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Analysis of colorectal cancer morphology in relation to sex, age, location and family history

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Structured abstract

Background: Studies of colorectal cancer (CRC) have suggested different mechanisms of carcinogenesis in men and women, young and old patients, right- and left sided tumors and sporadic and familial tumors. This might be reflected in morphology.

Methods: CRCs from 1613 patients operated 2004-2006 in Sweden were histologically reviewed. Morphology was correlated to sex, age groups, location and family history.

Results: Tumors in the right colon were larger, of higher stage, more often poorly differentiated, more mucin producing, more had often a peritumoral lymphocytic infiltrate and a high level of tumor infiltrating lymphocytes, and more seldom had an infiltrating margin than tumors in the left colon and rectum ($p < 0.0001$ for most features). Young patients (<60 years) more seldom had multiple tumors but more often had perineural invasion, an infiltrative tumor margin, and high-stage tumors. Three features, TILs, medullary tumors and invasive tumor margin, were related to sex. Only vascular invasion was related to familiality.

Conclusion: Location is the factor that has the most influence on tumor morphology. The results support the idea that different carcinogenic mechanisms may be involved in the right and left colon. Age is the most important determinant for the presence of multiple tumors and is a crucial factor for the aggressiveness of the disease.

Keywords: Colorectal cancer histopathology location familial

Introduction

The etiology of CRC is today considered to be influenced by environmental risk factors on a background of constitutional and acquired genetic variations. Strong genetic risk factors are known to confer a high risk of CRC in syndromes such as familial adenomatous polyposis (FAP) and Lynch syndrome. However, these syndromes explain less than 5% of all CRC. The remaining part is contributed by genetic risk factors of much smaller magnitude, as have been shown in studies of CRC as a complex disease [1]. Studies have suggested different mechanisms for tumor development in men and women, young and old patients and right- and left-sided tumors, as well as familial and sporadic tumors.

Studies of CRC and sex have shown female patients to be older and to have more proximal and more poorly differentiated tumors than males [2, 3]. Two retrospective studies have reported more advanced stages of cancer in women compared to men [2, 4].

Studies of the clinicopathological profile of CRC in relation to age have shown contradictory results. Fairley et al [5] reported that patients younger than 50 years had less localized and more distant disease, as well as a higher rate of poorly differentiated cancers. On the other hand, Heys et al [6] concluded in a review that patients younger than 45 years had a stage of disease similar to that in older patients but that they had had four times as many mucinous tumors, a feature associated with increased risk of local recurrence. In a retrospective population-based study from Iceland, individuals younger than 50 years more often had non-polypoid cancers, an infiltrating tumor border, vessel invasion, and lymph node metastases. Both younger men and older women showed a relatively high frequency of right-sided tumors [7].

When comparing CRCs in different locations, right-sided lesions show more aggressive features than left-sided as reflected in morphology and stage. Poor differentiation, mucinous type, larger size, higher TNM stage, vessel invasion, and expanding tumor border occur more frequently in right-sided lesions, while annular and polypoid growth and infiltrating tumor border are more common in left-sided lesions [7]. Conversely, poorly differentiated adenocarcinomas and mucinous adenocarcinomas are more frequently seen in the right colon than in the left [8]. Right sided cancers also show a higher frequency of lymph-node-positive disease [9]. The frequency of right- and left-sided colon cancer differs by age, as noted above, with pronounced age-location differences in females [7]. Most CRCs are located in the sigmoid and rectum, but since the 1980s there has been a trend towards a change in distribution, with an increasing proportion of proximal tumors [10, 11].

The majority of CRCs are sporadic and occur in individuals over 65 years of age [12]. Based on familial clustering it is estimated that 15-25% of CRCs have a potentially identifiable genetic cause, and among these are FAP and Lynch syndrome [12]. A high percentage of tumors in Lynch syndrome show microsatellite instability (MSI), which is also observed in 12-17% of sporadic CRCs [13]. A

unique histopathological phenotype with medullary or mucinous features, poor differentiation, a high number of tumor-infiltrating lymphocytes, and peritumoral Crohn-like lymphocytic infiltrate has been identified for MSI-tumors, as well as a better prognosis and a different response to chemotherapy [14, 15, 13]. Most cases of familial CRC do not fulfil the Amsterdam criteria for Lynch syndrome [16]. The morphology of the non-Lynch, non-FAP familial type of colorectal cancer has, according to our knowledge, not yet been studied.

The aim of this study was to provide a detailed and systematic histopathological characterization of CRC in a large population-based cohort, the assumption being that the morphology could reflect different mechanisms of carcinogenesis. To that end, we compared the morphology of tumors in men and women, in different age groups, in different anatomical locations, and in sporadic and familial cases in order to isolate the effect of these four factors.

Subjects, materials & methods

Patients

The recruitment of patients was done by the surgeon at surgery or after the surgery by using data provided by Regional Oncologic Centers. Letters were sent to the patients for invitation to the study. All patients who submitted an interest to participate were contacted over the telephone for informed consent and inclusion in the study. A family history of cancer was taken from all study participants and all CRC diagnoses in the family were verified by medical records or death certificates.

In total, 4585 patients were diagnosed with CRC in 14 hospitals in Middle-Sweden during the years 2004-2006. General hospitals, district general hospitals and university clinics participated. Of the 2410 patients that were excluded, 639 died before they could be asked to participate or before blood testing could be performed and 1680 patients declined to participate or withdrew their consent during the study. Another 81 patients were not included for various reasons. In 1613 of the remaining 2175 patients, tumors were available for re-reviewing and constitutional DNA was available for genetic analysis (used in a previous study) [17]. Thus, 1613 was the number of tumors included in the analysis. When comparing the morphology of sporadic and familial cases of CRC, known cases of FAP (0.1%, n=2) or Lynch syndrome according to Amsterdam criteria (0,6%, n=9) or genetic testing (0.1%,n= 1) were excluded from the analysis. Familial CRC was defined as patients with one or more first- or second-degree relatives with CRC.

Pathology

All tumors were re-reviewed according to a standardized protocol, which included information about the patients' sex, age at operation, name of hospital and pathology department, date of diagnosis,

date of our re-evaluation, and name of re-reviewing pathologist. Tumor location and information about multiple synchronous tumors was gathered from the original pathology report as well as from the Regional Oncologic Center registry. Information on whether the patient had received pre-operative chemo- and/or radiotherapy (for rectal cancers) was obtained from the clinical history on the pathology referral sheet and from lists provided by the Regional Oncologic Centers.

All macromorphological parameters, including tumor size in three dimensions, were obtained from the original pathology report, as all CRCs in Sweden are examined in a standardized way according to a nationwide protocol. The number of positive and negative lymph nodes was noted, as well as the number of blocks taken (including large sections).

In all cases Hematoxylin and eosin (H&E) stained slides were obtained from the pathology department. When slides could not be found in the archives new sections were prepared from paraffin blocks. In some cases additional immunohistochemical stainings (mainly CK7, CK20, CDX2 and neuroendocrine markers) were available. In 0.4% of cases (n=6) only biopsy material was available since the patient never underwent surgery or the blocks from the surgical specimen were impossible to obtain. In an additional 2.0% of cases (n=33), the specimen consisted of a polypectomy or local resection. In many of these cases parameters such as peritumoral lymphocytic reaction, vascular or perineural invasion, co-existing polyps or tumor margin were impossible to assess, according to below.

The micromorphological parameters assessed were tumor grade, stage, medullary features, mucin production, mucin type, Crohn-like peritumoral lymphocytic reaction, tumor infiltrating lymphocytes (TILs), desmoplasia, tumor necrosis, vascular invasion, perineural growth, co-existing polyps, budding and type of tumor margin. Attempts to diagnose any synchronous pathology of colon or rectum (e. g., inflammatory bowel disease, ulcers, fistulas, diverticulae etc) were made, but because adequate information was lacking in most cases no further analysis regarding this aspect was undertaken.

Pure neuroendocrine tumors, recurrent carcinomas, metastases, gastrointestinal stromal tumors and other tumors of the colon or rectum were excluded. However, adenocarcinomas with some neuroendocrine differentiation were included in the study.

In cases with complete regression of tumor due to preoperative chemo-/radiotherapy (i. e., no tumor tissue for assessment) an attempt was made to obtain the preoperative biopsy sample for examination.

In the analysis of necrosis, desmoplastic reaction, and budding, rectal cancers were excluded because, in most cases, they had received preoperative radiotherapy, which might have altered the morphology and affected the results. The Dworak regression grade for preoperatively treated tumors was not used.

Initially a small proportion of the cases (1.2%, n=20) was re-evaluated by two experienced gastrointestinal pathologists (S.G., N.P.) in order to establish consensus regarding the histopathological parameters. The remainder were examined by only one pathologist (S.G.).

Tumor stage was recorded according to both the American Joint Committee on Cancer (AJCC) classification and the TNM system [18]. T1 tumors were not subclassified into Sm 1-3 and T3 tumors not into a-d because this was not common practice in Sweden when the study started. For the same reason the R system for residual tumor (R0, R1, R2, RX), L for lymphatic invasion (L0, L1, LX) and V for venous invasion (V0, V1, VX) was not used. However, vascular invasion (lymphatic and/or blood vessel) was noted (Figure 1A).

Tumor grade was determined according to the WHO classification [19]. Both the predominant and the second most common grade were noted in order to give a correct picture of the morphology. Tumors were classified into two grades of differentiation: poor (including poorly differentiated and undifferentiated) and other (moderately and well-differentiated). This was done due to the general difficulty in distinguishing moderately and well-differentiated tumors and because the distinction between poor and moderate differentiation is the most relevant clinically when it comes to prognosis and management [20].

Medullary carcinomas (sheets of undifferentiated epithelial cells with vesicular nuclei, prominent nucleoli, abundant pink cytoplasm, and prominent infiltration by intraepithelial lymphocytes) were coded separately. Mucin production was divided into three categories: 0%, 0-50% and >50% of tumor area. Tumors containing more than 50% extracellular mucin were classified as mucinous and per definition low-grade according to Swedish consensus criteria and the WHO criteria[19]. Mucin type was noted as extracellular and/or signet-ring type (tumor cells with a large mucin vacuole filling the cytoplasm and displacing the nucleus).

Lymphocytic reaction was recorded as Crohn-like if there were at least 4 nodular aggregates of lymphocytes deep to the advancing tumor margin in low power (x4) field [21]. TILs were categorized as ≤ 30/10 high-power field (HPF) and >30/10 HPF from H & E sections, always counting in the deeper half of tumor and avoiding adenomas, intramucosal carcinomas, and early invasive tumor components [22, 15, 23].

Desmoplasia was defined as a hypocellular intense fibrous reaction around infiltrating tumor tissue and was scored as present or absent. Tumor necrosis was defined as presence of cell detritus and inflammatory cells within glandular lumina, and was scored as present or absent.

Vascular invasion was recorded when unequivocal tumor aggregates where found in preformed spaces lined by endothelium indicating lymphatic or blood vessels. Perineural growth was defined as tumor cells infiltrating underneath the perineurium at the invasive margin of the tumor or deep to it.

Co-existing polyps were recorded (based on the original report) as none, not assessable/not stated, tubular, tubulovillous, villous or serrated adenoma, or hyperplastic polyp. Data were, however, missing in too many cases to allow further analysis. The number of polyps, as well as the degree of dysplasia, high-grade or low-grade, was, however, noted.

The presence or absence of budding, defined as detachment of single isolated cancer cells or a cluster of up to four cells, was recorded, as this has been shown to be an adverse prognostic factor [24]. Immunohistochemistry with cytokeratin and quantification of buds was, however, not performed.

Tumor margin was classified as dominantly circumscribed (advancing with even, rounded infiltration) or infiltrative (invading foci identified) [25].

Statistical analysis

The analyses were done using Statview 5.0 (SAS Institute, Cary, NC, USA) and PASW Statistics version 18 for Windows (SPSS, an IBM company, Chicago, IL, USA). Determination of the association between clinicopathological features and sex, age group, location, and family history was performed by univariate and multiple binary and multinomial logistic regression analysis for categorical outcomes and linear regression analysis for continuous outcomes. Results are presented as odds ratio (OR) from the logistic regression and as regression coefficient (b) from the linear regression. The significance level for statistical tests was set at 0.05.

In addition, factor analysis (extracting factors using principal components analysis) with variance maximizing (varimax) rotation was performed to form a concise description for all the variables included in the study. This analysis seeks a few underlying dimensions (factors) that account for patterns of variation among the variables in the study, in this case the clinicopathological parameters such as sex, age, location, and family history, as well as the morphological features. Variables with loadings of > 0.40 usually are applied as meaningful loadings on the factor. If a variable has a meaningful loading on more than one component, that variable should be ignored in the interpretation.

Results

The total number of tumors was 1613; 100 patients had multiple synchronous cancers. Of the patients 52.6% were men (n=849) and 47.4% women (n=764). The mean age for men was 69.3 years, with median 70.0 and range 31-92 years. The mean age for women was 68.5 years, with median 69.0 and range 27-95. Regarding age group, 18.8% (n=303) of the patients were younger than 60 years, 50.3% (n=812) were aged 60-75 years, and 30.9% (n=498) were older than 75 years. The percentage of tumors in each location were as follows: appendix 0.4% (n=7), caecum 14.4% (n=233), ascending colon 10.7% (n=172), hepatic flexure 4.0% (n=64), transverse colon 4.8% (n=78), splenic flexure 2.5%

(n=40), descending colon 3.7% (n=59), sigmoid colon 21.9% (n=354) and rectum 36,0% (n=581). Tumor location was unknown in 1.5% (n=25). Thus, 36.8% (n=594) of the tumors were located in the right colon (appendix through splenic flexure) and 25.6% (n=413) in the left colon (descending through sigmoid colon). Of the tumors 76.9% (n=1241) were sporadic and 20.6% (n=331) were familial. In 1.7% (n= 29) the family history was unknown.

Sex

The univariate comparison between men and women (Table 1) showed that female patients significantly more often had tumors with TILs >30/10 HPF (OR 1.607, p<0.001) and tumors of medullary type (OR 1.861, p=0.009). Women also showed a lower frequency of tumors with infiltrative margin (OR 0.770, p=0.009). In the multivariate analyses (Table 5a) significant differences remained only in TILs >30/10 HPF (OR 1.482, p=0.002).

Age

The univariate comparison between the three age groups (Table 2) showed that patients aged < 60 years had a significantly lower frequency of multiple tumors, mucin production (0-50%), and TILs >30/10 HPF compared to the reference group (>75 years). They, however, showed a higher frequency of AJCC stage III tumors, N1 and N2/N3 tumors, vascular and perineural invasion, and infiltrative tumor margin. The highest levels of significance (p<0.001 or p=0.001) were seen for the differences in multiple tumors (OR 0.145), infiltrative tumor margin (OR 2.379), TILs >30/10 HPF (OR 0.560) and perineural invasion (OR 1.892). In the multivariate analyses (Tables 5a and b), significant differences remained for multiple tumors (p=0.003), AJCC stage III (p<0.001), N2/N3 (p=0.004), perineural invasion (p=0.001), and infiltrative tumor margin (p<0.001). In addition, AJCC stage II and IV tumors and T4 tumors were significantly more common in the youngest age group.

In the univariate analyses (Table 2) patients aged 60-75 years showed a lower frequency of mucin production (0-50%), TILs >30/10 HPF, and medullary-type tumors compared to the reference group, but a higher frequency of infiltrative tumor margin. The highest significances were seen for mucin production (0-50%) (OR 0.678, p=0.003) and infiltrative tumor margin (OR 1.417, OR=0.003). None of these differences, however, remained significant in the multivariate analyses (Tables 5a and b).

Location

In the univariate comparison (Table 3) most of the histological features studied showed a significant difference between the left colon and rectum compared to the right colon (reference group).

The most significant differences (p<0.0001) between the left and right colon were seen in mean tumor diameter (1.138 cm smaller in left colon), T3 tumors (OR 0.356), proportion of poorly differentiated tumors (OR 0.223), mucin production (0-50%) (OR 0.327), mucinous type (>50%) (OR

0.336), TILs >30/10 HPF (OR 0.229), and medullary type (OR 0.112). All of the differences from the univariate analyses, except for the higher frequency of N2/N3 tumors in the left colon, remained significant in the multivariate analyses, where the highest level of significance ($p<0.0001$) was seen in tumor diameter, proportion of poorly differentiated tumors, Crohn-like reaction, TILs >30/10 HPF, medullary type, T3 tumors, and mucinous type (50%).

In the univariate comparison between rectum and right colon, most of the features listed in Table 3 showed highly significant ($p<0.0001$) differences. All of these significant differences remained in the multivariate comparison.

Family history

The only difference between the sporadic and the familial group seen in the univariate analyses (Table 4) was a higher frequency of vascular invasion in the familial cases (OR 1.412, $p=0.016$). This difference remained significant in the multivariate comparison (OR 1.438, $p=0.012$) (Table 5a).

Factor analysis

All the dependent and independent variables could be grouped into six different factors (components), as shown in Table 6.

Discussion

Sex

In the univariate analyses tumors with TILs >30/10 HPF, medullary features, and circumscribed margin were more common in women than in men. The same was true for poorly differentiated tumors, although the OR did not reach significance ($p=0.056$). In the multivariate analyses only TILs>30/10 HPF remained significant. A high number of TILs, medullary features, circumscribed tumor margin, and poor differentiation are all features associated with MSI-positive tumors. The results support previous studies that have shown cancers with MSI-phenotype to be more common in women than in men [26, 15]. Differences in hormonal status between men and women could be a possible explanation. There is clinical evidence that estrogen protects against the development of CRC, but its exact role in carcinogenesis is not well understood. At least five isoforms of estrogen receptor beta (ERβ) are known and three of these are located in normal colon. Wong et al [27] showed that high ERβ1 expression was significantly associated with improved prognostic features such as lower grade, lower T stage, mucinous phenotype, and MSI-high (H) cancers. The authors suggested that ERβ1 expression is suppressed by estrogen and that decline in estrogen with menopause leads to an increase in ERβ1 expression and the subsequent development of MSI-H cancers. Older women have more MSI-cancers compared to younger women, in contrast to men,

where the frequency of MSI-cancers decreases with age. Thus, estrogen might preferentially protect against MSI-H cancers [28, 29].

Age

When comparing CRCs of different age groups we chose cut-off points at 60 and 75 years in order to get three groups of comparable size.

Multiple synchronous tumors were clearly much less common (OR 0.145, p<0.0001) in the youngest group (<60 years) compared to the reference group (>75 years). The results suggest that age is a crucial factor for this feature. This may be due to young patients having a better anti-tumorigenic immune response, which prevents them from developing multiple cancers. Also, they may not yet have accumulated as many mutations as older patients in their colonic mucosa. Alternatively, the tumors of the young patients may be more fast-growing so that they will cause symptoms and be diagnosed before additional tumors have developed.

Interestingly, patients aged less than 60 years showed more locally advanced tumors with more vascular and perineural invasion and infiltrative tumor margin. They also showed higher ORs for AJCC stage II-III, T4 and N2/N3 tumors than the reference group. The results indicate that younger patients have a more aggressive disease. Looking at the univariate analysis, we noted that their tumors displayed less mucin production, less Crohn-like lymphoid reaction, more seldom showed medullary features, and, as well, had a lower frequency of TILs. These features constitute the opposite of the MSI-phenotype seen in older patients [14, 15, 13]. None of these features, however, remained significant in the multivariate analysis. All in all, the patient's age seems to be correlated to tumor aggressiveness, rather than to morphology. The tumors of the young patients were more systemically advanced by the time of operation, thus indicating faster growth.

Location

Multiple tumors were much less common in rectum than in the right colon (OR 0.261, p<0.0001). This is probably for anatomical reasons: the short length of rectum and the narrow lumen result in symptoms and early discovery before any possible additional tumor could develop. The same anatomical factors probably explain why the tumors in left colon and rectum were smaller than the tumors in right colon. In addition to the larger lumen of the right colon, its bowel contents are also looser, which makes tumors in this local escape early detection by not causing symptoms such as obstipation.

The tumors in the rectum, and to a certain extent in the left colon, tended to be of lower AJCC- and T-stage than those in the right colon. This characteristic might also be explained by the fact that these tumors are detected earlier. Interestingly, the tumors in the left colon tended to be of higher N-stage (OR 1.474 for N2/N3, p=0.024) than tumors in both the right colon and the rectum. There is

no obvious explanation for this finding, as there were no significant differences in the number of lymph nodes or number of lymph node metastases between the tumors in the three locations (statistics not shown). The difference in the frequency of vascular invasion was also non-significant.
Mucinous tumors were common in the right colon compared to findings in both the left colon and the rectum. Because mucin production is a part of the morphological spectrum of MSI-tumors, which are more common on the right side, this is not surprising. The same was true for tumors with high number of TILs and medullary features, which are also characteristic of MSI-tumors (Figure 1B).
The frequency of signet-ring cell morphology parallels that of mucin production as a whole, with tumors showing this feature being significantly more common in the right colon. Signet-ring cell carcinomas are rare, comprising 0.7-2.6% of all colorectal carcinomas [30]. These tumors have been shown to present themselves at a higher T-stage and with a higher number of positive lymph nodes than both conventional adenocarcinomas and mucinous adenocarcinomas without signet ring cells. They also show a poorer prognosis with a higher distant recurrence rate and decreased survival [30]. Genetic and immunohistochemical studies have shown that signet-ring cell carcinomas arise through a separate genetic pathway showing disruption of the E-cadherin/beta-catenin complex involved in cell-to-cell adhesion. They also display a goblet-cell phenotype with positivity for intestinal trefoil factor (ITF) and mucin-2, as well as a pattern of alterations in growth kinase-related oncogenes (K-ras, BRAF), tumor suppressor genes (p53, p16), gene methylation, and Cox-2-expression different from that of conventional colorectal adenocarcinomas [30, 31].
The rectal tumors showed more perineural invasion, and an infiltrative tumor margin was more frequent in both rectal and left sided cancers, compared with findings in right-sided cancers (Figure 1C). Again, anatomical factors may lie behind this difference, as the rectum, which mainly consists of an outer longitudinal muscle without haustra and with its own mesentery, is innervated by a surrounding plexus of sympathic and parasympathic fibers. This, in turn, results in a high concentration of nerves close to the wall of the rectum. The limited space for luminal expansion in the rectum and left colon - because of the smaller diameter - may also force tumors in these locations to grow outward, hence causing a more infiltrative pattern.
For most morphological parameters the differences seem to be greatest between right-sided colon cancers and rectal cancers. In addition, most features show a gradient from right colon to left colon to rectum, as indicated by the ORs.
Most of the morphological features studied, such as multiple tumors, tumor diameter, grade of differentiation, mucin production, mucin type, TILs, medullary type, and tumor margin, seemed to be related to tumor location rather than to age group according to the multivariate analysis. AJCC-, T- and N-stage also showed significant correlations to location.
Different genetic abnormalities have been found in CRCs from different sites. Proximal CRCs are more often related to the MSI pathway, while distal cancers usually are associated with

chromosomal instability. Rectal cancer displays its own features with a significantly higher number of mutations than colon cancer, as well as the overexpression of nuclear beta-catenin, TP53, and Cox2 [32]. These differences, which might be attributed to different embryological development and physiological mechanisms, may be reflected in different morphological features.

Sporadic vs. familial

There were remarkably few differences in the morphology between sporadic and familial CRCs. Familial CRC, however, showed a higher frequency of vascular invasion.

In several studies vascular invasion was demonstrated to be prognostically significant by multivariate analysis, but no distinction was made between venous and lymphatic vessels [33]. Venous invasion has been found to be an independent prognostic factor in some univariate [34, 35] and multivariate analyses [36, 37, 38]. Similar results have been shown for lymphatic invasion [39, 40]. In some studies, location of the vascular involvement in extramural veins has been found to be of prognostic value [35]. The diagnosis of intravascular tumor growth is often difficult to make because fixational artefacts with retraction of tumor strands in fibrotic tissue can mimic vascular invasion. Reproducibility is low and interobserver variability is high. The frequency of vascular invasion is reported to vary from 10 to 89.5 % [41], with false negative rates between 10.5 and 29.6% if only H&E staining is used [42]. This frequency has also been shown to vary with number of blocks taken and if tangential sectioning is performed. In one study the frequency increased from 59% with two blocks examined to 96% with five blocks examined [43]. The assessment of vascular invasion may also be improved with immunohistochemical staining for endothelium or elastic tissue stains.

Considering the complexity of the issue as discussed above, and the retrospective nature of the study, as well as cost-, time- and labor-related aspects because of additional immunohistochemistry, we chose not to differentiate between venous and lymphatic invasion. Thus, our rate of vascular invasion, which is in the lower range of previously reported frequencies, might represent an underdiagnosis of this feature.

The finding of a higher frequency of vascular invasion in familial tumors is, however, interesting and raises the question whether tumors in the familial group have different biological properties, such as specific tumor antigens or adhesion molecules that influence the ability to invade vessel walls. Protein markers such as apoptosis protease activating factor-1 (APAF-1), mammalian sterile20-like kinase (MST1), urokinase plasminogen activator receptor (uPAR) and Raf-1 kinase inhibitor protein (RKIP) have been associated with vascular invasion [44]. The urokinase plasminogen activator (uPA)/uPAR system is associated with degradation and regeneration of the basement membrane and extracellular matrix and uPAR itself is involved in cell movement and adhesion. RKIP has recently been characterized as a metastasis suppressor gene and loss of it has been associated with an increased frequency of distant metastases in CRC [44]. The expression of vascular endothelial growth

factor C (VEGF-C) in CRC has been linked to lymphatic vessel invasion, probably by VEGF-C promoting dilatation of lymphatic vessels and thereby facilitating the invasion of cancer cells [45]. All in all, these findings may speak for a difference between sporadic and familial CRCs in the expression of proteins facilitating vascular invasion, but immunohistochemical comparison of the two groups is required.

One could expect that differences in vascular invasion between the two groups would be reflected in T and N stages. However, no such difference was evident. A higher frequency of vascular invasion should feasibly lead to more distant metastases, but M stage was not possible to assess in our material. A follow up of our patients after 5-10 years could perhaps reveal a correlation between vascular invasion and survival time, as has been shown in previous reports [38, 44].

In our study we did not perform MSI analysis on our patients and it could therefore be argued that we have not omitted possible cases of Lynch syndrome from our hereditary group. We, however, excluded 9 cases that fulfilled the Amsterdam criteria [16]. Mismatch-repair mutations have been found in up 90% of patients who meet these criteria [46]. We also excluded one case of Lynch syndrome that was diagnosed in a high-risk screening program. Judging from a previous study of a CRC population in Sweden [47] approximately 1.2% of the patients in our cohortshould have Lynch syndrome. Such a small proportion is not likely to have influenced the results much.

Factor analysis

We found that AJCC- and N-stage were in the same component (factor 1) together with vascular invasion, perineural invasion, budding, and tumor margin. This is not surprising because these are all features related to the extent of tumor spread and tumor aggressiveness. T-stage had a meaningful loading on two components and was therefore ignored in the interpretation. Mucin and mucin production were grouped into the same component (factor 2). Crohn-like peritumoral lymphocytic infiltrate is a part of the MSI spectrum, but in our analysis it was not grouped in the same component (factor 3) as the other MSI variables grade of differentiation (negative correlation to well/moderate), TILs, and medullary type. This finding supports the fact that peritumoral lymphoctic infiltration is a different entity from TILs and that it may have a different biological implication.

Desmoplastic reaction and Crohn-like peritumoral lymphocytic infiltration were grouped together (factor 4). Desmoplastic reaction is generally thought of as a feature favoring the host by encapsulating the tumor, but there are conflicting reports [48]. The fifth component (factor 5) consisted of age group and multiple tumors. This is in keeping with the multivariate analysis which showed that patients younger than 60 years had significantly fewer multiple tumors than the reference group.

In addition, our factor analysis showed a sixth component (factor 6), consisting of sex and age, which could be related to the fact that there was an age-related difference between men and women.

Location had a meaningful loading on both factors 4 and 5; however, this was not so high, at -0.41 and -0.44, respectively.

Because it seems reasonable that tumor morphology changes in relation to depth of invasion within the bowel wall, i.e., T-stage, we also performed such an analysis (Supplementary tables 1 and 2). A univariate analysis of T2, T3 and T4 tumors compared to T1 showed that high stage tumors were significantly larger and more often N1, poorly differentiated, and mucinous. They also showed Crohn-like lymphocytic reaction, desmoplasia, necrosis, vascular invasion, budding, and infiltrative tumor margin more often than T1 tumors ($p<0.0001$ for most features). In a multivariate comparison with our clinical parameters sex, age, localization and family history, T-stage was a significant factor for all morphological features possible to analyze except medullary type ($p<0.0001$ for T3 and T4 compared to T1 for the majority of features).

Conclusion

In this large and systematic study we have evaluated the clinicopathological characteristics of CRC in relation to sex, age, location, and family history. This is the largest study so far conducted on the morphology of familial non-Lynch, non-FAP patients. Our results suggest that tumor location is the factor having most influence on morphology, with left-sided colon cancers and rectal cancers clearly differing from right-sided colon cancers. The results are in line with tumors in different locations having different genetic and embryologic backgrounds as well as developing in different physiological settings. Age is the most important determinant for the presence of multiple tumors and an important factor for the aggressiveness of the disease, as indicated by AJCC-, T- and N-stage. The results could speak for a different mechanism of tumor development in young and old patients. Few morphological features are related to sex and almost none to family history.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics

This study was approved by the local Ethics Committee at Karolinska Institutet (no. KI Dnr 02-489).

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Table 1. Univariate comparison of clinicopathological features of male and female patients with colorectal cancer.

Feature	Men	Women	OR/b*	p-value for OR/b
> 1 tumors (%)	5.9	6.6	1.119	0.586
Tumor diameter (mean. cm)	4.8	4.7	-0.040	0.793
AJCC stage (%)				
I	17.7	19.3	rc	
II	39.6	39.6	0.919	0.550
III	39.1	37.2	0.874	0.344
IV	3.6	4.0	1.021	0.942
T (%)				
1	6.6	7.4	rc	
2	17.1	16.2	0.838	0.435
3	65.0	61.4	0.836	0.370
4	11.4	14.9	1.158	0.534
N (%)				
0	57.6	60.0	rc	
1	24.0	21.2	0.845	0.183
2 or 3	18.4	18.9	0.986	0.915
Proportion of poorly differentiated (%)**	10.2	13.2	1.349	0.056
Mucin production (%)				
0 %	59.5	59.5	rc	
0-50 %	25.6	25.6	0.998	0.989
>50% (mucinous type)	14.9	14.9	0.998	0.988
Mucin type, if mucinous (%)				
Only extracellular	83.4	85.4	rc	
Signet-ring component	16.6	14.6	0.859	0.488
Crohn-like lymphocytic reaction (%)	64.2	68.1	1.193	0.098
TILs				
≤30/10 HPF	81.3	73.0	rc	
>30/10 HPF	18.7	27.0	1.607	**<0.001**
Desmoplasia (%)	86.9	85.2	0.870	0.446
Necrosis (%)	72.1	70.4	0.920	0.552
Vascular invasion (%)	22.1	23.0	1.056	0.648
Perineural invasion (%)	15.6	16.8	1.092	0.521
Medullary type (%)	3.6	6.4	1.861	**0.009**
Budding (%)	42.5	45.8	1.143	0.298
Tumor margin (%)				
Circumscribed	48.4	55.0	rc	
Infiltrative	51.6	45.0	0.770	**0.009**

*Females vs males. Odds ratio for all features except tumor diameter where difference b (cm) is stated.
**In major tumor component.
rc=reference category.

Table 2. Univariate comparison of clinicopathological features of colorectal cancer in age groups <60, 60-75 and >75 years.

Feature	<60 yrs			60-75 yrs			>75 yrs
	Frequency*	OR/b**	p-value**	Frequency*	OR/b***	p-value***	Frequency*
> 1 tumors (%)	1.3	0.145	**<0.0001**	6.7	0.773	0.229	8.5
Tumor diameter (mean, cm)	4.8	-0.086	+0.624	4.7	-0.170	0.213	4.9
AJCC stage (%)							
I	13.9	rc		19.4	rc		19.6
II	35.0	1.231	0.351	40.9	1.037	0.819	40.1
III	46.3	1.768	**0.009**	36.1	0.994	0.969	36.8
IV	4.8	1.948	0.101	3.6	1.068	0.844	3.4
T (%)							
1	6.1	rc		7.4	rc		6.9
2	13.3	0.837	0.611	17.2	0.904	0.691	17.8
3	66.2	1.171	0.606	62.2	0.917	0.702	63.4
4	14.3	1.345	0.404	13.2	1.035	0.898	11.9
N (%)							
0	49.5	rc		60.9	rc		60.7
1	27.9	1.441	**0.041**	20.1	0.845	0.243	23.7
2 or 3	22.6	1.788	**0.003**	19.0	1.217	0.221	15.5
Proportion of poorly differentiated (%)†	13.6	1.027	0.899	9.8	0.710	0.054	13.3
Mucin production (%)							
0 %	62.5	rc		61.7	rc		54.1
0-50 %	22.3	0.628	**0.008**	23.7	0.678	**0.003**	30.7
>50% (mucinous type)	15.3	0.874	0.523	14.5	0.841	0.298	15.2
Mucin type, if mucinous (%)							
Only extracellular	81.4	rc		87.1	rc		82.1
Signet-ring component	18.6	1.044	0.884	12.9	0.678	0.113	17.9
Crohn-like lymphocytic reaction (%)	59.1	0.604	**0.001**	65.9	0.807	0.083	70.5
TILs							
≤30/10 HPF	82.3	rc		78.6	rc		72.2
>30/10 HPF	17.7	0.560	**0.001**	21.4	0.707	**0.009**	27.8
Desmoplasia (%)	90.1	1.813	0.052	86.7	1.309	0.165	83.3
Necrosis (%)	69.7	0.882	0.552	70.8	0.928	0.627	72.3
Vascular invasion (%)	28.1	1.423	**0.037**	21.1	0.971	0.834	21.5
Perineural invasion (%)	21.5	1.892	**0.001**	16.4	1.359	0.065	12.6
Medullary type (%)	3.7	0.487	**0.041**	3.9	0.511	**0.008**	7.3
Budding (%)	41.9	0.879	0.514	44.2	0.965	0.797	45.1
Tumor margin (%)							
Circumscribed	38.5	rc		51.2	rc		59.8
Infiltrative	61.5	2.379	**<0.0001**	48.8	1.417	**0.003**	40.2

*Except for tumor diameter where size (cm) is stated.

**OR and p-value for all features (except tumor diameter) present in age group <60 years vs. reference group (>75 years). For tumor diameter difference b (cm) is stated.

***OR and p-value for all features (except tumor diameter) present in age group 60-75 years vs. reference group (>75 years). For tumor diameter difference b (cm) is stated.

†In major tumor component.

rc=reference category.

Table 3. Univariate comparison of clinicopathological features of right sided colon cancers, left sided colon cancers and rectal cancers.

Feature	Right colon	Left colon			Rectum		
	Frequency*	Frequency*	OR/b**	p-value**	Frequency*	OR/b***	p-value***
> 1tumors (%)	8.1	8.7	1.089	0.711	2.2	0.261	**<0.0001**
Tumor diameter (mean, cm)	5.6	4.5	-1.138	**<0.0001**	4.0	-1.586	**<0.0001**
AJCC stage(%)							
I	12.1	15.8	rc		27.2	rc	
II	47.0	38.1	0.619	**0.016**	33.5	0.316	**<0.0001**
III	36.2	41.5	0.877	0.511	37.4	0.459	**<0.0001**
IV	4.8	4.6	0.741	0.383	1.9	0.181	**<0.0001**
T (%)							
1	3.4	8.6	rc		9.4	rc	
2	11.1	12.0	0.424	**0.011**	26.3	0.852	0.595
3	69.1	62.3	0.356	**<0.0001**	58.8	0.307	**<0.0001**
4	16.4	17.1	0.412	**0.006**	5.5	0.121	**<0.0001**
N (%)							
0	61.0	53.8	rc		60.4	rc	
1	22.2	24.5	1.250	0.163	22.0	0.999	0.997
2 or 3	16.7	21.7	1.474	**0.024**	17.7	1.086	0.687
Proportion of poorly differentiated (%)†	19.4	5.1	0.223	**<0.0001**	8.2	0.371	**<0.0001**
Mucin production (%)							
0 %	42.7	69.3	rc		69.5	rc	
0-50 %	35.8	19.0	0.327	**<0.0001**	20.2	0.348	**<0.0001**
>50% (mucinous type)	21.5	11.7	0.336	**<0.0001**	10.3	0.295	**<0.0001**
Mucin type, if mucinous (%)							
Only extracellular	78.6	88.8	rc		91.3	rc	
Signet-ring component	21.4	11.2	0.464	**0.014**	8.7	0.349	**<0.0001**
Crohn-like lymphocytic reaction (%)	83.6	73.6	0.548	**<0.0001**	42.1	0.142	**<0.0001**
TILs							
≤30/10 HPF	61.9	87.7	rc		86.3	rc	
>30/10 HPF	38.1	12.3	0.229	**<0.0001**	13.7	0.257	**<0.0001**
Desmoplasia (%)	86.6	85.2	0.889	0.522	ni	ni	ni
Necrosis (%)	69.6	73.5	1.210	0.184	ni	ni	ni
Vascular invasion (%)	23.2	20.5	0.853	0.311	23.3	1.005	0.973
Perineural invasion (%)	12.9	17.1	1.359	0.065	19.0	1.587	**0.005**
Medullary type (%)	11.8	1.5	0.112	**<0.0001**	0.2	0.013	**<0.0001**
Budding (%)	46.2	41.3	0.820	0.128	ni	ni	ni
Tumor margin (%)							
circumscribed	63.3	53.2	rc		37.9	rc	
Infiltrative	36.7	46.8	1.517	**0.001**	62.1	2.828	**<0.0001**

Right colon=caecum through splenic flexure. Left colon=descendens and sigmoid.

*Except for tumor diameter where size (cm) is stated.

**OR and p-value for all features (except tumor diameter) present in left colon vs. reference group (right colon). For tumor diameter difference b (cm) is stated.

*** OR and p-value for feature present (except tumor diameter) present in rectum vs. reference group (right colon). For tumor diameter difference b (cm) is stated.

†In major tumor component.

ni=Not included in comparison because of preoperative radiotherapy.

rc=Reference category.

Table 4. Univariate comparison of clinicopathological features in sporadic and familial colorectal cancer.

Feature	Sporadic	Familial	OR/b*	p-value for OR/b
> 1 tumors (%)	6.6	4.2	0.626	0.113
Tumor diameter (mean. cm)	4.7	4.9	+0.132	0.368
AJCC stage (%)				
I	19.2	16.3	rc	
II	39.3	39.1	1.175	0.380
III	37.3	41.9	1.325	0.122
IV	4.1	2.8	0.801	0.572
T (%)				
1	7.5	5.3	rc	
2	16.9	16.5	1.379	0.293
3	62.6	65.2	1.478	0.156
4	12.9	13.0	1.430	0.258
N (%)				
0	58.9	57.5	rc	
1	22.5	24.4	1.110	0.494
2 or 3	18.6	18.1	1.001	0.994
Proportion of poorly differentiated (%)**	11.8	10.1	0.833	0.369
Mucin production (%)				
0 %	60.1	58.8	rc	
0-50 %	24.7	29.0	1.200	0.202
>50% (mucinous type)	15.2	12.2	0.821	0.305
Mucin type, if mucinous (%)				
Extracellular	84.5	84.2	rc	
Signet-ring component	15.5	15.8	1.020	0.941
Crohn-like lymphocytic reaction(%)	65.6	68.4	1.135	0.346
TILs				
≤30/10 HPF	77.5	76.8	rc	
>30/10 HPF	22.5	23.2	1.036	0.810
Desmoplasia (%)	85.3	88.7	1.361	0.205
Necrosis (%)	71.0	71.1	1.003	0.987
Vascular invasion (%)	21.1	27.4	1.412	**0.016**
Perineural invasion (%)	16.1	17.3	1.093	0.591
Medullary type (%)	5.2	3.7	0.705	0.277
Budding (%)	44.3	43.3	0.964	0.817
Tumor margin (%)				
Circumscribed	52.4	48.0	rc	
Infiltrative	47.6	52.0	1.191	0.162

*Familial vs. sporadic. Odds ratio for all features except tumor diameter where difference b (cm) is stated.
**In major tumor component.
rc= Reference category.

Table 5a. Mutivariate analysis of clinicopathological features in relation to sex, age group, location and family history.

	>1 tumors (Yes vs. No)	Tumor diam. (cm)	Differen-tiation (Poorly vs. Other)*	Mucin type (Signet-ring component vs. Extra-cellular)	Crohn-like reaction (Yes vs. No)	TILs (>30/HPF vs. ≤ 30/10HPF)	Vascular invasion (Yes vs. No)	Perineural invasion (Yes vs. No)	Medullary type (Yes vs. No)	Tumor margin (Infiltrative vs. Circum-scribed)
Sex										
Male	–	–	–	–	–	rc	–	–	–	–
Female	–	–	–	–	–	**1,482** ** **p=0,002**	–	–	–	–
Age group										
>75 yrs	rc	–	–	–	–	–	–	rc	–	rc
60-75 yrs	0,912 p=0,683	–	–	–	–	–	–	1,375 p=0,059	–	1,238 p=0,078
<60 yrs	**0,204 p=0,003**	–	–	–	–	–	–	**1,953 p=0,001**	–	**2,076 p<0,0001**
Localization										
Right colon	rc	5,612	rc	rc	rc	rc	–	–	rc	rc
Left colon	1,139 p=0,586	**-1,138 p<0,0001**	**0,231 p<0,0001**	**0,484 p=0,021**	**0,554 p<0,0001**	**0,230 p<0,0001**	–	–	**0,115 p<0,0001**	**1,461 p<0,005**
Rectum	**0,308 p<0,0001**	**-1,576 p<0,0001**	**0,361 p<0,0001**	**0,346 p=0,001**	**0,140 p<0,0001**	**0,242 p<0,0001**	–	–	***	**2,603 p<0,0001**
Family history										
Sporadic	–	–	–	–	–	–	rc	–	–	–
Familial	–	–	–	–	–	–	**1,438 p=0,012**	–	–	–
Nagelkerke R Square†	0,068	0,051	0,070	0,044	0,198	0,138	0,006	0,012	0,219	0,081

Desmoplasia, necrosis and budding omitted from table because these features were not included in any multivariate model.

*In major tumor component.

** Odds ratio for all features except tumor diameter where mean diameter and difference b (cm) is stated.

***Odds ratio not possible to calculate because only one rectal cancer with medullary features present in the material.

†Or Adjusted R Square.

rc=Reference category.

- =Not included in model.

Table 5b. Multivariate analysis of clinicopathological features in relation to sex, age group, location and family history (continued).

	AJCC-stage (II,III,IV vs. I)		T-stage (T2,T3,T4 vs. T1)		N-stage (N1,N2/N3 vs. N0)		Mucinprod (0-50%, >50% vs. 0%)	
Age group								
>75 yrs		rc		rc		rc		
		1.263		0.921		0.863		
	II	p=0.156	T2	p=0.751	N1	p=0.313		
		1.225		1.128		1.339		
60-75 yrs	III	p=0.223	T3	p=0.607	N2/N3	p=0.078		
		1.360		1.591			–	
	IV	p=0.366	T4	p=0.100				
		1.721*		0.943		1.399		
	II	**p=0.021**	T2	p=0.872	N1	p=0.064		
		2.270		1.715		**1.799**		
<60 yrs	III	**p<0.0001**	T3	p=0.096	N2/N3	**p=0.004**		
		2.904		**2.353**				
	IV	**p=0.012**	T4	**p=0.026**				
Localization								
Right colon		rc		rc				rc
		0.617		**0.422**				**0.328**
	II	**p=0.017**	T2	**p=0.012**			0-50%	**p<0.0001**
		0.864		**0.356**				**0.344**
Left colon	III	p=0.473	T3	**p<0.0001**			>50%	**p<0.0001**
		0.708		**0.407**				
	IV	p=0.320	T4	**p=0.006**		–		
		0.287		0.854				**0.349**
	II	**p<0.0001**	T2	p=0.607			0-50%	**p<0.0001**
		0.422		**0.282**				**0.283**
Rectum	III	**p<0.0001**	T3	**p<0.0001**			>50%	**p<0.0001**
		0.157		**0.109**				
	IV	**p<0.0001**	T4	**p<0.0001**				
Nagelkerke R Square**		0.055		0.087		0.011		0.080

Sex and family history omitted from the table because these factors were not included in the multivariate model for AJCC-, T- and N-stage or mucin production.

AJCC-stage I, T1, N0 and 0% mucin are reference groups in analysis of AJCC-, T-, N-stage and mucin production.

* Odds ratio.

**Or Adjusted R Square.

rc=Reference category.

- =Not included in model.

Table 6. Factor analysis including both independent and dependent variables studied in relation to colorectal cancer morphology.

	Factor (component)					
	1	2	3	4	5	6
Sex	.089	.000	.100	-.021	.149	**.664**
Age group	-.088	-.043	.042	.010	**.601**	-.318
Localization	.031	-.213	-.279	-.406	-.438	-.184
Family history	-.005	.022	-.043	.052	-.262	**.529**
>1 tumor	-.020	.084	-.046	-.133	**.627**	.070
Tumor diameter	-.075	.192	.356	**.612**	-.144	-.066
AJCC-stage	**.711**	.095	.021	.384	-.061	-.201
T	.441	.116	.011	.675	-.094	-.041
N	**.707**	.134	.061	-.053	-.039	-.263
Differentiation	-.183	-.126	**-.815**	-.018	.085	.046
Mucin production	-.033	**.929**	.030	.117	.043	-.017
Mucin type	.027	**.894**	.133	.141	.065	-.007
Peritumoral*	-.179	.025	.113	**.496**	.328	.250
TILs	-.289	.151	**.571**	.028	.167	.164
Desmoplasia	.180	-.071	-.315	**.582**	-.074	.041
Necrosis	.074	-.542	.013	.486	-.044	-.117
Vascular invasion	**.531**	-.078	.121	.006	-.080	.163
Perineural invasion	**.626**	-.026	-.081	-.029	-.034	.167
Medullary type	-.031	-.090	**.854**	.049	.029	.020
Budding	**.506**	-.269	-.006	.144	.244	.069
Tumor margin	**.587**	-.012	-.302	.054	-.086	.041

Variables with loadings > 0,40 usually are applied as meaningful loadings on the factor. If a variable has a meaningful loading on more than one component, that variable should be ignored in the interpretation.

A minus (-) before the value indicates a negative correlation.

*=Peritumoral lymphocytic infiltration.

Factor 1=Variables related to aggressiveness and extent of tumor spread.

Factor 2=Variables related to mucin production/mucin type.

Factor 3=Variables related to MSI-type of CRC.

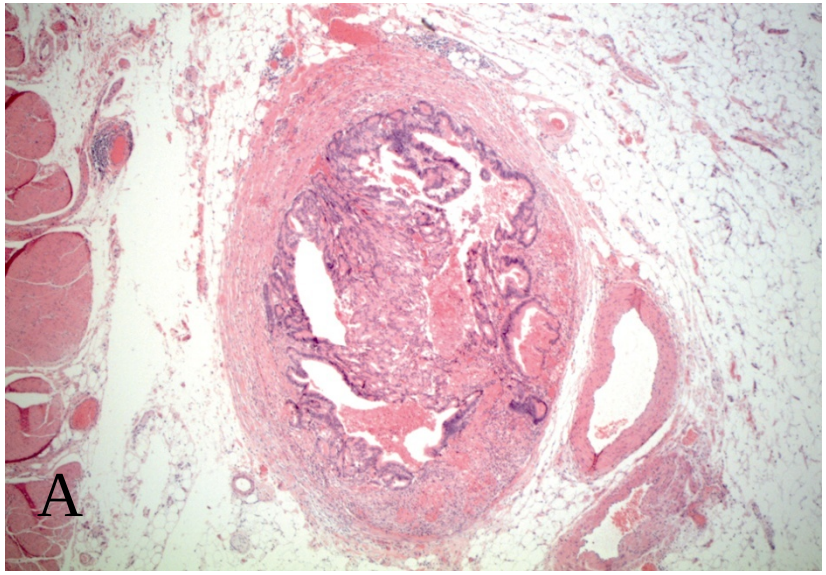
Factor 4=Tumor size, peritumoral lymphocytic infiltration and desmoplasia.

Factor 5=Age group and multiple tumors.

Factor 6=Sex and family history

Figure legend

Figure 1: A. Moderately differentiated colorectal cancer (CRC) showing venous invasion (x25, H & E staining). **B.** Right-sided CRC (caecum) displaying medullary features with poor differentiation, syncytial growth, tumor infiltrating lymphocytes (TILs) > 30/10 high-power field (HPF; not visible at this magnification), circumscribed tumor margin, and Crohn-like lymphoid reaction (x50, H & E staining). **C.** Left-sided CRC (sigmoid) with moderate differentiation, desmoplastic reaction, and infiltrative tumor margin (x25, H & E staining).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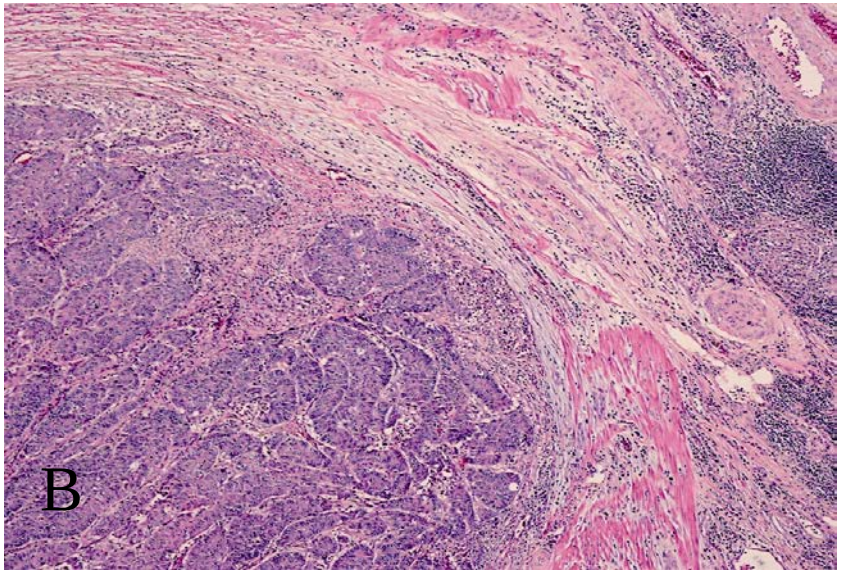
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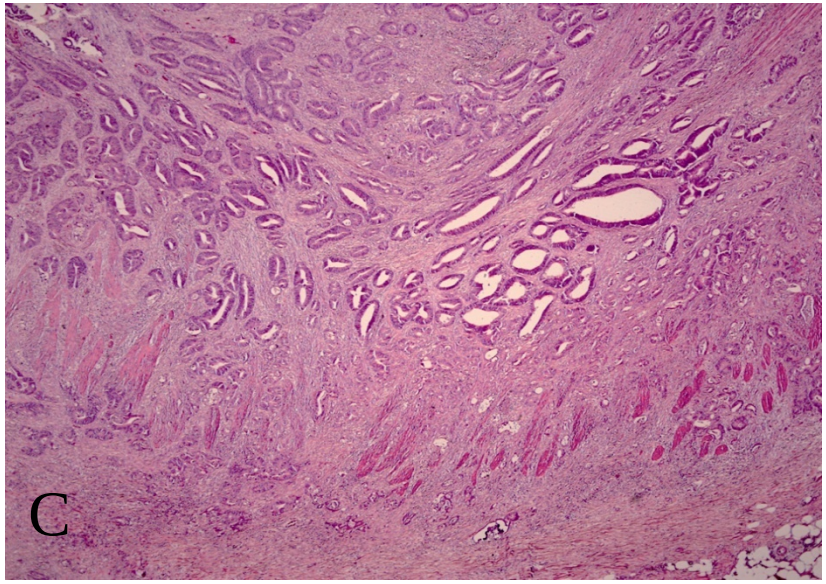
C