "PAGNI", General Hospital of Larissa "Koutlimbanio & Triantaphyllio", General Hospital of Volos "Achillopouleio", General Hospital of Trikala, General Hospital of Kozani "Mamatsio" and the Department of Hepatogastroenterology of the Clinical Pathological Physiology of the General Hospital of Athens "Laiko", all in Greece. These sites are located in the southern, central, and northern areas of Greece providing adequate generalizability.

4.3. STUDY PATIENTS

Patients scheduled for colonoscopy will be prospectively screened for eligibility. The workflow is shown in Fig.4

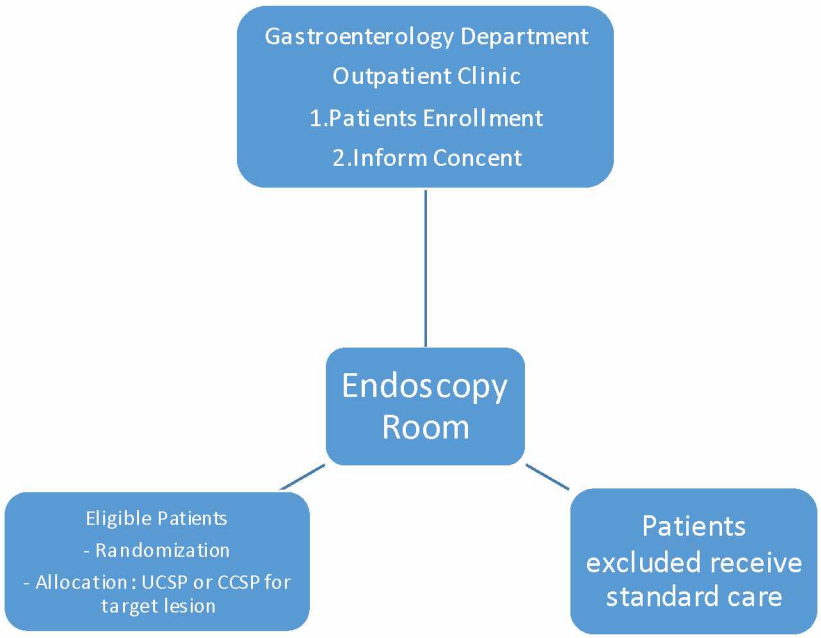


Figure 4. Study Workflow

4.3.1. INCLUSION CRITERIA

The inclusion criteria for this study are:

1. Age > 20years
2. Non pedunculated polyps (Paris classification Isp, Is, IIa, IIb)
3. Polyp size: diameter of 5 to 10mm
4. Endoscopic diagnosis of mucosal lesions – must not present malignant submucosal infiltration evidence (NICE 1, NICE 2A, BASIC)
5. Informed consent

Patients > 20 years of age with non-pedunculated polyps (Paris classification Isp, Is, IIa, IIb – figure 1) with a diameter of 5 to 10 mm. The endoscopic diagnosis of mucosal lesions will be based on their macroscopic appearance, Narrow-Band Imaging (NBI) findings or Blue Light Imaging (BLI), depending on the equipment of each Endoscopy Department. Endoscopic exemption will only be indicated for lesions that:

- ✓ According to the NICE (Narrow-Band Imaging International Colorectal Endoscopic Classification) are classified as type 1 or 2A. (Attached classification table - figure 2)
- ✓ According to the BASIC (Blue Light Imaging Adenoma Serrated International Classification) classification do not show evidence of malignant submucosal infiltration. (Attachment classification table - figure 3)
- ✓ Do not present macroscopic malignant submucosal infiltration evidence (deepening, ulceration / ulcer, abnormal vessels, irregular surface)

The size of the lesion will be determined according to its endoscopic appearance by comparing the lesion with the closed (2mm) or open (7mm) biopsy forceps and will be confirmed before resection by comparing it with the open snare (10mm).
Patients who receive antiplatelet / anticoagulant therapy are included in the study only if their therapy has been modified according to ESGE guidelines.
All patients will complete a written consent to participate in the study, after being thoroughly informed about the procedure.

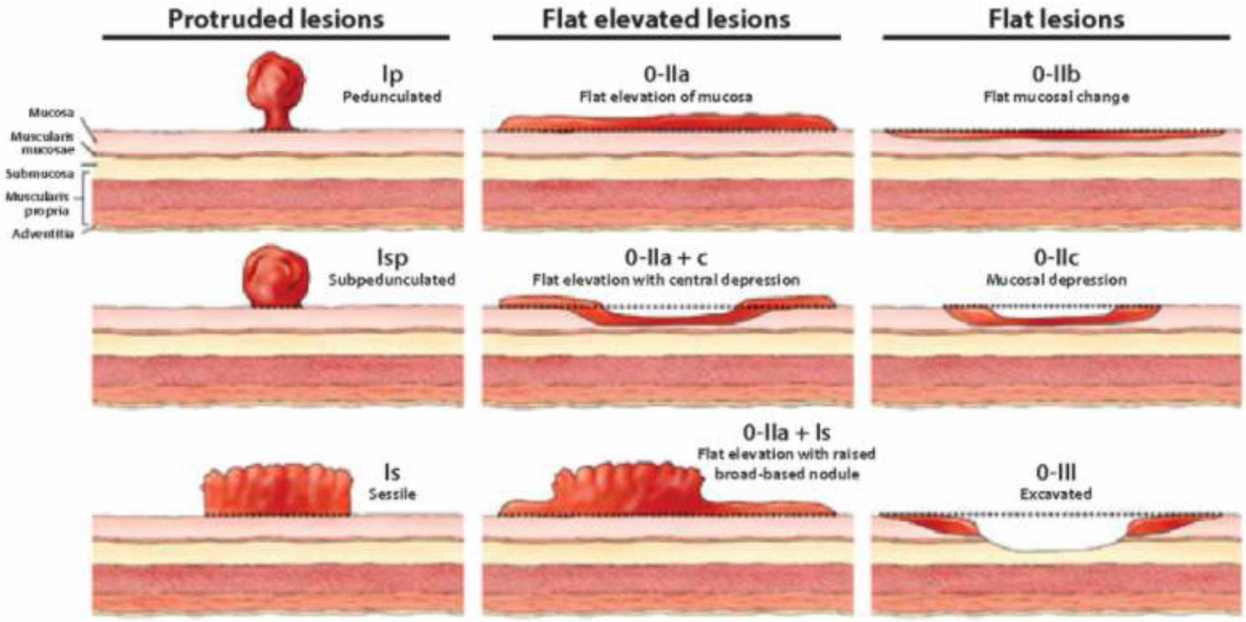


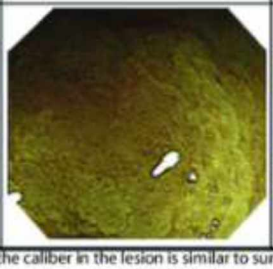
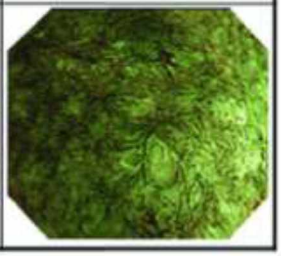
Figure 1 Paris classification for polyps

4.3.2. EXCLUSION CRITERIA

The exclusion criteria for this study are:

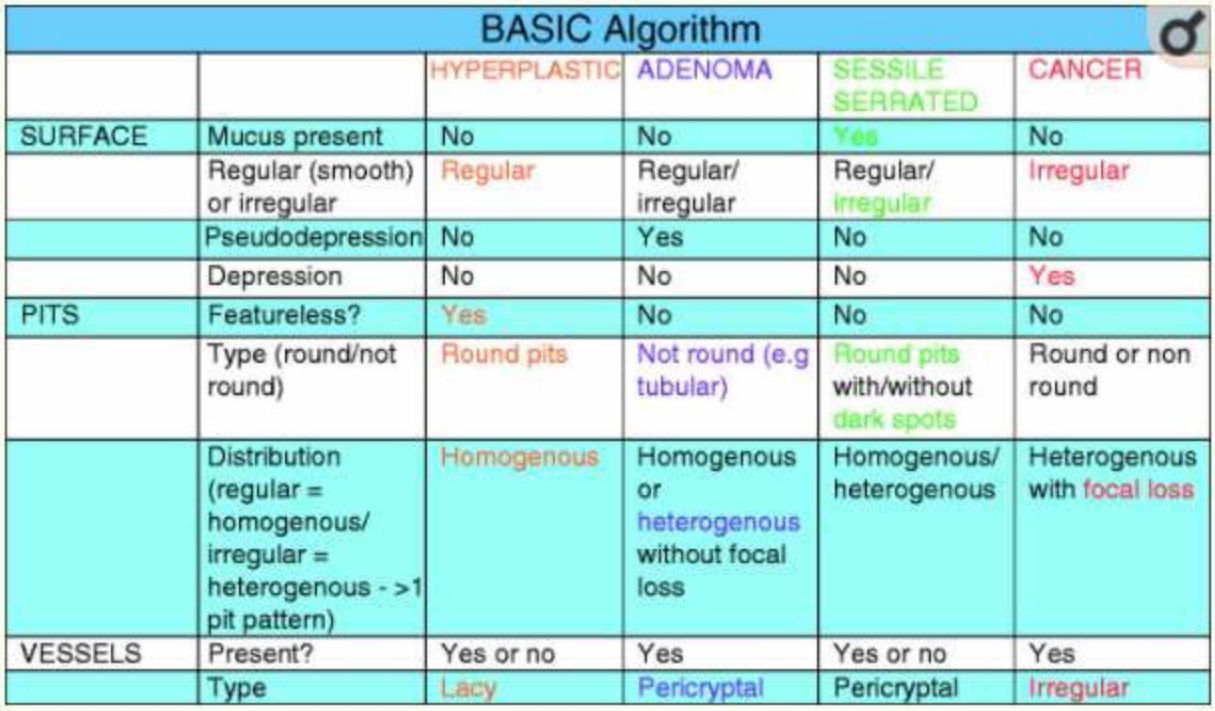
1. Age <20 years old
2. Pedunculated polyps
3. Lesions with macroscopic elements of high-grade dysplasia or submucosal infiltration
4. Sites with previous polypectomy
5. Patients with idiopathic inflammatory bowel disease
6. Patients with coagulation disorders
7. Patients with severe organ failure
7. Patients who during endoscopy will undergo any technique using electrocautery / electrocoagulation will not be eligible for participation in the study.

Japanese NBI Expert Team (JNET) classification

NBI	Type 1	Type 2A	Type 2B	Type 3
Vessel pattern	• Invisible [1]	• Regular caliber • Regular distribution [2] (meshed/spiral pattern)	• Variable caliber • Irregular distribution	• Loose vessel areas • Interruption of thick vessels
Surface pattern	• Regular dark or white spots • Similar to surrounding normal mucosa	• Regular (tubular/branched/papillary)	• Irregular or obscure	• Amorphous areas
Most likely histology	Hyperplastic polyp/ Sessile serrated polyp	Low-grade intramucosal [4] neoplasia	High-grade intramucosal [5] neoplasia/ Superficial [3] submucosal invasive cancer	Deep submucosal invasive cancer
Examples				

• 1. If visible, the caliber in the lesion is similar to surrounding normal mucosa.
• 2. Microvessels are often distributed in a punctate pattern and well-ordered reticular or spiral vessels may not be observed in depressed lesions.
• 3. Deep submucosal invasive cancer may be included.
• 4. Low-grade intramucosal neoplasia: low-grade dysplasia.
• 5. High-grade intramucosal neoplasia: high-grade dysplasia.

Figure 2.
Diagnostic performance of Japan NBI Expert Team classification for differentiation among noninvasive, superficially invasive, and deeply invasive colorectal neoplasia. Sumimoto K. Gastrointest Endosc. 2017 Oct;86(4):700-709. doi: 10.1016/j.gie.2017.02.018. Epub 2017 Feb 28. PMID: 28257790.



BASIC Algorithm					
		HYPERPLASTIC	ADENOMA	SESSILE SERRATED	CANCER
SURFACE	Mucus present	No	No	Yes	No
	Regular (smooth) or irregular	Regular	Regular/ irregular	Regular/ irregular	Irregular
	Pseudodepression	No	Yes	No	No
	Depression	No	No	No	Yes
PITS	Featureless?	Yes	No	No	No
	Type (round/not round)	Round pits	Not round (e.g tubular)	Round pits with/without dark spots	Round or non round
	Distribution (regular = homogenous/ irregular = heterogenous - >1 pit pattern)	Homogenous	Homogenous or heterogenous without focal loss	Homogenous/ heterogenous	Heterogenous with focal loss
VESSELS	Present?	Yes or no	Yes	Yes or no	Yes
	Type	Lacy	Pericryptal	Pericryptal	Irregular

Figure 3.
Optical diagnosis of colorectal polyps with Blue Light Imaging using a new international classification. Subramaniam S. United European Gastroenterol J. 2019 Mar;7(2):316-325. doi:10.1177/2050640618822402. PMID: 31080616

5. STUDY INTERVENTIONS

5.1. POLYPECTOMY

All endoscopic procedures will be performed with High Definition (HD) instruments. In both groups of patients, the use of a cap and the performance of the colonoscopy with water / air / CO_2 will be chosen according to the preferences of the physician. Additionally, in both groups, a 0.3mm dedicated cold snare will be used. Each polyp that will be introduced in the study will be recorded by taking a photo, if possible.

5.2. EXCISION PROCEDURE

The procedure of Underwater Cold Snare Polypectomy (UP) will be as follows:

1. Complete suction of air from the intestinal tract
2. Partial opening of the intestinal lumen by using sterile room temperature water via a water pump
3. Complete immersion of the lesion in water
4. Snaring of the lesion and a small amount of surrounding healthy tissue (1-2mm)
5. Excision

The procedure of Conventional Cold Snare Polypectomy (CCSP) will be as follows:

1. If the entry during the endoscopy has been made with water, the water will be aspirated, and the intestinal lumen will be re-stretched using air / CO2
2. Snaring of the lesion and a small amount of surrounding healthy tissue (1-2mm)
3. Excision

In both categories of patients, the objective will be the en bloc resection of the lesion including healthy tissue (about 2mm). If the en bloc resection fails, a piecemeal will be performed on as few ιστοτεμαχιδίων as possible.
The tissue specimen will be placed in a vial with formol (Vial 1).

5.3 TIME RECORDING

For UCSP: time interval measurement

- Δt1 (secs) with t0 the start of air suction / water sprinkling and t.final the placement of the snare in the working channel. In the case of water assisted colonoscopy, Δt1 is probably equal to 0 as the polyp may already be immersed in water. The positioning of a polyp in ablation position (6^{th} hour) is also included in Δt1.
- Δt2 (secs) with t0 the placement of the snare in the working channel and t.final the completion of the polypectomy

For CCSP: measurement of time intervals

- Δt1 (secs) in the case of colonoscopy with air / CO2 is equal to zero. In the case of water assisted colonoscopy, t0 is defined as the beginning of water aspiration and the beginning of dilation of the intestinal tract with air. The positioning of a polyp in ablation position ($6^{η}$ ώρα) is included in Δt1.
- Δt2 (secs) with t0 the placement of the snare in the working channel and t.final the completion of the polypectomy.

5.4. RESECTION POINT OVERVIEW

After the resection of the lesion, a careful review of the resection point for bleeding or perforation will follow.

- Bleeding that lasts over a period of < 60 seconds will not be treated with endoscopic hemostasis (endoscopic clips) and will be recorded as self-limited.
- Bleeding that will persist > 60 seconds is characterized according to the instructions of ESGE 2017 as intraprocedural bleeding and can be treated either by water sprinkling or by placing hemostatic clips. The use of electrocautery or electrocoagulation methods will not be permitted.

This will be followed by a meticulous evaluation of the resection margins with digital endoscopy and a photograph of the site after the polypectomy if possible.
Where residual damage is observed, it will be removed using Cold Snare or biopsy forceps, but once again without using electrocautery or electrocoagulation methods. In

any case, in the end of the procedure the resection should be judged as endoscopically complete.

5.5. BIOPSIES FROM RESECTION SITE

Often the resection margins cannot be clearly defined during the pathological examination for reasons such as : the destruction of the tissue particles during aspiration into the working canal of the endoscope, specimens' autolysis and orientation inability. In order to determine the R0 resection rate and the incomplete resection rate, additional biopsies will be taken from the resection site as follows :

Horizontal margins :

In order to determine the horizontal margins, 2-4 biopsies will be taken from the resection area (2 biopsies antidiametrically in polyps of 5-7 mm size, 4 biopsies in polyps of 8-10 mm size). One cup of forceps will be placed in the defect and the second in the macroscopically normal mucosa.

The received tissue pieces will be placed in a new vial with formol (Vial 1A)

At the end of the endoscopic removal there will be 2 vials.

5.6. MARKING OF LESION'S POSITION

In order to easily locate the point of the polypectomy in the repeated colonoscopy, a submucosal tattoo should be made 2-3 cm peripherally of the lesion. The tattoo will always be performed after the polypectomy and biopsy. For rectal or cecum polyps, positioning does not require a submucosal tattoo, and may be aided by photographic recording prior to polypectomy.

6. STUDY PROCEDURES

6.1. RANDOMIZATION

Randomization will be conducted at the patient level. If more than one polyp is diagnosed in a patient, then all will be resected by the same method. Specifically, the randomization process will be done by using the random numbers method of Microsoft Excel 2016. A research assistant, who will not participate in clinical practice, will allocate patients with a 1:1 ratio to the UCSP and CCSP group and will inform the endoscopist of the polypectomy method when a polyp is found. The Excel distribution table will not be accessible by the endoscopists. After written consent, patients will not be aware of the polypectomy method that will be applied (blinding)

6.2. PATHOLOGICAL EXAMINATION

Specimens will be placed in vials with 10% formol (a total of 2 vials as mentioned above) and will be sent to the Pathological Laboratory. The slides will be stained in the DAKO CoverStainer using the DAKO Hematoxylin&Eosin Staining Kit. The slides will be then evaluated by two independent experienced pathologists, under a Nikon Eclipse 50i and a Nikon Eclipse E400 optical microscope (4X). Dysplastic changes will be classified using the Vienna classification system. In case multiple tissue fragments were biopsied, the aforementioned parameters will be calculated cumulatively.

Pathologists examining the tissues will be blinded and will not know the resection method (CCSP or UCSP) of each specimen.
Vial 1:

- Determination of polypectomy margin according to Residual Tumor Classification
- Determination of percentage of muscularis mucosa included in specimen. The determination of the area containing muscularis mucosa layer will be done by measuring the lenght of muscularis mucosa mm^2 / length of specimen mm^2x100%. Using an ocular and stage micrometer, the length of the muscularis mucosa underlining a neoplastic lesion was measured, along with the maximum depth of the muscularis mucosae and the specimen's maximum diameter
- Presence or absence of submucosa
- Measurement of submucosa depth in μm (when submucosa is present in the specimen)

Vial 1A :

- Determination of horizontal margins: presence of residual damage or not

A Nikon 10X microscope lens is used for microscopic measurement.

6.3. BLINDING

This is a double blinded study. As it was mentioned above, pathologists and patients will be blinded. Blinding cannot be applied in the endoscopist level because of the nature of the intervention.

7. SAFETY ASSESSMENTS

7.1. RISKS

Regardless patients' participation in the study, those who are diagnosed with colon polyps will undergo polypectomy according to ESGE & ASGE guidelines in order to prevent malignancy in the future. UCSP and CCSP are both safe methods used in

everyday clinical practice. Although as polypectomy is an interventional procedure, adverse events may occur but they are not study-related.

- Intraprocedural bleeding - IPB (bleeding during polypectomy), is defined according to the guidelines of ESGE (2017) bleeding that occurs during polypectomy and lasts more than 60 secs or bleeding that requires endoscopic intervention. In these cases, the hemostasis will be performed by using hemostatic clips. Bleeding episode during polypectomy that lasts less than 60 secs will be characterized as self-limited.

- Post-procedural bleeding - PPB (bleeding after polypectomy), is defined according to the guidelines of ESGE (2017) bleeding that occurs up to 30 days after polypectomy, and leads to the need for unplanned medical evaluation, or as a visit to the emergency room, or as the need for hospitalization or invasive treatment (repeated endoscopy for hemostasis, transfusion, angiography, surgery).
  >Early post procedural bleeding (after polypectomy) is defined as rectal bleeding within 48 hours of polypectomy.
  > Late bleeding (after polypectomy) is defined as rectal bleeding from 48 hours to 30 days after polypectomy

New endoscopy is indicated in patients with two or more episodes of moderate / severe bleeding, drop in hemoglobin of at least 2 g / dl, and / or hemodynamic instability.

- Perforation: presence of air or intestinal contents outside the gastrointestinal tract [39] Perforation is a rare adverse events and occurs in polypectomies of large size lesions and when electrocautery is used.
- Abdominal pain not due to perforation [39]

All complications and treatment of them will be recorded.

7.2 FOLLOW UP FOR ADVERSE EVENTS

The aforementioned complications will be evaluated by the study physician of the Gastroenterology Department of each hospital, 30 days after the polypectomy with a phone call and clinical examination if needed. Patients will be able to communicate with a physician who participates in the study 24h/24h.

7.3. FOLLOW UP ENDOSCOPY

After 12 months, endoscopy will be repeated to check for recurrence of the lesion. The procedure will be done with a careful overview of the area of the previous resection. The area of the previous resection will be indicated by the tattoo or by the photo file or by the endoscopists descriptions at their reports. If the scar from the prior polypectomy can be

located, two biopsies will be conducted from the scar area to determine the occurrence or absence of a microscopic recurrence.

8. STATISTICAL CONSIDERATIONS

8.1. SAMPLE SIZE

It was estimated that to find a mean difference of 15% at the main endpoint of the study (rate of the area containing muscularis mucosa%) and based on the literature data provided by a relevant research [Ref: 38], 198 people will be required per group (total 396) in order to find differences in significance level 0.05.

8.2. DATA ANALYSIS

Absolute and relative frequencies, 95% confidence intervals and frequency graphs will be used to illustrate the qualitative variables of the study. The mean values, standard deviations, median values, intra-quadratic ranges (25th-75th percentage points). Histograms and boxplots will be used to describe the quantitative variables. The Kolmogorov - Smirnov test will be run to check the normality of the distributions. Pearson's chi-square and Fisher's exact test will be applied to compare the ratios between the study groups. On the other hand, the Student's t-test or the non-parametric Mann-Whitney test will be used to compare quantitative variables between the two groups, depending on whether the data follow the normal distribution. Moreover, linear or logarithmic models will be used to check for differences between the studied groups, taking into account other factors (e.g. demographic and clinical characteristics). In case of asymmetrical distribution, logarithmic transformations of the variables will be used. Significance levels will be bilateral and the statistical significance will be set at 0.05. The analysis will be run with the statistical program SPSS 24.0.

9. DATA QUALITY

9.1. DATA COLLECTION AND MANAGEMENT

A data collection form (source document) will be completed for each patient including all patient's details according to the study protocol. Designated, especially trained investigator staff will enter the data required by the protocol into the case report forms (CRFs) and into the database. The Primary Investigator must check and certify that CRFs are complete and accurate. According to GCP patients' data will be available for sponsor's essential documents too.

9.2. QUALITY ASSESSMENT

9.2.1 MONITORING

Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. Sponsor will appoint a person with appropriate training, scientific and clinical knowledge to monitor this clinical trial. Monitor will verify that the rights of human subjects are protected, the reported trial data are accurate, complete, and verifiable from source documents, the conduct of the trial is in compliance with the currently approved protocol, with GCP, and with the applicable regulatory requirements.

Monitoring plan: first visit before initiation of patients enrollment and following regular visits site according to an agreed monitoring plan every 4-6 weeks.

9.2.2. AUDIT

Systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analyzed and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s). An audit is essentially checking to determine that the monitor has properly ensured what must be ensured.

10. ETHICAL CONSIDERATIONS

10.1. ETHICAL COMPLIANCE

The study design conforms with ICH–GCP, the Declaration of Helsinki, and local ethical and legal requirements. The study was approved by the Committee of Bioethics and Ethics of the Medical School - National and Kapodistrian University of Athens, Greece (75 Mikras Asias Street , 11527, Goudi - Athens, Greece, bioethics@med.uoa.gr , +30 210-7462099) with application number 123456789 and it has also been registered at Clinical Trials.gov (NCT123456789). The study was approved by the Insitutional Review Boards of all the participant hospitals. This trial protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT). The SPIRIT checklist has been included as Additional File 1

10.2. INFORMED CONSENT

IRB-approved, written informed consent (witnessed, where required by law or regulation) was obtained from all participants during the visit at the out-patient clinic before enrollment in the study. All participants took a copy document, and the original document was kept as one of the essential documents for trial’s master file (TMF).

10.3. PUBLICATION POLICY

This protocol will be posted in clinicaltrials.gov which is a publicly accessible database. Study's results and outcomes will be submitted for publication in international medical journal and presented in medical conferences related to interventional gastrointestinal endoscopy and polypectomy techniques.

11. REFERENCES

1.Jia H, Pan Y, Guo X, et al. Water Exchange Method Significantly Improves Adenoma Detection Rate: A Multicenter, Randomized Controlled Trial. Am J Gastroenterol.2017. April;112(4):568–576. [PubMed] [Google Scholar]

2. Hsieh YH, Tseng CW, Hu CT, et al. Prospective multicenter randomized controlled trial demonstrating water exchange (WE), but not water immersion (WI), significantly increases adenoma detection compared with air insufflation (AI) even in propofol sedated patients. GIE. 2017. July;86(1):192–201. [Google Scholar]

3. Cadoni S, Falt P, Rondonotti E, et al. Water exchange for screening colonoscopy increases adenoma detection rate: a multicenter, double-blinded, randomized controlled trial. Endoscopy. 2017.

4. Shao PP, Bui A, Romero T, Jia H, Leung FW. Adenoma and Advanced Adenoma Detection Rates of Water Exchange, Endocuff, and Cap Colonoscopy: A Network Meta-Analysis with Pooled Data of Randomized Controlled Trials. Dig Dis Sci. 2020 May 25. doi: 10.1007/s10620-020-06324-0. Epub ahead of print. PMID: 32451757.

5. Liu Y, Huang QK, Dong XL, Jin PP. Water exchange versus air insufflation for colonoscopy: A meta-analysis. Saudi J Gastroenterol. 2018 Nov-Dec;24(6):311-316. doi: 10.4103/sjg.SJG_118_18. PMID: 30226480; PMCID: PMC6253909.

6. Binmoeller KF. Underwater EMR without submucosal injection: Is less more? Gastrointest Endosc. 2019;89:1117-1119

7. Binmoeller KF, Weilert F, Shah J, Bhat Y, Kane S. "Underwater" EMR without submucosal injection for large sessile colorectal polyps (with video). Gastrointest Endosc. 2012;75(5):1086-1091. doi:10.1016/j.gie.2011.12.022

8. Cammarota G, Cesaro P, Cazzato A, et al. The water immersion technique is easy to learn for routine use during EGD for duodenal villous evaluation: a single-center 2-year experience. J Clin Gastroenterol 2009; 43:244-8.

9. Binmoeller KF, Hamerski CM, Shah JN, Bhat YM, Kane SD, Garcia-Kennedy R. Attempted underwater en bloc resection for large (2-4 cm) colorectal laterally spreading tumors (with video). Gastrointest Endosc. 2015;81:713-8.

10. Kawamura T, Sakai H, Ogawa T, et al. Feasibility of Underwater Endoscopic Mucosal Resection for Colorectal Lesions: A Single Center Study in Japan. Gastroenterology Research 2018;11:274-279.

11. Wang AY, Flynn MM, Patrie JT, Cox DG, Bleibel W, Mann JA, et al. Underwater endoscopic mucosal resection of colorectal neoplasia is easily learned, efficacious, and safe. Surg Endosc. 2014;28:1348-54

12. Curcio G, Granata A, Ligresti D, Tarantino I, Barresi L, Liotta R, et al. Underwater colorectal EMR: remodeling endoscopic mucosal resection. Gastrointest Endosc. 2015;81:1238-42.
13. Uedo N, Nemeth A, Johansson GW, Toth E, Thorlacius H. Underwater endoscopic mucosal resection of large colorectal lesions. Endoscopy. 2015;47:172-4.
14. Amato A, Radaelli F, Spinzi G. Underwater endoscopic mucosal resection: The third way for en bloc resection of colonic lesions? United European Gastroenterol J. 2016;4:595-8.
15. Yamashina T, Uedo N, Akasaka T, Iwatsubo T, Nakatani Y, Akamatsu T, et al. Comparison of Underwater vs Conventional Endoscopic Mucosal Resection of Intermediate-size Colorectal Polyps. Gastroenterology. 2019 Apr 11.
16. Yen, A. W., Leung, J. W., Wilson, M. D., & Leung, F. W. (2019). Underwater versus conventional endoscopic resection of nondiminutive nonpedunculated colorectal lesions: a prospective randomized controlled trial (with video). Gastrointestinal Endoscopy. doi:10.1016/j.gie.2019.09.039
17. Li DF, Lai MG, Yang MF, Zou ZY, Xu J, Peng RM, Xiong F, Wei C, Xu Z, Zhang D, Wang LS, Yao J. Efficacy and Safety of Underwater Endoscopic Mucosal Resection for ≥10 mm Colorectal Polyps: Systematic Review and Meta-analysis. Endoscopy. 2020 Aug 6. doi: 10.1055/a-1234-8918. Epub ahead of print.
18. Ferlitsch M, Moss A, Hassan C, et al. Colorectal polypectomy and endoscopic mucosal resection (EMR): European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. Endoscopy 2017;49:270–97.
19. Takeuchi Y, Yamashina T, Matsuura N et al. Feasibility of cold snare polypectomy in Japan: a pilot study. World J. Gastrointest. Endosc. 2015; 7: 1250–6.
20. Rabeneck L, Paszat LF, Hilsden RJ et al. Bleeding and perforation after outpatient colonoscopy and their risk factors in usual clinical practice. Gastroenterology. 2008; 135: 1899–906, 1906.e1.
21. Gimeno-Garcia AZ, de Ganzo ZA, Sosa AJ, Pérez DN, Quintero E. Incidence and predictors of postpolypectomy bleeding in colorectal polyps larger than 10 mm. Eur. J. Gastroenterol. Hepatol. 2012; 24: 520–6.
22. Heldwein W, Dollhopf M, Rosch T et al. The Munich Polypectomy Study (MUPS): prospective analysis of complications and risk factors in 4000 colonic snare polypectomies. Endoscopy. 2005; 37: 1116–22.
23. Veitch AM, Vanbiervliet G, Gershlick AH et al. Endoscopy in patients on antiplatelet or anticoagulant therapy, including direct oral anticoagulants: British Society of Gastroenterology (BSG) and European Society of Gastrointestinal Endoscopy (ESGE) guidelines. Endoscopy. 2016; 48: 385–402.
24. Hassan C, Repici A, Sharma P et al. Efficacy and safety of endoscopic resection of large colorectal polyps: a systematic review and meta-analysis. Gut. 2016; 65: 806–20.
25. Fujiya M, Tanaka K, Dokoshi T et al. Efficacy and adverse events of EMR and endoscopic submucosal dissection for the treatment of colon neoplasms: a meta-analysis of studies comparing EMR and endoscopic submucosal dissection. Gastrointest. Endosc. 2015; 81: 583–95.

26. Heldwein W, Dollhopf M, Rosch T et al. The Munich Polypectomy Study (MUPS): prospective analysis of complications and risk factors in 4000 colonic snare polypectomies. Endoscopy. 2005; 37: 1116–22.
27. Rosen L, Bub DS, Reed JF 3rd, Nastasee SA. Hemorrhage following colonoscopic polypectomy. Dis. Colon Rectum. 1993; 36: 1126–31.
28. Burgess NG, Metz AJ, Williams SJ et al. Risk factors for intraprocedural and clinically significant delayed bleeding after widefield endoscopic mucosal resection of large colonic lesions. Clin. Gastroenterol. Hepatol. 2014; 12: 651.e1-3–61.e1-3.
29. Singh M, Mehta N, Murthy UK, Kaul V, Arif A, Newman N. Postpolypectomy bleeding in patients undergoing colonoscopy on uninterrupted clopidogrel therapy. Gastrointest. Endosc. 2010; 71: 998–1005
30. Pohl H, Srivastava A, Bensen SP, Anderson P, Rothstein RI, Gordon SR, Levy LC, Toor A, Mackenzie TA, Rosch T, Robertson DJ. Incomplete polyp resection during colonoscopy-results of the complete adenoma resection (CARE) study. Gastroenterology. 2013 Jan;144(1):74-80.e1. doi: 10.1053/j.gastro.2012.09.043. Epub 2012 Sep 25. PMID: 23022496.
31. Maruoka D, Arai M, Akizue N, Ishikawa K, Kasamatsu S, Taida T, Ishigami H, Okimoto K, Saito K, Matsumura T, Nakagawa T, Katsuno T, Kato N. Residual adenoma after cold snare polypectomy for small colorectal adenomas: a prospective clinical study. Endoscopy. 2018 Jul;50(7):693-700. doi: 10.1055/s-0043-124869. Epub 2018 Feb 7. PMID: 29415287.
32. Zhang Q, Gao P, Han B, et al. Polypectomy for complete endoscopic resection of small colorectal polyps. Gastrointest Endosc 2018;87:733–40

12. ABBREVIATIONS

ASGE : American Society for Gastrointestinal Endoscopy
CCSP : Conventional Cold Snare Polypectomy
CRF : Case Report Form
CRR : Complete Resection Rate
CS : Cold Snare
CSP : Cold Snare Polypectomy
EMR : Endoscopic Mucosal Resection
ESGE : European Society of Gastrointestinal Endoscopy
GCP : Good Clinical Practice
HS : Hot Snare
HSP : Hot Snare Polypectomy
IPB : Intraprocedural Bleeding
IRR : Incomplete Resection Rate
MM : Millimetre
UCSP : Underwater Cold Snare Polypectomy
UEMR : Underwater Endoscopic Mucosal Resection
UP : Underwater Polypectomy

PPB : Postprocedural bleeding
R0RR : R0 Resection Rate
RCT : Randomized Controlled Trial
SOP :Sponsor's Standard Operating Procedures
SPIRIT : Standard Protocol Items: Recommendations for Interventional Trials
TMF : Trial's Master File