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Risk Stratification for Covert Invasive Cancer Among Patients Referred for Colonic Endoscopic Mucosal Resection: A Large Multi-center Cohort

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Author contributions:

Author	**Nature of contribution**
Nicholas Burgess	Designed the study, collected data, analyzed data, wrote the manuscript and revised the manuscript after review by the co-authors.
Luke Hourigan	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Simon Zanati	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Gregor Brown	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Rajvinder Singh	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Stephen Williams	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Spiro Raftopoulos	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Donald Ormonde	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Alan Moss	Identified and recruited patients, performed procedures, collected data and critically reviewed the manuscript.
Karen Byth	Statistical analysis of data
Hema Mahajan	Collected and organized data, examined histological and pathogical specimens and critically reviewed the manuscript.
Duncan McLeod	Collected and organized data, examined histological and

	pathogical specimens and critically reviewed the manuscript.
Michael Bourke	Initiated, designed and led the study. Identified and recruited patients, performed procedures, collected data, co-wrote the manuscript and critically reviewed the manuscript.

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(The Australian Colonic Endoscopic Resection (ACE) study; ClinicalTrials.gov NCT02000141).

Background & Aims: Among patients with large colorectal sessile polyps or laterally spreading lesions, it is important to identify those at risk for submucosal invasive cancer (SMIC). Lesions with overt endoscopic evidence of SMIC are referred for surgery, although those without these features might still contain SMIC that is not visible on endoscopic inspection (covert SMIC). Lesions with a high covert SMIC risk might be better suited for endoscopic submucosal dissection (ESD) than for endoscopic mucosal resection (EMR). We analyzed a group of patients with large colon lesions to identify factors associated with SMIC, and examined lesions without overt endoscopic high risk signs to determine factors associated with covert SMIC.

Methods: We performed a prospective cohort study of consecutive patients referred for EMR of large sessile or flat colorectal polyps or laterally spreading lesions (20 mm or greater in size) at academic hospitals in Australia from September 2008 through September 2016. We collected data on patient and lesion characteristics, outcomes of procedures, and histology findings. We excluded serrated lesions from the analysis of covert SMIC due to their distinct phenotype and biologic features.

Results: We analyzed 2277 lesions (mean size 36.9 mm) from 2106 patients (mean age 67.7 years; 53.2% male). SMIC was evident in 171 lesions (7.6%). Factors associated with SMIC included Kudo V pit pattern, a depressed component (0–IIc), rectosigmoid location, 0–Is or 0–IIa+Is Paris classification, non-granular surface morphology, and increasing size. Following exclusion of lesions that were obviously SMIC or serrated, factors associated with covert SMIC were rectosigmoid location (odds ratio, 1.87; P=.01), combined Paris classification, surface morphology (odds ratios, 3.96–22.5), and increasing size (odds ratio, 1.16/10 mm; P=.012).

Conclusions: In a prospective study of 2106 patients who underwent EMR for large sessile or flat colorectal polyps or laterally spreading lesions, we associated rectosigmoid location, combined Paris classification and surface morphology, and increasing size with increased risk for covert malignancy. Rectosigmoid 0–Is and 0–IIa+Is non-granular lesions have a high risk for malignancy, whereas proximally located 0–Is or 0–IIa granular lesions have a low risk. These findings can be used to inform decisions on which patients should undergo ESD, EMR, or surgery. ClinicalTrials.gov no: NCT02000141

KEY WORDS: colon cancer, tumor, prognostic factor, prediction

Introduction

The prediction of submucosal invasive cancer (SMIC) is an integral part of the endoscopic evaluation of large colonic lesions. Lesions with a high risk of SMIC require careful decision making in order to select the best therapeutic modality and optimize outcomes for the patient. The gross morphology and surface characteristics of large colonic lesions have been shown to predict SMIC, but independently overestimate the risk in the majority of lesions and underestimate the highest risk lesions. Existing classification systems have used data derived primarily from Japanese cohorts and are typically single centre and retrospective[1,2]. Most studies enrol primarily small or diminutive lesions which can easily be entirely viewed lesions en-face in one image[3,4]. Several studies utilise specialized magnification endoscopes or chromoendoscopy, tools which are not available to the majority of endoscopists worldwide[1,5]. The Paris classification[6] is used to describe lesion morphology, however it was designed as a descriptive tool rather than a clinical risk stratification aide. Laterally spreading tumours (LSTs) have been characterised as granular, (LST-G) or non-granular (LST-NG), however this classification in isolation poorly predicts SMIC[1]. Overt SMIC is often readily manifest by a depressed or ulcerated component to the lesion or an

area of disrupted surface pit pattern. The Kudo classification of surface pit patterns strongly predicts SMIC[7,8], (Kudo V) but in lesions without these overt features, diminutive focal SMIC is often endoscopically undetectable. An accurate method of stratifying the risk of endoscopically non-visible, or "covert" malignancy in these lesions is required in order to guide resection choice. Any classification system should be simple to use and reflect clinical outcomes. In a prior study we evaluated risk factors for SMIC by univariable analysis in a cohort of 479 patients referred for EMR[9]. We identified Paris 0–IIa+c classification, LST-NG, and Kudo V as risk factors, and noted that the presence of multiple factors magnified risk.

In the current study we identify the key clinical and endoscopic features associated with SMIC within this large prospective multicentre cohort. We then excluded lesions with overt evidence of SMIC to identify factors associated with "covert" SMIC. Serrated lesions were excluded as they have biologic and phenotypic characteristics which fundamentally differ from conventional adenomas, and they also represent a minority of lesions. We have used the identified factors to develop a pragmatic schema for guiding endoscopic resection decisions. We also aim to examine whether these factors are associated with endoscopically curable superficial SMIC (SM1) or non-resectable deep SMIC (SM2/3).

Materials and Methods

Prospective observational multicentre data on consecutive patients referred to one of eight Australian academic hospitals for the management of large sessile and flat colorectal polyps or laterally spreading lesions (LSL) ≥20mm were analysed. The study period was from September 2008 to September 2016 and is registered as The Australian Colonic Endoscopic Resection (ACE) study (ClinicalTrials.gov NCT01368289 & NCT02000141). All lesions had been initially identified and referred by a nationally accredited consultant endoscopist. Institutional review board approval was obtained at each centre. Written informed consent was obtained from each patient on the day of the procedure. Data were recorded in a comprehensive centralized database.

All EMR procedures were performed by a study investigator or a senior therapeutic endoscopy fellow under direct supervision. All clinical investigators were gastroenterologists with significant prior colonic EMR experience after training in high-volume tertiary referral centers in Australia or overseas. Colonoscopy was performed using Olympus 180 or 190 series high-definition variable-stiffness colonoscopes (180/190 PCF/CF; Olympus, Tokyo, Japan). The EMR technique is standard across all centres, and has previously been described in detail[9]. Data collection included patient and lesion characteristics, procedural events and outcomes, complications and scheduled follow up at 14 days, 4-6 and 16 months. Data was prospectively collected at the time of patient admission, during, and then immediately after the procedure. Lesions were carefully examined in vivo by one of the study investigators at the initial endoscopy and classified according to Paris classification, Kudo Pit Pattern (KPP) and surface topography (LST-G, LST-NG or mixed). Paris classification is a consensus international standard for defining superficial gastrointestinal lesion morphology[6]. Elevated (>2.5mm above the surrounding normal mucosa) sessile lesions are described as Type 0-Is and sessile lesions under 2.5mm classed as 0-IIa (slightly elevated), 0-IIb (flat) or 0-IIc (slightly depressed). Excavated lesions are classed as 0-III. KPP is a classification of the endoscopic appearance of surface mucosal crypts[10]. A type V pattern is a disorganised pit pattern associated with invasive malignancy. Although initially described using magnification endoscopy, pit pattern can be discerned using high definition endoscopes although this has not been validated experimentally. Clinical follow-up for the index procedure was obtained at 14 days by structured telephone interview. Histology data was also collected at this time. All authors had access to the study data and reviewed and approved the final manuscript.

Pathological Analysis

Histologic specimens were analysed at their respective study centre pathology department. Results were then centrally collated on a prospectively maintained database. Surgical histology reports were obtained where patients had undergone surgical resection. In cases

where the underlying polyp type was not evident, primarily due to obliteration by invasive CRC, the case was reviewed and classified according to the morphology and molecular changes in the CRC.

Data and Analyses

SPSS statistical software (IBM Corp. 2012. IBM SPSS Statistics, Version 23.0. Armonk, NY) was used to analyze the data. All analyses were exploratory and two-tailed tests with a significance level of 5% were used throughout. No attempt was made to correct for multiple comparisons. Data analysis was per lesion, however for analyses that included patient level data where patients had two or more lesions resected in one procedure, the largest lesion was selected for analysis. Mann–Whitney U tests were used to test for differences in the distribution of age and lesion size. The Pearson χ^2 or the Fisher exact test was used to test for association between categorical variables and outcome. Multiple logistic regression with backward stepwise variable selection was used to identify the independent predictors of outcomes of interest. Candidate variables with P values for association that were equal to or less than 0.1 on univariable analysis were considered as potential risk factors in multiple logistic regression analysis. Backward stepwise variable selection was used to identify the best-fitting model and independent factors associated with SMIC. Odds ratios with 95% confidence intervals (95%CIs) from the model were used to quantify the extent of this association.

To determine factors associated with covert SMIC, lesions with endoscopically overt high risk features consistent with SMIC (Kudo V pit pattern, depressed Paris 0-IIc component) were excluded. Lesions with serrated histology or a serrated endoscopic appearance were excluded as they typically have characteristic endoscopic findings, a generally lower risk of SMIC and fundamentally different biological behaviour[11,12]. Non-dysplastic serrated lesions have identifiable features which allow them to be easily recognized[13]. Sessile serrated polyps with dysplasia (SSP-D) may be more likely to endoscopically resemble conventional adenoma, so prospective lesion assessment may result in these being misclassified[14–16]..

Moreover, SSP-D may be very rapidly progressive and represent only a small proportion of total lesions so it was thought that excluding these lesions would allow a more valid assessment of a group of conventional colon polyps with similar biological and phenotypic characteristics. SSPs require a separate assessment schema and malignancy risk is predicted by endoscopically recognizable dysplasia[14].

Following these exclusions, univariable and multivariable analyses were then repeated as above to generate the best-fitting model and independent predictors of covert SMIC.

Lesions with confirmed SMIC were examined to determine if the identified factors associated with covert SMIC were also associated with SM1 or SM2/3 invasion.

All authors had access to the study data and reviewed and approved the final manuscript.

Results

2693 lesions were assessed in the study period. 121 lesions had missing histological data, 89 lesions had incomplete Kudo classification data, 17 lesions had incomplete Paris classification data, 8 lesions had incomplete size data and 15 lesions had incomplete surface morphology data. 150 lesions had unclassifiable surface features, and 92 lesions had rare morphological type (Paris IIb, III). Some lesions had more than one missing data type, so in total 416 lesions with missing, unclassifiable or rare data were excluded (15.4%).

Overall 2277 lesions were assessed (mean size 36.9mm, splenic flexure and proximal 64.4%) in 2106 patients (mean age 67.7 years, 53.2% male). SMIC was evident in 171 lesions (7.6%). Cohort characteristics are presented in Table 1. **(Table 1)**

Factors associated with Submucosal Invasive Cancer

Univariable analysis indicated that patient factors including age, sex and ASA score were not predictive of SMIC risk. Key factors associated with SMIC were lesion characteristics including size, location, Paris classification, 0-IIc component and surface morphology.

Factors associated with SMIC on multivariable analysis are listed in Table 2. **(Table 2)** The strongest independent predictor was identification of a Kudo V pit pattern (Odds Ratio (OR) 12.1 (7.00-20.8) p<0.001). Size was an independent predictor of SMIC, however the effect was modest and only just reached significance. All other variables had similar odds ratios ranging from 1.92-2.84.

For lesions with Kudo type V pit pattern, the specificity for cancer prediction was 97.5% (95% CI, 96.7-98.1%), sensitivity 40.4% (95% CI, 33.3-47.8%), positive predictive value (PPV) 56.6% (95% CI, 47.7-65.0%) and negative predictive value (NPV) 95.3% (95% CI, 94.3-96.1%). Diagnostic accuracy was 93.2% (95% CI, 92.1-94.2%). For lesions with a Paris 0-IIc component, the specificity was 95.9% (95% CI, 95.0-96.7%), sensitivity 21.1% (95% CI, 15.6-27.8%), PPV 29.3% (95% CI, 22.0-37.8%) and NPV 93.7% (95% CI, 92.6-94.7%). Diagnostic accuracy was 90.3% (95% CI, 89.0-91.4%). The high specificity and limited sensitivity of these findings means that there remained a large proportion of bland appearing lesions with undefined SMIC risk.

Factors associated with "Covert" Submucosal Invasive Cancer

In order to determine the risk of SMIC in lesions without overt endoscopic evidence of invasion, we excluded lesions with Kudo type V pit pattern and Paris 0-IIc (depressed) components. Serrated lesions were also excluded.

Univariable analysis of this group identified the strongest remaining factors associated with covert SMIC; Paris classification, surface morphology, size and location. Multiple logistic regression indicated the best fitting factors were location, size and combined Paris classification and surface topography. **(Table 3).**

Assessing SMIC risk using these factors provided a stratification effect. **(Figure 1)** Proximal 0-IIa G or 0-Is G lesions had the lowest risk of SMIC (0.7% and 2.3%), whereas distal 0-Is NG lesions had the highest risk (21.4%). Increasing size had a minor effect when risk was

stratified per 10mm increase in size, however it had a more marked effect when the size cutoff was set at 50mm.

SMIC risk in the total cohort according to the risk factors identified in the multivariable model was then compared to the group with covert SMIC. **(Table 4).**

For granular lesions with SMIC, few display overt endoscopic evidence of submucosal invasion. As a result, the rates of SMIC are minimally changed when comparing the occult group with the total cohort. The rates of endoscopically overt SMIC are considerably higher for NG lesions, however despite this, for 0-Is NG or 0-IIa+Is NG lesions there remains a substantial (>10%) risk of SMIC even when lesions with overt evidence of SMIC are excluded. One lesion type that differs from the pattern is 0-IIa NG lesions. Once lesions with overt evidence of SMIC are excluded, these lesions have a low risk (4.2%) of harbouring underlying cancer.

All lesion types have a poor sensitivity for the diagnosis of SMIC, however the 0-Is NG and 0-IIa+Is NG lesions have specificities of 95.5% (95%CI 94.4-96.4) and 94.5% (95%CI 93.3-95.5) respectively.

(Table 5).

Superficial SMI – Potential for Curative Resection

Of the 138 lesions with covert SMIC, 64 were SM1 and potentially amenable to cure by endoscopic resection (41.6%, 3.7% of the total cohort). No lesion type identified by the multivariable model for covert SMIC was associated with a higher proportion of SM1 disease. **(Supplementary Table 1).** The analysis was also performed for the entire cohort, and there were similarly no associations with lesion type. **(Supplementary Table 2).**

Discussion:

The decision to undertake endoscopic resection of any colonic lesion hinges on the underlying risk of SMIC. For lesions with overt endoscopic signs of deep SMIC (Kudo V, Paris 0-IIc component), endoscopic resection is not recommended and surgical resection is favoured unless there are compelling comorbidities that preclude surgery. Deep SMIC is associated with higher rates of lymph node metastasis so even if ER is successful, surgical resection and removal of locoregional LNs is required to stage disease and reduce the risk of further metastatic spread[17]. Existing endoscopic predictors of SMIC are specific, but have poor sensitivity, meaning that a large proportion of lesions may harbour cancer without displaying overt evidence of this.

In this study we have defined the key risk factors for SMIC in a large prospective, multicentre, intention to treat cohort of lesions referred for EMR. The strongest predictor of SMIC was Kudo V pit pattern (OR 14.2; $p < 0.001$), and if this is present endoscopic resection is not advised. A depressed (0-IIc) component to the lesion is also an independent predictor of SMIC which may preclude resection. Separately to these overt endoscopic predictors of SMIC, four key variables (Distal location, increasing size, Paris type and surface morphology) are associated with elevated risk of occult cancer. Combining Paris type and surface morphology allows accurate stratification of SMI risk in large colonic lesions. Distally located 0-Is and 0-IIa+Is NG lesions have a high risk of SMIC whereas proximally located 0-Is G or 0-IIa G lesions a very low risk. The system is simple and easily applicable in clinical practice. It is pragmatic and readily adoptable, using existing assessment tools available to every endoscopist, and is derived from prospective, multicentre data. It helps endoscopists to grapple with the fact that the majority of colonic lesions encountered will be low risk. In fact, lesions with an SMIC risk of <5% make up ~75% of the cohort. Endoscopic resection strategy decisions can be more acutely focused on higher risk lesions.

Prediction of SMIC for LSL is currently flawed and a simple SMIC risk stratification system does not exist. Contemporary paradigms rely on identifying specific overt high risk factors such as 0-IIc morphology or KPP V in isolation[4,9]. An extension of the original NICE classification to identify deep SMIC has been developed using a small series of selected still images which demonstrated high (>90%) sensitivity and specificity when validated by trained novice raters[18]. Despite this, there are no published prospective clinical validation studies, and preliminary data from a Spanish prospective multicentre study of 824 lesions (546 sessile) showed that while specificity was satisfactory at 94.7% (95% CI, 92.8-96.7), sensitivity was poor at 60.0% (95% CI, 55.8-64.2). These results are similar to the results obtained in this study for the entire cohort assessing KPP V (specificity 97.1% (95% CI, 96.2-97.8%), sensitivity 41.4% (95% CI, 34.0-49.2%). It emphasises that when SMIC is not endoscopically evident, it is not possible to completely exclude. The perfect endoscopic test for SMIC is unlikely to be achieved as not all cancers present a visible face to the surface. Buried, or diminutive foci are essentially "covert" and endoscopists require an accurate and useful risk stratification system to advise patients and guide resection in this context.

Paris classification is used to describe lesion morphology, however it is cumbersome and rarely used by general endoscopists outside of research settings. Interobserver variation has been questioned, particularly for smaller polyps[19] and it does not specifically predict SMIC risk. Kudo et al. classified colon lesions ≥10mm as laterally spreading tumours (LSTs) and characterised them as granular, (LST-G) or non-granular (LST-NG)[20]. LST-NG is associated with SMIC, however this binary classification is overly simplistic and poorly predicts lesions at very high or very low risk of SMIC[21]. The Sano classification system identifies lesions at high risk of SMIC using narrow band imaging (NBI) (Olympus, Tokyo, Japan) assessment of the surface vasculature, however it has not been widely applied outside of initial validation studies[22,23]. Other features which may suggest submucosal invasion including probing the lesion with biopsy forceps to check for fixation, or submucosal injection to assess non-lifting. These factors have deliberately not been assessed in this

study as they may not be appropriate to perform for endoscopists who will subsequently refer to a tertiary centre for resection. Extensive biopsy or an unsuccessful attempt at resection of a lesion increases the difficulty for subsequent resection and is discouraged[24]. Non-lifting is strongly associated with deep submucosal invasive disease (SM3),[25] however lesions with superficial invasion (SM1 and SM2) may still lift well as the underlying submucosa is not completely obliterated and may still expand[26]. False positive non-lifting signs may also occur in the setting of submucosal fibrosis, biopsy or tattoo[24,27]. In our study, non-lifting was associated with SMIC, however not all lesions were subjected to the lifting test if EMR was not attempted. Non-lifting was observed in 20/2176 lesions, partial lifting in 155/2176 and good lifting in 2001/2176 lesions. SMIC was diagnosed in 4 non-lifting lesions (20%), 24 partially lifting lesions (15.5%) and 105 lesions with good lifting (5.2%), p=<0.001. Overall for any lesion with partial or non-lifting the unadjusted odds ratio for SMIC was 3.44 (95%CI 2.19-5.39), p=<0.001.

Expert endoscopists may have relied on a “gestalt” approach using several endoscopic risk factors together, however a risk stratification tool combining the strongest of these associated factors makes assessment more accessible and reproducible.

The risk of SMIC in 0-IIa NG lesions is low when there are no overt endoscopic predictors evident. The reason for this is likely that minimally elevated NG lesions are easy to comprehensively assess, as any SMIC is evident on the surface and not hidden by granular undulations or buried within a 0-Is nodule. Comprehensive inspection of large G lesions is often challenging due to these factors resulting in hidden disease, but the underlying SMIC risk is lower overall reducing the clinical impact. Size was relevant in the prediction of occult SMIC, however this was most prominent in lesions over 50mm in size, and in NG lesions with a 0-Is component (0-Is NG and 0-IIa+Is NG lesions). It is likely that NG lesions of this size are considerably more biologically advanced than granular lesions of a similar size. Size may be an additional factor compromising complete assessment of NG lesions

with a nodule, or extensive spreading granular lesions particularly if they also exhibit nodules. **(Figure 2.)**

Rates of SMIC in this study were markedly lower than in cohorts from "Eastern" investigators. The overall rate of SMIC was 8.3% in this study, 3.5% in G lesions and 11.1% in NG lesions. Yamada et al. report a rate of 29% in a cohort referred for endoscopic resection in Japan, 19% in G lesions and 39% in NG lesions. Other studies have reported similar rates of SMIC in Japanese populations[28–30]. This may represent biological differences or case selection, however it implies that the approach to endoscopic resection needs to be different in a Western setting. The differences in risk between distal and proximal lesions were clearly noted in this study, however are not as prominent in cohorts from Japan. There is clear evidence that there are biological differences depending on lesion location, however it may be that these are more pronounced in the West.

The prediction of endoscopically resectable disease is not clarified by this study. Stratifying SMIC risk according to the schema we have outlined does not delineate lesions with SM1 or SM2/3 disease. It may be that a depressed component to a lesion represents an endoscopically visible early superficial focus for disease, whereas a nodule represents an obscured deeper focus. Yamada et al. described risk factors for deep SMIC in 822 lesions ≥10mm in a series of patients undergoing en-bloc ESD. Lesions undergoing EMR or piecemeal ESD were excluded biasing the series toward higher risk lesions, and reducing the applicability for endoscopists faced with a lesion where the decision on resection modality is yet to be made. They demonstrated that a depressed component or a nodule both appear to be strongly associated with deep SM2/3 SMIC, however this was compared to all lesions rather than to SM1 disease[31]. Our analysis likewise showed that Kudo V and a depressed component were good predictors of any SMIC. Without tools to distinguish superficial versus deep invasion, it is impossible to clarify which lesions may fall into the narrow window where ESD is justified based on cost, safety and curative effectiveness.

This inability to accurately identify SM1 disease means that risk stratification for lesions without overt evidence of SMIC is vitally important for guiding resection decisions. The benefit of ESD lies in its potential for curative resection for SM1 early CRC. If all lesions in our cohort were subjected to ESD and assuming all ESD outcomes were perfect, the 64 patients that were diagnosed with SM1 disease (3.4%) would benefit from a curative resection and avoid surgery. ESD is however associated with a higher risk of surgery for adverse events over EMR (0.2% EMR[32], 1% ESD[33]) and curative resection rates are at best 90%[33]. This small net surgery sparing benefit must also take into account the significant resource, time and cost disadvantages of ESD, which in many time and budget-constrained health care systems would result in significantly reduced access to timely care for a large proportion of patients with large colonic LSL. An alternative strategy based on the risks of SMIC established in this study would favour a selective ESD approach. Where ESD is well established, with experienced operators, high success rates and low procedural adverse event risks, high risk lesions including distal 0-IIa NG lesions without overt evidence of SMIC, and all distal 0-Is or 0-IIa+Is lesions may be targeted by a primary ESD approach. This results in only 14% of patients undergoing ESD and ~10% of these ESDs resulting in a curative outcome. Where ESD is less well established, an approach targeting rectal NG lesions may be appropriate as the risks of ESD are lower and the surgical adverse event rates considerable for low rectal surgery[34].

The strengths of this study include the large number of advanced lesions enrolled, and the completeness of follow up data. The results represent “real-world” prospective assessment in a number of centres across Australia using standard colonoscopes without chromoendoscopy. Variation, or errors in assessment are possible in these settings, but the large dataset reduces the impact of error and makes the findings more applicable to general clinical practice in a Western environment. Lesions with ‘unclassifiable’ surface features were initially reported in the cohort, but as endoscopists have become more experienced over the course of the study these have declined in number. No ‘unclassifiable’ lesions have

been reported since 2014. Lesions with morphology consistent with serrated histology have been reported since an update to the study protocol in 2013 so this classification is not representative of all lesions in the cohort.

Lesion location and gross morphology are strong predictors of SMIC and allow the endoscopist greater confidence in decisions regarding resection or referral for surgery. Clinical predictors of SMIC in colonic lesions are well studied, but factors that improve clinical decision making are lacking, particularly in an era where the availability of colonic ESD is increasing. Distal non-granular lesions have a high risk of occult SMIC whereas proximal, granular 0-IIa lesions, after a careful assessment for features associated with SMIC, have a very low risk. 0-IIa NG lesions in the distal colon have a comparatively low risk of SMIC once overt features of malignancy are excluded. In lesions without overt evidence of SMIC, the risks can be stratified according to these factors and used to rationalise an approach to endoscopic resection based on local availability, expertise and adverse event rates. An informed and stratified approach is essential to mirror the disease process, choose the correct resection modality and thus minimise morbidity whilst optimising clinical outcomes.

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FIGURE LEGENDS:

Figure 1.

Figure 2.

(A) A 40mm 0-IIa G lesion in the ascending colon. If there are no overt features of SMIC, this lesion has a 0.7% risk of covert malignancy.
(B) A 50mm 0-IIa+Is G lesion at the hepatic flexure. If there are no overt features of SMIC, this lesion has a 4.2% risk of covert malignancy.
(C) A 50mm 0-Is NG lesion in the rectum. Despite having no overt features of SMIC, this lesion still has a 21.4% risk of covert malignancy.
(D) A 20mm 0-IIa NG lesion in the sigmoid colon. 0-IIa NG lesions have an overall 23.4% risk of SMIC, however if lesions with overt features of SMIC are excluded (KPP V, any 0-IIc component), the risk falls to 6.4%.
(E) A 40mm 0-IIa+c NG lesion in the distal transverse colon. This lesion has a central depressed component (0-IIc) (F) and on close inspection (including with narrow band imaging) there is a disrupted pit pattern (Kudo Pit Pattern Vn). (G) Lesions with overt endoscopic evidence of SMIC are not suitable for resection and should be referred for surgical management.

TABLES

Table 1. Characteristics of study patients and lesions

	Patient Characteristics
Patient Factors (n=2106)	
Age, years. mean, (SD)	67.7 (11.6) Range (18-95 years)
Sex Male Female	 1119 (53.2%) 983 (46.8%)
ASA (n, %) ASA 1 ASA 2 ASA 3 ASA 4 No data	 827 (41.1%) 895 (44.5%) 283 (14.1%) 7 (0.3%) 94 (4.5%)
Lesion factors (n=2277)	
Lesion Size, mm. mean, (SD)	36.9 (16.9) Range (20-180mm)
Lesion Location (n, %) Rectum <5cm Rectum >5cm Sigmoid Descending Colon Splenic Flexure Distal Transverse Mid Transverse Proximal Transverse Hepatic Flexure Ascending Colon Cecum Cecum ICV Involved Cecum Appendiceal Orifice Involved	 121 (5.3%) 304 (13.4%) 241 (10.6%) 98 (4.3%) 46 (2.0%) 62 (2.7%) 101 (4.4%) 95 (4.2%) 163 (7.2%) 543 (23.9%) 387 (17.0%) 92 (4.0%) 22 (1.0%)
Lesion Location (n, %) Rectum to Splenic flexure (Distal Colon) Distal transverse colon to Cecum (Proximal Colon)	 810 (35.6%) 1465 (64.4%)
Paris Classification (n, %) 0-IIa 0-Is 0-IIa + Is	 1218 (53.5%) 446 (19.6%) 613 (26.9%)
Any 0-IIc component (0-IIa+c, 0-IIc)	123 (5.4%)
Morphology (n, %) Granular (LST-G) Non-Granular (LST-NG) Mixed Consistent with Serrated Morphology	 1439 (63.2%) 583 (25.6%) 161 (7.1%) 94 (4.1%)
Kudo type (n, %) Kudo I Kudo II Kudo III Kudo IV Kudo V	 19 (0.8%) 232 (10.2%) 725 (31.8%) 1179 (51.8%) 122 (5.4%)

Histology	
Majority Polyp Histology (n, %) Tubular Adenoma Tubulovillous adenoma Villous Adenoma Sessile Serrated Polyp (SSP) Traditional Serrated Adenoma (TSA) Tubulovillous Adenoma with Serrated Component Invasive cancer only, no identifiable underlying polyp	 575 (25.3%) 1245 (54.7%) 59 (2.6%) 308 (13.5%) 30 (1.3%) 41 (1.8%) 19 (0.8%)
Submucosal Invasive Cancer (SMIC)	171 (7.6%)

Table 2. Risk of SMIC in all lesions according to study factors and best fitting multiple logistic regression model for factors associated with SMIC

	No SMIC	SMIC	p
Patient Factors (n=2106)			
Age, years. mean, (SD)	67.6 (11.6) Range (18-95 years)	68.4 (11.6) Range (27-91 years)	0.58
Sex Male Female	 1026 (91.7%) 908 (92.4%)	 93 (8.3%) 75 (7.6%)	 0.57
ASA (n, %) ASA 1 ASA 2 ASA 3 ASA 4	 755 (91.3%) 830 (92.7%) 259 (91.5%) 7 (100.0%)	 72 (8.7%) 65 (7.3%) 24 (8.5%) 0 (0.0%)	 0.66
Lesion factors (n=2277)			
Lesion Size, mm. mean, (SD)	36.5 (16.5) Range (20-160mm)	41.3 (20.7) Range (20-180mm)	**0.001**
Lesion Size 20-29.9mm 30-39.9mm 40-49.9mm 50+ mm	 717 (94.3%) 572 (93.2%) 360 (93.3%) 457 (88.4%)	 43 (5.7%) 42 (6.8%) 26 (6.7%) 60 (11.6%)	 **0.002**
Lesion Size 20-49.9mm 50+ mm	 1649 (93.7%) 457 (88.4%)	 111 (6.3%) 60 (11.6%)	 **<0.001**
Lesion Location (n, %) Rectum to Splenic flexure (Distal Colon) Distal transverse colon to Cecum (Proximal Colon)	 713 (88.0%) 1393 (95.0%)	 97 (12.0%) 74 (5.0%)	 **<0.001**
Lesion Location (n, %) Rectum Sigmoid Colon Descending colon to Splenic Flexure (Distal Colon) Distal transverse colon to Cecum (Proximal Colon)	 378 (88.9%) 201 (83.4%) 134 (93.1%) 1393 (95.0%)	 47 (11.1%) 40 (16.6%) 10 (6.9%) 74 (5.0%)	 **<0.001**
Lesion Location (n, %) Rectum and Sigmoid Colon (Rectosigmoid) Descending colon to Cecum	 579 (86.9%) 1527 (94.8%)	 87 (13.1%) 84 (5.2%)	 **<0.001**
Paris Classification (n, %) 0-IIa 0-Is 0-IIa + Is	 1158 (95.1%) 399 (89.5%) 549(89.6%)	 60 (4.9%) 47 (10.5%) 64 (10.4%)	 **<0.001**
No 0-IIc component Any 0-IIc component (0-IIa+c, 0-IIc)	2019 (93.7%) 87 (70.7%)	135 (6.3%) 36 (29.3%)	**<0.001**
Morphology (n, %) Granular (LST-G) Non-Granular (LST-NG) Mixed Consistent with Serrated Morphology	 1372 (95.3%) 510 (87.5%) 131 (81.4%) 93 (98.9%)	 67 (4.7%) 73 (12.5%) 30 (18.6%) 1 (1.1%)	 **<0.001**
Morphology (n, %) Granular (LST-G) Any Non-Granular component (LST-NG) Consistent with Serrated Morphology	 1372 (95.3%) 641 (86.2%) 93 (98.9%)	 67 (4.7%) 103 (13.8%) 1 (1.1%)	 **<0.001**

Kudo type (n, %) Kudo I Kudo II Kudo III Kudo IV Kudo V	 19 (100.0%) 228 (98.3%) 698 (96.3%) 1108 (94.0%) 53 (43.4%)	 0 (0.0%) 4 (1.7%) 27 (3.7%) 71 (6.0%) 69 (56.6%)	 **<0.001**
Kudo type (n, %) Kudo I-IV Kudo V	 2053 (95.3%) 53 (43.4%)	 102 (4.7%) 69 (56.6%)	 **<0.001**
Excluded Lesions			
Unclassifiable surface features*	132 (91.0%)	13 (9.0%)	
Rare morphological type (Paris IIb, III)*	86 (96.6%)	3 (3.4%)	

*Missing data on histology in each group: Unclassifiable surface features n=5; rare morphological type n=3,

Best Fitting Multiple Logistic Regression Model for factors associated with SMIC

Best fitting multiple logistic regression model	Adjusted OR	*P* value
Kudo type (n, %) Kudo I-IV Kudo V	 1 14.2 (8.57-23.4)	 **<0.001**
Size Per 10mm increase	 1.12 (1.01-1.23)	 **0.030**
Location Group Rectum and Sigmoid Colon (Rectosigmoid) Proximal Colon (Descending colon to Caecum)	 1.91 (1.31-2.79) 1	 **0.001**
Morphology (n, %) Granular (LST-G) Any Non-Granular (LST-NG) component Other (not reported as LST-G, NG or mixed)	 1 2.80 (1.89-4.16) 0.72 (0.09-5.20)	 **<0.001** **0.72**
Paris Classification 0-IIa 0-Is 0-IIa + Is	 1 2.73 (1.64-4.55) 2.49 (1.52-4.08)	 **<0.001** **<0.001**
Any 0-IIc component No 0-IIc component Any 0-IIc component	 1 1.80 (0.95-3.42)	 **0.07**

Table 3. Best fitting Multiple Logistic Regression model examining factors associated with Covert SMIC (Serrated histology, serrated surface features, Kudo type V and Paris 0-IIc lesions excluded)

Best fitting multiple logistic regression model for factors associated with Occult SMIC		
Best fitting multiple logistic regression model	**Adjusted OR**	***P* value**
Location Group		
Rectum and Sigmoid Colon (Rectosigmoid)	1.87 (1.16-3.02)	**0.010**
Proximal Colon (Descending colon to Caecum)	1	
Combined Paris and Surface Features		
0-IIa G	1	
0-Is G	3.96 (1.24-12.7)	**0.020**
0-IIa + Is G	6.11 (2.07-18.0)	**0.001**
0-IIa NG	5.97 (1.92-18.5)	**0.002**
0-Is NG	22.5 (7.07-71.6)	**<0.001**
0-IIa + Is NG	14.4 (4.53-45.5)	**<0.001**
Lesion Size		
Per 10mm increase	1.16 (1.04-1.31)	**0.012**

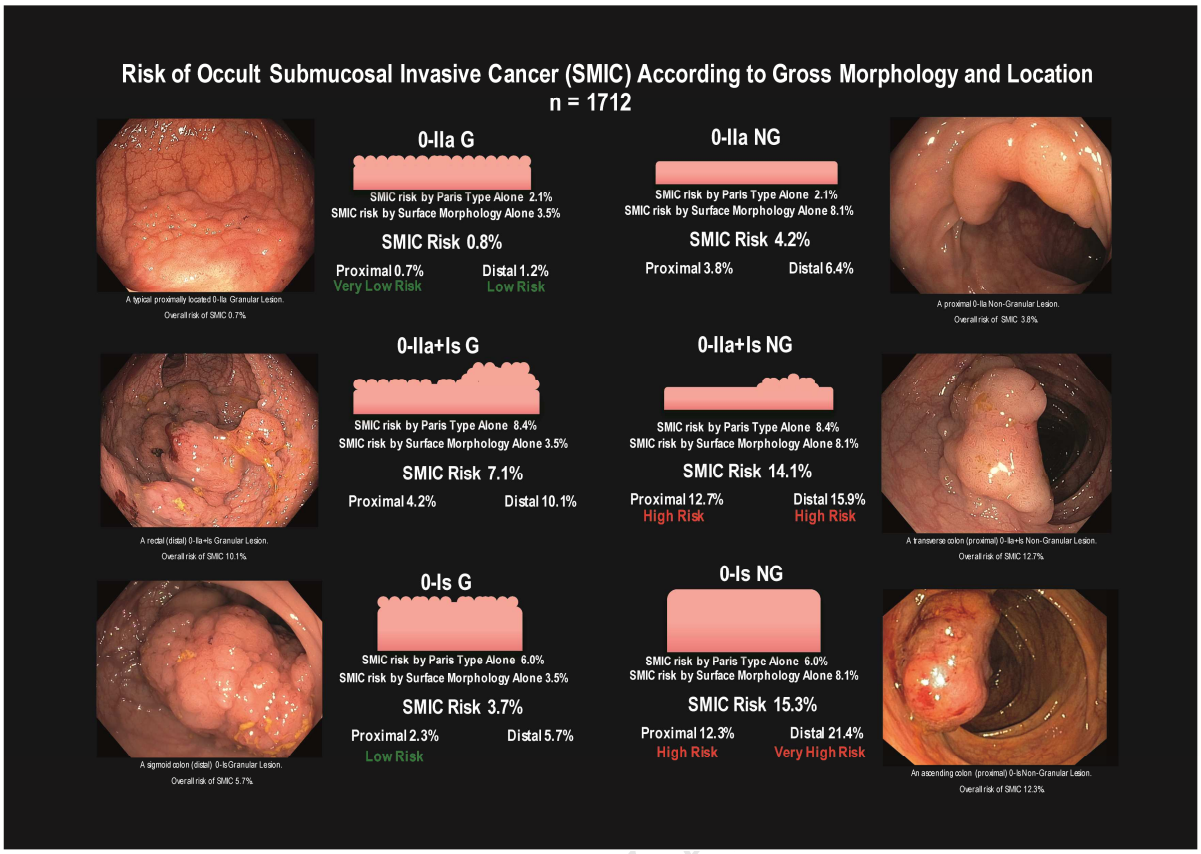
Table 4. Rates of SMIC in the total cohort and in lesions with potential covert SMIC according to location, morphology and surface characteristics.

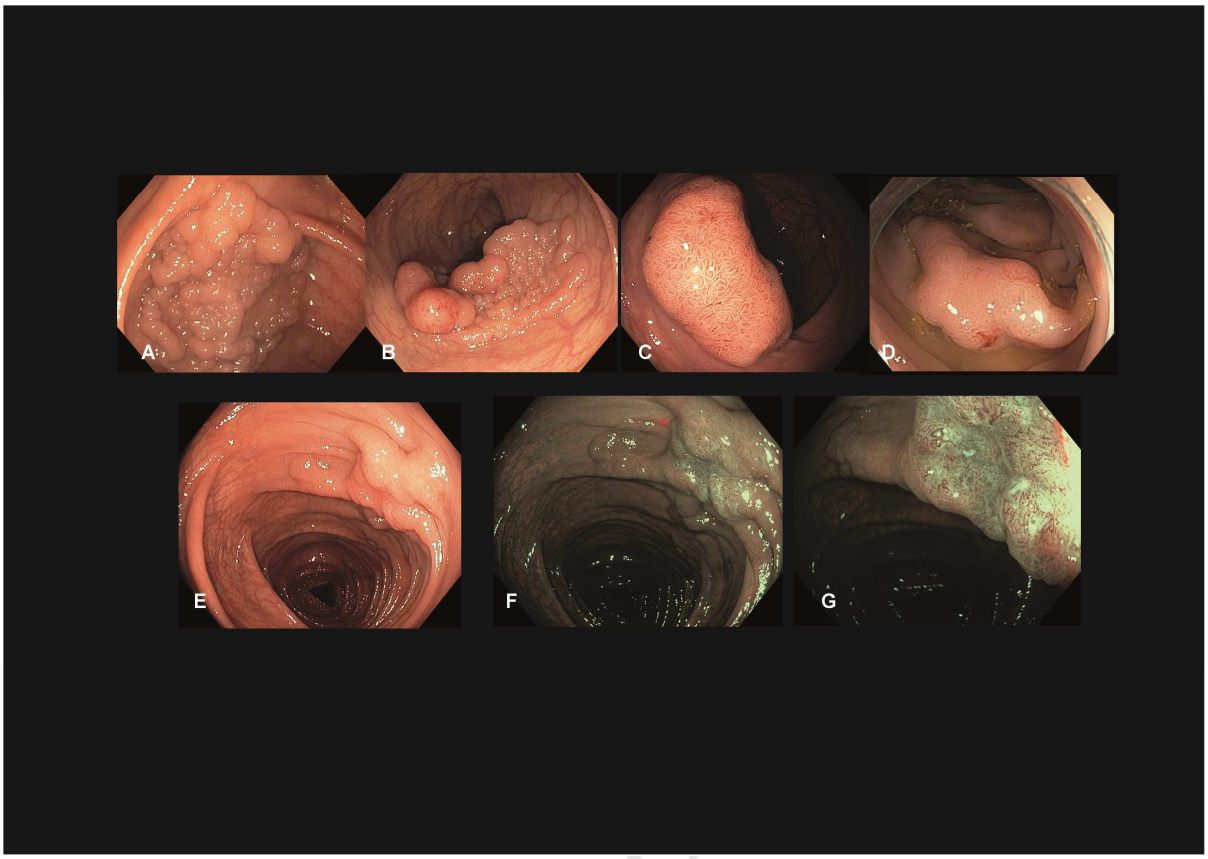
	Lesions with potential "Covert" SMIC (n=1712) **(Serrated histology, serrated surface features, Kudo type V and Paris 0-IIc lesions excluded)**			**Total Cohort (n=2277)**		
	Proportion of cohort	**Proximal Colon**	**RectoSigmoid Colon**	**Proportion of cohort**	**Proximal Colon**	**Rectosigmoid Colon**
0-IIa G	506 29.5%	3/422 **0.7%**	1/84 **1.2%**	634 27.8%	10/528 **1.9%**	1/106 **0.9%**
0-Is G	299 17.5%	4/176 **2.3%**	7/123 **5.7%**	328 14.4%	6/193 **3.1%**	13/135 **9.6%**
0-IIa + Is G	411 24.0%	9/212 **4.2%**	20/199 **10.1%**	477 20.9%	11/243 **4.5%**	26/234 **11.1%**
0-IIa NG	312 18.2%	10/265 **3.8%**	3/47 **6.4%**	496 21.8%	29/413 **7.0%**	19/83 **22.9%**
0-Is NG	85 5.0%	7/57 **12.3%**	6/28 **21.4%**	118 5.2%	14/73 **19.2%**	14/45 **31.1%**
0-IIa + Is NG	99 5.8%	7/55 **12.7%**	7/44 **15.9%**	130 5.7%	13/71 **18.3%**	14/59 **23.7%**

NB: Lesions not included in table from total cohort; 0-IIa Serrated surface n=88 (SMIC 1/85 Proximal, 0/3 Rectosigmoid), 0-IIa+Is Serrated n=6 (SMIC 0/5 Proximal, 0/1 Rectosigmoid)

Table 5. Diagnostic Performance of Lesion Classification Types for Covert SMIC.

	Sensitivity	**Specificity**	**Positive Predictive Value PPV**	**Negative Predictive Value NPV**	**Accuracy**
0-IIa G	5.73% (3.05-10.5)	**70.0% (67.8-72.1)**	1.70% (0.90-3.20)	89.1% (87.4-90.7%)	**64.6% (62.5-66.8%)**
0-Is G	11.5% (7.38-17.4)	**83.2% (81.4-84.9%)**	5.83% (3.72-9.02)	91.2 (89.7-92.5)	**77.3% (75.3-79.1)**
0-IIa + Is G	22.9% (17.1-30.1)	**77.4% (75.3-79.3)**	8.39% (6.12-11.4)	91.7 (90.2-93.0)	**72.8% (70.8-74.8)**
0-IIa NG	27.4% (21.0-34.8)	**79.5% (77.5-81.32)**	10.8% (8.1-14.2%)	92.4% (90.9-93.6)	**75.2% (73.2-77.1)**
0-Is NG	16.6% (11.6-23.2%)	**95.5% (94.4-96.4)**	25.0% (17.7-34.1)	92.7% (91.4-93.8)	**89.0% (87.5-90.3)**
0-IIa + Is NG	15.9% (11.0-22.5)	**94.5% (93.3-95.5)**	20.7% (14.4-28.7)	92.6% (91.2-93.7)	**88.0% (86.4-89.3)**





Supplementary Tables

Supplementary Table 1. Submucosal invasion according to lesion morphology and surface characteristics in the "covert" cohort (n=1892).

	SM1	SM2-3	p	Missing
0-IIa G	4 (44.4%)	3 (33.3%)		2 (22.2%)
0-Is G	4 (22.2%)	12 (66.7%)		2 (11.1%)
0-IIa + Is G	15 (41.7%)	15 (41.7%)		6 (16.7%)
0-IIa NG	22 (52.4%)	15 (35.7%)	p=0.36	5 (11.9%)
0-Is NG	9 (34.6%)	15 (57.7%)		2 (7.7%)
0-IIa + Is NG	10 (40.0%)	14 (56.0%)		1 (4.0%)
Totals	64 41.0%	74 47.4%		18 (11.5%) **N=138**

Supplementary Table 2. Submucosal invasion according to lesion morphology and surface characteristics in the entire cohort (n=2277).

	SM1	SM2-3	p	Missing
0-IIa G	5 (45.5%)	4 (36.4%)		2 (18.2%)
0-Is G	4 (21.1%)	13 (68.4%)		2 (10.5%)
0-IIa + Is G	16 (43.2%)	15 (40.5%)	p=0.53	6 (16.2%)
0-IIa NG	24 (50.0%)	18 (37.5%)		6 (12.5%)
0-Is NG	9 (32.1%)	16 (57.1%)		3 (10.7%)
0-IIa + Is NG	10 (37.0%)	15 (55.6%)		2 (7.4%)
Totals	68 (39.8%)	82 (48.0%)		21 (12.3%) **N=170**

NB: 1 lesion not included in this table: 0-IIa SM2/3 cancer with serrated surface appearance

STUDY PROTOCOL

Project Title: The Australian Colonic Advanced Mucosal Neoplasia and Endoscopic Resection Prospective Observational Study. (ACE)

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Protocol Version: 3.0

Background:

Colonoscopic polypectomy is well established as an effective way of reducing colorectal cancer mortality[1]. The majority of polyps detected and removed at colonoscopy are adenomas <10mm in size without advanced histology. These lesions have a low risk of progression to malignancy and are relatively easily removed by standard snare polypectomy with low complication rates[2]. Polyps that are sessile or flat and greater than 20mm in size are found in approximately 1% of all colonoscopies[3] and are more difficult to manage. These lesions, known as advanced mucosal neoplasia (AMN), have a high rate of advanced histology[4]. Traditionally they have been managed by referral for open or laparoscopic surgery, which is definitive, but invasive, costly and associated with a significant mortality risk in patients with advanced age or comorbidities[5]. Wide Field Endoscopic Mucosal Resection (WF-EMR) has emerged in recent years as an alternative to surgery that is now becoming the standard of care. It is an outpatient procedure which is effective, safe and less costly than surgery when delivered at a tertiary referral centre[6].

The Australian Colonic Endoscopic Mucosal Resection study (ACE), is a multicentre prospective observational study which examined WF-EMR of colonic AMN (Ethics approval No. HREC JH/TG 2008/9/6.1(2858)). This

project now has an extensive dataset from 8 leading colonic endoscopic resection centres in Australia on more than 1500 lesions resected over 4 years since June 2008.

The ACE study has been successful in addressing several aspects of the resection of AMN, resulting in several high profile papers in internationally recognised journals. The collection of this data has produced robust information on the efficacy of the procedure[4], recurrence rates[7], bleeding complications[8] and mortality when compared to surgery[5]. Single centre analysis of the ACE dataset at Westmead has also allowed insights into how to refine the procedure to improve outcomes. The target sign is now a recognised indication for the placement of clips to prevent perforation[9], CO2 insufflation for WF-EMR has been shown to be superior to air insufflation[10] and succinylated gelatin (Gelofusine®) has been shown to be superior to normal saline as a submucosal lifting agent[11].

There remain a number of unanswered questions regarding the endoscopic resection of large sessile lesions and expanding the ACE dataset in a new cohort of patients will allow these to be addressed. Enhancing the prediction of submucosal invasive cancer, advanced lesion classification, refinement of the assessment of deep injury, submucosal injectate constituents, the optimum electrosurgical resection methods, prevention and prophylaxis of bleeding, and subtype analyses of the different histological groups comprising AMN will be examined.

Literature Review:

The ACE study was initially designed to assess the efficacy of and complications related to WF-EMR of AMN. AMN is uncommon, but is an important subgroup of bowel lesions as it contains a high proportion of incipient and inevitable bowel cancers. Few centres internationally have published studies on the resection of AMN and there are only 3 prospective studies which have accrued more than 200 patients[12–14]. The focus of these studies was generally on technical efficacy, and data on complications or lesion subtypes was limited. Through its unique dataset and collection of rare but clinically important lesions, the ACE study has provided an insight into the technical aspects of resection, and valuable data to examine other aspects of AMN itself.

Since its inception in 2008, the ACE study has gathered data on over 1500 patients through a now well established tertiary referral service for the resection of AMN at 8 Australian major centres. The high throughput of cases and established research infrastructure means it has generated multiple internationally relevant studies and has adequate power to look at specific patient, lesion, technique and outcome subgroups. Due to these positive results, the study has now created several questions that could be addressed by maintaining the same structure, but incorporating other study centres and broadening the data collection. Technological advances in endoscopy have meant that real time prediction of lesion histology is becoming more accurate[15], and the ACE study is well placed to expand lesion assessment data to provide robust evidence on the appearance of large colonic lesions and prediction of submucosal invasive cancer. Important questions have arisen in recent years

as to what lesions are at high risk of progression to cancer[16], which patients may harbour these high risk lesions and what is the best way to identify them. The ACE study has the potential to focus on specific high risk lesion subtypes such as sessile serrated adenomas (SSAs) where there is little international data, and describe the histology of these lesions as well as link it to patient and procedural data. The endoscopic appearance of SSAs is also poorly described[17] and data will be collected on the prospective assessment of these lesions in the ACE study. "Missed" lesions and interval cancer are also an important emerging aspect of colon lesions[18,19], and detection of "missed" AMN may be valuable as an intermediate step before progression to cancer. Along with these new insights into AMN, new aspects of resection will also be assessed in this prospective cohort. A grading system for deep injury following WF-EMR will be added to the study and assessed to examine the effect of pro-active management of deep injury. Kudo[20] and Sano[21,22] grades will be prospectively assessed for their prediction of sub mucosal invasive cancer. Incremental improvement in refining the technique of WF-EMR by scrutinizing ACE outcomes means that the acceptability and availability of the procedure is improved internationally, and it is seen as a safe, efficacious and cost effective technique.

The ACE study has been valuable as a way of providing a base population for interventional studies. Several studies will tie in to the expanded ACE data. These studies will be independently submitted for HREC approval and review. A prospective randomised controlled trial of the use of a prophylactic polysaccharide powder to prevent bleeding is planned as well as an assessment of soft coagulation at the margins of the EMR defect to prevent recurrence.

Aims:

To enhance understanding of the risk factors for AMN, improve lesion assessment and prediction of submucosal invasive cancer, improve endoscopic resection efficacy, reduce complications of WF-EMR and improve the understanding of the progression of large lesions to cancer.

Methodology:

Project Design:

Prospective, observational multicentre study which aims to enrol all cases of AMN presenting to 8 academic endoscopy units across Australia.

Inclusion Criteria:

- Patients referred for endoscopic resection of a large sessile colonic polyp or laterally spreading tumour ≥20mm in size.
- Age > 18 years
- Able to give informed consent to involvement in the clinical study

Exclusion Criteria:

- Unable to provide informed consent for involvement

Method of Screening:

Patients referred to a study centre for colonic WF-EMR of a known sessile colonic polyp or laterally spreading tumour (LST) ≥20mm in size

Sequence of Procedures: (for flowsheet see Appendix 1.)

1. Patient is referred to one of 8 academic tertiary referral endoscopy units for removal of a large sessile colonic polyp or LST ≥20mm in size.
2. All patients referred to this service are routinely mailed an information pack about the EMR procedure. If the referral information indicates that the patient is potentially eligible for the trial, written information about the study is included in this pack for the patient to read in advance of their arrival for the procedure.
3. The patient reads the supplied information and consent form.
4. Once checked into the endoscopy suite on the day of the procedure, the patient is met by one of the investigators to discuss the risks and benefits of the procedure and the study. An interpreter is used to assist with the discussion if required.
5. If the patient agrees to participate, the informed consent form is signed and witnessed with the help of an interpreter if required.
6. If the patient decides not to participate, the colonoscopy and WF-EMR proceed as per usual.
7. Patient enters the endoscopy room and the procedure commences.
8. During the EMR procedure, data is recorded by the gastroenterology registrar or clinical research nurse regarding the technical aspects of the procedure.
9. The patient is moved to recovery for observation. They are observed for 2 hours in first stage recovery and at this stage are nil by mouth. They are then observed in second stage recovery while consuming clear fluids for 4 hours. They are examined by the proceduralist prior to discharge and provided with written post procedure information including a phone number to call in the event of any problems.
10. Overnight they remain on a clear fluid diet and resume a normal diet the following day.
11. Any adverse event is recorded prospectively on the data sheet as per the units standard practice. Adverse events include immediate or delayed bleeding, muscularis propria injury or perforation, persistent pain indicative of a serositis (inflammation of the outer layer of the bowel wall) or an unscheduled admission or readmission.
12. Patients are contacted by the research nurse by telephone 14 days following their procedure to assess ongoing symptoms and advise of any adverse events including admissions.

13. The formal histology results of the resected specimens are recorded on the follow-up data sheet. The slides are also reviewed as per usual endoscopy unit practice in the monthly gastroenterology unit histopathology meeting.
14. All patients return for a follow up procedure (scheduled colonoscopy) to check whether the lesion has been completely resected and to remove any recurrent or residual polyp. For the majority of patients this is at 5 months at the centre that performed the initial resection. A few patients will have lesions which are a low risk for recurrence and were resected "en-bloc", these patients may be booked for a 12 month follow up procedure, which may be performed by the referring institution.
15. Patients who have no, or low risk recurrence which is completely treated, are then followed up at 12 months, 3 years and 5 years at the referring institution. Endoscopy reports and histology are forwarded to the initial study centre for inclusion in the study. Patients with high risk or incompletely treated recurrence are managed by further endoscopic resection, or referral for surgery. This is based on the endoscopists assessment of the lesion and histology findings.
16. Patients who have significant adverse events may have their records for this event reviewed to create a more detailed picture of the complication.
17. The study outcomes will then be documented in manuscript form and submitted to a major internationally recognized peer reviewed journal for publication.
18. All participants will be mailed a letter outlining the results of the trial, and thanking them for their involvement.
19. All records of patients who participate in the trial will be marked so they are not destroyed by medical records for at least 15 years.

Non-lifting lesions

Lesions that are non-lifting will be removed using a stiff thin snare. The remaining tissue is removed by cold forceps avulsion and snare tip soft coagulation.

Data Security:

Patient data will be de-identified and a study code assigned to their information. The study code will however be able to be used to re-identify the patient for the purposes of linking follow up information. All data entry will be performed by the Clinical Research Nurse on a password secured Filemaker Database. This database is held on a Westmead Hospital internal server with regular automated backups. The database file is internally encrypted preventing patient data being visible by reading the source code. Paper datasheets collected are securely stored in a locked research office. The Clinical Research Nurse is the custodian of both electronic and paper data storage.

Participant Withdrawal From the Study:

In accordance with the Declaration of Helsinki and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Practice

Guidelines, a participant is free to withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or the institution. The Investigator may also withdraw the participant at any time in the interests of patient safety. Should a participant decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. Participants may be removed from the study if one or more of the following events occur:

- Withdrawal of consent
- Decision made by the investigators that removal from the study is in the patients best medical interest.
- Study stopped by ethics/regulatory authorities

The primary reason and additional reasons for withdrawal will be recorded in the participants medical record.

Statistics:

The ACE study aims to enrol patients for 10 years. At current rates of enrolment, this will result in 4000-5000 patients in the study. Comparison of quantitative variables will be performed by Students t-test and for qualitative variables by Pearson's χ^2 -test. A p value of < 0.05 will be considered significant. Statistical analyses will be performed with SPSS statistical software (IBM Corp. 2012. IBM SPSS Statistics, Version 21.0. Armonk, NY) with the help of an independent statistician.

Ethical Issues:

All patients will be managed according to established best practice according to international research and consensus on WF-EMR. Treatment does not differ according to whether or not the patient chooses to participate in the study.

The key ethical issues are:

1. Dependent Relationships
    - Most eligible participants will not be the regular patients of the investigators or the colonoscopists involved in the study. This is because the majority of the patients are referred from other medical specialists (Gastroenterologists or surgeons) to the tertiary referral service operated by the study centre Endoscopy Unit. Follow up after confirmed curative WF-EMR is with the referring specialist. Vigilance in explaining the voluntary nature of participation will be exercised for all patients. It will be emphasized that a decision not to enroll in the study will have no ramifications whatsoever for the patients care and ongoing relationship with the treating medical team.
2. Conflict of Interest
    - None of the investigators have financial conflicts of interest.

Potential Significance of the Study:

The ACE study has already produced internationally significant research output and the unique dataset is of considerable interest due to its potential to answer further questions about AMN and WF-EMR. The areas of research will cover epidemiological factors associated with large lesions, advanced lesion classification, refinement of the assessment of deep injury and subtype analyses of the different histological groups comprising AMN. The research has the potential to influence advice on screening and surveillance of colorectal polyps and in particular large lesions, to improve the ability of endoscopists to identify and resect AMN safely and to improve the worldwide acceptance of endoscopic resection of AMN as an alternative to surgery, reducing costs for healthcare systems.

Budget:

The cost of investigator time is free.

Funding for the project at is through the Westmead Hospital Endoscopy Research Fund.

Budget:

Ethics Committee Application Fee	$50.00
Total:	$50.00

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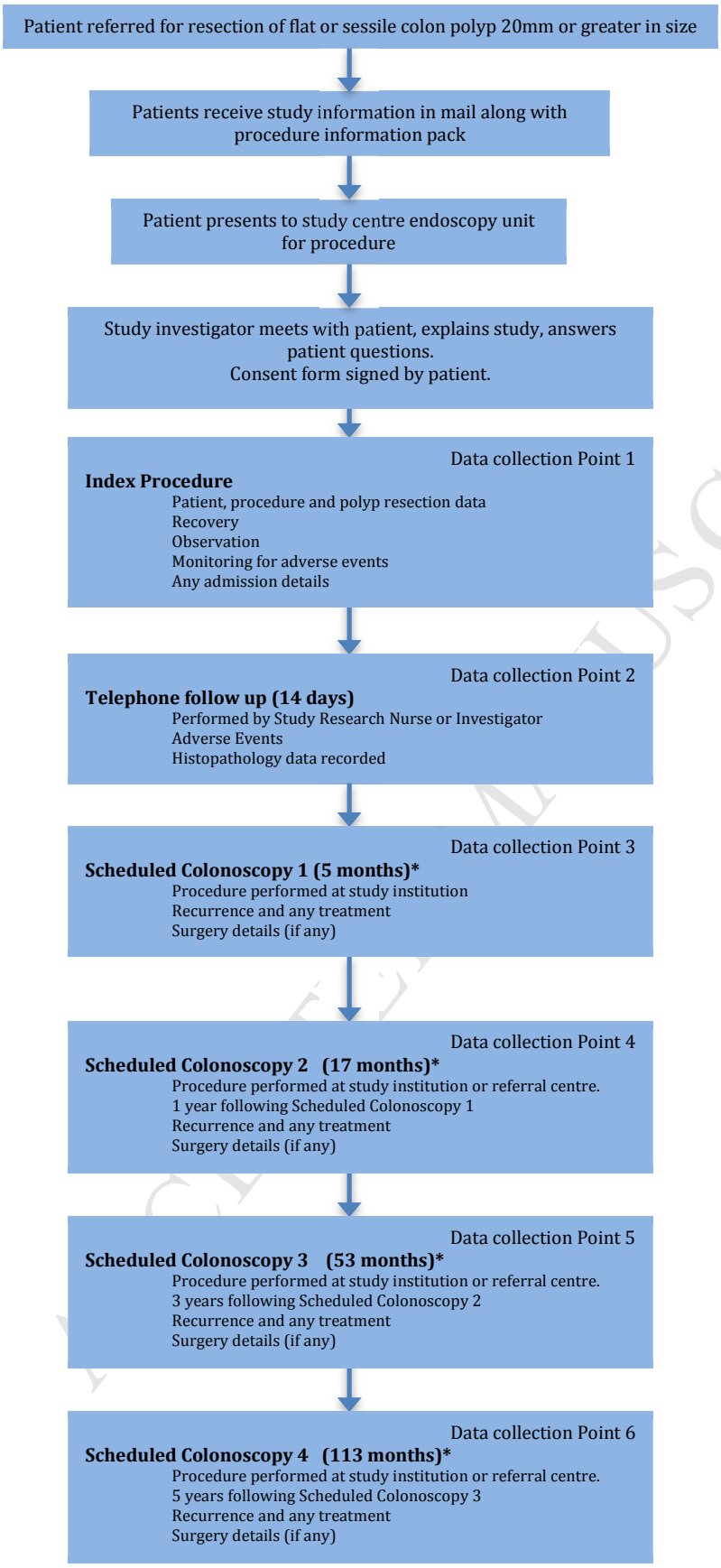
Appendix 1.

Study Flowchart:



NB: *=cumulative time from study entry to typical follow up for patients without recurrence. Follow up times are variable and are decided by the clinician at each procedure based on evidence of recurrence and histopathological findings. If recurrence occurs then typical subsequent follow up is 5 months, lesions without recurrence are followed up at 12 month, 3 year and then 5 year intervals.

Patients may be referred for surgery at any time point based on endoscopic findings or histology.