

Opinion paper

Study design of endoscopic polypectomy on clopidogrel (EPOC): A randomised controlled trial

Shara Ket^{a,*}, Andrew Metz^c, Alan Moss^{d,e}, Ravinder Ogra^f, William Tam^{g,h,i}, Robyn Secomb^a, John Reynolds^j, Peter R. Gibson^a, Gregor Brown^{a,b}

^a Department of Gastroenterology, Alfred Hospital and Monash University, Victoria, Australia

^b Epworth Health, Richmond, Victoria, Australia

^c Department of Gastroenterology, Melbourne Health, Victoria, Australia

^d Department of Endoscopic Services, Western Health, Melbourne, Australia

^e Department of Medicine – Western Health, Melbourne Medical School, The University of Melbourne, Victoria, Australia

^f Middlemore Hospital, Auckland, New Zealand

^g Royal Adelaide Hospital, South Australia, Australia

^h University of Adelaide, Australia

ⁱ Calvary North Adelaide Hospital, South Australia, Australia

^j Biostatistics Consulting Platform, Faculty of Medicine, Nursing & Health Sciences, Monash University, Melbourne, Australia



ARTICLE INFO

Keywords:

Clopidogrel
Prasugrel
Ticagrelor
Antiplatelet
Thienopyridine
Colonoscopy
Polypectomy
Bleeding

ABSTRACT

Concurrent cardiovascular disease and antiplatelet use (clopidogrel, prasugrel and ticagrelor) use poses a significant peri-endoscopic management challenge with a paucity of high-quality evidence available. Antiplatelet temporary interruption places patients at risk of serious cardiovascular thrombotic events. Continuing these agents potentially increases the risk of procedure related bleeding however this risk could be sufficiently mitigated by cold snare polypectomy and endoscopic clipping to manage intraprocedural bleeding, making routine colonoscopy on continued antiplatelet agents safe.

The EPOC trial will examine whether continuation of antiplatelet therapy (clopidogrel, prasugrel or ticagrelor) as single or dual therapy with aspirin, is inferior or superior to temporary interruption of antiplatelet therapy, current standard of care, with regard to the use of endoscopic rescue clips or clinically significant post-polypectomy bleeding after cold snare polypectomy of polyps ≤ 10 mm. EPOC is a parallel group, proceduralist-blinded randomized controlled trial comparing recruiting patients on antiplatelet therapy undergoing elective colonoscopy.

This trial is underway throughout Australia and New Zealand with a view to expanding to additional sites. 496 subjects in each arm are required for this study. EPOC is the first randomised controlled trial comparing temporary interruption with continuation of antiplatelet therapy in patients undergoing elective colonoscopy.

1. Introduction

Colonoscopic polypectomy has been associated with a 32% colorectal cancer mortality reduction over 30 years [1] and is routine practice worldwide for the prevention of colorectal cancer.

With an aging population, an increasing proportion of patients undergoing elective colonoscopy have concurrent cardiovascular disease requiring thienopyridine use such as clopidogrel, prasugrel and ticagrelor as single or dual antiplatelet therapy. This poses a peri-endoscopic management challenge balancing the risk of bleeding with

thromboembolic complications.

While predominantly retrospective and somewhat mixed, existing data on patient outcomes regarding the peri-endoscopic bleeding risk when thienopyridines are continued are favourable overall [2–5].

Temporary interruption for intervention or surgery is common with an incidence of 57.3% within a 2 year period following stent implantation [6]. Endoscopy accounts for approximately 40% of the minor surgical procedures requiring temporary interruption. 2.4% of these patients subsequently experiencing a thrombotic event [7]. Although the risk of thromboembolic events is small, it is a clinically significant

* Corresponding author. Department of Gastroenterology and Hepatology, The Alfred Hospital, Melbourne, Australia, 3004.

E-mail address: s.ket@alfred.org.au (S. Ket).

<https://doi.org/10.1016/j.conctc.2019.100479>

Received 7 May 2019; Received in revised form 20 October 2019; Accepted 24 October 2019

Available online 1 November 2019

2451-8654/© 2019 The Authors.

Published by Elsevier Inc.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

event for a patient.

The ASGE guidelines [8] and combined BSG and ESGE guideline [9] recommend continuing antiplatelet agents for low-risk procedures. Both guidelines consider colonoscopic polypectomy a high risk procedure however neither guideline differentiates between polypectomy of small polyps (e.g. ≤ 10 mm) and larger polyps. Small polyps are commonly found during low risk, routine colonoscopy [10] and practically speaking, the risk of bleeding following subcentimetre polypectomy is small [11] with the risk of bleeding increasing with increasing polyp size [12].

Modest data favours cold snare polypectomy over hot snare polypectomy [13] and combined with readily available, easy to use clips to manage intra-procedural bleeding, it may be that small polyps can be safely removed on antiplatelet agents, conferring the cardiovascular benefit of continuing antiplatelet therapy, whilst having small polyps removed without any clinically significant risk to the patient of post-procedural bleeding.

The purpose of this article is to describe the protocol for a randomised controlled trial in Australasia (EPOC - Endoscopic polypectomy on clopidogrel: a randomised controlled trial).

2. Method

2.1. Design overview

EPOC is a parallel group, proceduralist-blinded randomized controlled-trial comparing 2 different peri-endoscopic management strategies for patients on already receiving antiplatelet therapy (clopidogrel, prasugrel or ticagrelor) as single agent or in conjunction with aspirin, who are undergoing elective colonoscopy. Participants will be randomised at a 1:1 ratio to either “temporary interruption of antiplatelet therapy” or “continuation of antiplatelet therapy” (see Fig. 1).

2.2. Setting and participants

The trial will recruit at 20 academic hospitals throughout Australia and New Zealand. Participants are identified via site specific mechanisms which include endoscopy booking staff, gastroenterology clinics and triage systems. Eligible patients are screened and provided with a participant information and consent form. Once consent has been received, patients are then randomised by an associate investigator not involved in the endoscopic procedure to maintain proceduralist

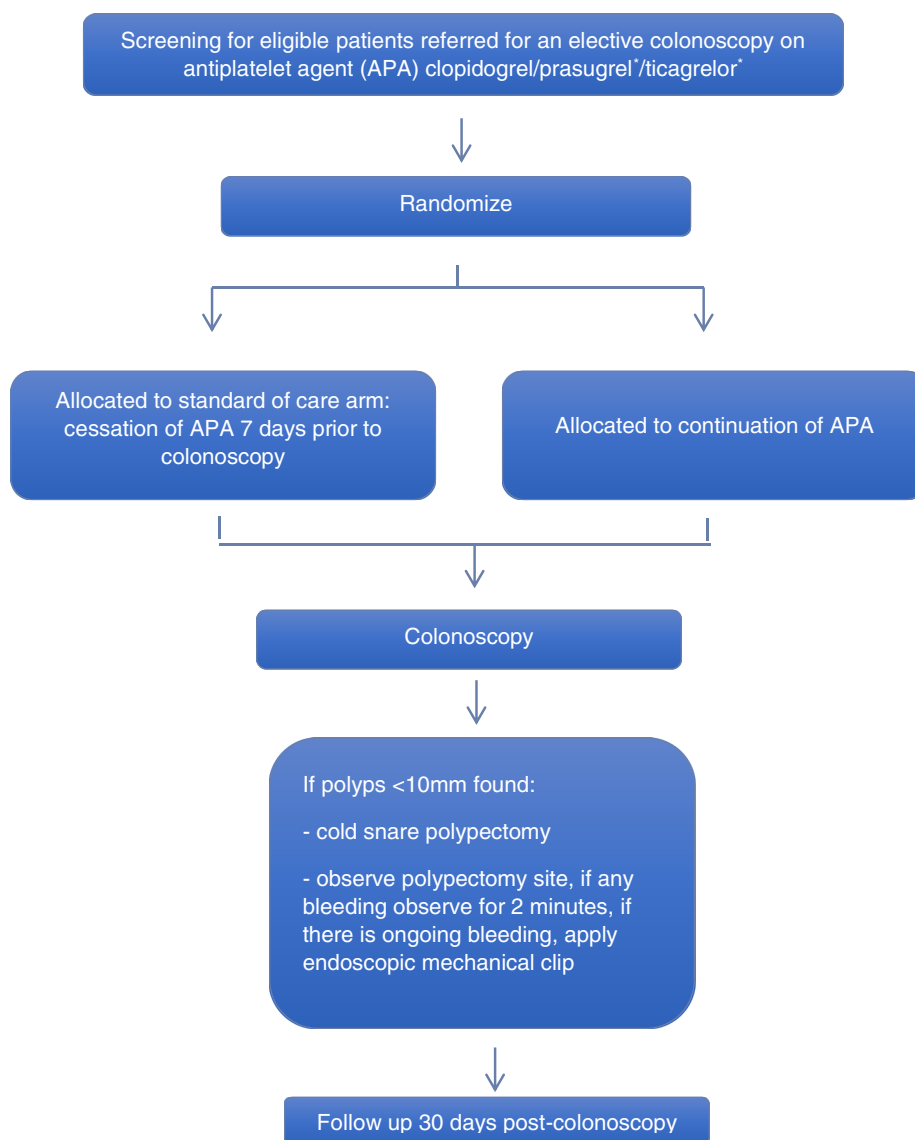


Fig. 1. Trial schema.

* To be included after a safety analysis.

blinding. Follow up will be performed at 30 days after the colonoscopy to assess for study outcomes or adverse events.

2.2.1. Inclusion criteria

- Any patient aged >18 years scheduled for routine colonoscopy who is
 - o On single agent antiplatelet therapy: clopidogrel or prasugrel or ticagrelor

OR.

- o On dual antiplatelet agent: aspirin *and* clopidogrel or prasugrel or ticagrelor

Initially the trial will commence with patients on clopidogrel ± aspirin which is a special condition for approval for this trial placed by the Alfred Hospital Human Research and Ethics Committee. This relates to assay studies of prasugrel and ticagrelor demonstrating significantly greater inhibition of platelet aggregation [14,15]. The addition of patients on prasugrel and ticagrelor will only occur after an interval safety analysis has been performed and approval obtained from the lead Human Research Ethics Committee.

2.2.2. Exclusion criteria

- Liver cirrhosis
- Chronic renal impairment (eGFR ≤ 30)
- History of a bleeding diathesis
- Thrombocytopenia of any cause (Platelet count ≤ 90)
- Other concurrent anticoagulation/antiplatelet agents
- Percutaneous coronary intervention
 - o with bare metal stent within the last 30 days
 - o with drug eluting stent within the last 12 months
- Acute coronary syndrome within the last 90 days
- Any other concern by treating physician(s)
- Inability to provide informed consent

2.3. Randomisation

Sites are provided with uniquely identified sealed envelopes containing randomisation instructions according to a computer-generated randomisation schedule, in a ratio of 1:1. Participants are allocated to one of two peri-endoscopic management strategies; “temporary interruption of antiplatelet therapy” or “continuation of antiplatelet therapy”.

2.4. Blinding

The proceduralist performing the procedure will be blinded to randomisation arms. The proceduralist will be more likely to use rescue endoscopic techniques if they are aware a patient is on clopidogrel. Patients will not be blinded as they will have to stop their clopidogrel.

2.5. Intervention

Colonoscopy bowel preparation and sedation will be as per usual practice for each site.

The trial will compare standard of care (temporary interruption of antiplatelet therapy) with continuation of antiplatelet therapy.

Colonoscopy and any polypectomy procedures will be performed by senior endoscopists or endoscopy fellows under the supervision of senior endoscopist.

2.5.1. Standard care arm

Standard of care involves temporary interruption of antiplatelet

therapy (clopidogrel or prasugrel or ticagrelor). Clopidogrel, prasugrel or ticagrelor will be ceased 7 days prior to colonoscopy and commencement 2 days after colonoscopy. If a patient is not usually on aspirin, low dose aspirin (75 mg–100 mg) orally daily will be commenced to bridge the period the patient is off antiplatelet medication. If a patient is on antiplatelet therapy plus aspirin, patient’s usual dose of aspirin is continued. If there are any concerns about temporary interruption of antiplatelet therapy, the endoscopist can liaise with the prescribing physician to decide whether it is appropriate to temporarily interrupt antiplatelet therapy, or the patient can be excluded from the trial.

2.5.2. Intervention arm

The intervention arm involves continuation of single or dual antiplatelet therapy (clopidogrel or prasugrel or ticagrelor) peri-endoscopically. This includes on the day of the procedure.

2.5.3. Polypectomy

If polyps are found, ultimately management is at the discretion of the endoscopist to ensure optimal patient care. However, to be included in the primary endpoint analysis cold snare polypectomy of polyps ≤10 mm is required. The polypectomy site will be observed for persistent bleeding over 2 min. If haemostasis has not occurred, adjunctive therapy in the form of mechanical clips will be used. In addition, a limit of up to 10 polyps during a single colonoscopy was decided to mitigate the risk of bleeding due to multiple polypectomies.

If polyps larger than 10 mm are encountered, management is at the discretion of the endoscopist with options including piecemeal cold snare, endoscopic mucosal resection, or rescheduling the procedure. If more than 10 polyps are removed or polyps >10 mm are removed, these patients will not be included in the safety analysis or primary endpoint.

2.6. Study endpoints

2.6.1. Primary endpoints

The primary aim of the study is to investigate whether or not “continuation of antiplatelet therapy” differs, in terms of bleeding, to “temporary interruption of antiplatelet therapy”. A composite primary end point will be used which captures both the intra-procedural and post-procedural risk of bleeding. This will include occurrence of the use of rescue endoscopic clips post-polypectomy to control immediate persistent intra-procedural bleeding, or major delayed bleeding, which must be symptomatically or clinically overt and is associated with an unplanned admission or re-admission to hospital for rectal bleeding or bleeding that directly contributes to death.

If continuation of antiplatelet therapy appears to be neither inferior nor superior to temporary interruption of antiplatelet therapy, a supplementary aim of the study is to investigate whether or not the two interventions are equivalent.

2.6.2. Secondary endpoints

Secondary endpoints are other bleeding and thromboembolic complications including non-major bleeding defined as any sign or symptom of PR bleeding that does not fit the above criteria; need for further intervention for bleeding such as endoscopic, surgical or radiological intervention; requirement for red blood cell transfusion; transient ischaemic attack defined as a brief neurological deficit caused by focal brain ischaemia, with clinical symptoms lasting greater than 1 h but less than >24 h; stroke defined as any new, focal neurologic deficit that persists for >24 h or any new, focal neurologic deficit of any duration and evidence of acute infarction on CT or MRI of the brain; myocardial infarction [16] defined as detection of a rise and/or fall of cardiac biomarker values with at least one value above the 99th percentile of the upper reference limit with at least one of the following i) symptoms of ischaemia, ii) new or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block, iii) development of

pathological Q waves in the ECG, iv) imaging evidence of new loss of viable myocardium with new regional wall motion abnormality or v) identification of an intracoronary thrombus by angiography; stent thrombosis defined by angiographic confirmation of thrombosis in the stent or within 5 mm proximal or distal to the stent, with or without occlusion, which is associated with at least one of the following, i) acute onset of ischaemic symptoms at rest or ii) ECG signs of acute ischaemia; other serious adverse event and a composite end point of major clinically significant bleeding and thromboembolic event.

2.7. Participant screening and selection

Potential trial participants will be identified prior to endoscopy when referrals for colonoscopy are received by endoscopy booking, at gastroenterology clinics and by triaging personnel. All other aspects of the colonoscopy will be managed in accordance with the hospital pathways and protocols. Eligible patients will be screened against inclusion criteria and exclusion criteria and then approached with a participant information and consent form. All participants are required to provide written informed consent prior to randomisation occurs. Follow up occurs at Day 30 via clinic or telephone to assess for study outcomes and adverse events.

2.8. Trial Steering Committee

A Trial Steering Committee will provide advice to the chief investigator on the progress of the trial in regards to recruitment rates; develop strategies to deal with any recruitment problems; monitor follow-up rates and review strategies to deal with problems including sites that deviate from the protocol; oversee the timely reporting of trial results; approve and comment on the main trial manuscript; approve and comment on any abstracts and presentations of any results during the running of the trial.

2.9. Data safety monitoring board

An independent data safety monitoring board (DSMB) has been appointed to review all serious adverse events, to interpret interim analysis of the primary endpoint and review any protocol amendments. The DSMB will review accumulating data approximately 6 monthly while the trial remains active.

2.10. Sample size and statistical method for the primary endpoint

We are aiming for a target of 992 patients in the Full Analysis Set which includes randomised patients who have had a polypectomy. We expect that this will require up to 2730 patients to be registered on the study and randomized (1:1) to the two treatment arms (intervention and control).

The proportion of patients in a treatment arm who experience a bleed will be calculated using the number of FAS patients in that treatment arm as the denominator. We conjecture that the proportion in the control arm (π_c) is 0.10 and we will test the null hypothesis that the two treatment arms have the same proportions ($H_0: \pi_t = \pi_c$) with a two-sided binomial test conducted at the 5% significance level ($\alpha = 0.05$). Under the specific alternative that the treatment arms differ by 0.06 or more ($|\pi_t - \pi_c| > 0.06$) we require at least 980 evaluable patients (490 in each treatment arm) in order for the two-sided test to have 80% power. To provide a trigger for modification or stopping of the trial, if one arm is evidently inferior to the other, we have made provision for two interim analyses, roughly equally spaced in terms of the number of accrued and evaluable patients. The Lan-DeMets (O'Brien-Fleming-like) alpha-spending function will be used to set the significance level (α) at the time of each interim analysis. With 992 evaluable patients (496 per arm), if the interim analyses occur precisely after $n = 331$ and $n = 661$ patients have been assessed then the respective alphas are 0.0002 and

0.0120, and, for the final analysis, $\alpha = 0.0463$ (in this way the cumulative alpha at the final analysis is 0.05), and, with a test based on the un-pooled estimate of the variance, 80% power is maintained (East 6, Cytel Inc., Cambridge, MA 02139, USA).

2.11. Data collection

A screening log of all identified patients on single or dual antiplatelet therapy will be recorded. The number of eligible patients and the number of patients giving informed consent will also be recorded.

All data will be recorded on paper data collection forms. Centres will send completed copies of these data collection forms to the data manager at the lead site, where they will then be entered into a trial database.

2.11.1. Baseline characteristics

Baseline clinical characteristics will be obtained from medical records which include age, gender, indication for antiplatelet agent, name of antiplatelet agent, use of concurrent NSAIDs or COX-2 inhibitors and indication for colonoscopy.

2.11.2. Endoscopic data

Endoscopic data will include date of procedure, polyp details including size, Paris classification, location, if there is any immediate bleeding post polypectomy, if there is any continued bleeding after 2 min, whether intraprocedural haemostasis was achieved and salvage techniques required to achieve this. Additionally, biopsies taken, the requirement of any other endoscopic intervention and any other peri-procedural complications will also be recorded.

Histological specimens will be treated and managed as per the usual standard of care for each site; histology and dysplasia will be recorded for each polyp.

2.11.3. 30 day follow up

The mode and date of follow up will be performed to ascertain if there were any bleeding, vascular or other complications. The presence of any bleeding complications will be recorded as minor, not requiring medical attention or significant, requiring medical attention. The need for admission, number of red packed cells transfused, and any intervention to control bleeding will also be included. Any type of vascular complication and resulting medical management will be recorded, as will any other complications including death.

2.12. Ethics

The trial received ethical approval from the following Ethics committees: Alfred Hospital Human Research and Ethics Committee HREC number HREC/16/Alfred/22 to conducting the study at public centres in Victoria, New South Wales and Queensland; Epworth Health Care reference number EF2016-128; Calvary Health Care Adelaide HREC number 17-CHREC-F001 and St John of God Health Care local reference number 1020. The trial has also been registered on the Australian New Zealand clinical Trials Registry (ANZCTR), registration number ACTRN12616000895482.

3. Discussion

Traditionally it has been assumed that medications prescribed for inhibiting platelet aggregation increases the risk of bleeding following invasive procedures such as colonoscopic polypectomy. After the emergence of colonoscopic polypectomy, it was common to stop aspirin due to concern about increased risk of post-polypectomy bleeding [17–19] however it was subsequently demonstrated that there was no increased risk [17] and current international guidelines recommend peri-endoscopic continuation of aspirin [8,9].

Medical management of cardiovascular disease now requires more

potent antiplatelet agents such as clopidogrel, prasugrel and ticagrelor. For colonoscopic polypectomy, irrespective of the size of the polyps, current guidelines recommend temporary interruption. This is not practical since approximately 24% of low risk procedures, where clopidogrel is recommended to continue, will have polyps found [20]. Given polypectomy is considered a high risk procedure many endoscopists choose to temporarily stop these agents prior to colonoscopy in anticipation of small polyps being found and resected.

Post polypectomy bleeding following removal of small polyps rarely has serious clinical sequelae and if further intervention is required, simple endoscopic haemostasis techniques are usually sufficient. Conversely, an atherothrombotic event manifesting as vascular associated ischaemia is a clinically significant event for a patient which may be more difficult to salvage clinically.

International guidelines have been based on expert opinion and moderate quality evidence however there are no clinical trials addressing this issue. This is the first randomised controlled trial addressing the question of whether antiplatelet therapy needs to be temporarily stopped or continued prior to elective colonoscopy using dedicated cold snare polypectomy methods. This study aims to determine whether the rate of use of intraprocedural endoscopic rescue clips or clinically significant post-polypectomy bleeding differs in patients continuing on clopidogrel, prasugrel or ticagrelor compared with those who temporarily cease them. Patients potentially benefit from continuing their antiplatelet medication with ongoing cardiovascular protective effects while still allowing safe removal of small polyps at the time of the colonoscopy.

Acknowledgments

We thank Olympus Australia who provided financial research support for this study. Shara Ket has a scholarship from Monash University for a PhD which includes managing this EPOC study.

Appendix

Australian Sites & principal co-investigators

Alfred Hospital, Jolimont Endoscopy Centre & Epworth Hospital, Richmond Brown G., St John of God Hospital, Geelong, Alexander S., Queen Elizabeth II (Jubilee) Hospital, Hewett D., Royal Melbourne Hospital, Metz A., Footscray Hospital, Sunshine Hospital & Sunbury Hospital, Moss A., Calvary North Adelaide Hospital, Tam W., Box Hill Hospital, Urquhart P., Austin Hospital, Vaughan R., Northern Hospital, Butt J., The Bays Hospital, La Nauze R., Peel Health Campus, Raftopoulos S.

New Zealand Sites & principal co-investigators

Middlemore Hospital, Auckland, New Zealand Ogra R., Christchurch Hospital, Christchurch Lim G.

Statistician

Associate Professor John Reynolds.

Data Safety Monitoring Board

Professor Finlay Macrae.
Professor Stuart Roberts.

Associate Professor William Kemp.

Trial Steering Committee

Associate Professor Andrew Metz.
Associate Professor Alan Moss.
Dr Ravinder Ogra.
Associate Professor William Tam.
Dr Shara Ket.

References

- [1] R. Nishihara, K. Wu, P. Lochhead, et al., Long-term colorectal-cancer incidence and mortality after lower endoscopy, *N. Engl. J. Med.* 369 (2013) 1095–1105.
- [2] S. Gandhi, N. Narula, W. Mosleh, et al., Meta-analysis: colonoscopic post-polypectomy bleeding in patients on continued clopidogrel therapy, *Aliment. Pharmacol. Ther.* 37 (2013) 947–952.
- [3] M. Singh, N. Mehta, U.K. Murthy, et al., Postpolypectomy bleeding in patients undergoing colonoscopy on uninterrupted clopidogrel therapy, *Gastrointest. Endosc.* 71 (2010) 998–1005.
- [4] L.A. Feagins, F.S. Uddin, R.E. Davila, et al., The rate of post-polypectomy bleeding for patients on uninterrupted clopidogrel therapy during elective colonoscopy is acceptably low, *Dig. Dis. Sci.* 56 (2011) 2631–2638.
- [5] L.A. Feagins, R. Iqbal, W.V. Harford, et al., Low rate of postpolypectomy bleeding among patients who continue thienopyridine therapy during colonoscopy, *Clin. Gastroenterol. Hepatol.* 11 (2013) 1325–1332.
- [6] R. Mehran, U. Baber, P.G. Steg, et al., Cessation of dual antiplatelet treatment and cardiac events after percutaneous coronary intervention (PARIS): 2 year results from a prospective observational study, *Lancet* 382 (2013) 1714–1722.
- [7] M. Schoos, J. Chandrasekhar, U. Baber, et al., Causes, timing, and impact of dual antiplatelet therapy interruption for surgery (from the patterns of non-adherence to anti-platelet regimens in stented patients Registry), *Am. J. Cardiol.* 120 (2017) 904–910.
- [8] R.D. Acosta, N.S. Abraham, V. Chandrasekhara, et al., The management of antithrombotic agents for patients undergoing GI endoscopy, *Gastrointest. Endosc.* 83 (2016) 3–16.
- [9] A.M. Veitch, G. Vanbiervliet, A.H. Gershlick, 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* 48 (2016) 385–402.
- [10] D.K. Rex, J.L. Pettrini, T.H. Baron, et al., Quality indicators for colonoscopy, *Am. J. Gastroenterol.* 101 (2006) 873–885.
- [11] W. Heldwein, M. Dollhopf, T. Rosch, et al., The Munich Polypectomy Study (MUPS): prospective analysis of complications and risk factors in 4000 colonic snare polypectomies, *Endoscopy* 37 (2005) 1116–1122.
- [12] N.G. Burgess, A.J. Metz, S.J. Williams, et al., Risk factors for intraprocedural and clinically significant delayed bleeding after wide-field endoscopic mucosal resection of large colonic lesions, *Clin. Gastroenterol. Hepatol.* 12 (2014) 651–661, e1-3.
- [13] A. Horiuchi, Y. Nakayama, M. Kajiyama, et al., Removal of small colorectal polyps in anticoagulated patients: a prospective randomized comparison of cold snare and conventional polypectomy, *Gastrointest. Endosc.* 79 (2014) 417–423.
- [14] P.A. Gurbel, K.P. Bliden, K. Butler, et al., Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study, *Circulation* 120 (2009) 2577–2585.
- [15] B. Zhu, M.B. Efron, M.P. Kulkarni, et al., The onset of inhibition of platelet aggregation with prasugrel compared with clopidogrel loading doses using gatekeeping analysis of integrated clinical pharmacology data, *J. Cardiovasc. Pharmacol.* 57 (2011) 317–324.
- [16] K. Thygesen, J.S. Alpert, A.S. Jaffe, et al., Third universal definition of myocardial infarction, *Circulation* 126 (2012) 2020–2035.
- [17] A.J. Hui, R.M. Wong, J.Y. Ching, et al., Risk of colonoscopic polypectomy bleeding with anticoagulants and antiplatelet agents: analysis of 1657 cases, *Gastrointest. Endosc.* 59 (2004) 44–48.
- [18] H. Nakajima, H. Takami, K. Yamagata, et al., Aspirin effects on colonic mucosal bleeding: implications for colonic biopsy and polypectomy, *Dis. Colon Rectum* 40 (1997) 1484–1488.
- [19] M.D. Basson, L. Panzini, R.H. Palmer, Effect of nabumetone and aspirin on colonic mucosal bleeding time, *Aliment. Pharmacol. Ther.* 15 (2001) 539–542.
- [20] R.L. Barclay, J.J. Vicari, A.S. Doughty, et al., Colonoscopic withdrawal times and adenoma detection during screening colonoscopy, *N. Engl. J. Med.* 355 (2006) 2533–2541.