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Histologic Model For Pedunculated T1 Colorectal Carcinomas

Hospitals: 13

Pedunculated T1 CRC: 708

Metastasis: 5.2%

	AUC	Low-risk T1 CRC	High-risk T1 CRC	Missed metastasis
Conventional model 1 (ASGE/ESGE)	0.67	43%	57%	1.3%
Conventional model 2 (JSCCR)	0.64	35%	65%	1.2%
Our new model	0.83	68%	32%	1.3%

Gastroenterology

Title: Histologic Factors Associated With Need for Surgery in Patients With Pedunculated T1 Colorectal Carcinomas

Short Title: Histologic Model For Pedunculated T1 CRC

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Abbreviations: ASGE: American Society for Gastrointestinal Endoscopy; AUC: area under the curve; CI: confidence interval; CRC: colorectal cancer; ESMO: European Society for Medical Oncolocy; H&E: haematoxylin-eosin; IQR: interquartile range; JSCCR: Japanese Society for Cancer of the Colon and Rectum; LNM: lymph node metastasis; LASSO: least absolute shrinkage and selection operator; muscularis mucosa: MM; NPV: negative predictive value; OR: odds ratios; PDC: poorly differentiated clusters; PPV: positive predictive value.

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Data availability: An online version of the LASSO model is available at: http://t1crc.com/calculator/

ABSTRACT

Background & Aims: Most patients with pedunculated T1 colorectal tumors referred for surgery are not found to have lymph node metastases, and were therefore unnecessarily placed at risk for surgery-associated complications. We aimed to identify factors associated with need for surgery in patients with pedunculated T1 colorectal tumors.

Methods: We performed a cohort-nested matched case–control study of 708 patients diagnosed with pedunculated T1 colorectal tumors at 13 hospitals in The Netherlands, from January 1, 2000 through December 31, 2014, followed for a median 44 months (interquartile range, 20–80 months). We identified 37 patients (5.2%) who required surgery (due to lymph node, intramural, or distant metastases). These patients were matched with patients with pedunculated T1 colorectal tumors without a need for surgery (no metastases, controls, n=111). Blinded pathologists analyzed specimens from each tumor, stained with hematoxylin and eosin. We evaluated associations between histologic factors and patient need for surgery using univariable conditional logistic regression analysis. We used multivariable LASSO regression to develop models for identification of patients with tumors requiring surgery, and tested the accuracy of our model by projecting our case-control data towards the entire cohort (708 patients). We compared our model with previously developed strategies to identify high-risk tumors: conventional model 1 (based on poor differentiation, lymphovascular invasion, or Haggitt level 4) and conventional model 2 (based on poor differentiation, lymphovascular invasion, Haggitt level 4, or tumor budding).

Results: We identified 5 histologic factors that differentiated cases from controls: lymphovascular invasion, Haggitt level 4 invasion, muscularis mucosae type B (incompletely or completely disrupted), poorly differentiated clusters and tumor budding, which identified patients who required surgery with an area under the curve (AUC) value of 0.83 (95% CI, 0.76 – 0.90). When we used a clinically plausible predicted probability threshold of 4.0% or more, 67.5% of patients (478/708) were predicted to not need surgery. This threshold identified patients who required surgery with 83.8% sensitivity (95% CI, 68.0 – 93.8) and 70.3% specificity (95% CI, 60.9 – 78.6). Conventional model 1 and 2 identified patients who required surgery with lower AUC values (AUC, 0.67; 95% CI, 0.60 – 0.74; *P*=.002 and AUC, 0.64; 95% CI, 0.58 – 0.70; *P*<.001, respectively) than our LASSO model. When we applied our LASSO model with a predicted probability threshold of 4.0% or more, the percentage of missed cases (tumors mistakenly assigned as low risk) was comparable (6/478, 1.3%) to that of conventional model 1 (4/307, 1.3%) or conventional model 2 (3/244, 1.2%). However, the percentage of patients referred for surgery based on

our LASSO model was much lower (32.5%, n=230) than that for conventional model 1 (56.6%, n=401) or conventional model 2 (65.5%, n=464).

Conclusions: In a cohort-nested matched case–control study of 708 patients with pedunculated T1 colorectal carcinomas, we developed a model based on histologic features of tumors that identifies patients who require surgery (due to high risk of metastasis) with greater accuracy than previous models. Our model might be used to identify patients most likely to benefit from adjuvant surgery.

KEY WORDS: CRC; submucosal invasive; colon cancer; prognostic factor

INTRODUCTION

The introduction of population-based screening programs for colorectal cancer (CRC) has led to a higher incidence of early stage CRCs.[1] Endoscopic resection is an attractive treatment for T1 CRC due to substantially lower morbidity and mortality rates as compared to surgery.[2] Whether endoscopic treatment can be considered curative depends on the risk of incomplete resection and the risk of lymph node metastasis (LNM). An important difference between non-pedunculated and pedunculated T1 CRCs is that pedunculated lesions are especially amenable for endoscopic treatment, since these polyps can be removed relatively easy with en-bloc snare polypectomy with a risk of incomplete resection of less than 3%.[3] The risk of LNM has been reported to be as low as 3-7% in pedunculated T1 CRC, whereas this risk is about 7-14% in non-pedunculated T1 CRC.[4-7] When doubt exists on the completeness of endoscopic resection, but the risk of LNM is considered to be low, innovative new techniques and devices for endoscopic full-thickness resection may be promising alternatives for major surgery.[8, 9] Nonetheless, lymph node dissection is required when LNM are suspected. To date, no good prediction rule exists to adequately determine the risk for LNM in pedunculated T1 CRC. As a result, up to 46-76% of patients with pedunculated T1 CRC end up having surgery.[4, 7, 10, 11]

Up to now, only a limited number of studies have evaluated risk factors for LNM specifically for pedunculated T1 CRC.[4, 5, 7, 12, 13] These studies included only 2 to 10 patients with LNM, and lack long-term follow-up data. Haggitt level 4 invasion, poor differentiation, and lymphovascular invasion have been associated with LNM, and most guidelines therefore recommend considering surgery in the presence of one of these features. [14-16] As a result, a high proportion of patients are exposed to the risks of surgery without any clinical benefit. Assigning weights to different risk factors might result in a more

refined risk estimation, allowing individualized predictions that could be of clinical value in the weighted balance between risks and benefits of surgery in patients with pedunculated T1 CRC.

In the present multicenter study, we aimed to develop a prediction model to better estimate the indication for surgery in pedunculated T1 CRC, by reviewing currently used and new histological risk factors on hematoxylin-eosin (H&E) stained slides by expert pathologists. A second aim was to compare the diagnostic performance of our developed model with the currently used decision rules for additional surgery in existing guidelines.[14-16]

METHODS

Study design and source population

This was a cohort-nested matched case control study. The study was conducted in the T1 CRC registration cohort, initiated by the Dutch T1 CRC Working Group, a collaboration of Dutch hospitals of which 13 hospitals participated in this study (12 non-academic and 1 academic hospital). All patients with T1 CRC diagnosed between January 1, 2000 and December 31, 2014 in these hospitals were identified using the Netherlands Cancer Registry. The electronic medical records were reviewed. Exclusion criteria included patients with hereditary predisposition for CRC, inflammatory bowel disease, synchronous advanced CRC (defined as advanced CRC in the previous 5 years before detection of T1 CRC, or elsewhere in the colorectum at the time of detection of T1 CRC), non-CRC related death within one year after treatment, non-adenocarcinoma (e.g. carcinoid), neo-adjuvant radiotherapy, and missing endoscopy or pathology reports. Next, the endoscopy reports were reviewed. All patients with flat or sessile T1 CRCs were additionally excluded, resulting in a cohort of patients with pedunculated T1 CRC (i.e., the endoscopist stated the morphology of the polyp was pedunculated, either by reporting the presence of a stalk or by reporting Paris 0-Ip classification in the endoscopy report).[17] Additional patient and tumor characteristics were collected from the endoscopy, surgery and radiology reports. Follow-up in these patients was performed according to routine clinical care.

This study was approved by the Medical Ethics Review Committee of the University Medical Center Utrecht (approval for data-collection, reference number: 15-487; approval for histological review, reference number 15-716), and performed in accordance with the Helsinki Declaration. The study conforms to the TRIPOD statement for studies developing a prediction model.[18]

Outcome definition

The study aim was to better estimate the indication for surgery. We used a composite endpoint for this, in which pedunculated T1 CRCs with either LNM, intramural or distant metastasis were identified as cases. Pedunculated T1 CRCs with LNM have an indication for surgery assuming that an adequate lymph node dissection prevents further spread. Pedunculated T1 CRCs that develop an intramural or distant metastasis have an indication for surgery assuming that these might have resulted from missed LNM, either because no surgery or an inadequate lymphadenectomy was performed. LNM was defined as ≥1 positive lymph node(s) in the resection specimen as reported in the pathology report. Intramural metastasis was defined as malignant tissue at the site of the anastomosis after surgery, or at the polypectomy site after endoscopic resection only when margins were free of cancer tissue. This was defined as such, because in the latter situation tumor remains, even though the tumor free margins of the polypectomy specimen suggested completeness of resection, and thus polypectomy alone was insufficient in these cases. Distant metastasis was defined as metastasis to extra-colonic organs, bone or peritoneum as confirmed with imaging or histology. For the readability, we use the term 'metastasis' for this composite endpoint throughout this paper.

Control selection

Pedunculated T1 CRC patients without metastasis were matched as controls for the cases on a 3:1 ratio (Supplementary Figure S1). Controls were matched (without replacement) by treatment method (primary endoscopic resection vs secondary surgical resection vs primary surgical resection) and tumor location (colon vs rectum or rectosigmoid), while using incidence density sampling (i.e., controls were selected from all those patients who have accrued at least the same length of follow-up as the duration of case occurrence).[19] For patients only treated with endoscopic resection, controls were additionally matched by R0-resection margins, as all cases were also R0-resections (i.e., to prevent selection bias). The researchers that performed the matching procedure (YB, LMGM) were blinded for the original histological reports and not involved in the histological review process.

Pathology review and histological factors

Candidate histological factors for review included histological factors evaluated in previous pedunculated T1 CRC studies as well as new promising factors only evaluated in non-pedunculated T1

CRC.[4, 5, 7, 10, 12, 13, 20-26] Selected factors were differentiation grade, submucosal invasion depth according to the Haggitt classification, lymphovascular invasion, tumor budding, poorly differentiated clusters (PDC) and the condition of the muscularis mucosae (MM). A quantitative evaluation of invasion depth and invasion width were not included, since proper assessment of these factors is highly dependent on specimen handling (e.g., adequate transverse sections).[5, 10] The definitions, methods of assessment and grading of the histological factors are presented in Supplementary Table S1. If the Haggitt classification could not be determined (e.g. due to piecemeal resection), this factor was considered absent.

The H&E stained slides of the selected cases and controls were collected from the participating hospitals. The median number of slides per polyp was 5 (IQR 2 - 8); all polyps were totally embedded. Slice thickness varied between 3 and 4 µm (information obtained from the pathology reports and participating centers). Two pathologists with special expertise in gastrointestinal pathology (MML & GJAO) reviewed the H&E slides, both blinded for the clinical characteristics and the original histological report. The slides were mutually reviewed to confirm the diagnosis T1 CRC, and excluded if the diagnosis could not be confirmed. T1 CRC was defined as invasion through the muscularis mucosae and into, but not beyond, the submucosa.[27] To optimize the reproducibility of histologic assessment, the first 30 cases (randomly selected) were also mutually reviewed on the candidate predictors. Consensus was reached on the interpretation of the definitions of the new parameters (i.e., status of the muscularis mucosa, PDC and tumor budding). After this set, the pathologists felt confident that a single pathologic evaluation would suffice as long as low confident cases would be discussed. Therefore, histological factors in the remaining set were reviewed by MML, and discussed with GJAO in case of uncertainty. To test the reproducibility, two pathologists (MGR and PD) from two high volume community hospitals were invited to review another set of 30 randomly selected cases in which the inter observer agreement with the study pathologist's assessment (i.e, assessment by MML which was optionally discussed with GJAO) was estimated (see Supplementary Methods).[28]

Statistical analysis

All analyses were performed using IBM SPSS Statistics version 21 (SPSS Inc., Chicago, IL, USA), MedCalc version 15.0 (MedCalc Software, Ostend, Belgium), and R version 3.2.2 (particularly packages penalized, pROC, irr). A two-sided p-value ≤0.05 was considered significant.

Model development

In view of the small number of patients in some histological factor categories, we binned some of these variables before further analysis, based on previous literature and current guidelines (Supplementary Table S1): differentiation grade: poor vs. good/moderate; invasion depth: Haggitt 4 vs. 1-3 or un-assessable; lymphovascular invasion: present vs. absent; tumor budding: grade 2-3 vs. grade 1; PDC: grade 2-3 vs. grade 1; status of the MM: type B vs. A.[10, 14-16, 22-25] We then evaluated the association between the histological factors and the outcome with uni- and multivariable conditional logistic regression analyses, acknowledging the matched design (i.e., stratified Cox regression models with time held constant and matched case-control sets as strata). Univariable results were reported as odds ratios (OR) with 95% confidence intervals (CI). All candidate histological factors were included in multivariable modeling. However, in view of the small number of events relative to the number of factors, and to obtain an optimal model with as few factors as possible, we used LASSO (L1 penalized least absolute shrinkage and selection) regression for multivariable analyses.[29] This is a logistic regression model that penalizes the absolute size of the coefficients of a regression model, based on the value of lambda. With larger penalties, the estimates of weaker factors shrink towards zero, so that only the strongest predictors remain in the model. Details regarding all steps performed can be found in the Supplementary Methods. We took into account the sampling fraction of controls for each matching factor thus extrapolating the findings developed in the nested case-control set to the entire cohort. We reported the resulting prediction rule enabling the calculation of the predicted probability of the outcome in new patients, the apparent and cross-validated area under the curve (AUC), calibration plots, and accuracy measures (i.e. sensitivity, specificity, negative and positive predictive value (NPV/PPV)) according to different predicted probability thresholds defining high-risk T1 CRC (and thus surgical referral).

During the above described multivariable modeling, we observed an unanticipated negative association between tumor budding and metastasis. As tumor budding and PDC are closely related phenomena which may have contributed to this unexpected observation, we additionally analyzed our data by combining PDC and budding as one variable (positive in the presence of either tumor budding grade 2-3 or PDC grade 2-3 and negative otherwise), similar to previous studies.[30-33] We therefore

report on two models: LASSO model 1 that considers PDC and budding separately, and LASSO model 2 that combines these entities.

Comparison with conventional models

The diagnostic performance of the decision rules in existing guidelines was calculated, defined as 'conventional models', in order to compare it with our LASSO-derived models.[14-16] In conventional model 1, high-risk T1 CRC was defined as T1 CRC with either poor differentiation, lymphovascular invasion, or Haggitt level 4, according to the European Society for Medical Oncology (ESMO) and the American Society for Gastrointestinal Endoscopy (ASGE).[14, 15] In conventional model 2, high-risk T1 CRC was defined as T1 CRC with either poor differentiation, lymphovascular invasion, Haggitt level 4, or tumor budding, since budding has been implemented as indicator for additional surgery in the Japanese Society for Cancer of the Colon and Rectum (JSCCR guidelines).[16] In the absence of all these features it was considered a low-risk T1 CRC. Receiver operating characteristics (ROC)-curves were plotted for all models, and curves were compared using the DeLongs test. Sensitivity and specificity were compared with the McNemar test. After adjustment for the sampling fraction (in the same manner as described above), absolute outcome probabilities were calculated.

RESULTS

Study population

In total, 2253 patients with T1 CRC were identified in the Netherlands Cancer Registry. Among these, 708 eligible patients with pedunculated T1 CRCs were selected. A study flowchart and baseline characteristics of the source population can be found in Supplementary Material Figure S1 and Table S2. Median follow-up time of these 708 patients was 44 months (IQR 20 - 80).

Within our pedunculated T1 CRC cohort, we identified 23 patients with LNM (3.3%; 95%CI 2.2 – 4.8%), 17 patients with distant metastasis (2.4%; 95%CI 1.5 – 3.8%), and 4 patients with intramural metastasis (0.6%; 95%CI 0.2 – 1.4%). Metastases during follow-up were detected after a median duration of 21 months (IQR 9 – 49). Two patients had both LNM and distant metastasis, resulting in an overall metastasis rate of 5.9% (N=42; 95%CI 4.4 – 7.9%). Among those cases, 5 cases were excluded because H&E slides were not available or too devastated (N=3), or because the slides were revised as ≥T2 CRC (N=2). This resulted in a final 37 cases, matched 1:3 with 111 control patients (Supplementary

Material Figure S1). Baseline characteristics of cases and controls are presented in Table 1. Median follow-up time was 48 months (IQR 20 - 78) in cases and 51 months (IQR 27 - 84) in controls. Within the secondary surgery group, piecemeal resection was performed in 18 controls (32.1%), and 5 cases (26.3%), p=0.63.

Histological factors in cases vs controls

The presence of histological factors in cases vs. controls is shown in Table 2. Cases more often had pedunculated T1 CRCs with Haggitt level 4 invasion (31.3% vs. 13.4%, p=0.02), were more often positive for tumorbudding (45.9% vs. 27.9%, p=0.04) and PDC (62.2% vs. 23.6%, p<0.001), more often showed lymphovascular invasion (73.0% vs. 33.3%, p<0.001), and more often showed MM type B (97.3% vs. 72.2%, p=0.001), compared to controls. A trend was observed towards poorer differentiation (37.8% vs 22.5%, p=0.07). Corresponding univariate OR, sensitivity, specificity and AUC are provided in Table 2.

Model development

Using multivariable LASSO regression, we identified a total of 5 out of 6 histological factors that differentiated cases from controls: lymphovascular invasion, Haggitt level 4 invasion, PDC, MM type B and tumor budding (LASSO model 1), yielding a cross-validation AUC of 0.82 (95%CI 0.76 – 0.87). Differentiation grade did not contribute. In this model, an unexpected negative association was observed between tumor budding and metastasis. Repeating LASSO-analysis while replacing tumor budding and PDC for 'any kind of budding' (i.e, positive in the presence of ≥5 cancer clusters in the stroma irrespective of number of cells per cluster, and negative otherwise), resulted in a prediction model based on 4 histological factors: MM type B, lymphovascular invasion, Haggitt level 4 invasion, and any kind of budding (LASSO model 2), yielding a cross-validation AUC of 0.80 (95%CI 0.74 – 0.88). The regression coefficients, LASSO-derived multivariate ORs, and the intercept of both LASSO models are presented in Table 3. The plots of the cross-validation AUC according to the penalty and the coefficient profile plots can be found in the Supplementary Material (Figure S2-S3). Both models showed good calibration (Supplementary Figure S4).

As an illustration, we depicted a score chart with the estimated predicted probability for metastasis using LASSO model 2 (Table 4). For example, a pedunculated T1 CRC with MM type A,

Haggitt level 4 invasion, no lymphovascular invasion and no budding (PDC or tumor budding) had an estimated 1.4% risk for metastasis, whereas this risk increased to 5.0% in the presence of lymphovascular invasion.

Model performance and comparison with conventional models

We next evaluated the diagnostic performance of the LASSO models. In our data, LASSO model 1 (i.e., the model with tumor budding and PDC separately) and LASSO model 2 (i.e., the model with tumor budding and PDC combined) yielded (apparent) AUCs of 0.84 (95%CI 0.77 – 0.91) and 0.83 (95%CI 0.76 – 0.90), respectively. The performance was compared with conventional model 1 (i.e., high risk in the presence of either lymphovascular invasion, poor differentiation or Haggitt level 4 invasion) and conventional model 2 (i.e., high risk in the presence of the aforementioned factors combined with tumor budding) (Figure 1). The AUC of conventional model 1 (0.67; 95%CI 0.60 – 0.74) was significantly lower than both LASSO model 1 (p<0.001) and LASSO model 2 (p=0.002). Similarly, the AUC of conventional model 2 (0.64; 95%CI 0.58 – 0.70) was significantly lower than both LASSO model 1 (p<0.001) and LASSO model 2 (p<0.001).

Sensitivity and specificity of the models were calculated (Figure 2). For the LASSO models, sensitivity and specificity were calculated for three clinically plausible probability thresholds for metastasis (≥3.0%, ≥4.0% and ≥5.0%). For example, at a ≥4.0 threshold, sensitivity of LASSO model 1 (86.5%; 95%CI 71.2 – 95.5) and LASSO model 2 (83.8%; 95%CI 68.0 – 93.8) did not significantly different from the sensitivity of conventional model 1 (89.2%; 95%CI 74.6 – 97.0) and conventional model 2 (91.9%; 95%CI 78.1 – 98.3). However, specificity of LASSO model 1 (69.4%; 95%CI 59.9 – 77.8) and LASSO model 2 (70.3%; 95%CI 60.9 – 78.6) was significantly better than the specificity of conventional model 1 (45.1%; 95%CI 35.6 – 54.8, p<0.001) and conventional model 2 (36.0; 95%CI 27.1 – 45.7, p<0.001) (Figure 2).

Clinical implications in entire cohort

To provide more insight in the clinical implications, we translated the findings in the development (nested-matched) set to the entire cohort of 708 pedunculated T1 CRCs. The number of patients tested positive (i.e., high-risk T1 CRC), the number of patients inappropriately not referred for surgery (i.e., missed cases), and the number of high-risk patients in whom metastases were observed (i.e., PPV) are

presented in Table 5. For the LASSO models, different clinically plausible predicted probability thresholds for surgery are presented. For example, using LASSO model 2 at a ≥4.0% threshold, the percentage of patients with an indication for surgery despite a negative test result (i.e., low-risk T1 CRC with metastasis) was comparable (1.3%; 6/478) to conventional model 1 (1.3%; 4/307) or conventional model 2 (1.2%; 3/244). However, the percentage of patients with high-risk T1 CRC was much lower (32.5%; N=230) than when using conventional model 1 (56.6%; N=401) or conventional model 2 (65.5%; N=464).

Inter observer agreement

Two pathologists were invited to test the inter observer agreement in a random sample of 30 cases (Supplementary Table S4). Using LASSO model 1 at a 4% threshold, they agreed with the study pathologist's assessment in 87% and 97% of cases with a kappa of 0.73 (substantial) and 0.93 (almost perfect) for pathologist 1 and 2, respectively. For LASSO 2, agreement was observed in 73% and 87% with a kappa of 0.48 (moderate) and 0.68 (substantial) for pathologist 1 and 2, respectively.

DISCUSSION

Clinicians encounter T1 CRCs with increasing frequency and are faced with the difficulty of selecting candidates who will benefit from surgery in an increasing proportion of CRC patients.[1] To date, a simplified risk stratification is used that divides patients into low- and high-risk groups based on histological risk factors.[14, 15] Although it is known that some histological factors (e.g. lymphovascular invasion) are stronger and more robustly associated with metastasis than others (e.g. poor differentiation), conventional risk models do not take this into account.[14-16] Moreover, conventional models do not stratify based on polyp morphology, whereas the risk of LNM is much lower in pedunculated than non-pedunculated T1 CRC.[4-7] The two major steps forward of our strategy is that histological risk factors were weighted and compiled into a final estimate, and that this was done specifically for pedunculated T1 CRC. We incorporated both currently used and novel promising histological factors. Our model – developed in a cohort with an almost 4-fold higher number of metastases than in any earlier study on this topic[4, 5, 7, 12, 13] – has the potential to reduce the number of unnecessary surgical referrals substantially with a comparable risk of missing metastasis in patients tested negative (low-risk T1 CRC). The weighted approach has the advantage over currently used

models that the quantitative evaluation of the individual patient's risk for metastasis facilitates clinicians to weigh this risk against the risk for surgical morbidity and mortality.

Our model was developed using a large Dutch multicenter cohort. Previous prediction models have shown that estimates of the model's performance can be influenced in several ways when assessed in a new population.[18] The regression coefficients can be incorrect, often seen as a result of statistical overfitting. We however performed several steps to minimize this risk (i.e., internal validation using cross-validation and shrinkage of coefficients at model development). Moreover, the model's performance also depends on the case-mix to which the model is applied. In our cohort, the background risk of metastasis in pedunculated T1 CRC was 5.2%, which is comparable to incidence rates of metastasis in pedunculated T1 CRC reported in cohorts outside the Netherlands (all Asian cohorts).[4-7] To further enhance the reproducibility of our model, we evaluated all histological factors according to previously described (consensus) definitions. Moreover, the calculation of our model does not need sophisticated assessment strategies, as all risk factors can be evaluated on H&E stained slides readily available in laboratories throughout the world. Furthermore, the observation that the inter observer agreement was reasonably good supports that our model can be applied by other pathologists.

We used a cohort-nested matched case control design and composite endpoint, which is an efficient approach to study associations when the outcome is rare. A composite endpoint of LNM, intramural and distant metastasis was used rather than LNM alone as endpoint for two reasons. First, it increased the robustness of our analysis. Although the present T1 CRC cohort is one of the largest to date, only 23 eligible pedunculated T1 CRC patients with LNM could be identified. Second, it enabled us to identify pedunculated T1 CRCs with high vs. low metastatic potential. The presence of LNM is one of the strongest prognostic factors in CRC.[34] The concept of a sequential progression of tumor cells, in which LNM are precursors of distant metastases, forms the basis of the TNM staging system.[34] In this way, lymph node dissection, which is the primary reason to proceed to surgery in T1 CRC, prevents seeding of distant metastasis. An alternative model is that distant metastases arise independently of LNM, supported by a recent study that observed genetically distinct origins of LNM and distant metastasis, indicating that both types of metastatic lesions likely originate from distinct sub clones in the primary tumor.[35] In this view, using a composite endpoint to identify biologically aggressive pedunculated T1 CRCs in which endoscopic resection alone is insufficient seems more appropriate. To explore the magnitude of potential bias introduced by using a composite endpoint, we compared

the predicted probabilities for LNM and metastasis during follow-up separately, showing similar estimates (Supplementary Material Table S3).

Ideally, the model would not miss any patients with metastasis. Our study shows that in all scenarios, irrespective whether conventional or our models are used, patients with metastasis will be missed, unless the threshold is set at a value with a very low specificity. However, even after surgery there is still a 0.3-4.5% risk that patients with T1 CRC develop metastasis.[2, 36] Surgery therefore decreases the risk of metastasis, but is not a curative treatment for each patient. Our model is the first that enables the calculation of the individual T1 CRC patient's risk for metastasis, facilitating to weigh this risk against the risk of surgical mortality based on the patient's profile (age, condition and comorbidity).[37, 38] For example, a patient <75 years old with ASA-score I, has an estimated surgical mortality and morbidity risk of 0.1 – 1.0% and 20 – 24% respectively, vs. 7.5 – 9.4% and 36 – 37% respectively for a patient ≥75 years with an ASA-score III-IV. As a consequence, the threshold for surgical referral when using our model for these patients needs to be adjusted.[37, 38]

In our study, tumor budding was associated with metastasis in univariate analysis (OR 1.9), however, this positive association was no longer present in multivariate analysis. Although unanticipated, previous work of Ueno et al., conducted in stage II-III CRCs, also showed a univariate association between tumor budding and metastasis (HR 4.6) which disappeared when correcting for PDC.[22] The authors' explanation was the difficulty to assess tumor budding on H&E-stained slides, necessitating immuno-histochemical staining for accurate identification of single cancer cells and small cell clusters. We also experienced this difficulty, and especially the quantification of the number of cells per undifferentiated cluster was problematic. The observation of Ueno et al. that the intra-observer reproducibility for PDC was much better than for tumor budding further supports this notion.[22] In addition, Barresi et al. evaluated both tumor budding and PDC in 101 T1 CRC specimens, and observed no association between tumor budding and metastasis, whereas PDC appeared to be a strong independent factor.[39] Both PDC and tumor budding are associated with epithelial-mesenchymal transition, an important process in the initiation of metastasis.[40] It therefore seems rather arbitrary to use a cut-off of 5 cells per cluster to define whether it is called tumor budding or PDC. Interpreting these factors as one phenomenon, as done in LASSO model 2, therefore seems logical from a biological perspective.[22, 39] A validation study should confirm whether interpreting these factors as one entity indeed results in comparable accuracy, as we observed.

Both the qualitative (i.e., sm1 vs sm2-3) and quantitative (i.e., ≥1000 µm) evaluation of invasion depth have been associated with LNM in sessile T1 CRCs.[20] Quantitative assessment of invasion depth is however much more complicated in pedunculated T1 CRC (e.g. due to differences in the length of stalks). An additional disadvantage is that its assessment is highly dependent on tissue handling and processing. Although we therefore decided not to incorporate this parameter as candidate predictor in our model, we did find it interesting to explore its value in pedunculated T1 CRC. The quantitative evaluation of invasion depth (according to the method as proposed by Ueno et al[10]) could be evaluated in 33 cases and 98 controls (inadequate sectioning in 25%), and was not associated with metastasis (median 6.0 mm [IQR 4.0 – 8.5] in cases vs 4.8 mm [IQR3.0 – 8.0] in controls, p=0.16, OR 1.05 [95%CI 0.92 – 1.19]). This is in line with previous studies that quantitatively evaluated invasion depth in pedunculated T1 CRC, and limits the chance that this factor would have contributed to the diagnostic accuracy of our model.[5, 13] Instead, we evaluated the Haggitt classification, and confirmed previous studies that this factor per se is insufficient for risk stratification.[7, 13, 41] For an adequate assessment, it is essential that both the endoscopist and pathologist handle the polyp in such a way that accurate information concerning the level of invasion can be obtained, as underlined by Haggitt et al.[12] If malignancy is suspected, the stalk should be snared at the base, so that the pathologist can distinguish Haggitt 3 from Haggitt 4 invasion.

We observed that the risk for metastasis was very low in pedunculated T1 CRC with MM type A. This is in line with four previous studies, which observed a metastasis risk of 0% (0/44), 0% (0/18), 0% (0/43) and 2.4% (1/41) when the MM was preserved.[21, 25, 42, 43] Taken these and our study together, the proposed strategy of Miyachi et al to be very conservative with the decision to perform additional surgery when the MM is intact seems justified.[25]

Our study flowchart depicts that approximately 42% of T1 CRCs were pedunculated, comparable to previous studies reporting proportions between 40-60%.[11, 44] A relative high proportion of patients was excluded due to missing reports or early death, however, evaluation of characteristics of these patients does not suggest major concerns regarding selection bias (data not shown). Study characteristics of the source population were also comparable to previous pedunculated T1 CRC studies.[4, 5, 7, 13] We extrapolated the findings developed in the nested case-control set to the entire cohort by updating the intercept so that the average predicted probabilities of the model corresponded with the average risk for metastasis in the source population, i.e. pedunculated T1 CRC. It should

however be noted that this prevalence remains an estimate. Pathologists experience diagnostic difficulty distinguishing pseudo-invasion and high-grade dysplasia from T1 CRC, which might have resulted in an underestimation of the metastasis risk.[45] It is however very difficult to account for this, since diagnosis of T1 CRC in pedunculated lesions is a contemporary challenge even for expert pathologists, and a golden standard does not exist.[45, 46] The incidence of 5.2% used for our analysis seems justified, since there were also cases revised as T2 CRC, and this is in good agreement with previous reported incidence rates varying between 3 – 7%.[4-7]

Ikematsu et al showed recurrence rate to be significantly higher in patients with high-risk T1 rectal cancer treated with endoscopic resection without adjuvant surgery (16.2%) as compared to similarly treated high-risk T1 colon cancer (1.4%).[2] This difference was not observed for other treatment groups. As surgical outcomes differ between colon and rectum cancer, confirmation of this finding would be very relevant. In a previous study of our research group considering 877 patients treated with endoscopic resection, we did not observe an association between incomplete resection (i.e., local recurrence or residual tumor) and rectal location when adjusting for other clinical factors.[3] In the current study, only 1 high-risk rectum T1 cancer treated with endoscopic resection alone developed recurrence, which would indicate a 5.7% recurrence risk for high risk T1 rectal cancer vs 3.4% for similarly treated high-risk T1 colon cancer when extrapolating these findings to the entire cohort (data not shown). These percentages are however not robust as they are based on very small case numbers. Moreover, our (nested) matched case-control design on both treatment and localization hampers to adequately evaluate the effect of matching factors on outcome. Nevertheless, it would be interesting to incorporate tumor localization - and perhaps other factors as well - in future studies to attempt to further improve and refine risk stratification in these patients.

Our study has some limitations. Most important, despite that our cohort is the largest pedunculated T1 CRC cohort to date, the absolute number of patients with metastasis was still low. For this reason, we used all cases for the development of the model, and used cross-validation for interval validation, together with a statistical analysis approach specifically useful to allow robust multivariable prediction modeling in small datasets. However, before considering implementation of our model in general practice, the model needs to be formally validated in another cohort, preferably prospectively but at least in another independent set of archival slides. This formal validation will possibly also clarify which of the two LASSO models is most suitable for risk stratification. A second limitation is that we

used archival slides, resulting that tissue processing was not standardized. This might however also enhance generalization of our results. Slice thickness and orientation vary throughout the world, and our cohort hereby reflects real time practice. Importantly, most of the parameters incorporated in our model could be assessed even when the specimen was fragmented or inadequately handled. Moreover, although tissue handling was not standardized, the pathologic assessment itself was fully standardized. Established definitions were used, and only H&E stained slides were evaluated (including in the cases in which additional stainings were available) to prevent variability in assessment introduced by the availability of additional stainings. Nevertheless, although the inter observer agreement was reasonably good, it should be noted that histological assessment will always be subject to inter observer variation. Finally, although baseline characteristics were comparable, we cannot exclude that - as in any case-control study - there are differences between the groups that were not accounted for. Moreover, the number of LNs retrieved during surgery was relatively low.[14] As low LN yield has been associated with increased risk for recurrence, this might have resulted in an underestimated incidence of metastasis. The number of retrieved LNs was however equally distributed over cases and controls and the estimated risk for metastasis did not significantly differ between patients with low (<12) and high (≥12) LN yield (data not shown), making it less likely that this has caused a significant bias.

In conclusion, current guidelines (ESMO, ASGE, JSCCR) provide a weak recommendation to consider surgery in patients with pedunculated T1 CRC in the presence of one of the histological high-risk factors, resulting in a high proportion of patients referred for surgery without any benefit. This multicenter study is not only the first to evaluate the diagnostic performance of the decision rules in existing guidelines in a large multicenter cohort, but is also the first to evaluate an alternative strategy. We propose two models with a significantly better specificity while maintaining comparable sensitivity, thus having the potential to safely reduce unnecessary surgery substantially.

FIGURES

Figure 1. Receiver operating characteristic curves for metastasis in pedunculated T1 CRC for the two conventional models and the two LASSO-derived models.

Dashed line is reference line. Abbreviations: AUC: area under the curve; CI: confidence interval; LASSO: least absolute shrinkage and selection operator.

Figure 2. Comparison of the sensitivity and specificity between the two conventional models and the two LASSO models for different predicted probability thresholds (≥3.0%, ≥4.0% and ≥5.0%). Error bars indicate the 95%confidence interval. Abbreviations: LASSO: least absolute shrinkage and selection operator; ns: not significant.

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Table 1. Baseline characteristics of cases (i.e., with metastasis) and matched controls (i.e. without metastasis) with pedunculated T1 colorectal cancer

	Cases N=37	Controls N=111	p-value
Male gender, n (%)	17 (45.9)	60 (54.1)	0.39
Age in years, median (IQR)	67.3 (62.9 – 75.1)	65.6 (60.0 – 75.3)	0.56
ASA-score, n (%) [a]			
- **ASA 1**	10 (27.0)	37 (33.6)	0.54
- **ASA 2**	19 (51.4)	57 (51.8)	
- **ASA 3 – 4**	8 (21.6)	16 (14.5)	
Polyp size in mm, median (IQR) [b]	20 (15 – 30)	20 (15 – 30)	0.81
Polyp location, n (%)			
- **Colon**	29 (78.4)	87 (78.4)	Matched
- **Rectum or rectosigmoid**	8 (21.6)	24 (21.6)	
Overall treatment, n (%)			
- **Primary endoscopy**	9 (24.3)	27 (24.3)	Matched
- **Secondary surgery**	19 (51.4)	57 (51.4)	
- **Primary surgery**	9 (24.3)	27 (24.3)	
Number of retrieved LNs, median (IQR) [c]	7 (4 – 10)	6 (4 – 10)	0.64

Abbreviations: ASA: American Society of Anesthesiologists; IQR: interquartile range; LN: lymph node
a. ASA score: missing in one control
b. Polyp size: missing in 2 cases and 13 controls
c. Only for pedunculated T1 CRC removed with surgical resection, missing in 1 control patient

Table 2. Histological risk factors in cases (i.e., with metastasis) vs matched controls (i.e. without metastasis) with pedunculated T1 colorectal cancer

	Cases (N=37)	Controls (n=111)	p-value	Univariate odds ratio (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	AUC (95%CI)
Differentiation grade, n (%)							
- Good or moderate	23 (62.2)	86 (77.5)	0.07	Reference	37.8%	77.5%	0.58
- Poor	14 (37.8)	25 (22.5)		2.4 (1.0 – 5.7)	(22.5 – 55.2)	(68.6 – 84.9)	(0.46 – 0.70)
Haggitt level, n (%)							
- Haggitt 1 – 3	22 (68.8)	84 (86.6)	0.02	Reference	31.3%	86.6%	0.59
- Haggitt 4	10 (31.3)	13 (13.4)		3.1 (1.0 – 9.8)	(16.1 – 50.1)	(78.2 – 92.7)	(0.47 – 0.71)
- Unable to determine	5	14					
Tumor budding, n (%)							
- Negative	20 (54.1)	80 (72.1)	0.04	Reference	46.0%	72.1%	0.59
- Positive	17 (45.9)	31 (27.9)		1.9 (1.0 – 3.9)	(29.5 – 63.1)	(62.8 – 80.2)	(0.48 – 0.70)
Poorly differentiated clusters, n (%)							
- Negative	14 (37.8)	84 (76.4)	<0.001	Reference	62.2%	76.4%	0.69
- Positive	23 (62.2)	26 (23.6)		4.9 (2.0 – 9.5)	(44.8 – 77.5)	(67.3 – 83.9)	(0.59 – 0.80)
- Unable to determine	-	1					
Lymphovascular invasion, n (%)							
- Absent	10 (27.0)	74 (66.7)	<0.001	Reference	73.0%	66.7%	0.70
- Present	27 (73.0)	37 (33.3)		4.8 (2.1 – 11.0)	(55.9 – 86.2)	(57.1 – 75.3)	(0.60 – 0.80)
Muscularis mucosa, n (%)							
- Type A	1 (2.7)	30 (27.8)	0.001	Reference	97.3%	27.0%	0.63
- Type B	36 (97.3)	81 (72.2)		16.5 (2.1 – 129.7)	(85.8 – 99.9)	(19.0 – 36.3)	(0.53 – 0.72)

Abbreviations: AUC: area under the curve; CI: confidence interval

Table 3. LASSO-derived multivariate models of histologic predictors for metastasis in pedunculated T1 colorectal cancer

	Model with tumor budding and PDC separately (LASSO model 1)		Model with tumor budding and PDC combined (LASSO model 2)	
	Regression coefficient	Lasso-derived Multivariate OR[4]	Regression coefficient	Lasso-derived Multivariate OR[4]
Differentiation grade [1]				
- Good/moderate	Reference	Reference	Reference	Reference
- Poor	0.00	1.00	0.00	1.00
Haggitt level				

- Haggitt 1 – 3 [2]	Reference	Reference	Reference	Reference
- Haggitt 4	1.45	4.26	1.42	4.14
Tumor budding				
- Negative	Reference	Reference	-	-
- Positive	-0.66	0.52	-	-
PDC				
- Negative	Reference	Reference	-	-
- Positive	1.30	3.67	-	-
Any kind of budding (tumor budding or PDC) [3]				
- Negative (<5 cancer clusters in the stroma)	-	-	Reference	Reference
- Positive (≥5 cancer clusters in the stroma)	-	-	0.57	1.76
Lymphovascular invasion				
- Absent	Reference	Reference	Reference	Reference
- Present	1.28	3.60	1.31	3.69
Status of the muscularis mucosa				
- Type A	Reference	Reference	Reference	Reference
- Type B	2.31	10.1	1.84	6.28
Intercept	-6.13		-5.68	
Cross validation AUC (95%CI)	0.82 (0.76 – 0.87)		0.80 (0.74 – 0.88)	

Abbreviations: AUC: area-under-the-curve; LASSO: least absolute shrinkage and selection operator; OR: odds ratio; PDC: poorly differentiated clusters.

1. Regression coefficient of differentiation grade was zero in both LASSO models, indicated that this factor did not contribute to the differentiation of cases from controls.
2. Also includes unable to determine Haggitt level.
3. Positive in the presence of either tumor budding or PDC and negative otherwise.
4. Confidence interval are not provided because they cannot be reliably estimated in penalized estimation methods such as LASSO analysis.[47]

Note: The predicted probability for metastasis can be calculated using the following formula: $P = (1/[1 + \exp(-1 * (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_k X_k))]) * 100$, in which β_0 is the intercept, and β_k is the regression coefficient for predictor X_k (the different histological factors that contribute to the differentiation of cases and controls in LASSO regression-analysis). For example, a pedunculated T1 CRC with poor differentiation, lymphovascular invasion and musc. mucosa type B in the absence of the other factors has a risk of 7.3% in LASSO model 1 (1/[1 + exp(-1 * (-6.13 + (1.28 + 2.31))]) and 7.4% in LASSO model 2 (1/[1 + exp(-1 * (-5.68 + (1.31 + 1.84))]).

Table 4. Example of the LASSO-derived predicted probability (%) of metastasis in pedunculated T1 CRC for LASSO model 2 based on histological risk factors.

		Muscularis mucosa type A		Muscularis mucosa type B	
		Any kind of budding -	Any kind of budding +	Any kind of budding -	Any kind of budding +
Haggitt 1-3	LVI -	0.3	0.6	2.1	3.7
	LVI +	1.2	2.2	7.4	12.3
Haggitt 4	LVI -	1.4	2.4	8.2	13.6
	LVI +	5.0	8.5	24.8	36.8

Abbreviations: LASSO: least absolute shrinkage and selection operator; LVI: lymphovascular invasion. – indicates absence; + indicates presence.
For example, a pedunculated T1 CRC with muscularis mucosa type A, Haggitt level 4 invasion, no lymhovascular invasion and no budding (PDC or tumor budding) has a 1.4% risk of metastasis. The diagnostic accuracy for different thresholds can be found in Table 5.
Note: For ease of use the table has been colour coded. It displays a green (low risk for metastasis) to red (high risk for metastasis) gradient, with the shade of the colour representing the value in the cell.

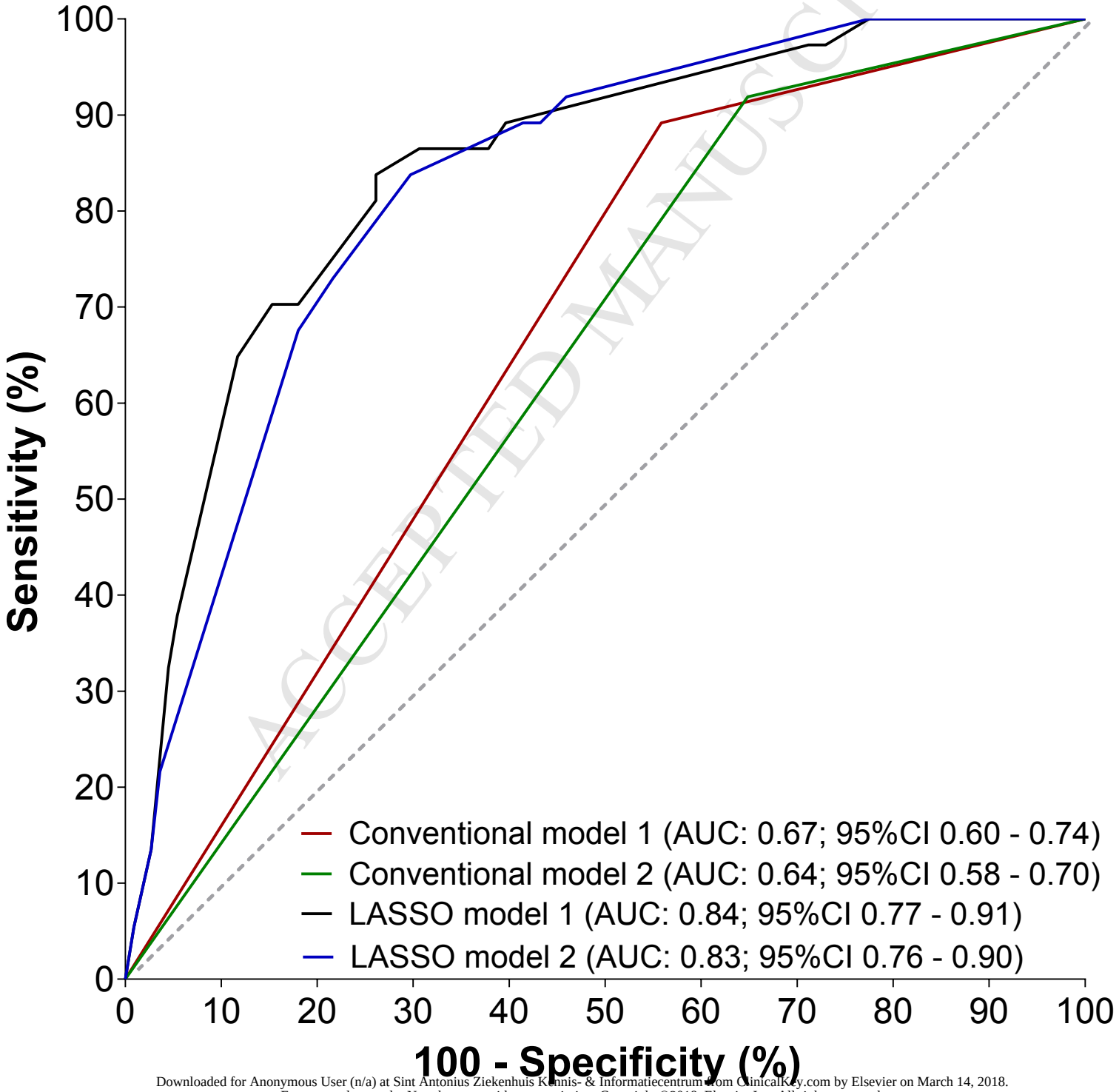
Table 5. Estimated diagnostic accuracy and surgical referral rate for the two conventional models and the two LASSO models (based on varying predicted probability thresholds for metastasis), for 708 patients with pedunculated T1 colorectal cancer

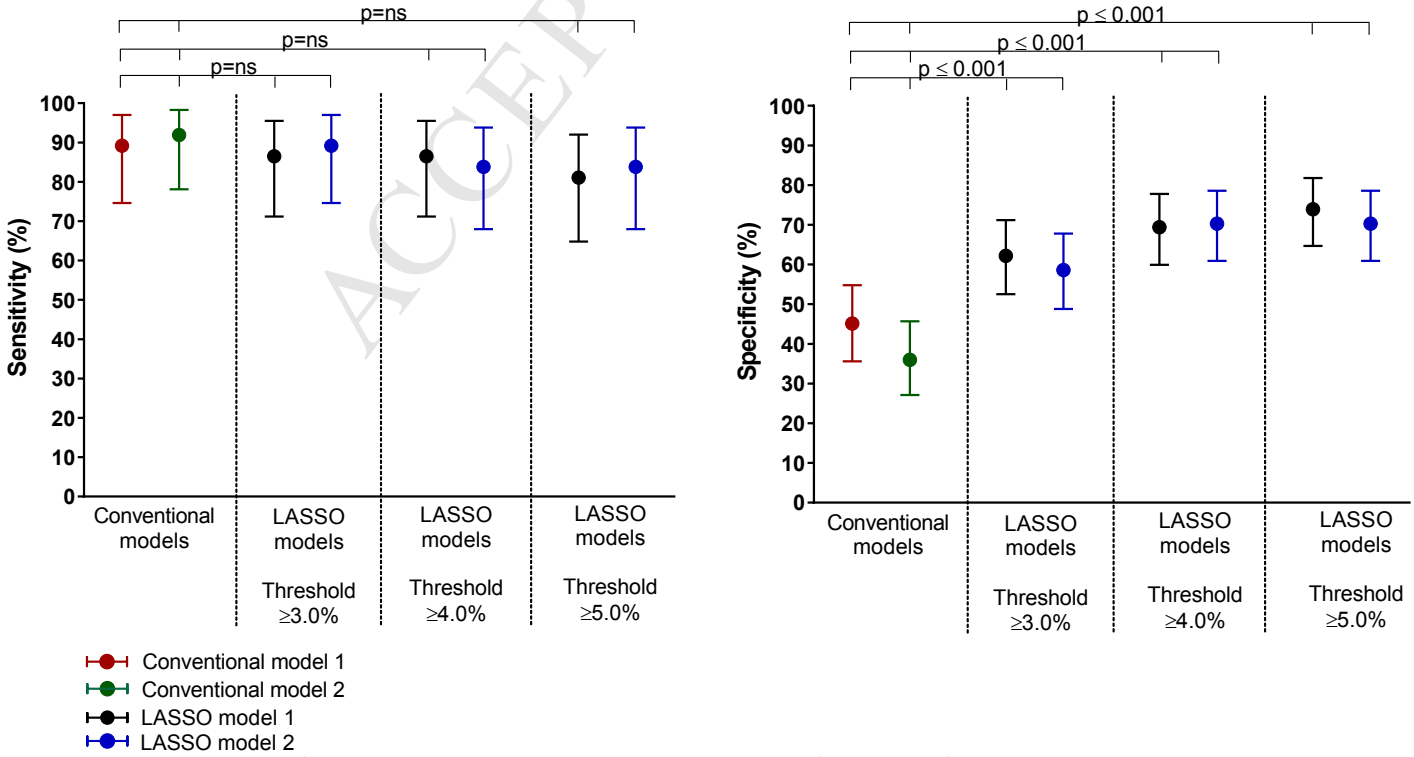
Model [a]	Threshold	High-risk T1 CRC			Low-risk T1 CRC			Missed cases		Predictive value	
		Total, n (%) [b]	TP (n) [c]	FP (n) [c]	Total (%) [b]	TN (n) [c]	FN (n) [c]	LNM (n)	M [d] (n)	PPV (%)	NPV (%)
Conventional model 1	Presence of one of the high-risk factors	401 (56.6)	33	368	307 (43.4)	303	4	2	2	8.2	98.7
Conventional model 2		464 (65.5)	34	430	244 (34.5)	241	3	2	1	7.3	98.8
LASSO model 1	≥ 1.5 %	520 (73.4)	36	484	188 (26.6)	187	1	0	1	6.9	99.5
	≥ 2.5 %	299 (42.2)	33	266	409 (57.8)	405	4	2	2	11.0	99.0
	≥ 3.0 %	286 (40.4)	32	254	422 (59.6)	417	5	3	2	11.2	98.8
	≥ 4.0 %	237 (33.5)	32	205	471 (66.5)	466	5	3	2	13.4	98.9
	≥ 5.0 %	205 (29.0)	30	175	503 (71.0)	496	7	4	3	14.6	98.6
LASSO model 2	≥ 1.5 %	557 (78.7)	37	520	151 (21.3)	151	0	0	0	6.6	100.0
	≥ 2.5 %	311 (43.9)	33	278	397 (56.1)	393	4	3	1	10.6	99.0
	≥ 3.0 %	311 (43.9)	33	278	397 (56.1)	393	4	3	1	10.6	99.0
	≥ 4.0 %	230 (32.5)	31	199	478	472	6	3	3	13.4	98.8

					(67.5)						
	≥ 5.0 %	230 (32.5)	31	199	478 (67.5)	472	6	3	3	13.4	98.8

Abbreviations: CRC: colorectal cancer; LASSO: least absolute shrinkage and selection operator; LNM: lymph node metastasis; NPV: negative predictive value; PPV: positive predictive value.

a. Conventional model 1: High risk in the presence of: lymphovascular invasion, poor differentiation or Haggitt level 4 invasion; Conventional model 2: High risk in the presence of: lymphovascular invasion, poor differentiation, Haggitt level 4 invasion or tumor budding. LASSO model 1: Model with tumor budding and poorly differentiated clusters separately (Table 3), LASSO model 2: model with tumor budding and poorly differentiated clusters considered as one entity (Table 3).
b. Percentage of total cohort of 708 patients
c. TP: true positive; i.e. metastasis correctly referred for surgery; FP: false positive, i.e. surgical overtreatment; TN: true negative, i.e. endoscopic resection appropriate treatment; FN: false negative, i.e. missed metastasis (inappropriately not referred for surgery)
d. M indicates metastasis during follow-up





SUPPLEMENTARY MATERIAL

Supplement to: Histologic Factors Associated With Need for Surgery in Patients With Pedunculated T1 Colorectal Carcinomas

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** Both senior authors contributed equally to the work.*

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Supplementary Methods

Model development

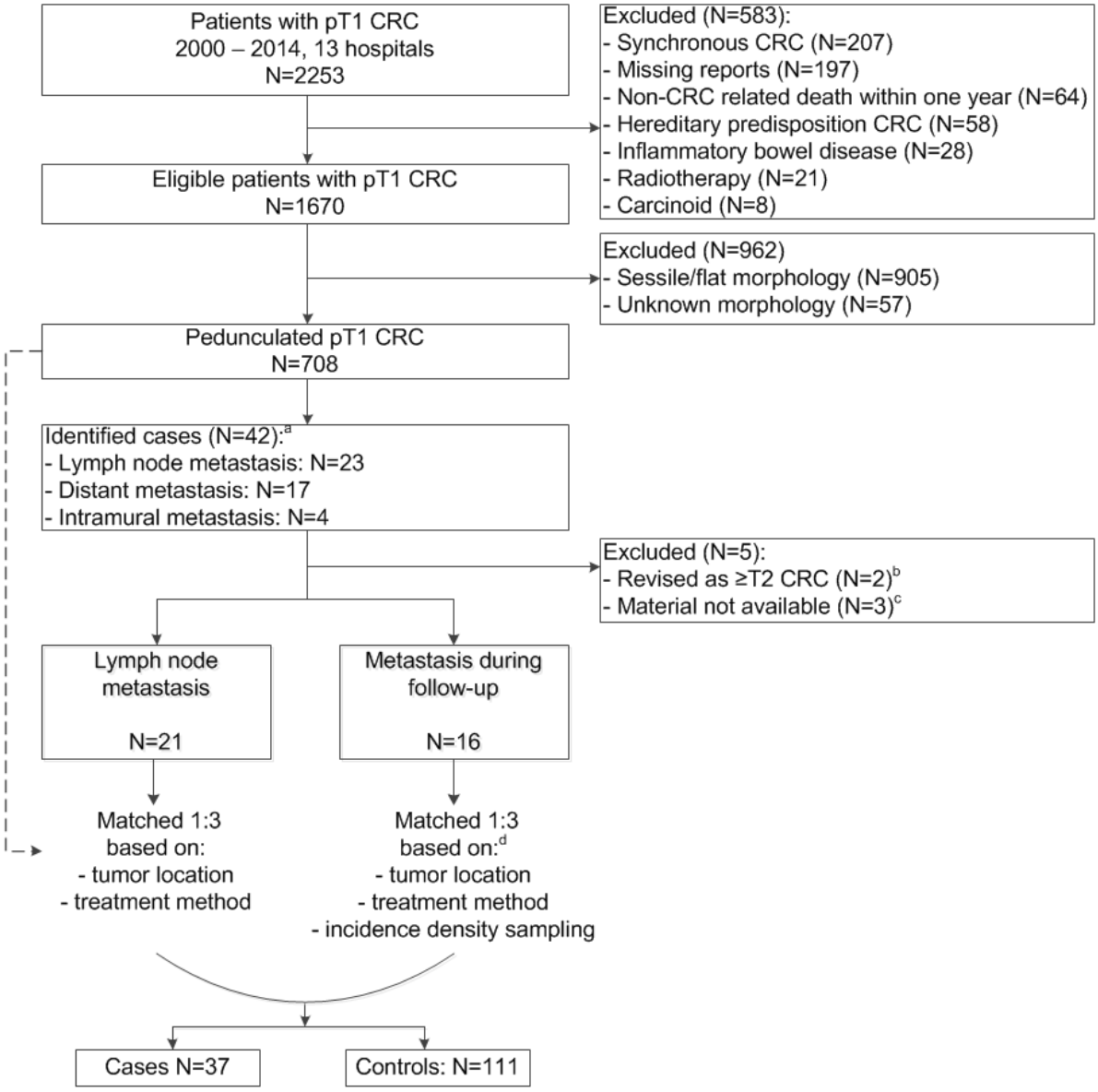
We evaluated the association between the histological factors and the outcome with uni- and multivariable conditional logistic regression analyses, acknowledging the matched design. In view of our limited number of metastasis in relation to the number of potential histological predictors, we conducted a penalized analysis for the development of the prediction model, named L1 penalized least absolute shrinkage and selection (LASSO) logistic regression, augmented with 5-fold cross-validation for internal validation. This is a logistic regression model that penalizes the absolute size of the coefficients of a regression model, based on the value of lambda (λ). With larger penalties, the estimates of weaker factors shrink towards zero, so that only the strongest predictors remain in the model. We identified the optimal λ penalty under five-fold cross-validation, maximizing the cross-validation area under the receiver operating characteristic curve (AUC). Matched cases and controls were kept together. We then refitted a penalized model with that λ penalty on the full data, resulting in a final set of regression coefficients (i.e. log(ORs)) for the histological factors). As a final step, we updated the intercept of that model so that the average predicted probabilities of the model corresponded with the average risk for metastasis, by taking into account the sampling fraction of controls for each matching factor thus extrapolating the findings developed in the nested case-control cohort to the entire cohort.

Inter observer agreement

To test whether application of the LASSO models was reproducible, we invited two pathologists (MGR and PD) from two high volume community hospitals to review a set of 30 randomly selected cases. The correlation between the estimated risk for metastasis of the study pathologist's assessment (i.e., the assessment by expert pathologist MML, which was discussed with expert pathologist GJAO in case of uncertainty) vs the two external pathologists when using the LASSO models was calculated with use of the intraclass correlation (ICC). We additionally calculated the agreement between the pathologists when using an estimated metastasis risk ≥4% as threshold for surgical referral with Cohen's kappa (between two pathologists) and Fleiss kappa (between three pathologists). Agreements measures were: 0.00 – 0.20: slight; 0.21 – 0.40: fair; 0.41 – 0.60: moderate; 0.61 – 0.80: substantial; 0.81-1.00: almost perfect, as proposed by Landis et al.[1]

Supplementary Results

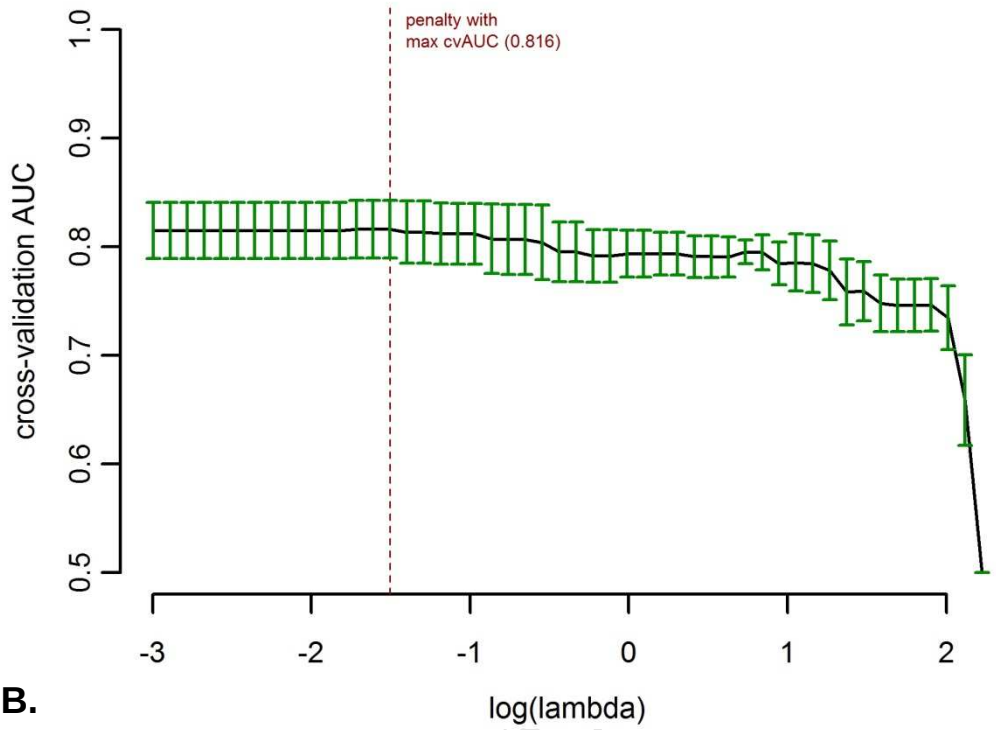
Figure S1. Flowchart of inclusion and matching



a. Two cases with LNM also had distant metastasis
b. One case with both LNM and distant metastasis and one case with LNM
c. Two cases with distant metastasis
d. For cases with metastasis after endoscopic resection, controls were additionally matched by R0-resection margins, as all cases were also R0-resections (i.e., to prevent selection bias)

Figure S2. Plot of the cross-validation area under the curve according to the penalty for (A) LASSO model 1 (i.e. model with tumor budding and PDC separately) and (B) LASSO model 2 (i.e. model with tumor budding and PDC combined). The green error bars indicate the standard error. The red line indicates the optimal penalty (i.e. the penalty with the maximum cross-validation AUC).

A.



B.

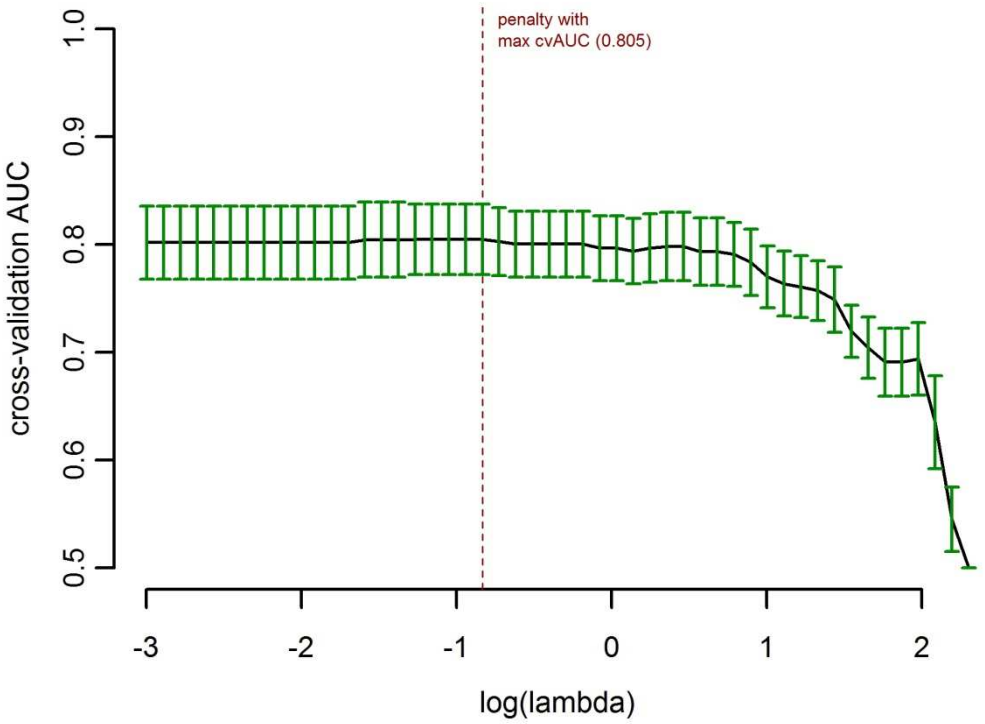
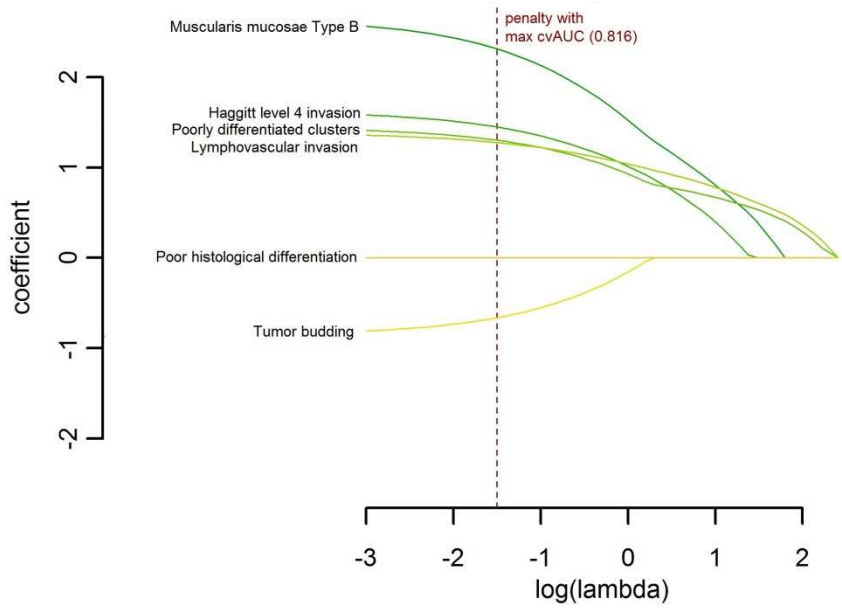


Figure S3. Coefficient profile plots showing how size of the coefficients of the histological factors shrink with increasing value of the lambda (λ) penalty, with the factors and their regression coefficients selected for the model based on the optimal λ (i.e. the λ with the maximum cross-validation AUC) for (A) LASSO model 1 and (B) LASSO model 2. Abbreviations: cvAUC: cross-validation area under the curve; PDC: poorly differentiated clusters. The exponential function of the regression coefficient on the y-axis ($e^{coefficient}$) is the multivariate odds ratio.

A.



B.

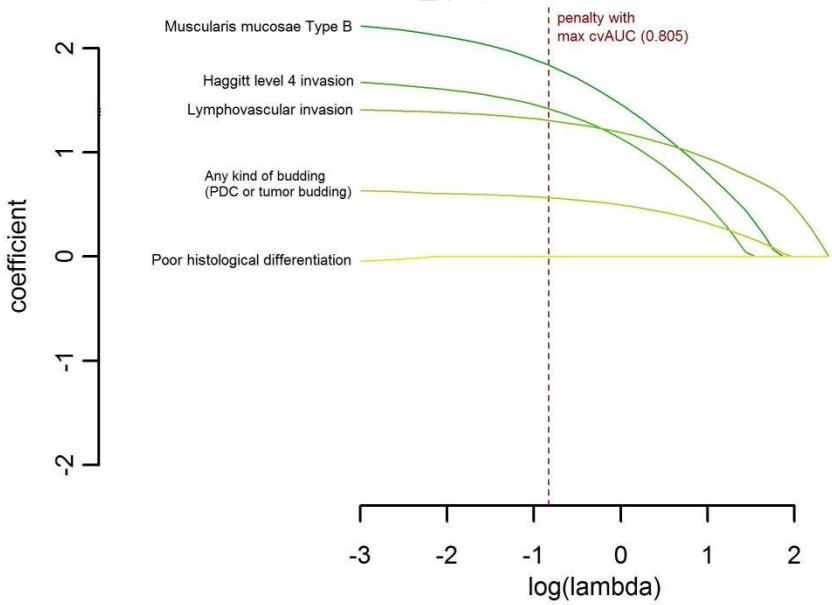


Figure S4. Calibration plots presenting the predicted probability against the observed risk for metastasis for (A) Cross validation plot LASSO model 1 (B) Apparent plot LASSO model 1 (C) Cross validation plot LASSO model 2 (D) Apparent plot LASSO model 2. Solid grey line shows perfect calibration (i.e. perfect agreement between predicted and observed metastasis risk); solid black line shows the smoothed calibration curve; dotted line is the top 5% of the predicted probabilities; histograms depict predicted probability distribution of patients with (top) or without (bottom) an adverse outcome.

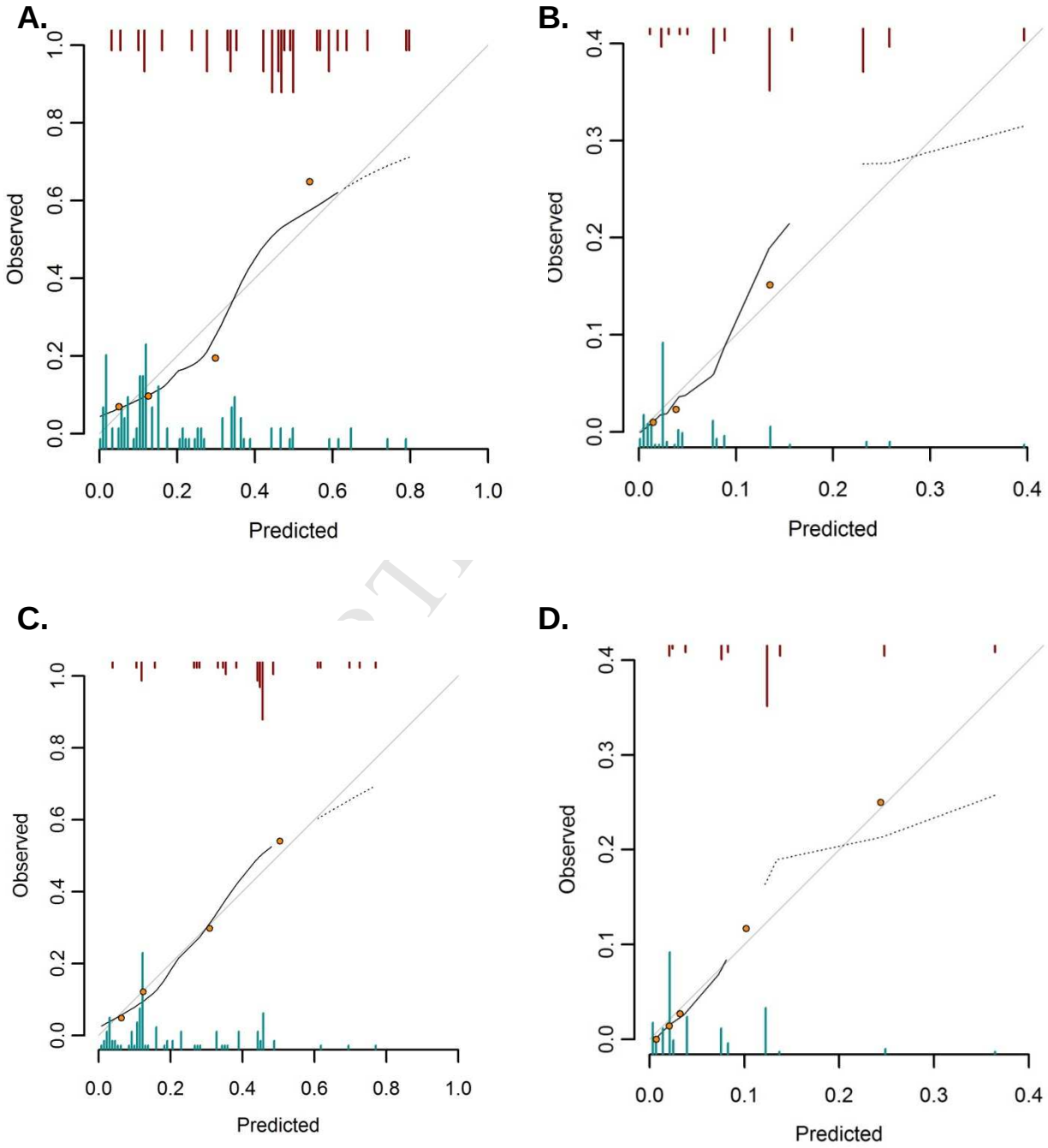


Table S1. Definitions, method of assessment and grading of the histological factors

Factors	Method of assessment and grading		Ref
Differentiation grade	Assessed for the tumor as a whole according to the WHO classification of tumors: Good: exhibits glandular structures in >95% of the tumor Moderate: exhibits glandular structures in 50-95% of the tumor Poor or undifferentiated: exhibits glandular structures in <50%	Poor vs. good/moderate	2
Invasion depth	Assessed according to the method as proposed by Haggitt et al: Level 1: invasion of the submucosa but limited to the head of the polyp Level 2: invasion extending into the neck of polyp Level 3: invasion into any part of the stalk Level 4: invasion beyond the stalk but above the muscularis propria In polypectomy specimens, Haggitt level 3 invasion with positive resection margins was considered Haggitt level 4 invasion.	Haggitt 4 vs. Haggitt 1-3 or unassessable[1]	3
Lymphovascular invasion	The presence of cancer cells within endothelial-lined channels. Distinction between lymphatic-invasion and blood vessel-invasion was not made, because of the used H&E staining. Cases in which venous invasion was clearly present were considered positive.	Presence vs. absence	4
Tumor budding	Cancer cell nest consisting of 1-4 cells that infiltrates the interstitium at the invasive margin. After selecting one field where budding was the most intensive, the number of buddings was counted in a field measuring 0.785 mm^2 observed through a 209 objective lens (WHK 109 ocular lens). The grade was defined as follows: Grade 1: 0-4 buds Grade 2: 5-9 buds Grade 3: 10 or more buds	Positive (G2-3) vs. negative (G1)	5,6
Poorly differentiated clusters (PDC)	Cancer clusters in the stroma composed of ≥5 cancer cells and lacking a gland-like structure. After selecting one field where PDC was the most intensive, the number of PDCs was counted in a field measuring 0.785 mm^2 observed through a 209 objective lens (WHK 109 ocular lens). The grade was defined as follows: Grade 1: 0-4 PDCs Grade 2: 5-9 PDCs Grade 3: 10 or more PDCs	Positive (G2-3) vs. negative (G1)	7
Status of the muscularis mucosa	The status of the muscularis mucosa was classified as: Type A: shattered but aligned muscularis mucosa Type B: incompletely or completely disrupted muscularis mucosa (this was considered as default)	Type B vs. Type A	8

Abbreviations: CRC: colorectal cancer; ITBCC: international tumor budding consensus conference; mm: millimeter; WHO: world health organization

1. In absence of adequate transverse sections this could not be assessed.

Table S2. Baseline characteristics of the source population (i.e., 708 pedunculated T1 CRCs)

Baseline characteristics of 708 pedunculated T1 CRCs	
Male gender, n (%)	403 (56.9)
Age in years, median (IQR)	69.0 (62.6 – 76.5)
ASA-score, n (%)	
- **ASA 1**	228 (32.8)
- **ASA 2**	339 (48.8)
- **ASA 3 – 4**	128 (18.4)
- **Missing**	13
Polyp size in mm, median (IQR)	20 (15 – 30)
Missing, n	58
Polyp location, n (%)	
- **Colon**	542 (76.7)
- **Rectum or rectumsigmoid**	165 (23.3)
- **Missing**	1
Overall treatment, n (%)	
- **Primary endoscopy**	372 (52.5)
- **Secondary surgery**	217 (30.6)
- **Primary surgery**	119 (16.8)
Number of retrieved LNs, median (IQR) [a]	5 (2 – 10)
Missing, n	7

Abbreviations: ASA: American Society of Anesthesiologists; IQR: interquartile range; LN: lymph node
a. Only for pedunculated T1 CRC removed with surgical resection

Table S3. Predicted probabilities and percentage of high-risk patients among the patients with lymph node metastasis vs metastasis during follow-up vs controls

	Lymph node metastasis (N=21)	Metastasis during follow-up (N=16)	Controls (N=111)
	Estimated predicted probability for metastasis, median (IQR)		
LASSO model 1	13.0 (7.3 – 22.5)	13.0 (7.3 – 22.5)	2.2 (1.1 – 7.3)
LASSO model 2	12.2 (7.7 – 13.5)	12.2 (7.3 – 12.2)	2.1 (2.1 – 7.3)
	Percentage of high-risk T1 CRCs, %		
Conventional model 1	90.5%	87.5%	55.0%
Conventional model 2	90.5%	93.8%	64.0%

Table S4. Inter observer agreement in a set of 30 randomly selected cases

		Agreement between the absolute estimated metastasis risk (%) between pathologists			Agreement between pathologists when using an estimated metastasis risk ≥4% as threshold for surgical referral			
		Intraclass correlation (95%CI)	p-value	Strength of correlation	Overall agreement	Kappa[1] (95%CI)	p-value	Strength of agreement
LASSO model 1	Pathologist 1 with study pathologist's assessment	0.50 (0.17 – 0.72)	0.002	Moderate	87%	0.73 (0.48 – 0.98)	<0.0001	Substantial
	Pathologist 2 with study pathologist's assessment	0.80 (0.63 – 0.90)	<0.001	Substantial	97%	0.93 (0.80 – 1.07)	<0.0001	Almost perfect
	Overall agreement[2]	0.57 (0.36 – 0.74)	<0.001	Moderate	89%	0.78 (0.59 – 0.95)	<0.001	Substantial
LASSO model 2	Pathologist 1 with study pathologist's assessment	0.67 (0.42 – 0.83)	<0.001	Substantial	73%	0.48 (0.18 – 0.78)	0.004	Moderate
	Pathologist 2 with study pathologist's assessment	0.64 (0.38 – 0.81)	<0.001	Substantial	87%	0.68 (0.39 – 0.98)	0.0001	Substantial
	Overall agreement[2]	0.60 (0.41 – 0.77)	<0.001	Moderate	78%	0.54 (0.29 – 0.77)	<0.001	Moderate

Note: The study pathologist's assessment was the assessment by expert pathologist MML, which was discussed with expert pathologist GJAO in case of uncertainty

1. Cohen's kappa was used to calculate the agreement between two pathologists, Fleiss kappa was used to calculate the agreement between three pathologists.
2. Agreement between the study pathologist's assessment, pathologist 1 and pathologist 2
3. Agreements measures were: 0.00 – 0.20: slight; 0.21 – 0.40: fair; 0.41 – 0.60: moderate; 0.61 – 0.80: substantial; 0.81-1.00: almost perfect, according to Landis et al.[1]

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