

Impact of Formalin Fixation on Colonic Polyp Size and Post-Polypectomy Surveillance Thresholds: Evidence from a Prospective Paired Analysis

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Abstract

Background

Post-polypectomy surveillance is anchored to polyp size, with a 10-mm cut-off separating a return to screening from 3-year follow-up and a 5-mm cut-off defining the diminutive category. For most polyps the only numerical size record available to the clinician is the pathologist's post-fixation measurement, yet whether formalin fixation shifts lesions across these thresholds is disputed.

Methods

Colorectal polyps ≥ 5 mm removed by snare polypectomy during routine colonoscopy and retrieved intact were measured fresh by the endoscopist and again after 8–24 h in 10% neutral buffered formalin by a blinded pathologist, using identical millimetric rulers. Comparative analyses were restricted to neoplastic lesions; unaided in-vivo visual assessments were examined descriptively.

Results

Sixty-three polyps from 53 patients were analysed; 55 (87.3%) were neoplastic. Mean size fell from 10.09 to 8.98 mm (reduction 1.11 mm, 95% CI 0.86–1.36; 13.7% per lesion; $p < 0.001$); 67.3% shrank and none enlarged. Shrinkage was greater in lesions < 10 mm than ≥ 10 mm (18.3% vs. 8.3%; $p = 0.005$) and in sessile than pedunculated lesions (17.3% vs. 7.5%; $p = 0.008$). Four of the eight lesions measuring 10–11 mm when fresh fell below 10 mm, and the number classified as diminutive rose from 7 to 15. In-vivo assessment showed wider dispersion and twice the threshold discordance.

Conclusions

Formalin fixation produces a small but directional size reduction that disproportionately affects small and sessile polyps and can shift lesions across both decision thresholds. Recording a fresh ex-vivo measurement at polypectomy is a simple, no-cost step that standardises the reference point.

1. Background

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer death worldwide, with more than 2 million new cases and approximately 918,000 deaths in 2024 [1]. Most sporadic CRC arises through the adenoma–carcinoma sequence, and colonoscopic detection with complete polypectomy interrupts this pathway and reduces both incidence and mortality [2]. Once a polyp has been removed, its pathological features—size, dysplasia grade, villous architecture and morphology—determine the recommended surveillance interval [3].

Among these features, size occupies a uniquely threshold-driven position. European Society of Gastrointestinal Endoscopy (ESGE) guidance stratifies patients around a 10-mm cut-off: complete removal of one to four adenomas smaller than 10 mm with low-grade dysplasia permits a return to screening, whereas any adenoma of 10 mm or more mandates surveillance at three years [3]. A second, lower boundary operates at 5 mm, below which lesions are classified as diminutive and become the target of optical-diagnosis strategies such as resect-and-discard and diagnose-and-leave [4]. A single millimetre near either boundary can therefore change management. The guidelines do not, however, specify which measurement should serve as the operative value, and no gold standard for sizing colorectal polyps has been established [3, 5]. The same lesion may accordingly receive a different surveillance interval depending on whether the in-vivo estimate, the fresh ex-vivo specimen or the post-fixation pathological value is used—a methodological gap that matters most precisely where measurement precision is most consequential.

The reliability of each of these measurements is contested. Visual assessment during endoscopy is operator-dependent and subject to both inter- and intra-observer variability, producing systematic over- and underestimation [6–11]; in some series this reclassified a substantial proportion of borderline lesions across the 10-mm threshold [10]. Several tools have been developed to make in-vivo sizing more objective, including comparison against an opened snare or biopsy forceps, the virtual scale endoscope that projects a measurement grid onto the mucosa [12, 13], and, more recently, artificial-intelligence systems able to estimate polyp size in real time without any physical reference object [14, 15]. These technologies remain under validation, are not yet universally available and address only the in-vivo value. In everyday practice the pathology report—generated after formalin fixation—remains the only numerical size record available to the clinician for most polyps.

Formalin preserves tissue architecture but is recognised to alter dimensions across many tissue types [16], and this matters most in organs where a metric diameter or margin directly drives staging or management. In colorectal resection specimens, Lam et al. reported margin reductions of roughly 10–15% after fixation [17], and a comparable diameter loss with potential tumour understaging has been demonstrated for renal tumours [18]. Radical prostatectomy specimens shrink by a mean of about 4.5% after fixation and processing, and planimetric tumour-volume calculations are routinely corrected for this effect [19, 20]. In breast cancer the tumour mass itself changes little, but the distance from tumour to resection margin decreases appreciably—enough to alter the interpretation of margin status and decisions about re-excision [21]. No consensus has emerged on whether staging should rest on the fresh or the fixed measurement, and because shrinkage is tissue- and organ-specific, organ-specific data are required.

If a comparable effect applies to intact colonic polyps, it could move a subset of lesions across the 10-mm boundary. The polyp-specific literature is limited and has reached mixed conclusions. Gopalswamy et al. found no significant effect of fixation and directed their criticism instead at the unreliability of endoscopic estimation [22]. Turner et al., comparing in-situ, pre-fixation and post-fixation measurements, likewise reported no significant difference between the in-situ and post-fixation values, although their

pre-fixation measurements were significantly larger than their post-fixation ones [23]. In neither study was fixation-related shrinkage of intact polypectomy specimens the primary object of enquiry, and in neither was the change related explicitly to the surveillance cut-offs. The extent to which fixation alters classification at clinically relevant thresholds therefore remains uncertain. We measured each polyp both fresh and after formalin fixation in order to quantify this shrinkage and to determine its potential to alter size-based surveillance classification.

2. Methods

2.1. Study design and setting

This single-centre study was conducted in the Endoscopy Unit of Karaman Training and Research Hospital between 1 January 2025 and 31 March 2026. All colonoscopies and polypectomies were performed on routine clinical indications; no procedure was undertaken for the purposes of the study and no aspect of patient management was altered by participation. Within this routine workflow, size measurements were recorded prospectively at the time each specimen was obtained, and the accumulated measurements were subsequently analysed. Consecutive patients in whom an eligible polyp was identified were included. Reporting follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [26]; the completed checklist is provided as Additional file 1.

2.2. Inclusion and exclusion criteria

Colonic polyps ≥ 5 mm removed by snare polypectomy and retrieved intact with an endoscopic retrieval net were included. Polyps were excluded if they fragmented during resection or retrieval, if fixation duration fell outside the 8–24 h window, or if a protocol deviation in measurement occurred (measurement by an operator other than the two designated study assessors).

2.3. Measurement protocol

Each lesion was measured at two sequential time points using identical rulers graduated to 1 mm. Fresh (ex-vivo) size was measured immediately after retrieval by the endoscopist, who placed the specimen on gauze and recorded the maximal dimension; this served as the reference standard. The specimen was then immersed in 10% neutral buffered formalin, the institution's routine fixative. Post-fixation size was measured after 8–24 h of fixation by a single consultant pathologist as part of the routine macroscopic protocol. All post-fixation measurements were performed by the same pathologist in order to eliminate inter-observer variability, and the pathologist was blinded to the fresh value.

Colonoscopies were performed by four endoscopists. The measurement protocol, however, was carried out by two fixed observers throughout: every fresh ex-vivo measurement was made by the same investigator and every post-fixation measurement by the same pathologist. The paired comparison on which this study rests is therefore unaffected by variation between endoscopists.

For sessile lesions the largest diameter was recorded and for pedunculated lesions the total height, using the same dimension at every time point. The paired difference between fresh and post-fixation values defined shrinkage, expressed in millimetres and as a percentage of the fresh size. Lesions were categorised as neoplastic (conventional and serrated adenomas, intramucosal carcinoma) or non-neoplastic (hyperplastic, inflammatory and fibroepithelial polyps). Patient demographics, colonoscopy indication, lesion location, macroscopic morphology and histopathological diagnosis were recorded concurrently. Recorded data were verified against the original colonoscopy reports and the endoscopy unit specimen log.

2.4. In Vivo Visual Assessment

Endoscopists also recorded an in-vivo assessment of lesion size in the routine procedure report. These assessments were made by unaided visual inspection, without the use of an opened snare, biopsy forceps or any other reference object, and they did not form part of the study measurement protocol. They were retrieved from the source documents for descriptive comparison only. Where the report gave a single approximate figure for two or more lesions, or expressed size as a range rather than a per-lesion value, no in-vivo size was assigned and the lesion was treated as missing for this comparison; seven lesions were handled in this way. Because the assessments were made by four different endoscopists outside a standardised protocol and showed marked digit preference, they were analysed descriptively and no hypothesis testing was performed on them.

2.5. Statistical analysis

The required sample size was estimated with G*Power 3.1.9.6 (Heinrich-Heine-Universität, Düsseldorf, Germany). Assuming a moderate effect ($d = 0.5$), $\alpha = 0.05$ and power = 0.95 for a paired comparison, a minimum of 54 lesions was required; recruitment continued until 63 evaluable specimens were obtained. All analyses were performed in jamovi (version 2.6; The jamovi project, Sydney, Australia).

An overall descriptive shrinkage estimate was calculated for the entire cohort, including non-neoplastic polyps. Because post-polypectomy surveillance recommendations apply specifically to neoplastic lesions, the primary comparative analysis, all subgroup analyses (size class and morphology) and the threshold-reclassification analyses were restricted to neoplastic polyps; non-neoplastic polyps were retained for the overall estimate rather than excluded. Neoplastic lesions were analysed as a single group. Post-polypectomy surveillance recommendations apply to neoplastic polyps as a class, and formalin-induced shrinkage is a biophysical process determined by tissue bulk and architecture rather than by dysplasia grade; the prespecified subgroup variables were therefore lesion size and macroscopic morphology. Analysis by histological subtype was not prespecified and was not attempted, because the number of lesions within the individual subtypes was insufficient to support it. Distribution normality was assessed with the Shapiro–Wilk test. Paired fresh versus post-fixation dimensions were compared with the paired-samples t-test and the Wilcoxon signed-rank test; the standardised effect size (Cohen d_z) and the 95% confidence interval (CI) for the mean difference are reported. The correlation between fresh and post-fixation size was assessed with the Pearson correlation coefficient. The effect of size class (< 10

vs. ≥ 10 mm) and morphology (sessile vs. pedunculated) on percentage shrinkage was tested with the independent-samples t-test. Clinical impact was quantified as the proportion of lesions reclassified across the 10-mm surveillance threshold and, as a prespecified exploratory analysis defined in the approved study protocol, across the 5-mm diminutive threshold. Agreement between the in-vivo assessment and the fresh measurement was summarised with the median and interquartile range of the paired difference, the Bland–Altman limits of agreement and the Spearman rank correlation, without significance testing. A two-sided $p < 0.05$ was considered significant.

Generative artificial intelligence was used during the preparation of this work. Claude (Anthropic) was used to assist with statistical computation and verification, to generate the figures and tables, to support the literature search, and for language editing and structuring of the text. It was not used to generate, alter or interpret primary data; all measurements were recorded by the investigators, and all statistical output and all references were independently reviewed and verified by the authors, who take full responsibility for the content.

3. Results

3.1. Cohort characteristics

Patient and polyp selection are shown in Fig. 1. Seventy-eight polyps were collected from 68 patients. Fifteen polyps were excluded (four fragmented, two non-epithelial benign tumours [lipoma, leiomyoma] and nine measured off-protocol), each from a different patient, leaving 63 polyps from 53 patients for analysis. Mean patient age was 64.2 ± 9.9 years (range 42–87) and 28 patients (52.8%) were men. Forty-five patients contributed a single polyp, six contributed two and two contributed three.

Lesions were most often left-sided (33, including 20 sigmoid or rectosigmoid), followed by the anorectum (16) and the right colon (14). Thirty-nine lesions (61.9%) were sessile and 24 (38.1%) pedunculated. Fifty-five (87.3%) were neoplastic—50 conventional adenomas with low-grade dysplasia, three serrated lesions, one adenoma with high-grade dysplasia and one intramucosal adenocarcinoma—and eight were non-neoplastic. Characteristics are shown in Table 1.

Table 1
Characteristics of the analysed patients and polyps.

Characteristic	Value
Patient characteristics (n = 53)	
Age, years, mean $\pm$ SD (range)	64.2 $\pm$ 9.9 (42–87)
Sex, n (%)	
Male	28 (52.8)
Female	25 (47.2)
Polyps per patient, n (%)	
One	45 (84.9)
Two	6 (11.3)
Three	2 (3.8)
Indication for colonoscopy, n *	
Faecal occult blood test positive	11
Faecal occult blood test negative	8
Macroscopic rectal bleeding	8
Polyp characteristics (n = 63)	
Location, n	
Right colon (ascending 10, transverse 4)	14
Left colon (descending 13, sigmoid/rectosigmoid 20)	33
Anorectum	16
Morphology, n (%)	
Sessile	39 (61.9)
Pedunculated	24 (38.1)
In-vivo visual assessment available, n (%)	56 (88.9)
Fresh size class, n	
<10 mm	34
$\geq$ 10 mm	29
SD, standard deviation; TA, tubular adenoma; TVA, tubulovillous adenoma; VA, villous adenoma. * Indication data were available for 27 of the 53 patients.	

Characteristic	Value
Patient characteristics (n = 53)	
Histology, n	
Adenoma, low-grade dysplasia (TA/TVA/VA)	50
Serrated lesion	3
Adenoma, high-grade dysplasia	1
Intramucosal adenocarcinoma	1
Non-neoplastic (hyperplastic 5, inflammatory 2, fibroepithelial 1)	8
<i>SD, standard deviation; TA, tubular adenoma; TVA, tubulovillous adenoma; VA, villous adenoma. * Indication data were available for 27 of the 53 patients.</i>	

3.2. Overall shrinkage

Across the whole cohort (n = 63, including the eight non-neoplastic polyps), mean size decreased from 10.10 ± 4.71 mm fresh (median 8, interquartile range [IQR] 7–12) to 9.02 ± 4.79 mm after fixation (median 8, IQR 5–11), a mean reduction of 1.08 mm (95% CI 0.84–1.32), corresponding to a mean per-lesion shrinkage of 13.0% (pooled 10.7%; paired t-test $p < 0.001$). Forty-one of 63 polyps (65.1%) decreased in size and none enlarged. In the eight non-neoplastic lesions considered separately, the mean reduction of 0.88 mm did not reach significance ($p = 0.064$), supporting the restriction of the comparative analyses to the 55 neoplastic polyps, to which all subsequent results refer.

3.3. Formalin-induced shrinkage in neoplastic polyps

Both fresh and post-fixation size distributions deviated from normality (Shapiro–Wilk $p < 0.001$). Among the 55 neoplastic polyps, mean size decreased from 10.09 ± 4.78 mm fresh (median 8, IQR 7–12) to 8.98 ± 4.95 mm after fixation (median 8, IQR 5–11). The mean absolute reduction was 1.11 mm (95% CI 0.86–1.36), significant by both the paired t-test ($t = 8.78$; $p < 0.001$) and the Wilcoxon signed-rank test ($p < 0.001$), with a large effect size (Cohen $d_z = 1.18$). Mean per-lesion shrinkage was $13.7 \pm 13.4\%$ (pooled 11.0%). Thirty-seven of 55 neoplastic polyps (67.3%) decreased in size, 18 (32.7%) were unchanged and none enlarged (Fig. 2). Fresh and post-fixation measurements were strongly correlated (Pearson $r = 0.98$; $p < 0.001$). The size change was small and strictly unidirectional, ranging from 0 to 3 mm; most affected polyps shrank by 1–2 mm.

3.4. Determinants of shrinkage

Shrinkage was significant within every subgroup examined, but its magnitude varied. Percentage shrinkage was greater in small than in large lesions: those < 10 mm (n = 30) shrank by 18.3% versus 8.3% for those ≥ 10 mm (n = 25; $p = 0.005$ for the between-subgroup difference). It was also greater in sessile than in pedunculated lesions (17.3% [n = 35] vs. 7.5% [n = 20]; $p = 0.008$). Notably, the paired fresh-versus-

fixed reduction remained significant even in the least affected subgroup, pedunculated lesions ($p = 0.002$), indicating that fixation-related shrinkage was present throughout the cohort and differed between subgroups in degree rather than in kind (Fig. 3, Table 2).

Table 2

Fresh versus post-fixation size and shrinkage. The overall cohort figure is shown for reference; comparative and subgroup analyses are restricted to neoplastic polyps.

Group	n	Fresh, mm	Fixed, mm	Shrinkage, %	p, Within group *	p, Between subgroups †
All polyps (reference) ‡	63	10.10	9.02	13.0	< 0.001	—
Neoplastic — all	55	10.09	8.98	13.7	< 0.001	—
<10 mm	30	6.73	5.60	18.3	< 0.001	0.005
≥10 mm	25	14.12	13.04	8.3	< 0.001	
Sessile	35	8.69	7.43	17.3	< 0.001	0.008
Pedunculated	20	12.55	11.70	7.5	0.002	
<i>Values are means. Shrinkage is the mean per-lesion percentage reduction (%). * Paired t-test comparing the fresh and post-fixation measurements within each group; the Wilcoxon signed-rank test gave the same conclusion in every group. † Independent-samples t-test comparing percentage shrinkage between the two subgroups of each pair. ‡ The whole-cohort row (including eight non-neoplastic polyps) is provided for reference only.</i>						

3.5. Impact on the 10-mm surveillance threshold

Fixation moved a meaningful subset of neoplastic lesions across the guideline threshold. Of the 25 neoplastic polyps measuring ≥ 10 mm when fresh, four (16.0%) fell below 10 mm after fixation, reducing the number classified as ≥ 10 mm from 25 to 21. The effect was concentrated at the boundary: among the eight neoplastic lesions measuring 10–11 mm fresh, four (50.0%) dropped below 10 mm, whereas none of the 17 lesions measuring ≥ 12 mm crossed the threshold. Had post-fixation size been used as the operative value, these patients would have qualified for a longer surveillance interval than their true (fresh) size warranted (Fig. 4, Table 3).

Table 3
Clinical impact of formalin-induced shrinkage at the two size-based decision thresholds (neoplastic polyps, n = 55).

Parameter	Value
10-mm surveillance threshold	
Polyps $\geq$ 10 mm — fresh measurement, n	25
Polyps $\geq$ 10 mm — post-fixation measurement, n	21
Reclassified from $\geq$ 10 mm to $<$ 10 mm, n (%)	4 (16.0)
Borderline lesions measuring 10–11 mm when fresh, n	8
of which reclassified below 10 mm, n (%)	4 (50.0)
Lesions $\geq$ 12 mm when fresh crossing the threshold, n	0
5-mm diminutive threshold (exploratory)	
Polyps $\leq$ 5 mm — fresh measurement, n	7
Polyps $\leq$ 5 mm — post-fixation measurement, n	15
Lesions measuring 6–9 mm when fresh, n	23
of which reclassified to $\leq$ 5 mm, n (%)	8 (34.8)
Lesions measuring exactly 6 mm when fresh, n	5
of which reclassified to $\leq$ 5 mm, n (%)	3 (60.0)
<i>All lesions reclassified across the 5-mm threshold were sessile.</i>	

3.6. Exploratory Analysis at the 5-mm Diminutive Threshold

A parallel effect was observed at the lower boundary, and it was proportionally larger. The number of neoplastic lesions classified as diminutive ($\leq$ 5 mm) rose from seven when fresh to fifteen after fixation; across the whole cohort the corresponding figures were nine and seventeen. Of the 23 neoplastic polyps measuring 6–9 mm when fresh, eight (34.8%) were reported as $\leq$ 5 mm after fixation, and three of the five lesions measuring exactly 6 mm fell to 5 mm. Every lesion reclassified in this way was sessile, consistent with the morphology effect described above (Table 3).

3.7. In-Vivo Visual Assessment Compared with the Fresh Measurement

An in-vivo visual assessment was available for 56 of the 63 lesions (88.9%); in the remaining seven the procedure report gave an approximate figure covering more than one lesion or expressed size as a range, and no per-lesion value could be assigned. The lesions without a recorded assessment were

larger than those with one (mean fresh size 15.3 vs. 9.4 mm), so this comparison is weighted towards smaller polyps and is presented descriptively only.

Central agreement with the fresh measurement was good: the median difference was 0.0 mm and the two values agreed to within 2 mm in 82.1% of lesions. Dispersion, however, was far wider than that seen with fixation, with Bland–Altman limits of agreement of – 4.8 to + 7.5 mm against a total observed range of 0 to 3 mm for fixation-related change (Table 4). Among the 49 neoplastic lesions with a recorded assessment, the in-vivo value disagreed with the fresh measurement across the 10-mm threshold in nine (18.4%), compared with four (8.2%) for the post-fixation value in the same lesions, and the in-vivo disagreements ran in both directions whereas every post-fixation discordance was downward.

Table 4
In-vivo visual assessment compared with the fresh ex-vivo measurement.

Parameter	Value
Lesions with an in-vivo assessment available, n (%)	56 (88.9)
Median difference (in-vivo – fresh), mm (IQR)	0.0 (0.0 to + 2.0)
Limits of agreement (Bland–Altman), mm	–4.8 to + 7.5
Lesions within 2 mm of the fresh measurement, n (%)	46 (82.1)
Assessments ≥ 15 mm recorded as a multiple of five (digit preference), n/N	13/13
Lesions falling on the opposite side of the 10-mm threshold from the fresh measurement, n (%) †	
In-vivo assessment vs. fresh measurement	9 (18.4)
Post-fixation vs. fresh measurement	4 (8.2)
<i>IQR, interquartile range. † Among the 49 neoplastic lesions for which an in-vivo assessment was available. Every assessment of 15 mm or more was recorded as a multiple of five, indicating rounded visual judgement rather than calibrated measure</i>	

4. Discussion

In this prospective paired study of intact polypectomy specimens, formalin fixation produced a small but consistent and significant reduction in size—about 1 mm, or roughly 13% of fresh diameter—that was highly reproducible and never reversed, both across the whole cohort and in the surveillance-relevant neoplastic subgroup on which we focused our analyses. The effect was not uniform: among neoplastic polyps it was proportionally largest in the small, sessile lesions that cluster around the decision thresholds. It reclassified half of the borderline 10–11 mm lesions below the 10-mm cut-off, and it more than doubled the number of lesions reported as diminutive. To our knowledge, this is the first study in which fixation-related shrinkage of intact polypectomy specimens is the primary object of enquiry and in which its capacity, on its own, to move lesions across the size-based decision thresholds is quantified.

Our findings are consistent with a broad literature on fixation-related tissue shrinkage in other organs. Lam and colleagues prospectively measured 46 colorectal resection specimens before and after fixation and found that the distal resection margin shortened by a mean of 14.7% and the radial margin by 10.5%; they concluded that shrinkage should be taken into account whenever tumour-free margins are narrow [17]. Working on renal tumours, Tran and co-workers demonstrated that fixation and processing reduced tumour diameter by a mean of 11.4%, and that this was sufficient to shift individual tumours from pT1b to pT1a—an explicit example of measurement-driven understaging—which led them to propose a correction factor for pathological reporting [18]. A comparable phenomenon has been documented in the upper aerodigestive tract, where nasal mucosa and cartilage shrank significantly between resection and fixation [16]. The magnitude we observed for whole colonic polyps (approximately 10–14%) is strikingly close to these figures, which argues for a shared biophysical mechanism—cross-linking and dehydration of predominantly mucosal tissue—rather than an organ-specific artefact.

The prostate and breast literature is instructive because it shows how the same phenomenon is handled once it is accepted. In radical prostatectomy specimens, fixation and processing were shown to cause a mean linear shrinkage of about 4.5%, and this correction is now routinely applied when tumour volume is calculated planimetrically [19, 20]. Horn and Naugler, studying breast specimens, reported that although the tumour mass itself changed little after fixation, the distance from the tumour to the resection margin shrank appreciably—enough to alter the interpretation of margin status and, potentially, the decision to re-excite [21]. In both fields the response has been to quantify the effect and correct for it rather than to ignore it. Colonic polyps, where a 10-mm number carries comparable decision weight, have not yet received the same treatment.

Our results contrast with the earlier polyp-specific literature only in part, and the discrepancy is smaller than it first appears. Gopalswamy and colleagues, in a study that remains widely cited, compared endoscopic, fresh and post-fixation measurements and concluded that polyp size was not significantly affected by formalin fixation, directing their criticism instead at the inaccuracy of in-vivo endoscopic estimation [22]. Turner and co-workers subsequently measured 107 colorectal polyps in situ, before fixation and after fixation, taking the pre-fixation value as their gold standard—a design very close to ours [23]. They found no significant difference between in-situ and post-fixation ruler measurements, and on that basis endorsed the post-fixation value for routine reporting because in-situ estimates were the more variable of the two. It is this comparison that is usually quoted. Their data nevertheless also showed that pre-fixation measurements were significantly larger than post-fixation measurements, which is precisely the effect we report here. Read in full, their study therefore corroborates rather than refutes the existence of fixation-related shrinkage; what differed was the emphasis, since their concern was the unreliability of in-vivo estimation rather than the fate of the specimen after it reaches the pot.

Several factors may explain why this effect has been treated as negligible. Earlier series were smaller, frequently recorded size in 0.1-cm increments that would mask a change of the order of 1 mm, and were not powered to detect an effect of the modest magnitude we observed; none related the change explicitly to the surveillance cut-offs. The central message of that literature—that endoscopic estimation

is unreliable—is not in dispute. Anderson and colleagues documented systematic endoscopic overestimation of polyp size, Moug and co-workers showed that endoscopists' estimates should not by themselves determine surveillance, and Schoen and colleagues argued on this basis that the pathological measurement is preferable to the endoscopic estimate [7–9]. What our data do not support is the further inference sometimes drawn from those studies: that the post-fixation value can be treated as interchangeable with true lesion size. When shrinkage is directional and concentrated near a decision threshold, even a mean effect of 1 mm has disproportionate classification consequences.

The greater proportional shrinkage of smaller and sessile lesions is mechanistically plausible: a fixed absolute loss at the specimen surface represents a larger fraction of a small polyp's diameter, and broad-based, mucosa-dominant sessile lesions lack the fibrovascular stalk that gives pedunculated polyps additional structural bulk. This is precisely the subgroup for which the size rules are most consequential, because small, flat adenomas are the lesions triaged between a return to screening and 3-year surveillance. Our threshold analysis makes this concrete: every reclassification we observed at the 10-mm cut-off arose from the narrow 10–11 mm band, while no lesion of 12 mm or more crossed it.

The exploratory analysis at the 5-mm boundary deserves comment because the proportional effect there was substantially larger. More than a third of the lesions measuring 6–9 mm when fresh were reported as diminutive after fixation, and the diminutive group as a whole more than doubled in size. This matters for a different reason than the 10-mm finding. The diminutive category underpins optical-diagnosis strategies such as resect-and-discard and diagnose-and-leave, and it is also the category in which endoscopic and pathological size estimates are most often compared when those strategies are validated [4]. If the pathological measurement is used as the comparator in such validation work, systematic post-fixation shrinkage will make endoscopists appear to overestimate size more than they in fact do. This finding was prespecified in our study protocol but should be interpreted as hypothesis-generating: 5 mm is not itself an ESGE surveillance threshold, and the number of lesions in the relevant band was small.

Our secondary comparison with the in-vivo visual assessment is informative despite its descriptive status. Unaided assessment performed better than the older literature might suggest: it agreed with the fresh measurement to within 2 mm in more than four-fifths of lesions, and the median difference was zero. What distinguished it was not systematic bias but dispersion. The limits of agreement spanned nearly 12 mm, against a total range of 0 to 3 mm for fixation-related change, and the in-vivo value disagreed with the fresh measurement across the 10-mm threshold more than twice as often as the post-fixation value did. The two errors are therefore different in kind. Fixation produces a small, unidirectional and predictable reduction; in-vivo estimation produces a larger, bidirectional and unpredictable scatter. A correction factor can in principle be applied to the first but not to the second. Seen this way, the fresh ex-vivo measurement is the only one of the three that is subject to neither, which is the practical case for recording it. Two caveats limit how far this comparison can be pushed: the assessments were made by four endoscopists outside any standardised protocol and without a reference object, and the seven lesions for which no per-lesion value could be retrieved were the larger

ones, so the comparison is weighted towards small polyps and probably flatters in-vivo estimation rather than the reverse.

These observations expose a standardisation gap. The ESGE framework anchors surveillance to size but does not define the measurement reference point [3], so the same lesion may receive different intervals depending on whether the endoscopic, fresh or post-fixation value is used. Our data argue that the fresh ex-vivo measurement is the most defensible reference: it avoids both the subjectivity of in-vivo estimation and the shrinkage of fixation, is trivial to obtain with a ruler at retrieval, and adds negligible cost or time. Where only the pathological measurement is available, clinicians should recognise that it may understate true size by roughly 10–15% and exercise particular caution for lesions reported just below a threshold.

This study has several limitations. It was conducted at a single centre, and the fixed measurement protocol that strengthens its internal validity also constrains how directly the estimate transfers to other settings. All fresh measurements were made by one investigator and all post-fixation measurements by one pathologist using identical rulers; this removed inter-observer variability but does not guarantee that an effect of this magnitude would be detected with equal precision where several observers are involved, and it leaves any systematic bias of either observer uncorrected. Fixation duration was likewise confined to a narrow 8–24 h window, and centres using longer fixation may observe greater shrinkage. Set against this, the outcome measured here is a physical property of fixed tissue rather than a clinical endpoint, and 10% neutral buffered formalin is a standard reagent, so the principal determinants of the effect are unlikely to differ fundamentally between centres; the magnitude we observed is also closely comparable to that reported independently for colorectal resection margins, renal tumours and nasal tissue [16–18].

All lesions were removed by a single standard snare technique with electrocautery, which ensured procedural uniformity but limits direct generalisability to specimens obtained by cold snare polypectomy, now recommended for lesions below 10 mm [24, 25]. Because thermal coagulation causes some immediate desiccation at the resection surface, the fresh measurement of an electrocautery specimen may already incorporate a small thermal effect; if so, the shrinkage we report is likely to be a conservative estimate of what would be observed in cold-snare specimens, and confirmation in such a series is warranted. A further limitation is that 18 of the 63 lesions came from eight patients who contributed more than one polyp, so the assumption of independence between lesions is not strictly met; the degree of clustering was modest, but a multilevel analysis in a larger series would be preferable. The series was also too small to support comparison across histological subtypes: a single adenoma with high-grade dysplasia, one intramucosal carcinoma and three serrated lesions cannot sustain such an analysis, and detecting a moderate difference between subtypes would require of the order of 64 lesions per group. In-vivo visual assessments were available for only 56 lesions and were recorded without a reference object, so the comparison presented here is descriptive and cannot substitute for a formal accuracy study using a calibrated in-vivo method. We did not assess the additional shrinkage introduced by tissue processing and microtomy, which may compound the fixation effect. Finally, we modelled surveillance impact using size alone, whereas interval assignment also incorporates dysplasia, lesion

number and histology. Multicentre studies using calibrated digital measurement, incorporating the full processing pathway and large enough to compare histological subtypes, are needed to establish a correction factor of general applicability – rather than to establish the existence of the effect, for which the present data and the wider fixation literature already provide consistent evidence.

5. Conclusion

Formalin fixation reduces colonic polyp size by a small but significant, directional margin that is proportionally greatest in small, sessile lesions near the size-based decision thresholds. It can therefore lengthen the recommended surveillance interval for a subset of patients whose true lesion size warrants closer follow-up, and it can move lesions into the diminutive category to which optical-diagnosis strategies are applied. Recording a fresh ex-vivo measurement at the time of polypectomy is a simple, no-cost step that standardises the reference point for size-based decisions and reduces the risk of threshold misclassification.

Abbreviations

CI, confidence interval; CRC, colorectal cancer; ESGE, European Society of Gastrointestinal Endoscopy; IQR, interquartile range; SD, standard deviation; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; TA, tubular adenoma; TVA, tubulovillous adenoma; VA, villous adenoma.

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and was approved by the Local Scientific Medical Research Ethics Committee of Karamanoğlu Mehmetbey University Faculty of Medicine (reference number 05-2026/13, 2 June 2026). All colonoscopies and polypectomies were performed on routine clinical indications; no procedure was undertaken for the purposes of the study and no aspect of patient management was altered by participation. Data collection began on 1 January 2025 within this routine workflow and the approval was granted while the work was already under way, the delay being administrative; the protocol reviewed by the committee describes the prospective recording of fresh and post-fixation measurements in full and covers the entire study period of 1 January 2025 to 31 March 2026. Written informed consent for colonoscopy and polypectomy, including consent for the use of the resulting clinical data for research purposes, was obtained from all patients as part of routine institutional practice. Because the study involved no additional intervention beyond routine care and used only measurements taken from specimens already removed on clinical indication, no study-specific consent was required.

Consent for publication

Not applicable. The manuscript contains no data relating to any identifiable individual.

Clinical trial number

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of large language models

Generative artificial intelligence was used during the preparation of this work, as described in the Methods. Claude (Anthropic) assisted with statistical computation and verification, with the generation of figures and tables, with literature searching and with language editing and structuring of the text. It was not used to generate, alter or interpret primary data. No patient-identifiable data were entered into the tool. All statistical output and all references were independently reviewed and verified by the authors, who take full responsibility for the content of this manuscript.

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Author Contribution

S.A.K. conceived the study, performed all fresh ex-vivo measurements, curated the data, carried out the statistical analysis, prepared the figures and wrote the first draft. G.V. performed all post-fixation measurements and the histopathological assessment and contributed to the study design and to revision of the manuscript. A.A., M.B. and P.A. contributed to data acquisition. P.A. supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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Not applicable

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author on reasonable request.

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Figures

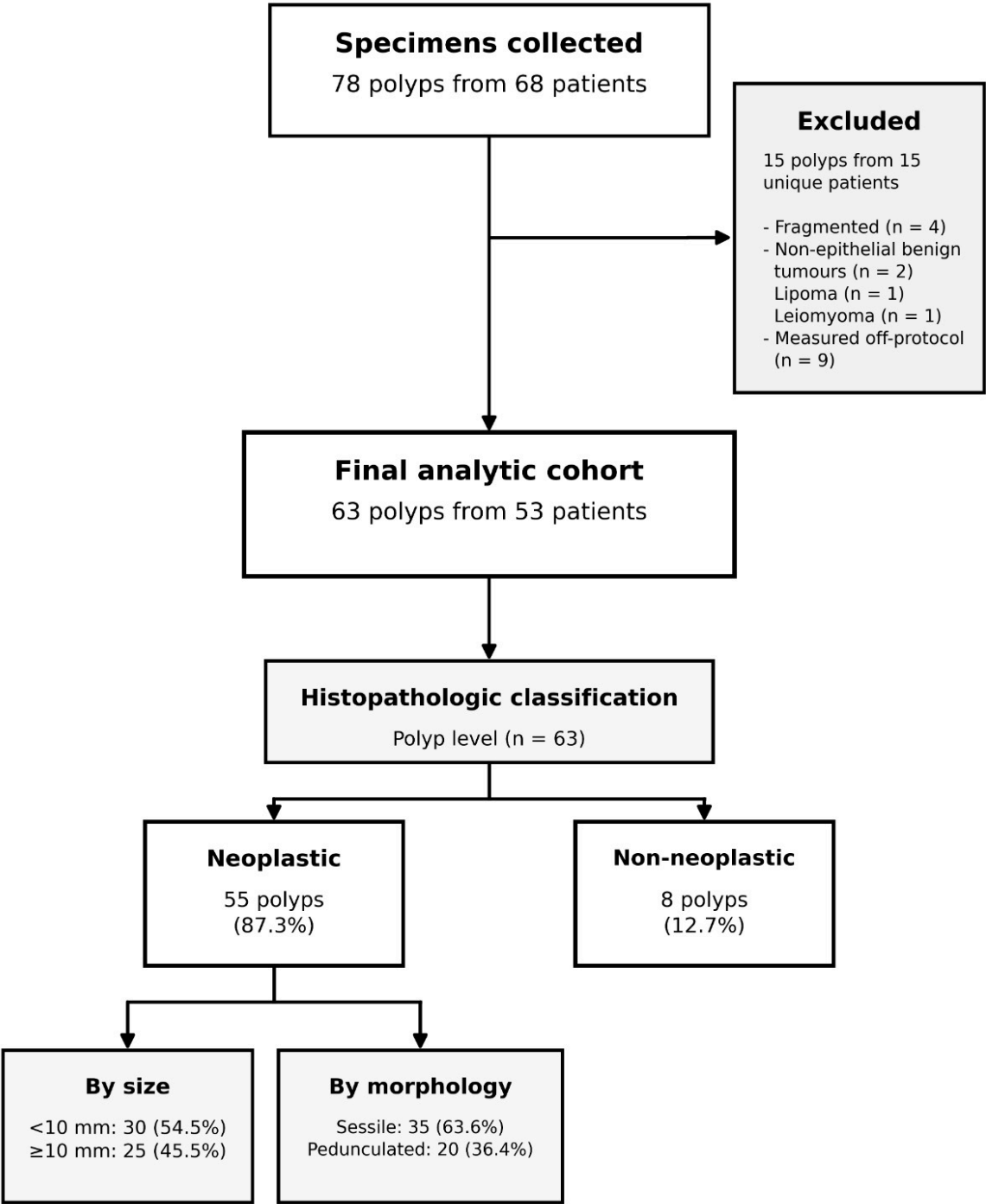


Figure 1

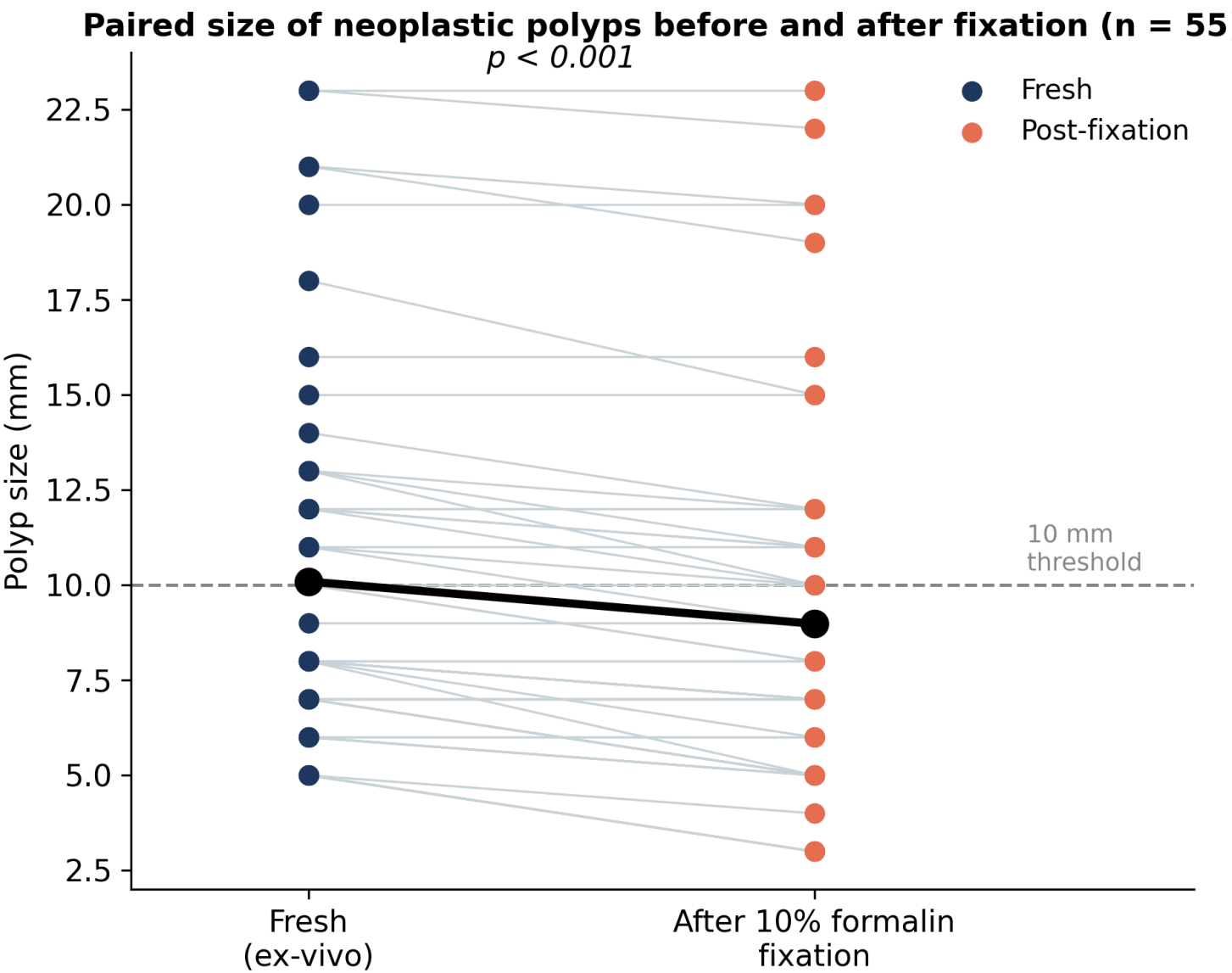


Figure 2

Paired size of neoplastic polyps before and after formalin fixation (n = 55). Each thin line links the fresh and post-fixation measurement of one polyp; the heavy line joins the group means (10.09 → 8.98 mm). The dashed line marks the 10-mm surveillance threshold (paired t-test $p < 0.001$).

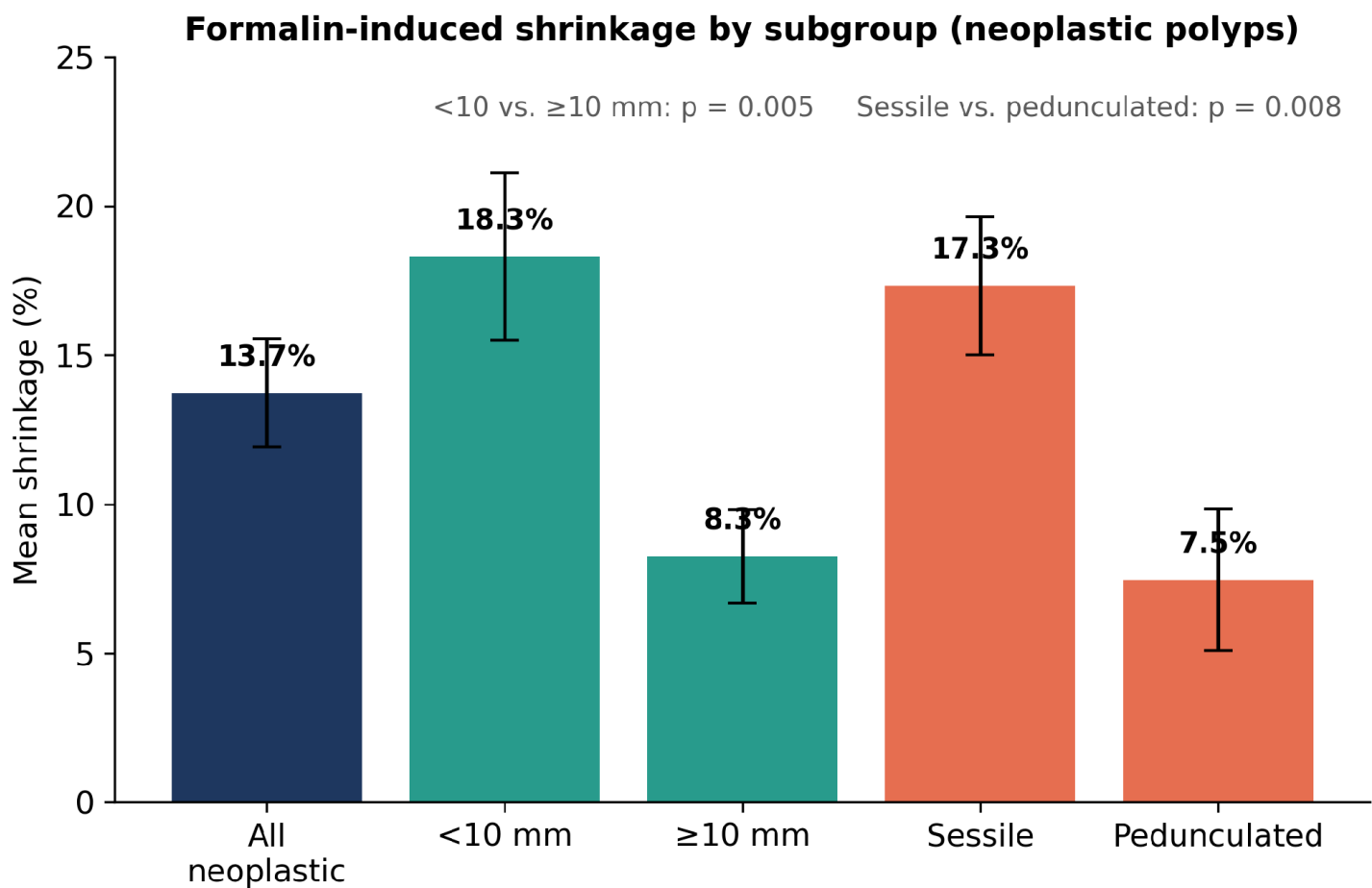


Figure 3

Mean percentage shrinkage by subgroup (neoplastic polyps, n = 55). Error bars denote the standard error of the mean. Smaller (<10 mm) and sessile lesions shrank proportionally more than larger and pedunculated lesions.

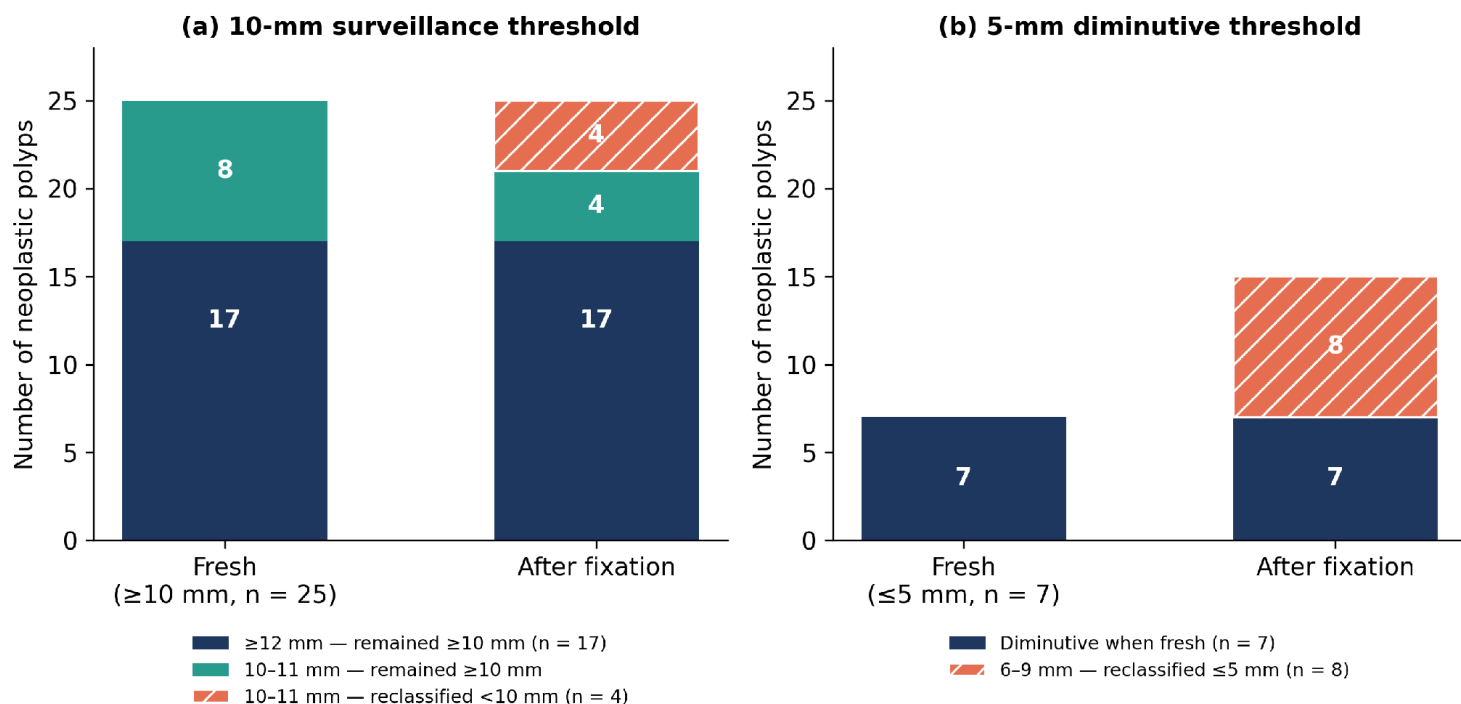


Figure 4

Reclassification of neoplastic polyps across the two size-based decision thresholds. (a) At the 10-mm surveillance threshold, all four reclassifications to <10 mm arose from the eight borderline (10–11 mm) lesions; none of the 17 polyps measuring ≥12 mm when fresh crossed the threshold. (b) At the 5-mm diminutive threshold, the number of lesions reported as ≤5 mm rose from seven to fifteen, the eight additional lesions all having measured 6–9 mm when fresh.

Supplementary Files

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