Diploma Thesis

PROGNOSTIC VALUE OF TUMOR GRADING IN COLORECTAL CANCER

Systematic analysis of primary and metastatic tumor tissue

submitted by

Annika Resch

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Graz, August 2015

Affidavit

I herby declare that the following thesis has been written by myself and without any assistance from third parties. For preparation of this thesis no other sources than those indicated in the thesis itself have been used.

Please note that parts of this thesis have already been published in the peer-reviewed journal “Journal of Clinical Pathology”:

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Graz, August 2015 Annika Resch eh

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Abbreviations

CRC	Colorectal Cancer
NOS	Not Otherwise Classified
WHO	World Health Organization
TNM	Tumor Node Metastasis
AJCC	American Joint Committee on Cancer
UICC	Union for International Cancer Control
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
PET-CT	Positron Emission Tomography – CT
LVI	Lymphovascular Invasion
MSI	Microsatellite Instability

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Abstract

Background: The identification of parameters, which are associated with disease progression or, on the contrary, favorable outcome is crucial for the management of cancer patients. In a large cohort of patients with colorectal cancer, we systematically compared the prognostic value of traditional grading based upon histological features, that is, gland formation alone, with grading based upon both histological and cytological features ("alternative grade"). In addition, we aimed to assess the clinicopathological significance of tumor differentiation of metastatic lymph node tissue ("lymph node grade") in patients with AJCC/UICC stage III disease.

Patients and Methods: Traditional and alternative tumor grade were evaluated in a cohort of 330 patients with colorectal adenocarcinoma (not otherwise specified, NOS). Both grades were related to various clinicopathological features and to progression-free and cancer-specific survival applying univariate and multivariate testing. 145 patients of the cohort were eligible for the evaluation of lymph node metastases, comparing the clinicopathological significance of primary tumor and corresponding lymph node grades.

Results: Traditional and alternative tumor grades were significantly associated with T and N classification, tumor size, lymphovascular invasion, progression-free and cancer-specific survival. In Cox's proportional hazards regression models, the alternative grade was superior to the traditional tumor grade and was significantly associated with cancer-specific survival, independent of patients' age and gender, T and N classification, and lymphovascular invasion. The lymph node grade was significantly associated with N classification, tumor size, and lymphovascular invasion. Patients with lymph node grade G1 had better progression-free and cancer-specific survival. Multivariate analysis proved lymph node grade to be superior to primary tumor grade in predicting outcome and identified lymph node grade as predictor of cancer-specific survival (but not of progression-free survival), independent of T classification, lymphovascular invasion, as well as patients' age and gender.

Conclusion: The alternative tumor grade, which was based upon both histological and cytological features proved to be superior to the traditional tumor grade. In addition, we identified the lymph node grade as promising novel prognostic parameter for patients with AJCC/UICC stage III disease, superior to primary tumor grade. Additional studies are warranted to validate these new findings.

Zusammenfassung

Hintergrund: Die Identifikation von Parametern, welche eine Krankheitsprogression oder eine günstige Prognose erwarten lassen, ist von großer Bedeutung im klinischen Management von PatientInnen mit Krebs. In einer großen Kohorte von PatientInnen mit kolorektalem Karzinom wurde die Wertigkeit von „traditionellem“ Grading, basierend auf histologischen Eigenschaften, das heißt, nur das Ausmaß glandulärer Differenzierung berücksichtigend, mit einem „alternativen“ Grading, welches histologische und zytologische Eigenschaften berücksichtigt, verglichen. Zusätzlich wurde die prognostische Bedeutung der Tumordifferenzierung in Lymphknotenmetastasen („Lymphknotengrad“) bei PatientInnen im AJCC/UICC Stadium III untersucht.

PatientInnen und Methodik: Traditioneller und alternativer Tumorgrad wurden in einer Kohorte von 330 PatientInnen mit kolorektalem Adenokarzinom untersucht. Beide Tumorgrade waren signifikant mit verschiedenen klinischen und pathologischen Tumorparametern und mit dem progressions- und dem krebsspezifischen Überleben assoziiert (unter Anwendung von univariater und multivariater Analyse). Weiters wurden Untersuchungen an den Präparaten von 145 PatientInnen mit Lymphknotenmetastasen durchgeführt, um die prognostische Bedeutung der Tumordifferenzierung in Lymphknotenmetastasen mit der in den korrespondierenden Primärtumoren zu vergleichen.

Ergebnisse: Traditioneller und alternativer Tumorgrad waren signifikant mit der T und N Klassifikation, Tumorgröße, lymphovaskulärer Invasion und auch mit dem progressions- und dem krebsspezifischen Überleben assoziiert. In der multivariaten Analyse war das alternative dem traditionellen Grading überlegen und zeigte eine signifikante Assoziation mit dem krebsspezifischen Überleben, unabhängig von Alter und Geschlecht, T und N Klassifikation sowie dem Vorhandensein einer lymphovaskulären Invasion. Der Lymphknotengrad war signifikant mit der N Klassifikation, Tumorgröße und lymphovaskulärer Invasion assoziiert. PatientInnen mit einem niedrigen Lymphknotengrad wiesen ein signifikant günstigeres progressions- und dem krebsspezifischen Überleben auf. In der Multivariatanalyse war der Lymphknotengrad dem korrespondierenden Primärtumorgrad in der Prognosevorhersage überlegen. Der Lymphknotengrad wurde als unabhängiger prognostischer Faktor im Bezug auf das krebsspezifische Überleben ermittelt, unabhängig von T Klassifikation, lymphovaskulärer Invasion, Alter und Geschlecht.

Schlussfolgerung: Der alternative Tumorgrad, welcher sowohl histologische als auch zytologische Eigenschaften berücksichtigt, war dem traditionellen Tumorgrad überlegen. Weiters zeigte die Überlebensanalyse, dass das Grading an der Lymphknotenmetastase ein vielversprechender neuer prognostischer Parameter für PatientInnen im AJCC/UICC Stadium III darstellt, der sich auch gegenüber dem Grading am Primärtumor überlegen präsentierte. Weitere Studien zur Bestätigung unserer Ergebnisse sind wünschenswert.

Introduction

Cancer is the leading cause of death in economically developed countries and second in developing countries (1) accounting for about 12.7 million cancer cases and 7.6 million cancer deaths estimated to have occurred in 2008, with numbers increasing due to population aging and growth as well as the cancer-associated lifestyle (i.e. smoking, physical inactivity and nutrition). Lung cancer in men and breast cancer in females are not only the most commonly diagnosed cancers but also responsible for the majority of cancer related deaths for each sex (Figure 1A-B). (2–4)

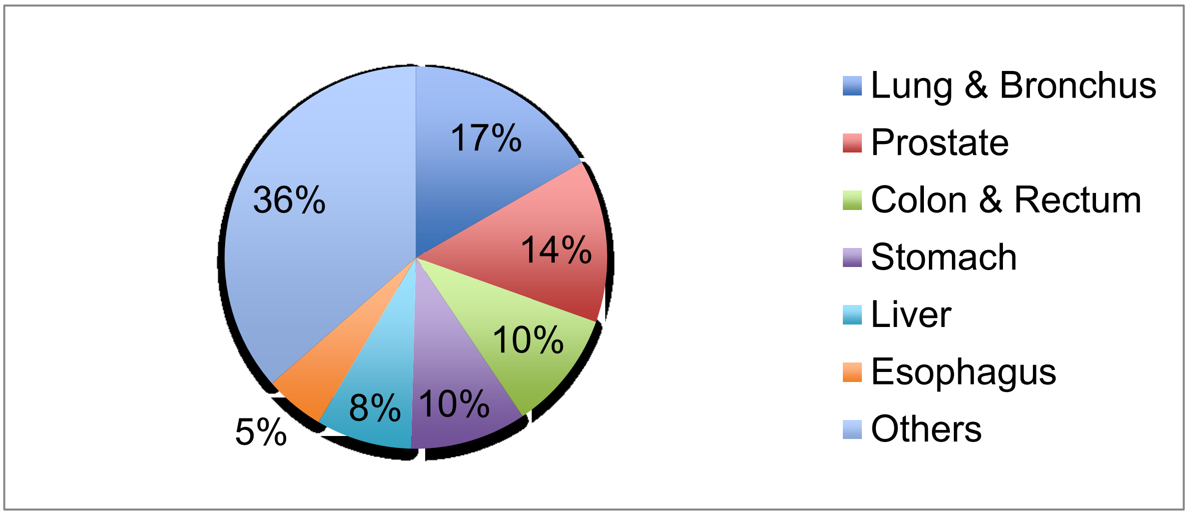

Figure 1A: Estimated new cases worldwide in men; data from Jemal et al. (2)

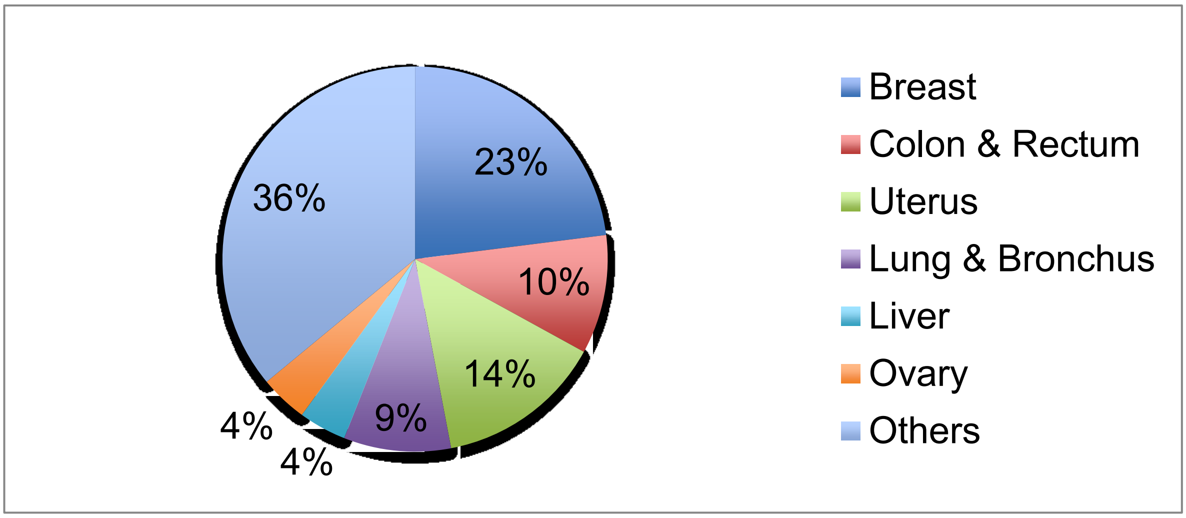

Figure 1B: Estimated new cases worldwide in women; data from Jemal et al. (2)

Epidemiology

Colorectal cancer (CRC) is the leading cause of deaths among gastrointestinal tumors (5) with incident rates being higher in men than in women and strongly increase with age. CRC is the third most commonly diagnosed cancer in men (after lung and prostate cancer) and the second in women (after breast cancer). The median age at diagnosis is about 70, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008. (2) The highest incidence rates are found in North America, Europe, and Oceania, whereas the lowest rates are found in Africa and South-Central Asia. Rates are considerably higher in males than in females. (2) Rapid increase in earlier low-risk countries is observed, which has been ascribed to the so-called western lifestyle. Remarkably, incident and death rates start to stabilize or decrease in high-risk and high-income countries as a result of improved screening and awareness, enabling early detection and accurate treatment. (2,6,7)

In the European Union, approximately 92,900 deaths in men and 75.500 in women have been estimated for 2014, accounting for about 12.5 and 13.0% of all cancer related deaths. (8) This numbers are expected to increase in 2015 with 95,900 predicted deaths from CRC in men and 76,700 in women, corresponding to 12.5 and 13% of all cancer related deaths 2015 (Figure 2A-B). (9) Whereas in the United States an estimated number of 136,830 new cancer cases and 50,310 deaths from CRC are expected for 2014 with more than 60% of cases and 70% of deaths occur in those aged 65 years or older. (10)

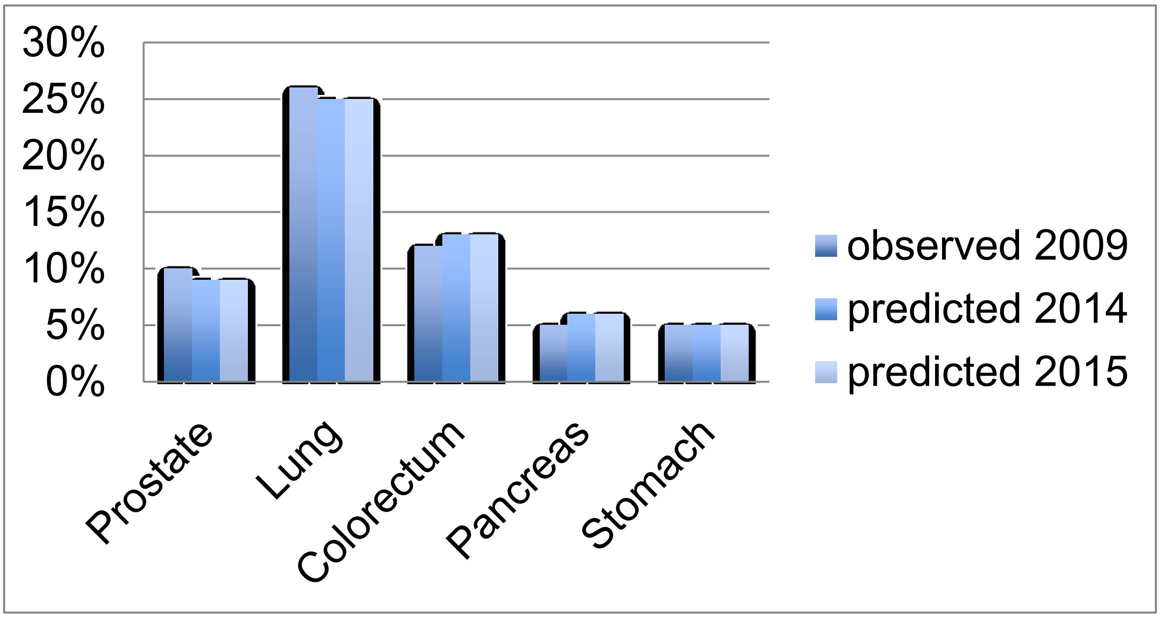

Figure 2A: Observed cancer related deaths in men in the year 2009 compared to estimated cancer related deaths in men predicted for the years 2014 and 2015; data from Malvezzi et al. (8,9)

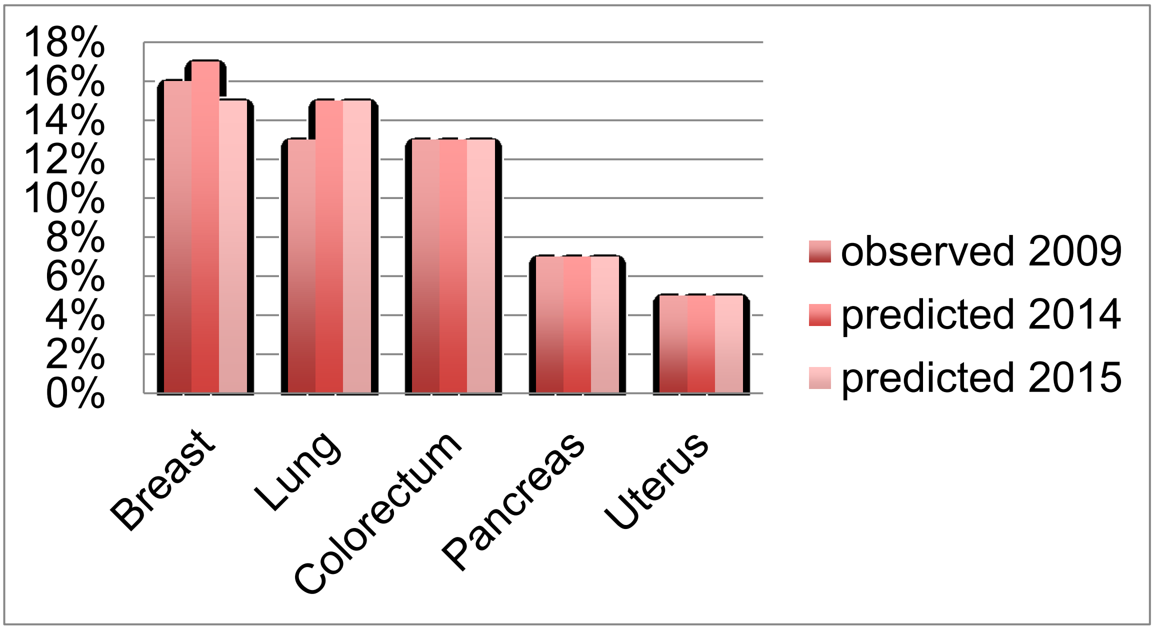

Figure 2B: Observed cancer related deaths in women in the year 2009 compared to estimated cancer related deaths in women predicted for the years 2014 and 2015; data from Malvezzi et al. (8,9)

Risk Factors

CRCs vary in terms of clinicopathological and tumor characteristics as well as risk factors depending on their location within the colon or rectum, suggesting clear etiologies and carcinogenic processes. (10) The most frequently diagnosed tumor location is the proximal colon (42%), followed by the rectum (28%), with distribution varying with sex and age (Figure 3). The median age at diagnosis for colon cancer are 69 years in men, 73 years in females and 63 years and 65 years for rectum cancer, respectively. CRC survival rates are slightly higher for rectal than for colon cancer. This may be due to the fact that rectal cancers are more likely to be diagnosed at a localized stage compared to colon cancers. Furthermore, patients aged younger than 65 have higher 5-year survival rates than those aged 65 years or older. (10)

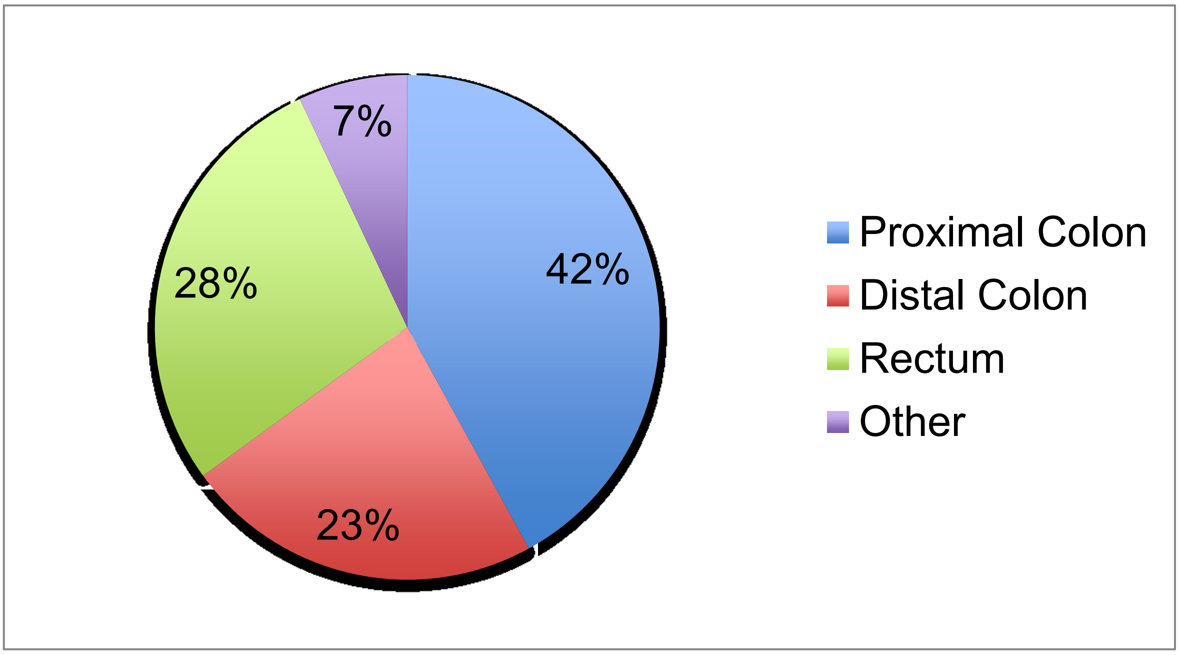

Figure 3: Colorectal cancer incidence rates by tumor location; data from Siegel et al. (10)

The clinicopathological correlation of colorectal carcinomas is depending on the localization. Rectal cancer and cancer located in the distal colon may characteristically cause rectal bleeding and blood on the stool. Carcinomas of the proximal colon are most likely associated with a change in bowel habits, obstipation, diarrhea or narrowing of the stool, lasting for more than a few days as well as weight loss. (6)

Compared to other cancer types such as lung cancer no isolated risk factor can be defined for CRC. Besides age and male sex following risk factors have been established: family history of CRC, inflammatory bowel disease, smoking, obesity, diabetes and excessive consumption of alcohol and red/processed meat. Furthermore, evidence suggests that infections with *Helicobacter pylori*, *Fusobacterium* spp, among others, might increase the risk for CRC (6)

Risk reduction includes preventive factors such as physical activity, hormone replacement therapy, and aspirin, as well as routine screening colonoscopy and endoscopic removal of precancerous lesions. This screening method represents a strong tool of prevention since thereby the development of CRC through the adenoma-carcinoma sequence can be interrupted, which occurs in more than 70% of colorectal adenomas (Table 1). (6)

Table 1: Risk and prevention factors in colorectal cancer

	Risk
Sociodemographic Factors	
Older age	+++
Male sex	++
Medical Factors	
Family History	++
Inflammatory bowel disease	++
Diabetes	+
Helicobacter pylori infection	(+)
Large bowel endoscopy	- -
Hormone replacement therapy	-
Aspirin	-
Statins	(-)
Lifestyle Factors	
Smoking	+
Excessive alcohol consumption	+
Obesity	+
Physical activity	-
Diet Factors	
High consumption of red/processed meat	+
Fruit and vegetables	(-)
Cereal fibre and whole grain	(-)
Fish	(-)
Dairy products	(-)

Modified after Brenner et al. (6)

Histology

Colorectal adenocarcinomas are defined by invasion through the muscularis mucosae into the submucosa. Most CRCs are gland-forming, that is, adenocarcinomas (not otherwise specified, NOS) (Figure 4).

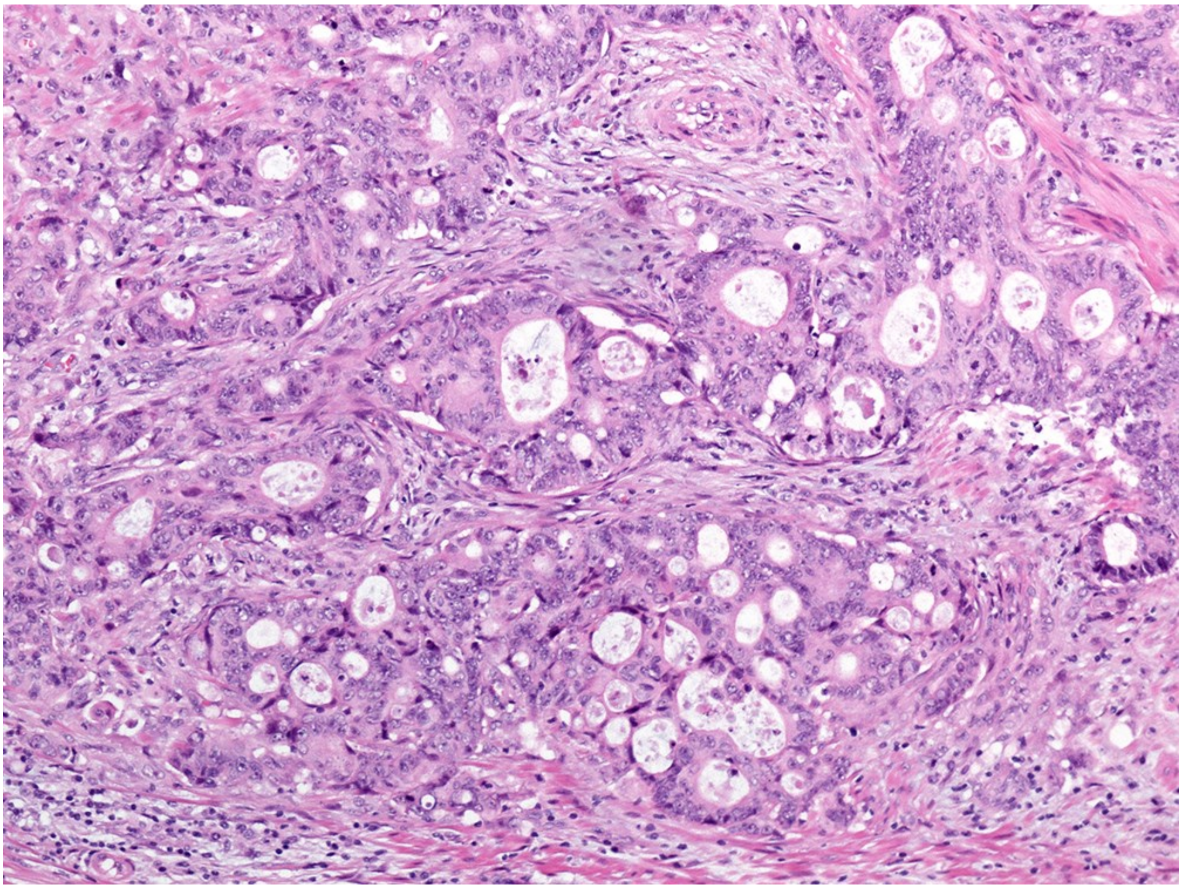

Figure 4: Adenocarcinoma not otherwise specified (NOS). (original x100)

The current World Health Organization (WHO) classification of Tumors of the Digestive System includes the following histological variants: mucinous adenocarcinoma, signet-ring cell carcinoma and medullary carcinoma, as well as micropapillary adenocarcinoma. (11)

The mucinous adenocarcinoma constitutes 4%–19% of CRC worldwide. (12–16) The designation is used when more than 50% of the lesion is composed of pools of extracellular mucin that contain malignant epithelium as acinar structures, layers of tumor cells, or individual tumor cells including signet ring cells (Figure 5A). Carcinomas with mucinous areas of less than 50% are categorized as having a mucinous component. (17,18)

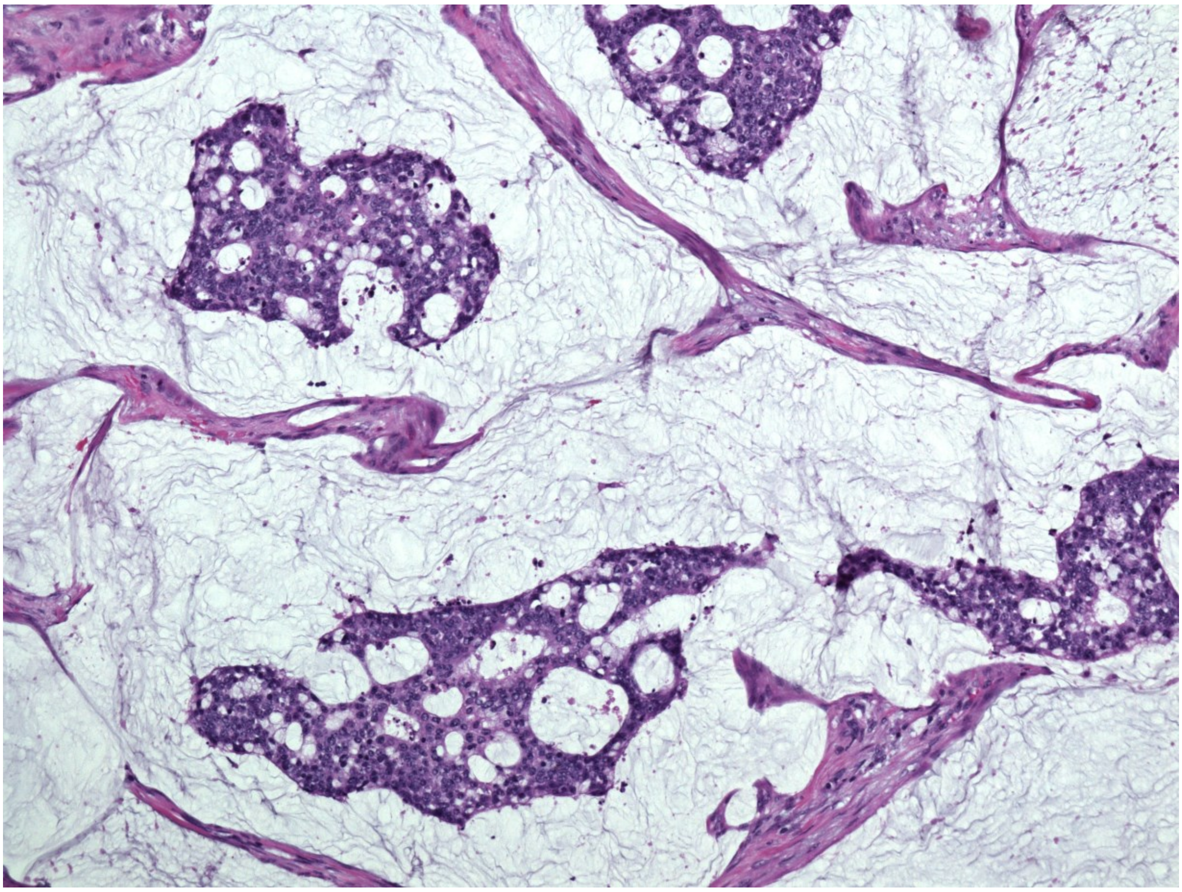

Figure 5A: Mucinous adenocarcinoma: >50% of the lesion is composed of pools of extracellular mucin. (original x100)

About 1% of CRC are signet-ring cell carcinomas. (19–21) This subtype is defined by the presence of more than 50% of tumor cells with prominent intracytoplasmic mucin, typically with displacement and molding of the nucleus (Figure 5B). Carcinomas with signet ring cells covering less than 50% of the tumor are categorized as adenocarcinoma with a signet ring cell component. (17,18)

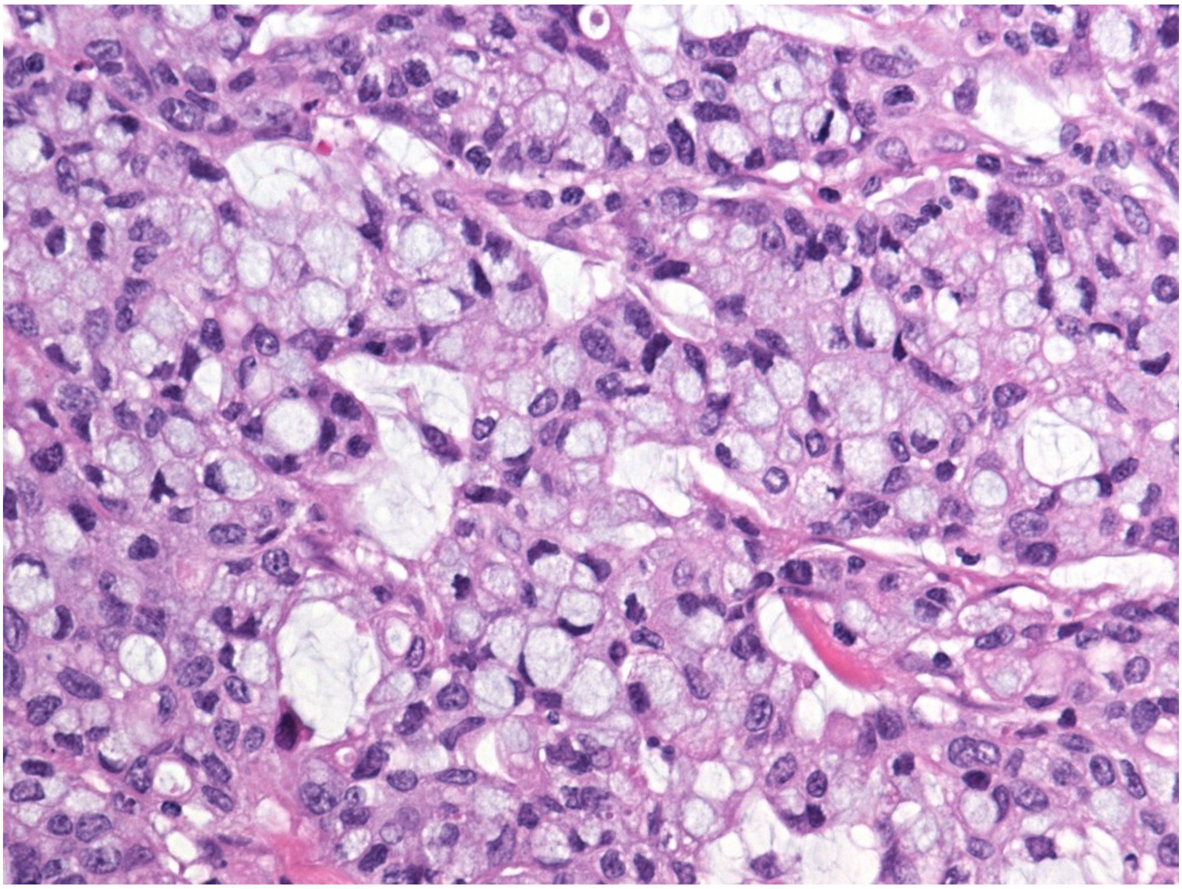

Figure 5B: Signer-ring cell carcinoma: >50% of tumor cells display prominent intracytoplasmic mucin. (original x100)

The medullary carcinoma is a rare mainly right-sided subtype that is characterized by sheets of malignant cells with vesicular nuclei with prominent nucleoli, and abundant eosinophilic cytoplasm exhibiting prominent infiltration by intraepithelial lymphocytes (Figure 5C). (17,18)

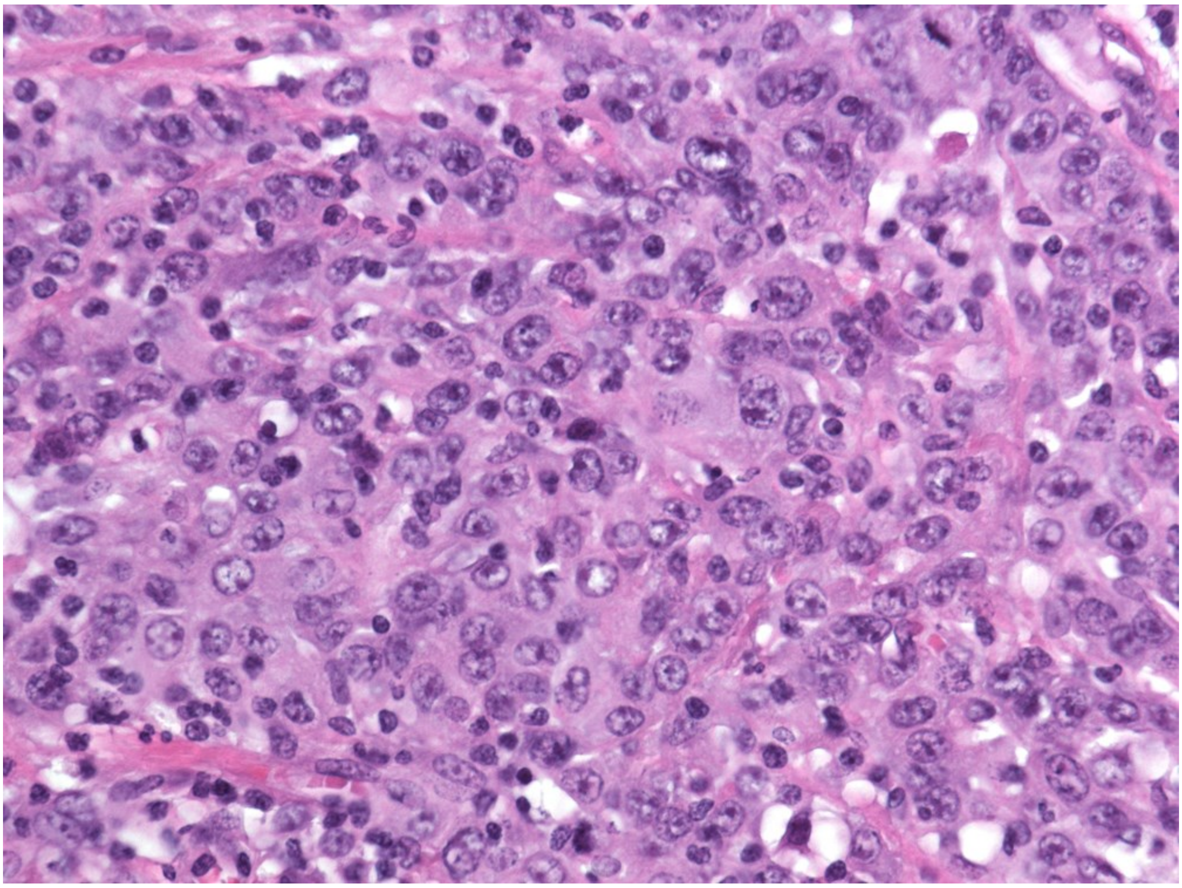

Figure 5C: Medullary carcinoma: the tumor is characterized by sheets of malignant cells with vesicular nuclei and prominent intratumoral inflammation. (original x100)

Micropapillary adenocarcinoma is a rare tumor that is defined by small papillary and/or angular clusters of tumor cells, accompanied by stromal spaces mimicking vascular channels (Figure 5D). The pattern is mainly seen as a minor component of conventional adenocarcinoma. (17) Upon immunohistochemistry, micropapillary adenocarcinoma shows a characteristic “inside-out” staining-pattern, i.e. reversed polarity for MUC1 (EMA) and villin. (18,22)

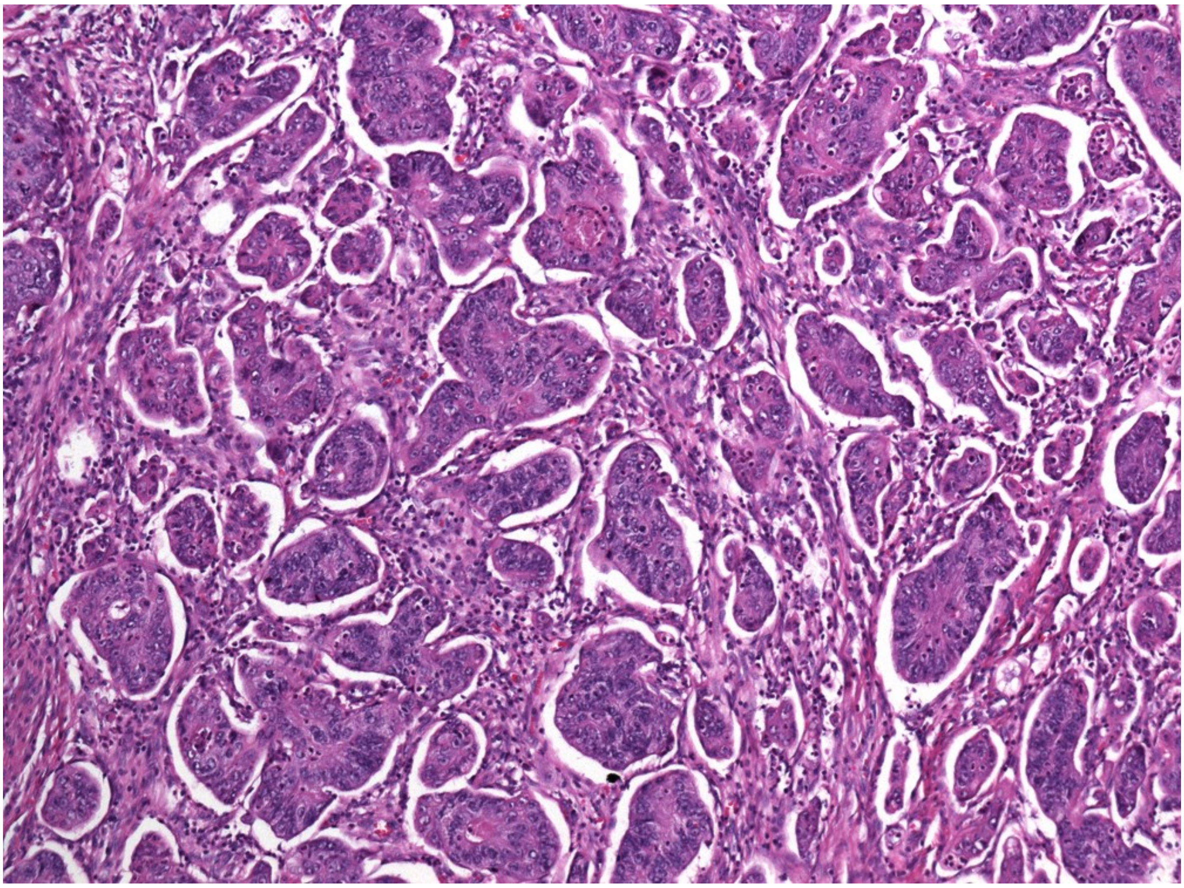

Figure 5D: Micropapillary adenocarcinoma: the tumor is defined by small papillary and/or angular clusters of tumor cells, accompanied by stromal spaces mimicking vascular channels. (original x100)

Tumor Staging

The process of staging aims to assess the extent (and the location) of cancer in the patient's body. The tumor node metastasis (TNM) system was developed and standardized by the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) in order to obtain a global system of cancer staging. (23) Thereby, the TNM system provides the basis for uniform, i.e. standardized patient care worldwide. Within the TNM system cancers are classified considering the primary tumor, that is size and/or depth of invasion (T classification), lymph node involvement (N classification) and the present or absence of distant metastases (M classification).

In CRC, the T classification includes 5 categories (T0-T4) depending upon the depth of invasion (Figure 6A-D). The categories N0, N1 and N2 stratify the tumors according to the number of involved regional lymph nodes (Figure 7A-B). Specifically, N1a refers to 1 involved node, N1b to 2 or 3 involved nodes, N2a to 4 to 6 involved nodes, and N2b to seven or more involved nodes. In CRC the M classification is defined as follows: The category M1a refers to metastatic spread confined to one organ (liver, lung, ovary, non-regional lymph nodes), while the category M1b refers to metastatic spread in more than one organ or the peritoneum. The combination of different values for T, N, and M allows the stratification of patients into tumor stage I to IV. (11,24)

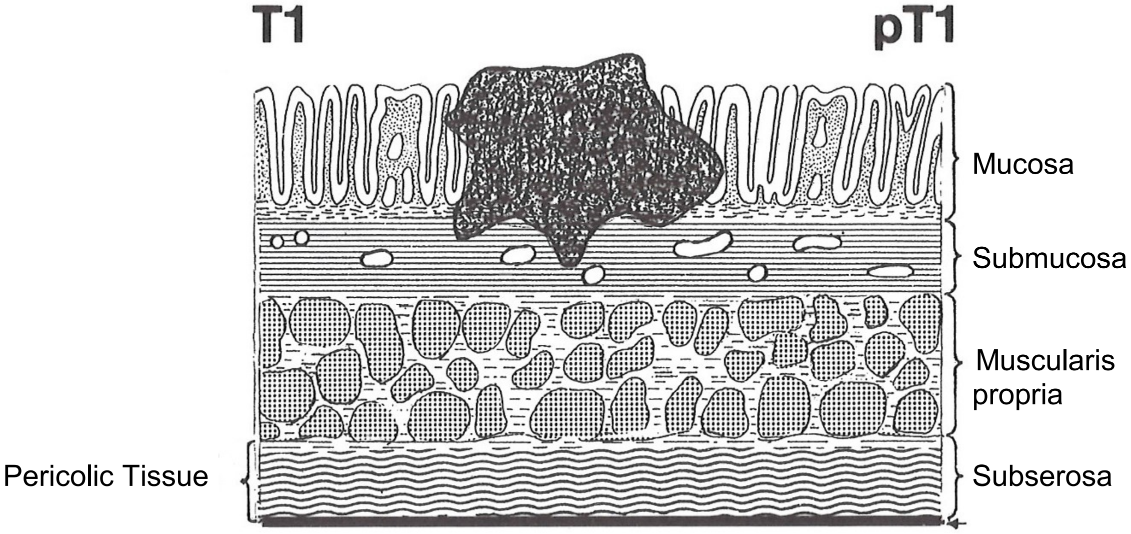

Figure 6A: Tumor invades the submucosa; modified after Wittekind et al. (25)

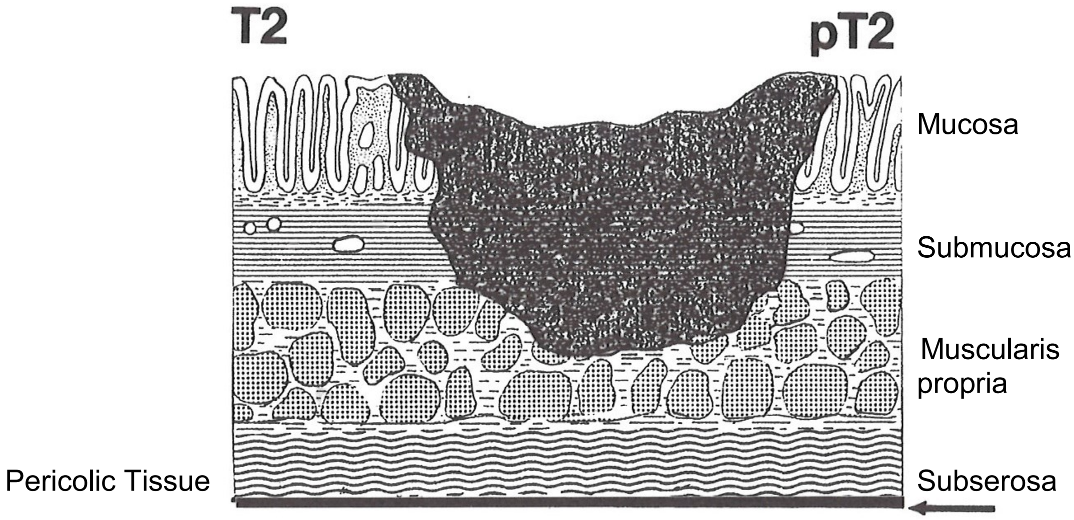

Figure 6B: Tumor invades beyond the submucosa into the muscularis propria; modified after Wittekind et al. (25)

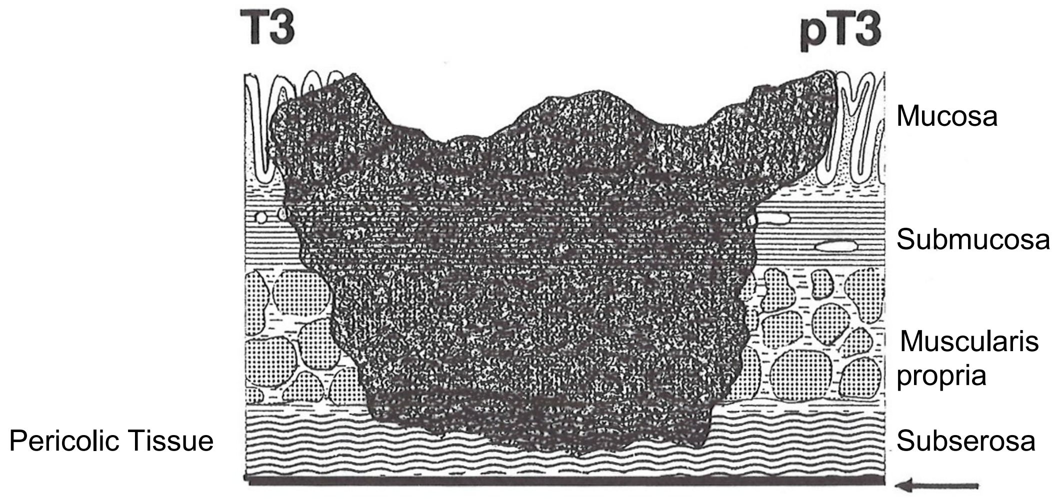

Figure 6C: Tumor invades throughout the muscularis propria into the subserosa or into non-peritonealized pericolic or perirectal tissues; modified after Wittekind et al. (25)

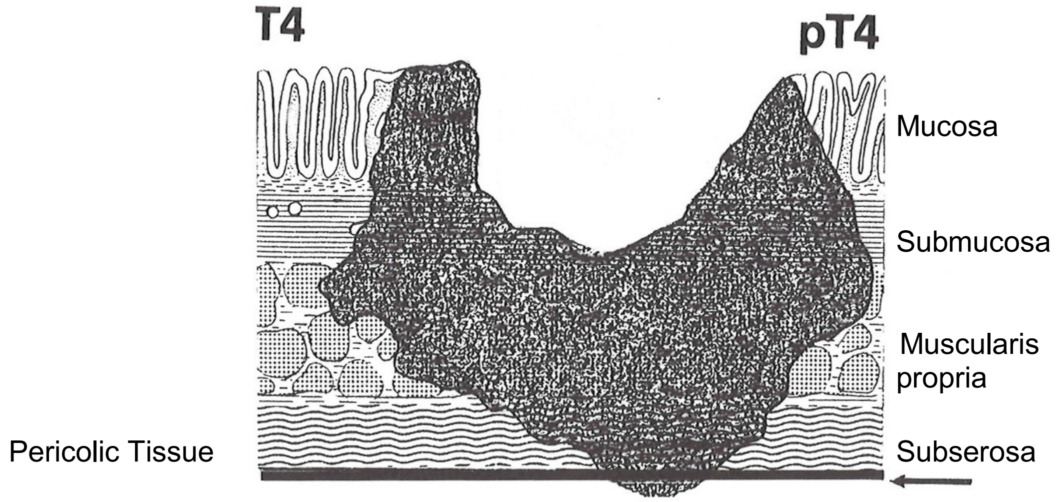

Figure 6D: Tumor directly invades other organs or structures and/or visceral peritoneum; modified after Wittekind et al. (25)

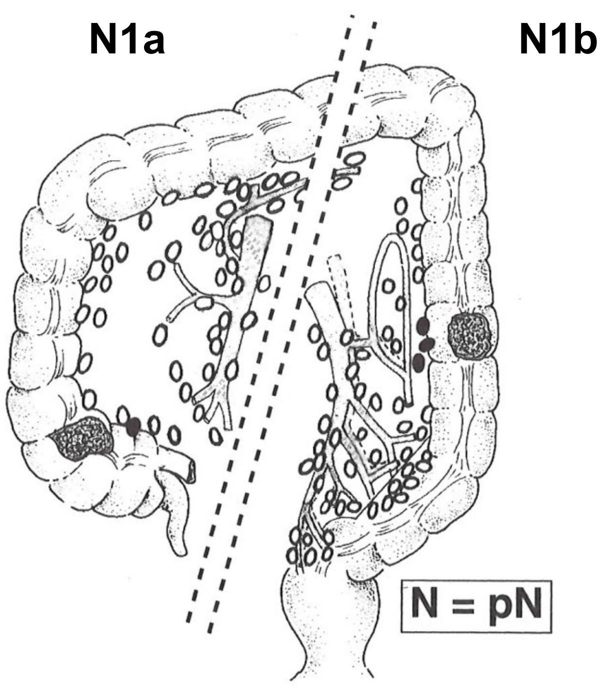

Figure 7A: Metastasis in 1-3 regional lymph nodes; modified after Wittekind et al. (25)

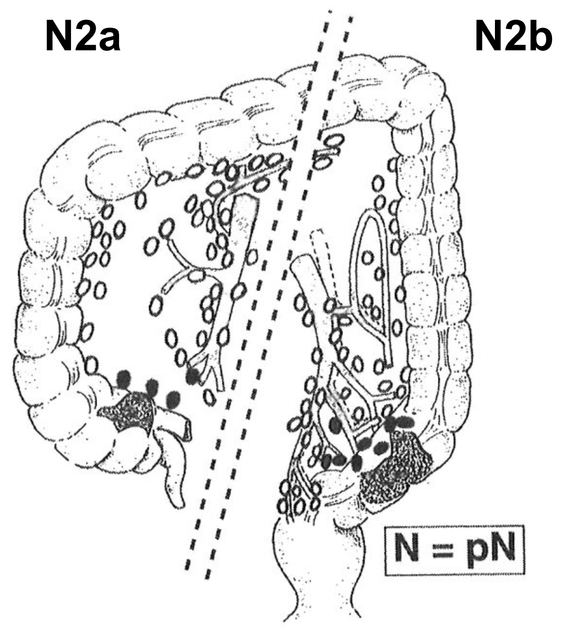

Figure 7B: Metastasis in 4 or more regional lymph nodes; modified after Wittekind et al. (25)

Prognostication

Over the past decades the prognosis of patients with CRC has improved, in particular in high-income countries. (6) Facing the increase in cancer incidence and therapeutic options, current research aims to optimize the accuracy of prognostic stratification to treat every single patient according to his/her individual risk profile. The diagnosis of CRC is an interdisciplinary assignment. After the initial diagnosis, which is based upon the histological work-up of the biopsy samples taken during colonoscopy, additional staging investigations including abdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography CT (PET-CT) are applied to assess the extent of disease. (6,26)

Clinicopathological staging according to the AJCC/UICC TNM system is regarded as the strongest prognostic parameter for patients with early-stage disease and those with advanced disease, where as for intermediate stage of disease the situation is less clear. (23,27) The evaluation of lymph nodes is of eminent importance in this process, since, to a great extent, the indication for adjuvant therapy, which is primarily based on 5-fluorouracil, is determined by the presence of positive nodes. Due to a high risk of recurrence, adjuvant therapy is currently recommended for all stage III CRC patients. (28–30)

In the last decade, several studies attempted to refine lymph node staging in node-positive (AJCC/UICC stage III) CRC and the minimum number of lymph nodes available for histological evaluation. Recommending a minimum number of 12 nodes that should be examined. Moreover, the lymph node ratio, and the presence of extracapsular invasion were identified as additional factors indicating adverse outcome. (31) However, patients with the same stage of disease may experience significantly different clinical outcomes. (5,32) Hence, a considerably high number of patients with AJCC/UICC stage III disease will not develop systemic cancer spread, and adjuvant chemotherapy will only cause side effects in this subgroup. On contrary, some patients with AJCC/UICC stage II disease will develop disease progression, but these patients, according to current national and international guidelines, do not usually receive adjuvant treatment, which might however be beneficial to them. (23,33–35)

It is of note that tumor stage does only indicate how far the tumor has progressed. Tumor stage, however, does not reflect the biological aggressiveness of disease. (36) Stage-independent prognostic parameters may help to overcome this dilemma and may serve as valuable tools for outcome prediction. Histological parameters, which can be used for prognostication, include histologic type and grade, the presence of tumor thrombi in endothelium-lined channels, that is lymphovascular invasion (LVI), perineural invasion, status of the surgical resection margins, and tumor border configuration. (37) More recently, molecular markers have entered the stage. Among these, the presence or absence of microsatellite instability (MSI), in conjunction with the BRAF status, have been used as prognostic variables, while the prognostic significance of RAS (KRAS, NRAS, HRAS) is less clear. (33,34,38)

Tumor grade is a traditional prognostic parameter, which is easily assessable on hematoxylin and eosin (H&E)-stained slides. Grading is a measure of cell anaplasia or dedifferentiation and is based on the resemblance of the tumor to the tissue of origin. The score (of tumor grade) increases with the loss of cellular differentiation, thereby reflecting how much the neoplastic cells differ from their non-neoplastic counterparts. Grading has deep clinical impact, in that the loss of differentiation during tumor progression has repeatedly been associated with tumor aggressiveness, thereby indicating poor patients' outcome.

Tumor grading does only apply for adenocarcinomas NOS. All other morphological variants carry their own prognostic significance and grading is usually not performed. (11) Carcinomas are sometimes heterogeneous, and grading should be based upon the least differentiated component, not including the leading front of invasion. According to the current WHO classification, (11) grading follows a three-tier approach and classifies tumors into well differentiated (G1) (Figure 8A), moderately differentiated (G2) (Figure 8B) and poorly differentiated (G3) (Figure 8C) lesions, depending on the extent of glandular appearance. (39)

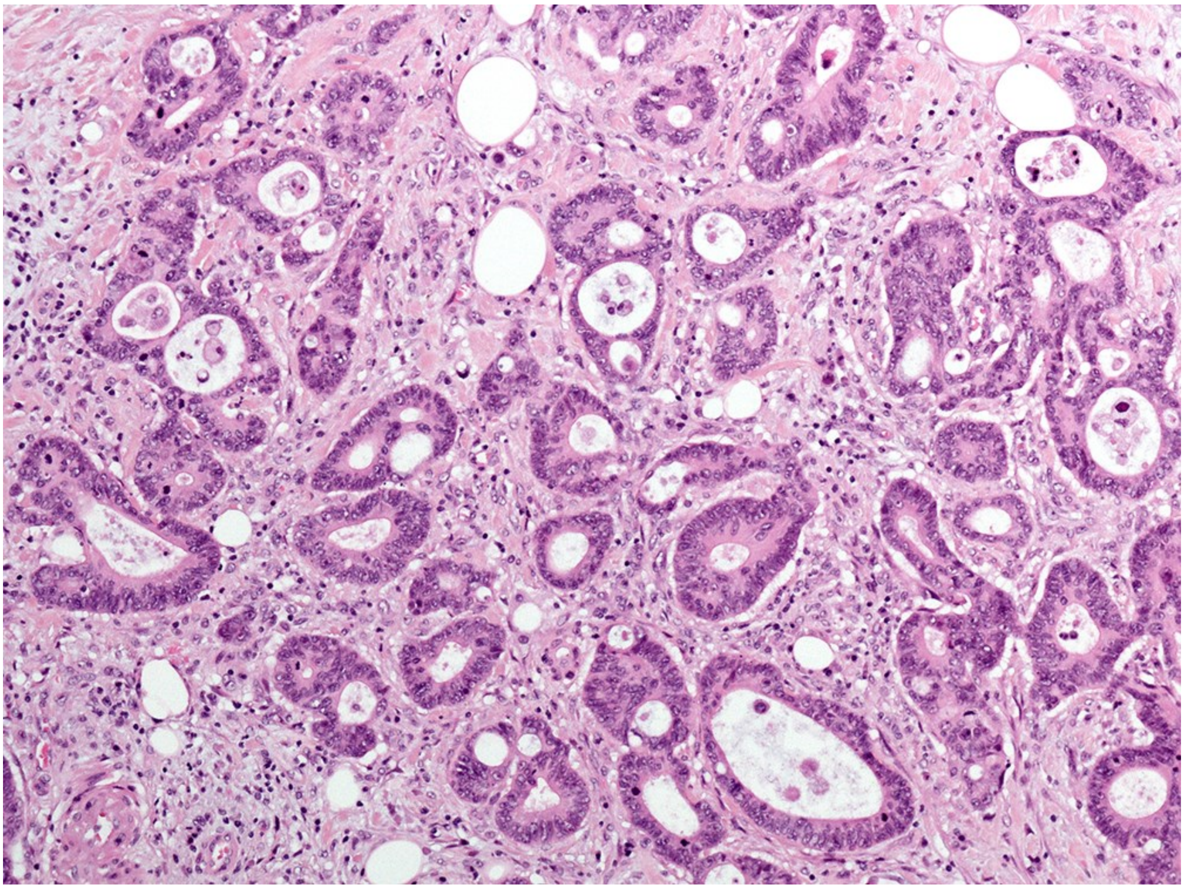

Figure 8A: Well differentiated adenocarcinoma not otherwise specified (NOS) (G1), >95% gland formation. (original x100)

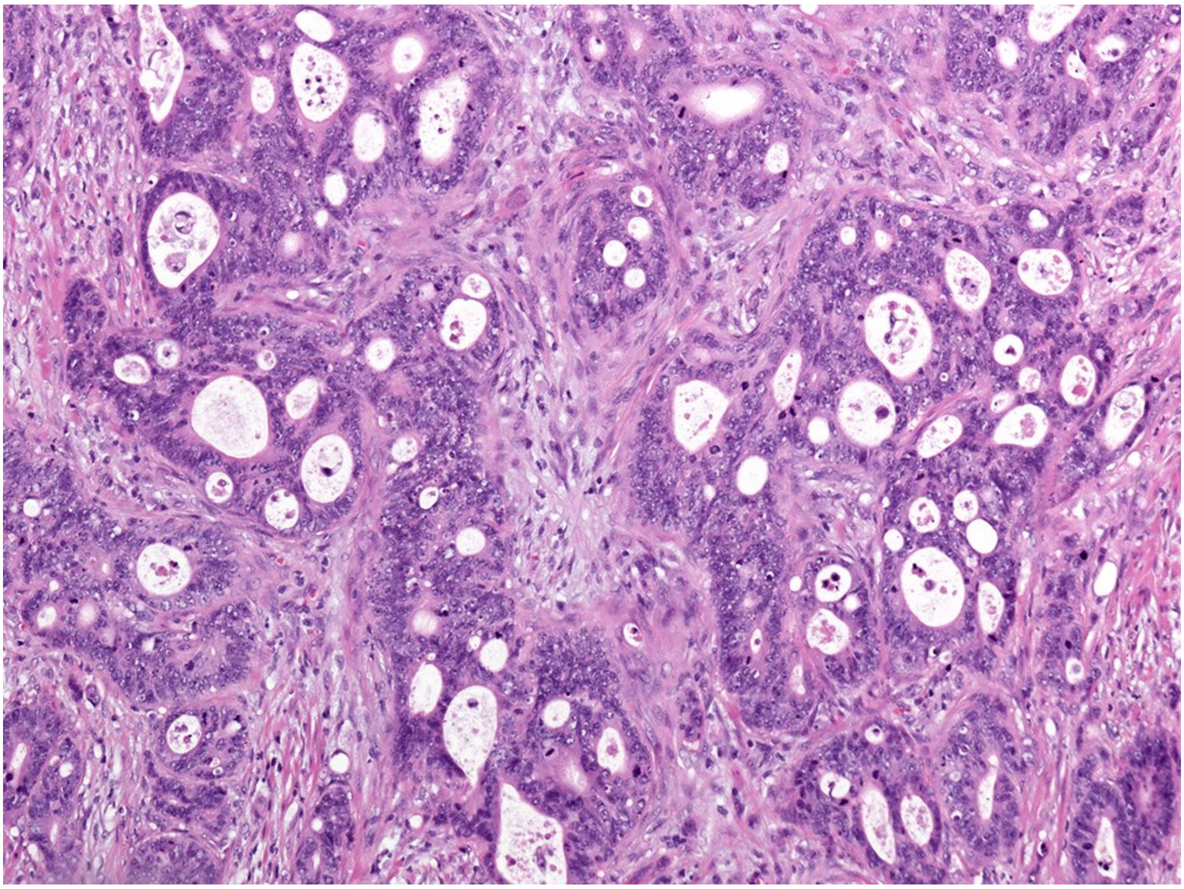

Figure 8B: Moderately differentiated adenocarcinoma not otherwise specified (NOS) (G2), 50-95% gland formation. (original x100)

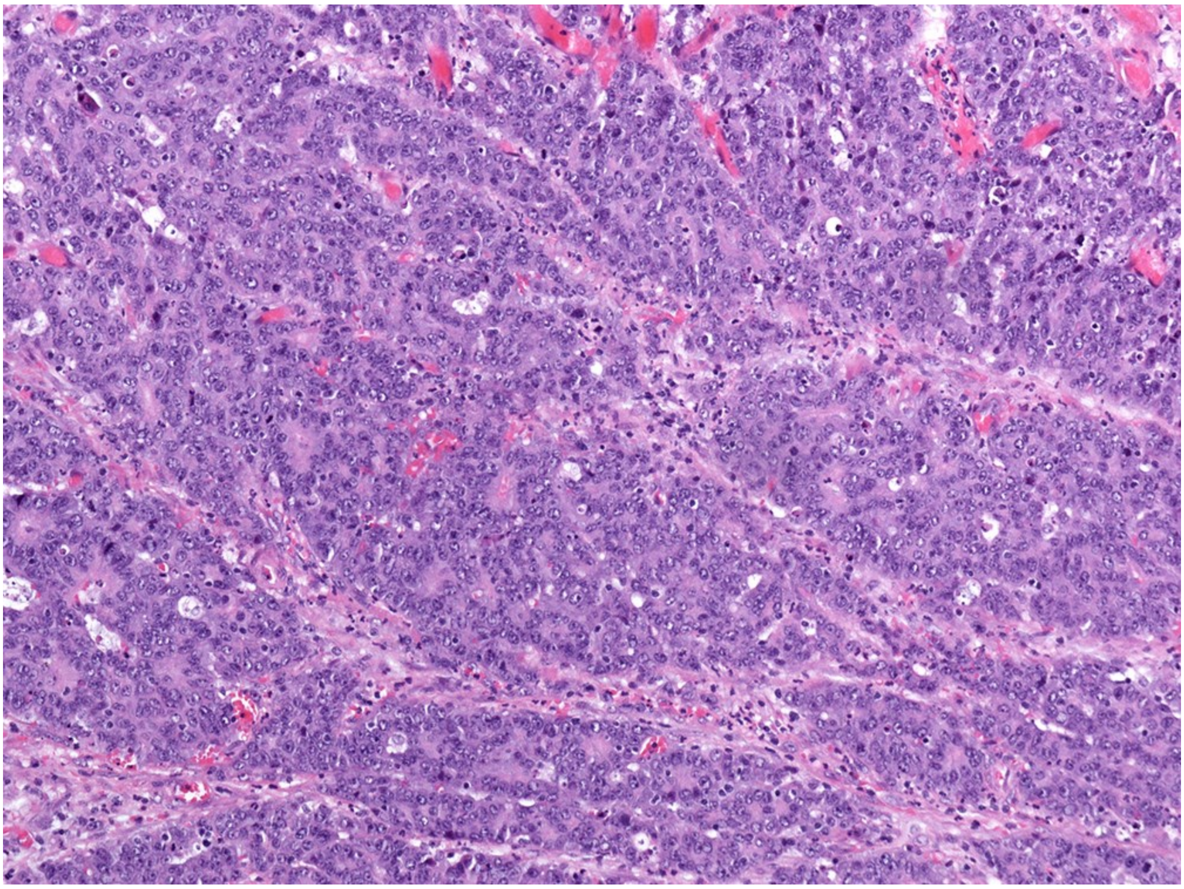

Figure 8C: Poorly differentiated adenocarcinomas not otherwise specified (NOS) (G3), >0-49% gland formation. (original x100)

In detail, adenocarcinomas NOS displaying more than 95% gland formation are considered grade 1, those with between 50 and 95% gland formation considered grade 2, and those with less than 50% gland formation considered grade 3 (Table 2). Cytological changes, such as nuclear atypia are not considered in the WHO classification. (11) This is in contrast to endometrial adenocarcinomas, for which the presence of bizarre nuclear atypia prompts upgrading the tumor by one grade. (40)

Table 2: Traditional (WHO) grading of colorectal adenocarcinomas based upon the extent of glandular appearance

Criterion	Differentiation category	Numerical grade
> 95% gland formation	Well differentiated	1
50-95% gland formation	Moderately differentiated	2
> 0-49% gland formation	Poorly differentiated	3

Aims

Our study aimed to refine the criteria of cancer grading in a large cohort of patients with CRC, specifically adenocarcinoma NOS, aiming to optimize outcome prediction. The study had two different parts.

In the *first*, we compared the traditional grading, based solely on histological features, that is the extent of glandular appearance (as mentioned above) with an alternative grading system, which also takes into account cytological, that is, nuclear features, as currently in use for endometrial cancer.

In the *second*, we analyzed whether the lymph node grade, that is, the differentiation of metastatic tumor deposits in regional lymph nodes differs from that of corresponding primary tumors. It is of note, that the differentiation of metastatic tumor tissue within regional lymph nodes, that is, the lymph node grade has never been systematically analyzed, and its prognostic significance, in particular compared with that of the traditional primary tumor grade is totally unclear. Specifically, we associated the lymph node grade with different histological parameters and evaluated the lymph node grade as potential prognostic variable, comparing its prognostic significance with that of the corresponding primary tumor grade.

Patients and Methods

Patient' selection and follow-up assessment

During the period from January 1, 1984 to December 31, 2005, a total of 7909 CRCs from 7564 patients (4095 males, 3469 females; ratio 1.2:1) were identified in the local CRC database of the Institute of Pathology (Medical University of Graz, Austria). Of these, 400 (5%) patients were randomly sampled from January 1992 through December 2000 with the aim of obtaining identical adjuvant treatment modalities as well as a minimum of 5 years' follow-up. (13,41,42)

Decision for adjuvant chemotherapy was guided by AJCC/UICC stage: stage I and II patients did not receive adjuvant therapy, while stage III patients were given 5-fluorouracil/folinic acid based chemotherapy according to the Mayo Clinic protocol. (13,41–43) Patients with rectal cancer received adjuvant radiotherapy.

The following patients were excluded: (i) those who underwent endoscopic polypectomy for low-risk T1 cancer due to missing data regarding nodal status; (ii) patients who underwent neoadjuvant chemotherapy due to presumptive treatment-related changes in T classification; (iii) patients with synchronous or metachronous secondary CRC; and (iv) patients with competitive invasive cancers originating from other sites if metastatic deposits were not assessed by histology. (13,41,42)

In all, 381 patients out of 400 (95.2%) were included for review pathology. Among these, 330 patients (189 [57.3%] males, 141 [42.7%] females; Figure 9) with adenocarcinomas NOS were identified. Mean and median ages were 68.6 and 69.8 years, respectively. The age of the youngest patient was 33 years and that of the oldest patient 92 years. The age distribution of our cohort is illustrated in Figure 10.

Adenocarcinomas NOS were right-sided (caecum to transverse colon) in 81 (24.5%) patients, left-sided (flexura lienalis to sigmoid colon) in 100 (30.3%) or were diagnosed in the rectum in 149 (45.2%) patients, respectively.

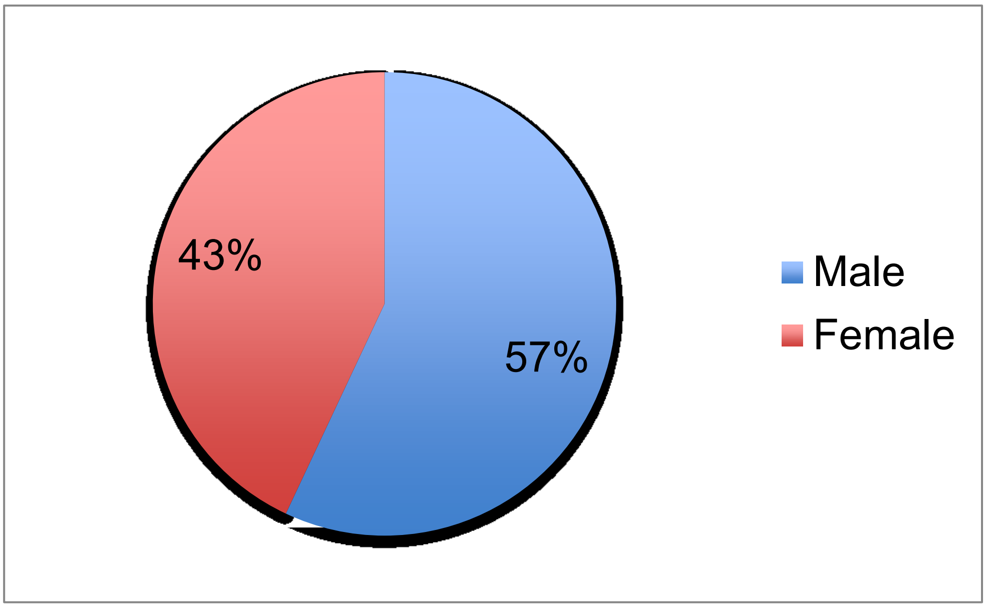

Figure 9: **Gender Distribution in our cohort of 330 patients with adenocarcinoma not otherwise specified (NOS).**

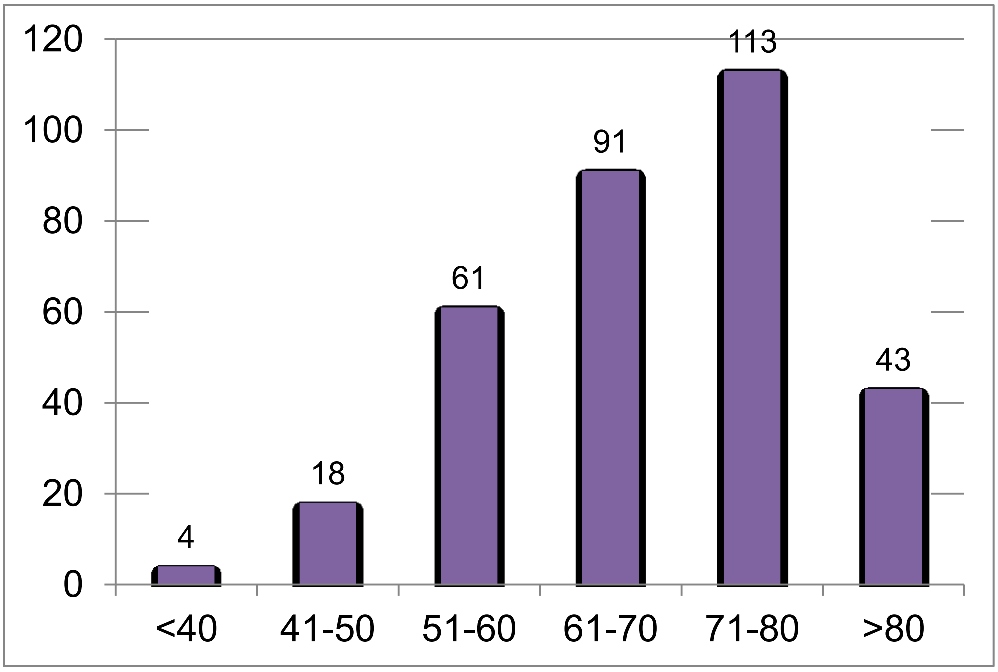

Figure 10: **Age distribution in our cohort of 330 patients with adenocarcinomas not otherwise specified (NOS).**

The histological diagnoses (according to the WHO classification; 11) of the tumors of the remaining 51 patients were as follows: 44 mucinous adenocarcinomas, 3 signet-ring cell carcinomas and 4 other types of carcinoma (medullary, adenosquamous, and so-called “mixed adenoneuroendocrine carcinoma”, MANEC). In pursuance of the aims of our study, these rare entities were excluded from further analysis (Figure 11).

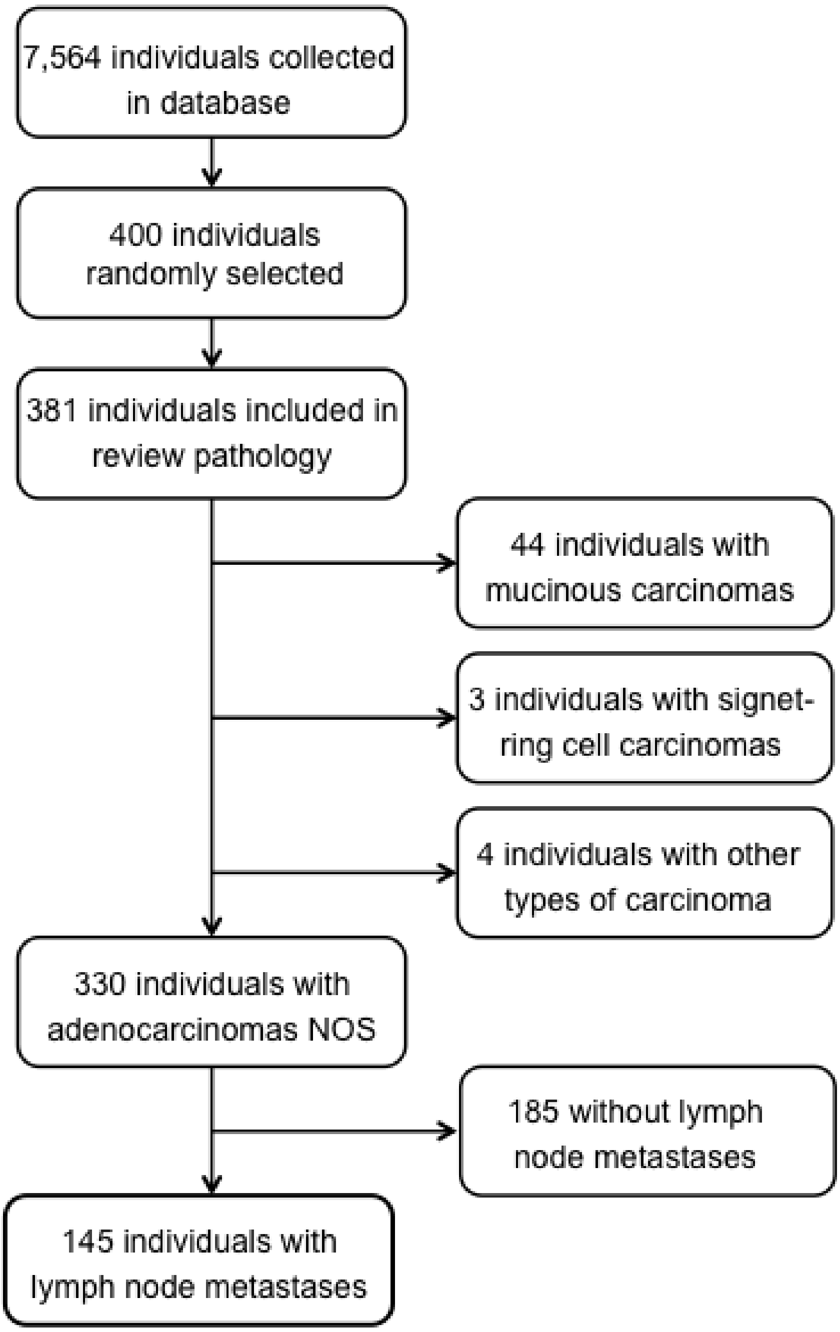

Figure 11: Study cohorts eligible for further evaluation regarding alternative tumor grade and lymph node grading.

Follow-up included laboratory testing, considering blood count, liver enzymes and the tumor markers carcinoembryonic antigen and carbohydrate antigen 19-9 at 3-month intervals (extended to 6 months after 3 years), chest X-ray and abdominal ultrasound at 6-month intervals (extended to 12 months after 3 years). Patients with rectal cancer underwent CT every 12 months. (13,41,42,44) Progressive disease was defined as either local recurrence, that is any detectable recurrent disease at the resection site, or systemic recurrence, that is hematogenous metastatic cancer spread to distant organs, such as liver, lungs and peritoneum. (13,41,42,44)

The second part of our study that aimed to asses the role of lymph node grade in regard to its association with different histological parameters and prognosis in patients with AJCC/UICC stage III CRC, was by definition restricted to node positive cancer cases and patients without lymph node metastases were consequently excluded from analysis.

In all, 145 patients were eligible for the evaluation of lymph node grade (Figure 11). These included 92 males (63%) and 53 females (37%) (Figure 12). Mean and median ages of this cohort were 68.8 and 68.4 years, respectively. The age of the youngest patient was 40 years and that of the oldest patient 92 years. Figure 13 illustrates the age distribution of this second cohort.

Institutional review board approval was obtained from the Ethics Committee of the Medial University of Graz, Austria (04/2007).

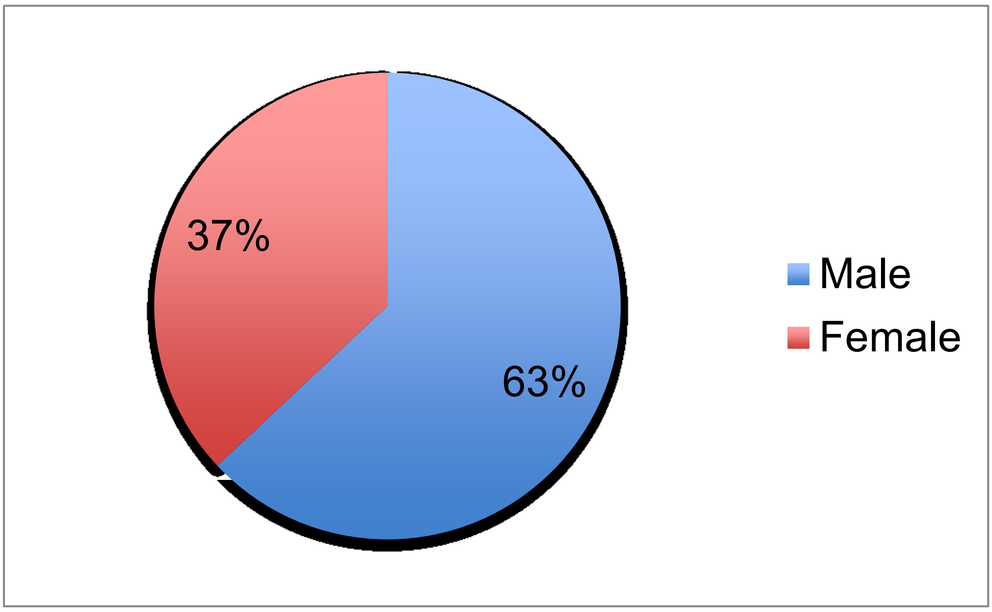

Figure 12: **Gender distribution in our cohort of 145 patients with node-positive CRC (AJCC/UICC stage III).**

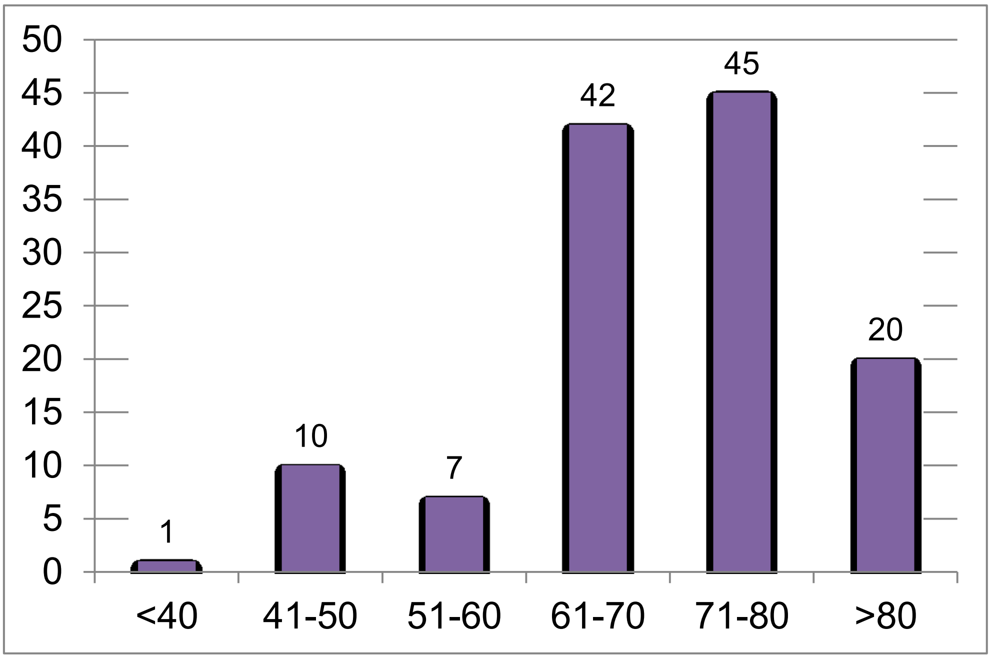

Figure 13: **Age distribution in our cohort of 145 patients with node-positive CRC (AJCC/UICC stage III).**

Histopathology

All available histological slides were independently reviewed by two gastrointestinal pathologists (Dr. Marion J. Pollheimer and Dr. Cord Langner) who were blinded to clinical data, in particular follow-up information. Discrepancies were resolved by simultaneous re-examination of the slides using a double-headed microscope. (13,41,42)

T and N classification were assessed according to the AJCC/UICC 2009 edition of the TNM classification. (24)

Grading was guided by the WHO guidelines, assessing the extend of glandular appearance, as illustrated in Table 2. (11)

In the first part of our study, we additionally applied an alternative grading system on all tumors, which takes into account cytological changes, thereby adopting the WHO endometrial cancer grading system (40): In this alternative system the presence of bizarre nuclear atypia, that is, marked nuclear pleomorphism and anaplasia justified upgrading the tumor by one grade (i.e. from 2 to 3 or from 3 to 4). Examples are illustrated in Figure 14A-B.

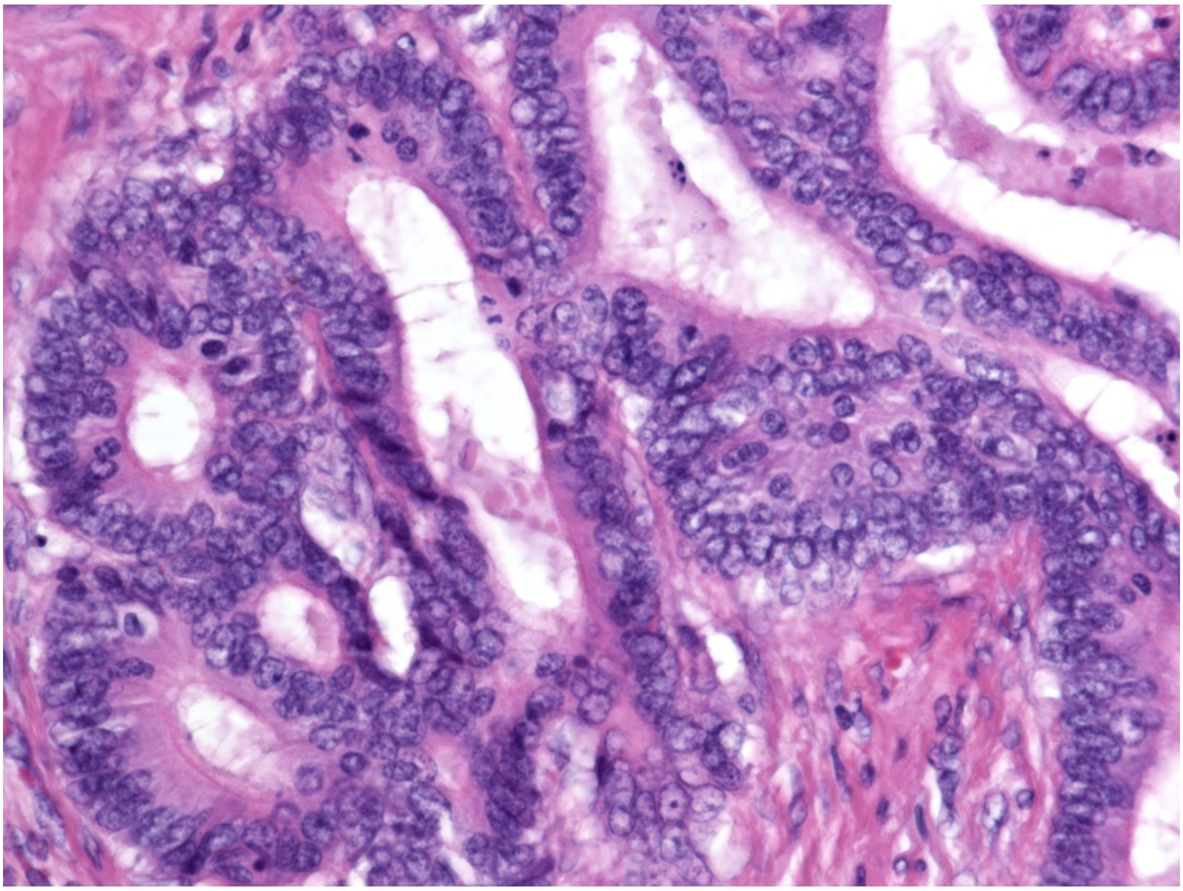

Figure 14A: Gland-forming adenocarcinoma with low-grade nuclear changes, qualifying for traditional WHO and alternative grade G1. (original x200)

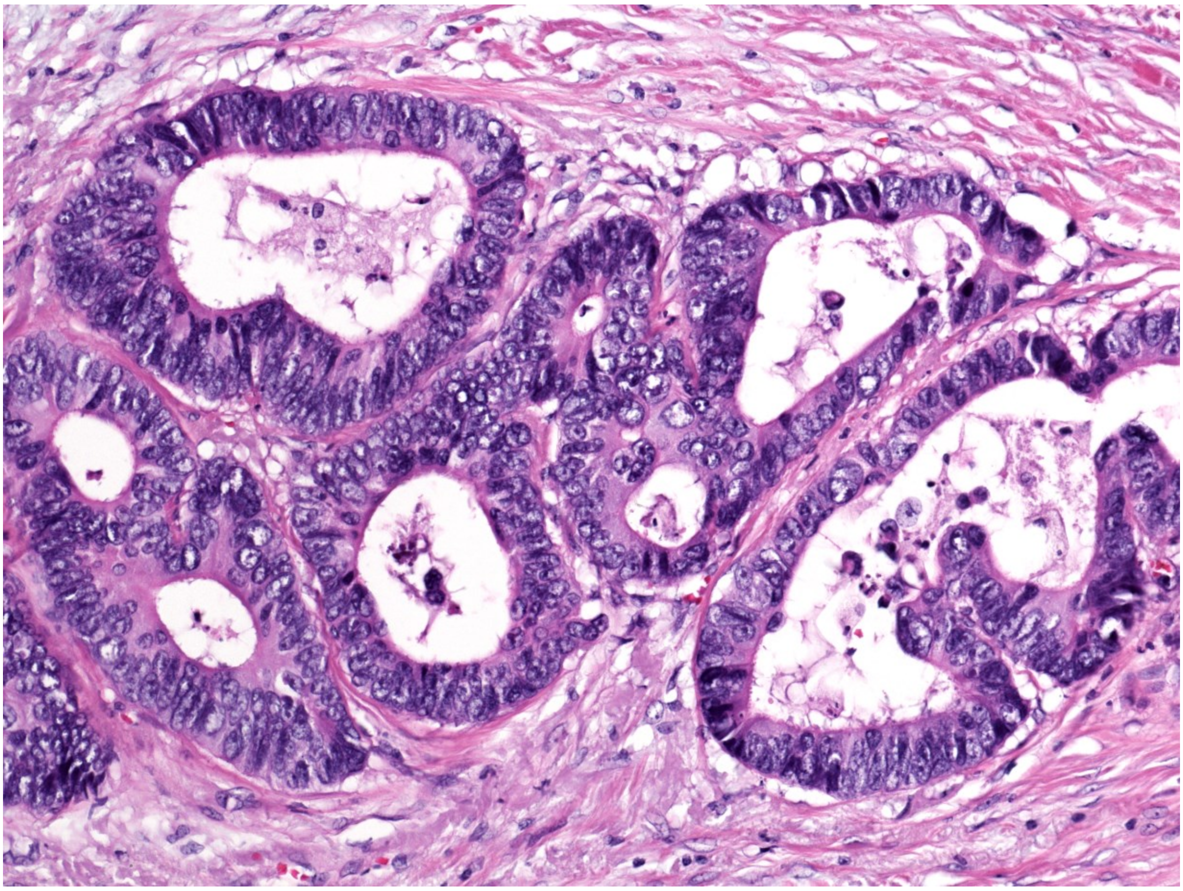

Figure 14B: Gland-forming adenocarcinoma with high-grade nuclear changes, prompting upgrading the tumor by one grade, that is, G2 in the alternative system. (original x200)

In the second part of our study, grading was performed separately for primary tumors and corresponding lymph node metastases. This part strictly followed the current WHO guidelines (11), not taking into account cytological, that is, nuclear changes.

LVI was diagnosed when tumor cells or tumor cell thrombi were observed within an endothelium-lined space. (45) Special care was taken to differentiate endothelial cells from retraction artifacts lined by fibroblasts. Therefore, the presence of a clear-cut endothelial lining was an important requirement for diagnosis, as retraction space artifacts are particularly common in CRC. Immunohistochemistry for endothelial cells was not done, in keeping with standard practice. (41,46)

Statistical analysis

Associations of tumor grade, that is, the current WHO grade, the alternative primary tumor grade and the lymph node grade, with other tumor parameters, such as T and N classification, LVI, tumor size and location were analyzed using the χ^2-test.

To assess concordance between primary tumor and lymph node grade the Somer's D rank-order correlation coefficient was used (the higher the D value [range -1 to 1] the more concordant pairs exist).

Progression-free (disease-free) survival was defined as the time (measured in months) from the date of surgical resection of the primary tumor to the date of diagnosis of recurrent disease, including both local and systemic recurrences. Cancer-free survival was defined as the time from the date of surgical resection to tumor-related death. Cause of death was determined by treating physicians and/or by chart review and was corroborated by death certificates if available. Survival rates were determined by the Kaplan-Meier method and differences between groups were evaluated by the log-ranked test. Hazard rations were calculated using Cox's proportional hazards regression models (multivariate testing). (13,41)

All reported tests were two-sided and p Values were significant at levels <0.05. All statistical evaluations were performed by Dr. Lars Harbaum, Department of Oncology, Hematology, BMT with Section Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; using SPSS statistics version 20 (IBM, Armonk, New York, United States).

Results

Tumor characteristics

Regarding the cohort of patients with adenocarcinomas NOS, disregarding the nodal status, that is, tumors with and without lymph node metastasis, traditional (WHO) tumor grades were G1 in 121 (36.7%), G2 in 138 (41.8%), and G3 in 71 (21.5%) cases, respectively.

The inclusion of cytological changes in assessment, that is, the alternative tumor grade, caused upgrading in 30 (24.8%) cases with traditional G1, 84 (62.7%) cases with traditional G2, and 61 (85.9%) cases with traditional G3, respectively. Traditional and alternative tumor grades were significantly associated with T and N classification, tumor size, and LVI. Data are presented in Table 3.

Table 3: Association of the traditional (WHO) and the alternative tumor grade with different pathological features

		N	Traditional tumor grade			p Value	Alternative tumor grade				p Value
			G1 (n = 121)	G2 (n = 138)	G3 (n = 71)		G1 (n = 91)	G2 (n = 84)	G3 (n = 94)	G4 (n = 61)	
T classification	T1	27	23 (85.2%)	3 (11.1%)	1 (3.7%)	<0.001	20 (74.1%)	6 (22.2%)	0 (0.0%)	1 (3.7%)	<0.001
	T2	66	36 (54.5%)	22 (33.4%)	8 (12.1%)		31 (47.0%)	13 (19.7%)	15 (22.7%)	7 (10.6%)	
	T3	185	54 (29.2%)	85 (45.9%)	46 (24.9%)		36 (19.5%)	55 (29.7%)	55 (29.7%)	39 (21.1%)	
	T4	52	8 (15.4%)	28 (53.8%)	16 (30.8%)		4 (7.7%)	10 (19.2%)	24 (46.2%)	14 (26.9%)	
N classification	N0	185	94 (50.8%)	66 (35.7%)	25 (13.5%)	<0.001	74 (40.0%)	52 (28.1%)	37 (20.0%)	22 (11.9%)	<0.001
	N1a	42	11 (26.2%)	22 (52.4%)	9 (21.4%)		8 (19.0%)	14 (33.4%)	12 (28.6%)	8 (19.0%)	
	N1b	33	9 (27.3%)	13 (39.4%)	11 (33.3%)		4 (12.1%)	7 (21.2%)	14 (42.4%)	8 (24.3%)	
	N2a	33	4 (12.1%)	21 (63.6%)	8 (24.3%)		3 (9.1%)	5 (15.1%)	19 (57.6%)	6 (18.2%)	
	N2b	37	3 (8.2%)	16 (43.2%)	18 (48.6%)		2 (5.4%)	6 (16.2%)	12 (32.4%)	17 (46.0%)	
Tumor size	≤4.5 cm	204	80 (39.2%)	95 (46.6%)	29 (14.2%)	<0.001	64 (31.4%)	51 (25.0%)	67 (32.8%)	22 (10.8%)	0.002
	>4.5 cm	126	41 (32.5%)	43 (34.1%)	42 (33.4%)		27 (21.4%)	33 (26.2%)	27 (21.4%)	39 (31.0%)	
LVI	negative	194	98 (50.5%)	73 (37.6%)	23 (11.9%)	<0.001	76 (39.2%)	56 (28.9%)	43 (22.1%)	19 (9.8%)	<0.001
	positive	136	23 (16.9%)	65 (47.8%)	48 (35.3%)		15 (11.0%)	28 (20.6%)	51 (37.5%)	42 (30.9%)	
Tumor location	Right	81	28 (34.6%)	29 (35.8%)	24 (29.6%)	0.125	21 (25.9%)	15 (18.6%)	24 (29.6%)	21 (25.9%)	0.175
	Left	100	42 (42.0%)	44 (44.0%)	14 (14.0%)		29 (29.0%)	32 (32.0%)	28 (28.0%)	11 (11.0%)	
	Rectum	149	51 (34.2%)	65 (43.6%)	33 (22.2%)		41 (27.5%)	37 (24.8%)	42 (28.2%)	29 (19.5%)	

Regarding the second part of our study lymph node grades were G1 in 77 (53.1%), G2 in 41 (28.3%), and G3 in 27 (18.6%) cases, respectively. Tumor grades of primary tumors and corresponding lymph node metastases were largely concordant: The Somer's D coefficients was D=0.639 (p<0.001). Data are presented in Table 4.

When lymph node grade was associated with different tumor characteristics, significant associations were noted for N classification, LVI, and tumor size. Data are presented in Table 5.

Table 4: Concordance between primary and corresponding lymph node grade

Primary tumor	**Lymph node metastasis**	
G1	G1	25 (92.6%)
27/145 (18.6%)	G2	2 (7.4%)
	G3	0 (0%)
G2	G1	50 (69.4%)
72/145 (49.7%)	G2	19 (26.4%)
	G3	3 (4.2%)
G3	G1	2 (4.3%)
46/145 (31.7%)	G2	20 (43.5%)
	G3	24 (52.2%)

Table 5: Correlation of lymph node grade with other pathological variables in AJCC/UICC stage III colorectal cancer

		N	Lymph node grade			p Value
			G1 (n = 77)	G2 (n = 41)	G3 (n = 27)	
T classification	T1	1	1 (100%)	0 (0%)	0 (0%)	0.52
	T2	13	9 (69.2%)	2 (15.4%)	2 (15.4%)	
	T3	92	51 (55.4%)	24 (26.1%)	17 (18.5%)	
	T4	39	16 (41%)	15 (38.5%)	8 (20.5%)	
N classification	N1a	42	28 (66.6%)	11 (26.2%)	3 (7.1%)	0.009
	N1b	33	19 (57.6%)	10 (30.3%)	4 (12.1%)	
	N2a	33	19 (57.6)	8 (24.2%)	6 (18.2%)	
	N2b	37	11 (29.7%)	12 (32.4%)	14 (37.8%)	
Tumor size	≤4.5 cm	81	49 (60.5%)	23 (28.4%)	9 (11.1%)	0.024
	>4.5 cm	64	28 (43.8%)	18 (28.1%)	18 (28.1%)	
LVI	negative	48	34 (70.8%)	11 (22.9%)	3 (6.3%)	0.004
	positive	97	43 (44.3%)	30 (30.9%)	24 (24.7%)	
Tumor location	Right	20	10 (50%)	7 (35%)	3 (15%)	0.88
	Left	66	36 (54.5%)	19 (28.8%)	11 (16.7%)	
	Rectum	59	31 (52.5%)	15 (25.4%)	13 (22%)	

Survival Analysis

Regarding the first part of our study, that is, the comparison between the traditional and the alternative tumor grade, follow-up data were available for 304 out of 330 (92.1%) patients. After a mean and median follow-up of 65 and 63 months, respectively, progressive disease was observed in 122 (40.1%) patients, including: 101 patients who died from cancer, 10 patients who currently are alive with metastatic disease, but also 6 patients who currently show no evidence of disease after metastasectomy, and finally 12 patients who initially presented in poor condition due to advanced disease and who died within 30 days from surgery. Mean time to progression was 15 months (median 7, range 0 - 87). 31 (10.2%) patients died from causes not related to CRC.

Both the traditional and the alternative tumor grade were significantly associated with progression-free and cancer-specific survival (each $p<0.001$; Figure 15). When analysis was restricted to tumors with AJCC/UICC stage II both parameters were no longer significant (data not shown).

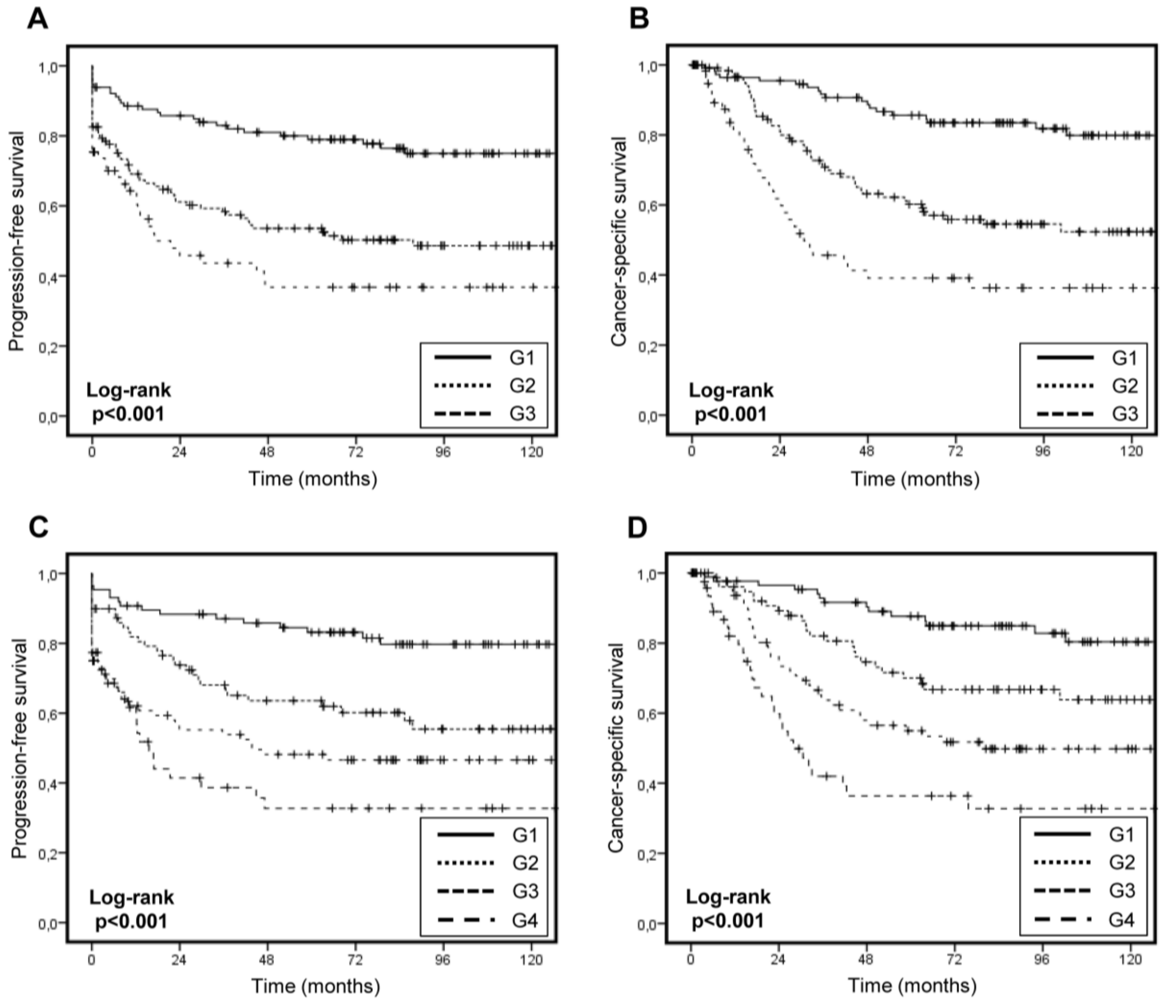

Figure 15: Progression-free (A, p<0.001; C, p<0.001) and cancer-specific (B, p<0.001; D, <0.001) survival related to the traditional (WHO) tumor grade (A, B) and the alternative tumor grade considering gland formation and cytological changes (C, D).

In a next step we compared the prognostic power of the traditional grade with that of the alternative grade in a multivariate analysis including both forms of tumor grade as variables. The results of these Cox's proportional hazards regression models indicate superiority of the alternative grading system, particularly with respect to the prediction of progression-free survival.

Regarding cancer-specific survival, the p Value of the alternative grade was clearly superior to that of the traditional tumor grade. However, statistical significance was not reached. Data are presented in Table 6.

Table 6: Cox's proportional hazards regression models comparing the prognostic significance of the traditional (WHO) and the alternative tumor grade, which considers both gland formation and cytological changes

		Hazard Ratio	**95% CI**	**p Value**
Progression-free survival	Traditional tumor grade (G1 vs. G2 vs. G3)	0.893	0.5-1.594	0.701
	Alternative tumor grade (G1 vs. G2 vs. G3 vs. G4)	1.799	1.184-2.734	0.006
Cancer-specific survival	Traditional tumor grade (G1 vs. G2 vs. G3)	1.422	0.768-2.263	0.263
	Alternative tumor grade (G1 vs. G2 vs. G3 vs. G4)	1.481	0.954-2.299	0.08

In the next step we evaluated whether the alternative grade represents an independent prognostic variable regarding progression-free and cancer-specific survivals. Our results applying the Cox' proportional hazards regression analysis prove the alternative grade to be a significant prognosticator with respect to cancer-specific survival (hazard ratio 1.37, 95% confidence interval 1.10-1.71; p=0.005), independent of patients' age and gender, T and N classification, and LVI. Likewise, patients with tumors with high alternative grade were more likely to develop disease progression (hazard ratio 1.22, 95% confidence interval 1.00-1.49), but this difference was not statistically significant (p=0.055). Data are presented in Table 7.

Table 7: Cox's proportional hazards regression models analyzing progression-free and cancer-specific survival of patients with colorectal cancer including alternative tumor grade

Progression-free survival				**Cancer-specific survival**			
	Hazard Ratio	95% CI	p Value		Hazard Ratio	95% CI	p Value
Age (≥70)	0.83	0.58-1.21	0.34	Age (≥70)	1.17	0.79-1.57	0.43
Female gender	1.27	0.87-1.84	0.22	Female gender	1.34	0.89-2.02	0.16
T3/T4 vs. T1/T2	2.01	1.09-3.71	0.026	T3/T4 vs. T1/T2	2.14	1.07-4.25	0.030
N1/2 vs. N0	3.18	2.00-5.06	<0.001	N1/2 vs. N0	3.16	1.92-5.10	<0.001
LVI + vs. LVI -	1.58	1.04-2.40	0.034	LVI + vs. LVI -	1.62	1.03-2.55	0.036
Alternative tumor grade (G4 vs. G3 vs. G2 vs. G1)	1.22	1.00-1.49	0.055	Alternative tumor grade (G4 vs. G3 vs. G2 vs. G1)	1.37	1.10-1.71	0.005

In the final step of this part of our study, we repeated the multivariate analysis now including not only the alternative grade but also the traditional grade. Results again indicate superiority of the alternative grade.

Specifically, we identified the alternative grade as independent prognostic variable regarding progression-free survival, but not regarding cancer-specific survival. For the traditional grade no independent impact on outcome was noted. Data are summarized in Table 8.

Table 8: Cox's proportional hazards regression models analyzing progression-free and cancer-specific survival of patients with colorectal cancer including both alternative and traditional tumor grade

Progression-Free Survival				Cancer-Specific Survival			
	Hazard Ratio	95% CI	p Value		Hazard Ratio	95% CI	p Value
Age (≥70)	0.84	0.58-1.21	0.34	Age (≥70)	1.17	0.79-1.75	0.43
Female gender	1.28	0.88-1.87	0.19	Female gender	1.34	0.89-2.01	0.16
T3/T4 vs. T1/T2	2.10	1.14-3.89	0.018	T3/T4 vs. T1/T2	2.11	1.06-4.22	0.035
N1/2 vs. N0	3.24	2.04-5.15	<0.001	N1/2 vs. N0	3.16	1.91-5.19	<0.001
LVI + vs. LVI -	1.64	1.07-2.50	0.023	LVI + vs. LVI -	1.03	1.03-2.54	0.038
Alternative tumor grade	1.57	1.04-2.35	0.031	Alternative tumor grade	1.30	0.85-2.00	0.224
Traditional tumor grade	0.67	0.38-1.18	0.161	Traditional tumor grade	1.09	0.60-1.97	0.778

In the second part of our study we focused on patients with node-positive tumors. Follow-up data were available for 128 out of 145 (88.3%) patients. After a mean and median follow-up of 45 and 31 months, respectively, progressive disease was observed in 85 (66.4%) patients: 70 patients who died from cancer, 6 patients who currently are alive with metastatic disease, but also 5 patients who currently show no evidence of disease after metastasectomy, and 4 patients who initially presented in poor condition due to advanced disease and who died within 30 days from surgery. Mean time to progression was 32 months (median 10, range 0 - 151). 5 (4.4%) patients died from causes not related to CRC.

Disease progression occurred in 42 out of 70 (54.5%) patients with lymph node grade 1 and 43 of 58 (63.2%) patients with lymph node grade 2/3 (p=0.031, log-rank test; Figure 16). Actuarial 5-year progression-free survival rates for patients with lymph node grade 1 or 2/3 were 39% and 24%, respectively. In addition, 32 out of 70 (45.7%) patients with lymph node grade 1 and 38 of 60 (63.3%) patients with lymph node grade 2/3 died of disease (p=0.008, log-rank test; Figure 16). Actuarial 5-year cancer-specific survival rates for patients with lymph node grade 1 or 2/3 were 54% and 33%, respectively. In contrast, primary tumor grade had no significant influence on both progression-free (p=0.21) and cancer-specific (p=0.07) survival.

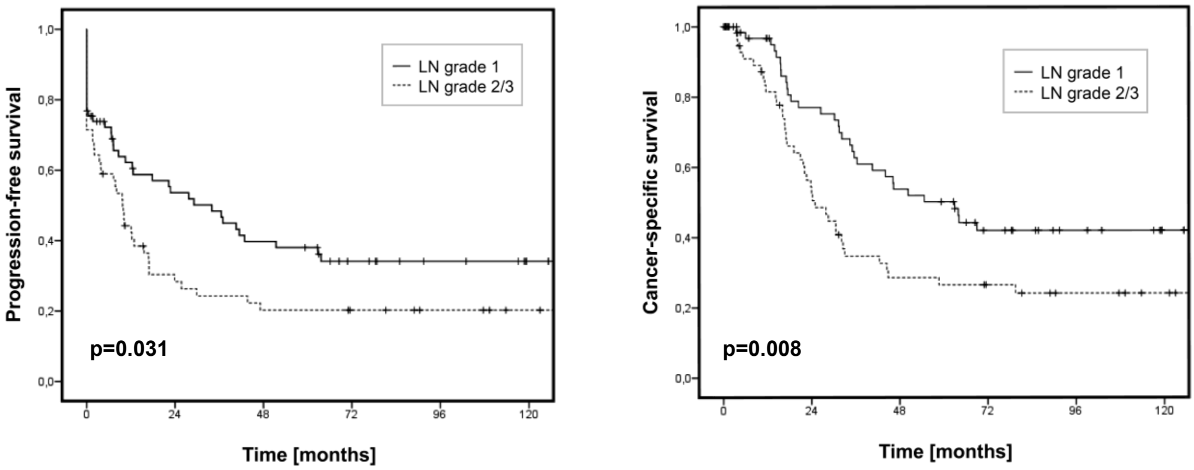

Figure 16: Progression-free (p=0.031) and cancer-specific (p=0.008) survival of patients with American Joint Committee on Cancer/Union for International Cancer Control stage III colorectal cancer related to lymph node grade.

To compare the prognostic power of the lymph node grad with that of the corresponding primary tumor grade, Cox's proportional hazards regression models were applied. The results of these multivariate analyses indicate superiority of lymph node grade in prognostication of patients with node-positive cancer, in particular regarding cancer-specific survival. Data are presented in Table 9.

Table 9: Cox's proportional hazards regression models comparing the prognostic significance of primary and lymph node grade in AJCC/UICC stage III colorectal cancer

		Hazard Ratio	**95% CI**	**p Value**
Disease-free survival	Primary tumor grade (G1 vs. G2/3)	0.84	0.45-1.53	0.55
	Lymph node grade (G1 vs. G2/3)	0.68	0.43-1.08	0.11
Cancer-specific survival	Primary tumor grade (G1 vs. G2/3)	0.69	0.34-1.39	0.31
	Lymph node grade (G1 vs. G2/3)	0.60	0.36-0.99	0.047

In addition, lymph node grade proved to be the only independent predictor of cancer-specific survival in multivariate analysis including patients' age and gender, T classification, lymph node grade and LVI. Thus our results suggest that patients with a higher lymph node grade are more likely to experience recurrent disease, while patients with a low lymph node grade are at lower risk and may not necessarily need adjuvant therapy. Data are presented in Table 10.

Table 10: Cox's proportional hazards regression models of patients with AJCC/UICC stage III colorectal cancer, analyzing progression-free and cancer-specific survival

	Progression-free survival			Cancer-specific survival		
	Hazard Ratio	95% CI	p Value	Hazard Ratio	95% CI	p Value
Age (>70)	1.04	0.67-1.63	0.86	1.54	0.95-2.49	0.079
Female gender	0.95	0.61-1.48	0.81	1.02	0.63-1.66	0.94
T3/T4 vs. T1/T2	1.44	0.61-3.42	0.41	1.51	0.60-3.84	0.39
LVI + vs. LVI -	1.19	0.72-1.97	0.49	1.16	0.68-1.98	0.60
Lymph node grade G1 vs. G2/3	0.71	0.44-1.12	0.14	0.55	0.33-0.90	0.018

Discussion

The steadily increasing number of new cancer cases worldwide represents a major health problem implementing a global burden on public health systems. Expecting that over the next few years cancer is going to be the leading cause of death in the United States (4) and CRC being the second to third leading cause of cancer related deaths, growing research activities in the field are urgently needed. (6)

Among other cancers, CRC occupies a special position since due to its well recognized multistep development over years (adenoma-carcinoma sequence) early detection is feasible and may result in curative resection of precursor lesions. Hence, colonoscopy is an integral part of population-based screening programs in many countries including Austria. Increasing awareness of the disease in the general population may additionally reduce risk factors and contribute to reduction of disease-related morbidity and mortality. (6,10)

In clinical practice, diagnosis and management of patients with CRC is a multidisciplinary assignment. Every patient needs to be treated according to her/his individual risk profile, which remains to be the most difficult challenge. (26) Five-year relative survival rates of 90.3%, 70.4%, and 12.5% have been calculated for patients with localized, regional and distant disease, respectively. (10)

The prognostication of CRC patients with AJCC/UICC stage III disease is a critical task since accurate risk assessment is pivotal for clinical decision-making and patient management. According to current guidelines, stage III patients usually receive adjuvant therapy; but only 30 to 50% develop distant metastasis within five years, mainly depending on the extent of regional lymph node spread. (23)

Hence, a significant number of patients with stage III disease may receive aggressive cancer treatment though they do not need it. This treatment, however, may cause severe side effects. This is of particular importance regarding our aging population, which, by nature, suffers from an increasing number of comorbidities. For these patients every additional treatment implements high risk of complication. In addition, unnecessary adjuvant therapies are costly, which has to be taken into consideration facing growing limitation of financial resources of public health care systems.

For this reason, the identification of prognostic parameters, that is, parameters associated with disease outcome, indicating either disease progression or, on the contrary, favorable outcome is a central aspect of current cancer research. The identification of such parameters or “biomarkers” might directly influence patient management, facilitating the identification of patients who would benefit from adjuvant treatment or, on the contrary, the identification of patients who do not need further therapy.

In this respect, the pathological evaluation of the resection specimen plays a central role. As indicated above, tumor grading represents one of the most traditional histological parameters in use for prognostication. (47–49) Still, the prognostic role of tumor grade appears to be less well defined than that of tumor stage.

This may be due to the fact that until today there is still no generally accepted consensus how to perform tumor grading. Thus, tumor grading in CRC is poorly standardized and prone to considerable interobserver variation, hampering the value of this marker. Most grading systems, including that of the current WHO classification (11), are based solely on the assessment of histological criteria, whereas cytological features, such as nuclear atypia are not considered. Despite the notion that gland-forming tumors may include low-grade and high-grade cytological atypia, the significance of this feature remains largely unclear.

In CRC, tumor grading does only apply for adenocarcinomas NOS. All other morphological variants carry their own prognostic significance and grading is usually not performed. (11) Despite all these limitations, the lack of standardization and completeness, tumor grade is generally regarded be a powerful, stage-independent prognostic factor (37).

Morson’s famous textbook of gastrointestinal pathology (50) refers to three different histological grades of malignancy, termed low, average and high. Specifically, adenocarcinomas are separated based upon the degree of differentiation of the tubules, the size and shape of cells and nuclei as well as the number of mitotic figures. Following this instruction, histological grade is related to tumor proliferation, presence of lymph node metastasis and survival.

The first edition of the World Health Organization (WHO) Classification of Intestinal Tumours, which was edited by Morson and Sobin in 1976 (51) also refers to three histological grades: well differentiated carcinomas are characterized by histological and cellular features that closely resemble normal epithelium of similar type, while poorly differentiated carcinomas show histological and cellular features that only barely resemble normal epithelium of similar type. Moderately differentiated carcinomas are “intermediate between well differentiated and poorly differentiated” tumors. Thus, again both histological and cytological features are considered, but tumors of intermediate grade are in fact not defined and no detailed manual for daily practice is provided.

In the second edition of the WHO classification dating from 1989 (52) the authors refer to four different tumor grades (well differentiated, moderately differentiated, poorly differentiated, undifferentiated), which are distinguished based upon the degree of cytological and architecture similarity to the presumed tissue of origin, as well as nuclear pleomorphism and mitotic activity. This classification is the first to consider intratumoral heterogeneity: When tumors show different grades of differentiation the higher grade should determine the final categorization.

In the third edition of the WHO classification dating from 2000 (53), again four grades are presented, which are now, however, solely based upon architectural criteria, as the authors believed that architectural criteria share lower interobserver variability than cytological criteria. In this classification, the percentage of the tumor showing formation of gland-like structures is used to define the grade: Well differentiated (grade 1) adenocarcinomas exhibit glandular structures in more than 95% of the tumor; moderately differentiated (grade 2) carcinomas have 50-95% glands, poorly differentiated (grade 3) carcinomas 5-50%, and undifferentiated (grade 4) carcinomas less than 5% glands, respectively. As in the previous edition grading should be based upon the least differentiated tumor component, not including the leading front of invasion. This scheme has been collapsed to a three-tiered system in the fourth edition (11), as described in detail above (Table 2).

It is of note that the assessment of differentiation is largely subjective, and CRCs have previously been shown to be markedly heterogeneous, with considerable impact on intra-/interobserver agreement (36,54,55). Nevertheless, studies assessing interobserver variation have rendered conflicting results, with some studies reporting only fair to moderate agreement (36,56), while others demonstrated more favorable agreement (56,57).

In a systematic web-based survey with 104 pathologists analyzing 20 cancer specimens, intraobserver agreement was substantial, while interobserver agreement was at best fair. However, in 17 of the 20 cases, over 90% of respondents were within one grade of each other, and in 15 of the 20 cases, more than 95% of respondents were within one grade of each other. (58) Interobserver agreement was not influenced by the type or complexity of the used grading system. Thus, kappa values were likewise unsatisfactory for both two-tiered and three-tiered systems. According to the authors of this survey, the main difficulty that pathologists face is separating moderately from poorly differentiated tumors. (58)

As indicated already above, intra/interobserver variation represents a central point of concern in diagnostic histopathology. The restriction to only three (G1 through G3) or two different grades (low-grade vs. high-grade) is a general principal that aims to reduce this variation. The current WHO guidelines go even further and refer only to histological features, as cytological features may be an additional source of diagnostic inaccuracy. (11) Disregarding cytological features, however, may reduce the prognostic power of tumor grading. This has been well recognized for endometrial cancer, (40) whereas the situation in gastrointestinal malignancies, in particular CRC, is largely unclear in this regard.

The project presented in this diploma thesis aimed to evaluate different tools to increase the prognostic significance of tumor grading. Specifically, this study had two parts.

In the first part, the alternative tumor grading based upon both histological features, that is, gland formation, as well as cytological features, such as nuclear pleomorphism and anaplasia, proved to be superior to grading based upon histological features alone. The alternative tumor grade was prognostic regarding cancer-specific survival independent of patients' age and gender, T and N classification, and LVI. Hence, our data suggest that a grading system that is comparable to current endometrial cancer grading (40) might improve the accuracy of prognostic stratification of patients with CRC. Validation of our finding by other independent centers is, however, inevitable.

In the second part of our study we investigated the lymph node grade. In fact, our study is the first to compare systematically the prognostic power of tumor grading in lymph node deposits compared with that in corresponding primary tumors. We were able to show that the lymph node grade is significantly associated with different primary tumor characteristics, such as N classification, tumor size, as well as, LVI. Survival analysis proved lymph node grade to be a significant novel prognostic parameter, which appeared superior to primary tumor grade. In addition, we were able to identify lymph node grade as the only significant predictor of cancer-specific survival in a multivariate analysis including patients' age, gender, T and N classification and LVI as diagnostic variables. Specifically, low lymph node grade may be used to identify those patients with AJCC/UICC stage III disease, who will experience comparably favorable outcome and may not necessarily need to receive adjuvant treatment. As comparable data regarding lymph node grade are not available in literature, independent validation of our findings is inevitable.

Our study has strengths and limitations. The investigated cohort of CRC patients is large, with a comparably long follow-up time. Moreover, this cohort is not selected, but represents a random sample of more than 7,500 CRC specimens diagnosed at the Institute of Pathology, Medical University of Graz, Austria. (13,41,42) It is of note that two experienced gastrointestinal pathologists performed review-pathology independently. However, several limitations deserve attention, which at least in part are inherent to all retrospective studies. By excluding patients with neoadjuvant chemotherapy, patients with synchronous or metachronous secondary CRC as well as patients with competitive invasive cancers originating from other sites if metastatic deposits were not assessed by histology we tried, however, to control the homogeneity of the study population. Nevertheless, the patients included in this study underwent surgical therapy by multiple surgeons from both academic and community settings. (13,41,42,59)

In conclusion, tumor grade is a well-established prognostic parameter, however with limitations regarding various definitions, that is, missing consensus how grading should be performed. We tried to refine the criteria of tumor grading in CRC and were able to show that the inclusion of cytological criteria in analysis significantly improves outcome prediction. Moreover, the lymph node grade offers an entirely novel tool for the risk stratification of patients with AJCC/UICC stage III disease and should be included routinely in the pathology report. Its application may significantly improve clinical decision-making and patient management.

References

1. United Nations Population Division. World Population Prospects, the 2008 Revision [Internet]. New York, United Nations. Available from: http://www.un.org/. 2008. Last accessed 10/30/2010.

2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.

3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin [Internet]. 2013;63:11–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23335087

4. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. CA Cancer J Clin. 2015;65(1):5–29.

5. Puppa G, Sonzogni A, Colombari R, Pelosi G. TNM staging system of colorectal carcinoma: A critical appraisal of challenging issues. Arch Pathol Lab Med. 2010;134(June):837–52.

6. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet [Internet]. Elsevier Ltd; 2014;383(9927):1490–502. Available from: http://dx.doi.org/10.1016/S0140-6736(13)61649-9

7. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010. p. 544–73.

8. Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2014. Ann Oncol. 2014;25(April):1650–6.

9. Malvezzi M, Bertuccio P, Rosso T, Rota M, Levi F, La Vecchia C, et al. European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women? Ann Oncol [Internet]. 2015; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25623049

10. Siegel R, Desantis C, Jemal A. Colorectal Cancer Statistics, 2014. CA Cancer J Clin. 2014;64:104–17.

11. Hamilton S, Bosman F, Boffetta P. Carcinoma of the colon and rectum. In: Bosman F, Carneiro F, Hruban R, Theise N, editors. World Health Organization Classification of Tumours of the Digestive System. Lyon: IARC Press; 2010. p. 131–81.

12. Xie L, Villeneuve PJ, Shaw A. Survival of patients diagnosed with either colorectal mucinous or non-mucinous adenocarcinoma: A population-based study in Canada. Int J Oncol. 2009;34:1109–15.

13. Langner C, Harbaum L, Pollheimer MJ, Kornprat P, Lindtner R a, Schlemmer A, et al. Mucinous differentiation in colorectal cancer--indicator of poor prognosis? [Internet]. Histopathology. 2012. p. 1060–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22348346

14. Stewart SL, Wike JM, Kato I, Lewis DR, Michaud F. A population-based study of colorectal cancer histology in the United States, 1998-2001. Cancer. 2006;107:1128–41.

15. Du W, Mah JTL, Lee J, Sankila R, Sankaranarayanan R, Chia KS. Incidence and Survival of Mucinous Adenocarcinoma of the Colorectum: A Population-Based Study from an Asian Country. Dis Colon Rectum. 2004;47:78–85.

16. Chew MH, Yeo SAE, Ng ZP, Lim KH, Koh PK, Ng KH, et al. Critical analysis of mucin and signet ring cell as prognostic factors in an Asian population of 2,764 sporadic colorectal cancers. Int J Colorectal Dis. 2010;25:1221–9.

17. Schneider NI, Langner C. Prognostic stratification of colorectal cancer patients: Current perspectives. Cancer Management and Research. 2014. p. 291–300.

18. Resch A, Schneider NI, Langner C. Pathological evaluation of colorectal cancer specimens: advanced and early lesions. Cesk Patol. 2015;51:12–22.

19. Hyngstrom JR, Hu C-Y, Xing Y, You YN, Feig BW, Skibber JM, et al. Clinicopathology and Outcomes for Mucinous and Signet Ring Colorectal Adenocarcinoma: Analysis from the National Cancer Data Base. Annals of Surgical Oncology. 2012. p. 2814–21.

20. Kang H, O'Connell JB, Maggard MA, Sack J, Ko CY. A 10-year outcomes evaluation of mucinous and signet-ring cell carcinoma of the colon and rectum. Dis Colon Rectum. 2005;48:1161–8.

21. Nitsche U, Zimmermann A, Späth C, Müller T, Maak M, Schuster T, et al. Mucinous and signet-ring cell colorectal cancers differ from classical adenocarcinomas in tumor biology and prognosis. Ann Surg [Internet]. 2013;258:775–82; discussion 782–3. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3888475&tool=pmcentrez&rendertype=abstract

22. Verdú M, Román R, Calvo M, Rodón N, García B, González M, et al. Clinicopathological and molecular characterization of colorectal micropapillary carcinoma. Mod Pathol. 2011;24:729–38.

23. Edge S, Byrd D, Compton C. AJCC Cancer Staging Manual. 7th ed. New York, Dordrecht, Heidelberg, London; 2010. 143-164 p.

24. Sobin S, Gospodarowicz M, Wittekind C. TNM Classification of Malignant Tumours. 7th ed. West Sussex: Wiley-Blackwell; 2010.

25. Wittekind C, Klimpfinger M, Sobin L. Kolon und Rektum. TNM-Atlas. 4th ed. Berlin, Heidelberg, New York: Springer; 2005. p. 101–13.

26. Schmoll HJ, Van cutsem E, Stein a., Valentini V, Glimelius B, Haustermans K, et al. Esmo consensus guidelines for management of patients with colon and rectal cancer. A personalized approach to clinical decision making. Ann Oncol. 2012;23:2479–516.

27. Washington MK. Colorectal carcinoma: Selected issues in pathologic examination and staging and determination of prognostic factors. Archives of Pathology and Laboratory Medicine. 2008. p. 1600–7.

28. Benson AB, Arnoletti JP, Bekaii-Saab T, Chan E, Chen Y-J, Choti M a, et al. Colon cancer. J Natl Compr Canc Netw [Internet]. 2011;9:1238–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22056656

29. Benson AB, Bekaii-Saab T, Chan E, Chen Y, Choti MA, Cooper HS, et al. Rectal cancer. J Natl Compr Canc Netw [Internet]. 2012;10:1528–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23221790

30. Benson AB, Bekaii-Saab T, Chan E, Chen YJ, Choti MA, Cooper HS, et al. Localized Colon Cancer, version 3.2013. JNCCN J Natl Compr Cancer Netw. 2013;11:519–28.

31. Resch A, Langner C. Lymph node staging in colorectal cancer: Old controversies and recent advances. World J Gastroenterol. 2013;19:8515–26.

32. Dotan E, Cohen SJ. Challenges in the management of stage II colon cancer. Seminars in Oncology. 2011. p. 511–20.

33. Sinicrope F a, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J, et al. Molecular Markers Identify Subtypes of Stage III Colon Cancer Associated With Patient Outcomes. Gastroenterology. 2014;

34. Phipps AI, Limburg PJ, Baron J a, Burnett-Hartman AN, Weisenberger DJ, Laird PW, et al. Association between molecular subtypes of colorectal cancer and patient survival. Gastroenterology. 2015;

35. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010;28:3219–26.

36. Quirke P, Dyson JE, Dixon MF, Bird CC, Joslin CA. Heterogeneity of colorectal adenocarcinomas evaluated by flow cytometry and histopathology. Br J Cancer. 1985;51:99–106.

37. Sagaert X. Prognostic biomarkers in colorectal cancer: Where do we stand? Virchows Archiv. 2014. p. 379–91.

38. Seppälä TT, Böhm JP, Friman M, Lahtinen L, Väyrynen VMJ, Liipo TKE, et al. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. Br J Cancer [Internet]. 2015;112:1966–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25973534

39. Turner J. Gastrointestinal Tract. In: Kumar V, Abbas A, Fausto N, Aster J, editors. Pathologic Basis of Disease. 8th ed. Philadelphia: Saunders Elsevier; 2010. p. 822–5.

40. Zaino A, Carinelli S, Ellenson L, Eng C, Katabuchi H, Konishi I, et al. Tumours of the uterine corpus: epithelial tumours and precursors. In: Kurman R, Carcangiu M, Herrington C, Young R, editors. World Health Organization Classification of Tumours of Female Reproductive Organs. Lyon: IARC Press; 2014. p. 121–54.

41. Betge J, Kornprat P, Pollheimer MJ, Lindtner RA, Schlemmer A, Rehak P, et al. Tumor Budding is an Independent Predictor of Outcome in AJCC/UICC Stage II Colorectal Cancer. Annals of Surgical Oncology. 2012. p. 3706–12.

42. Harbaum L, Pollheimer MJ, Kornprat P, Lindtner RA, Bokemeyer C, Langner C. Peritumoral eosinophils predict recurrence in colorectal cancer. Mod Pathol [Internet]. 2015;28:403–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25216222

43. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Goodman PJ, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. The New England journal of medicine. 1990.

44. Betge J, Schneider NI, Pollheimer MJ, Lindtner RA, Kornprat P, Schlemmer A, et al. Is there a rationale to record lymphatic invasion in node-positive colorectal cancer? Journal of Clinical Pathology. 2012. p. 847–50.

45. Betge J, Langner C. Vascular invasion, perineural invasion, and tumour budding: Predictors of outcome in colorectal cancer. Acta Gastro-Enterologica Belgica. 2011. p. 516–29.

46. Betge J, Langer C, Rehak P. Adjuvant chemotherapy improves survival in patients with American Joint Committee on Cancer stage II colon cancer. Cancer. 2012. p. 2184.

47. Grinnell RS. The grading and prognosis of carcinoma of the colon and rectum. Ann Surg. 1939;109:500–33.

48. Compton CC. Pathology report in colon cancer: What is prognostically important? Digestive Diseases. 1999. p. 67–79.

49. Barresi V, Reggiani Bonetti L, Ieni A, Caruso RA, Tuccari G. Histological grading in colorectal cancer: new insights and perspectives. Histol Histopathol [Internet]. 2015;11633. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26004398

50. Morson B, Dawson I, Spriggs A, Jones F. Gastrointestinal Pathology. 2nd ed. Oxford: Blackwell Scientific Publications; 1979.

51. Morson B, Sobin L. Histological Typing of Intestinal Tumours. Geneva: World Health Organization; 1976.

52. Jass J, Sobin L. World Health Organization, Histological Typing of Intestinal Tumours. Berlin, Heidelberg: Springer; 1989.

53. Hamilton S, Vogelstein B, Kudo S, Riboli E, Nakamura S, Hainaut P, et al. Carcinoma of the colon and rectum. In: Hamilton S, LA A, editors. Pathology and Genetics of Tumours of the Disgestive System. Lyon: IARC Press; 2000. p. 105–19.

54. Qualheim RE, Gall EA. Is histologic grading of colon carcinoma a valid procedure? A M A Arch Pathol. 1953;56:466–72.

55. Halvorsen TB, Seim E. Degree of differentiation in colorectal adenocarcinomas: a multivariate analysis of the influence on survival. J Clin Pathol. 1988;41:532–7.

56. Thomas GD, Dixon MF, Smeeton NC, Williams NS. Observer variation in the histological grading of rectal carcinoma. J Clin Pathol. 1983;36:385–91.

57. Vobecky J, Leduc CP, Devroede G, Madarnas P. The reliability of routine pathologic diagnosis of colorectal adenocarcinoma. Cancer. 1989;64:1261–5.

58. Chandler I, Houlston RS. Interobserver agreement in grading of colorectal cancers - Findings from a nationwide web-based survey of histopathologists. Histopathology. 2008;52:494–9.

59. Pollheimer MJ, Kornprat P, Pollheimer VS, Lindtner RA, Schlemmer A, Rehak P, et al. Clinical significance of pT sub-classification in surgical pathology of colorectal cancer. Int J Colorectal Dis. 2010;25:187–96.

Appendix

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Grading lymph node metastasis: a feasible approach for prognostication of patients with stage III colorectal cancer

Annika Resch,[1] Lars Harbaum,[2] Marion J Pollheimer,[1] Peter Kornprat,[3] Richard A Lindtner,[4] Cord Langner[1]

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[1]Institute of Pathology, Medical University of Graz, Graz, Austria
[2]Department of Oncology, Haematology, BMT with Section Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[3]Department of Surgery, Medical University of Graz, Graz, Austria
[4]Department of Surgery, Medical University of Innsbruck, Innsbruck, Austria

Correspondence to
Dr Cord Langner, Institute of Pathology, Medical University of Graz, Auenbruggerplatz 25, Graz A-8036, Austria; cord.langner@medunigraz.at

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ABSTRACT

This study aimed to assess the clinicopathological significance of tumour differentiation of metastatic lymph node tissue in patients with American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) stage III colorectal cancer. In a cohort of 145 patients, lymph node grades were G1 in 77 (53.1%), G2 in 41 (28.3%) and G3 in 27 (18.6%) cases, respectively. Despite differences in 77 (53.1%) cases, primary tumour and lymph node grade correlated significantly (Somer's D=0.639; p<0.001). Lymph node grade was significantly associated with N classification (p=0.009), tumour size (p=0.024) and lymphovascular invasion (p=0.004). Patients with lymph node grade G1 had better progression-free survival (p=0.031) and cancer-specific survival (p=0.008). Multivariable analysis identified lymph node grade as independent predictor of cancer-specific survival in this cohort. In conclusion, lymph node grade emerged as a promising novel prognostic variable for patients with AJCC/UICC stage III disease. Additional studies are warranted to validate this new finding.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide implicating a high rate of cancer-related deaths.[1] Outcome prediction based on tumour stage reflected by the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) tumour, node, metastases (TNM) system is currently regarded as the strongest prognostic parameter.[2] The evaluation of lymph nodes is of eminent importance in this process, since, to a great extent, the indication for adjuvant therapy is determined by the presence of positive nodes.[3] In the last decade, several studies attempted to refine lymph node staging in node-positive (AJCC/UICC stage III) CRC, and the number of involved nodes, the lymph node ratio and the presence of extracapsular invasion were identified as additional factors indicating adverse outcome.[4]

The differentiation of metastatic tumour tissue within regional lymph nodes, that is, the lymph node grade has never been systematically analysed, and its prognostic significance, in particular compared with that of the traditional primary tumour grade, is unclear. The present analysis aims to assess the role of lymph node grade in regard to its association with different histological parameters and prognosis in patients with AJCC/UICC stage III CRC.

PATIENTS AND METHODS

Patient selection and follow-up

Patients with node-positive adenocarcinomas were recruited from our previous study analysing the prognostic significance of lymphatic invasion in node-positive CRC.[5] In brief, the following patients had been excluded: (1) patients with rectal cancer who underwent neoadjuvant chemotherapy due to presumptive treatment-related changes in tumour classification and grading; (2) patients with synchronous or metachronous secondary CRC and (3) patients with competitive invasive cancers originating from other sites if metastatic deposits were not assessed by histology. The study cohort comprised 92 men (63%) and 53 women (37%) (ratio 1.7:1) with a mean age of 68.8 (median 68.4, range 40.4–92.3) years. All patients were given 5-fluorouracil/folinic acid according to the Mayo Clinic regimen.[6] Patients with rectal cancer received adjuvant radiotherapy.

Follow-up included laboratory testing at 3-month intervals for the first 3 years and 6-month intervals thereafter. Chest X-ray and abdominal ultrasound were performed at 6-month intervals for the first 3 years and yearly thereafter. Patients with rectal cancer underwent pelvic CT every 12 months. Patients were followed after resection until death or time of last follow-up. Disease progression was defined as local tumour recurrence or development of distant metastasis.

Institutional Review Board approval was obtained from the Ethics Committee of the Medial University of Graz, Austria.

Pathological evaluation

All histological slides were independently re-evaluated by two investigators (MJP and CL) who were blinded to clinical data. Discrepancies which occurred in 18 cases (12%) were resolved by simultaneous re-examination of the slides using a double-headed microscope.

Tumours located in the caecum to transverse colon were defined as right-sided cancers, and tumours located in the left colonic flexure to rectosigmoid junction as left-sided cancers. Tumour stage was assessed according to the AJCC/UICC 2009 issue of the TNM classification.[7] Grading was performed separately for primary tumours and corresponding lymph node metastases, according to the WHO guidelines, assessing the extent of glandular appearance.[8] Lymphovascular invasion (LVI), including both venous and lymphatic invasion, was considered positive when tumour cells or tumour cell thrombi were observed within an endothelium-lined space.[9]

Table 1 Association between primary and corresponding lymph node grade

		Lymph node metastasis		
		G1	G2	G3
Primary tumour				
G1	27/145 (18.6%)	25 (92.6%)	2 (7.4%)	0 (0%)
G2	72/145 (49.7%)	50 (69.4%)	19 (26.4%)	3 (4.2%)
G3	46/145 (31.7%)	2 (4.3%)	20 (43.5%)	24 (52.2%)

Statistical analysis

Associations between lymph node grade and T and N classification, LVI, tumour size and location were analysed using the χ^2 test. To assess concordance between primary and lymph node grade, the Somer's D rank-order correlation coefficient was used (the higher the D value (range −1 to 1) the more concordant pairs exist). Cause of death was determined by attending physicians and/or by chart review and was corroborated by death certificates if available. Progression-free (disease-free) and cancer-specific survival was investigated using the Kaplan–Meier method and compared by the log-rank test. For multivariable testing a Cox's proportional hazards regression model was performed.[10] Proportional hazard assumption was evaluated graphically with log-minus-log graphs applying the survival function versus survival time for each variable. All reported test were two-sided and p values were significant at levels <0.05. All statistical analyses were performed using SPSS statistics V.20 (IBM, Armonk, New York, USA).

RESULTS

Tumour characteristics

Lymph node grades were G1 in 77 (53.1%), G2 in 41 (28.3%) and G3 in 27 (18.6%) cases. Primary tumour and lymph node grade differed in 77 (53.1%) cases; Somer's D coefficient was D=0.639 (p<0.001; table 1). Lymph node grade was significantly associated with N classification, LVI and tumour size (table 2).

Survival analysis

Follow-up data were available for 128 of 145 (88.3%) patients. After a mean and median follow-up of 45 and 31 (quartile range 14–70) months, progressive disease was observed in 85 (66%) patients: 70 patients who died from cancer, six patients who currently are alive with metastatic disease, but also five patients who currently show no evidence of disease after metastasectomy, and four patients who initially presented in poor condition due to advanced disease and who died within 30 days of surgery. Specifically, 68 patients demonstrated systemic disease progression, 13 patients local disease progression and four patients both systemic and local disease progression. Systemic progression occurred to the liver in 51 patients, to the lungs in 10 patients, to the peritoneum in 10 patients and to both liver and lungs in one patient, respectively. Mean time to progression was 32 months (median 10, IQR 0–62). Five (4%) patients died from causes not related to CRC.

Disease progression occurred in 42 of 70 (54.5%) patients with lymph node grade 1 and 43 of 58 (63.2%) patients with lymph node grade 2/3 (p=0.031, log-rank test; figure 1A). Actuarial 5-year progression-free survival rates for patients with lymph node grade 1 or 2/3 were 39% and 24%, respectively. In addition, 32 of 70 (45.7%) patients with lymph node grade 1 and 38 of 60 (63.3%) patients with lymph node grade 2/3 died of disease (p=0.008, log-rank test; figure 1B). Actuarial 5-year cancer-specific survival rates for patients with lymph node grade 1 or 2/3 were 54% and 33%, respectively. In contrast, primary tumour grade had no significant influence

Table 2 Association of lymph node grade with other pathological variables in AJCC/UICC stage III colorectal cancer (analysed using the χ^2 test)

		Lymph node grade			
	n	G1 (n=77)	G2 (n=41)	G3 (n=27)	p Value
T classification					
T1	1	1 (100%)	0 (0%)	0 (0%)	0.520
T2	13	9 (69.2%)	2 (15.4%)	2 (15.4%)	
T3	92	51 (55.4%)	24 (26.1%)	17 (18.5%)	
T4	39	16 (41%)	15 (38.5%)	8 (20.5%)	
N classification					
N1a	42	28 (66.6%)	11 (26.2%)	3 (7.1%)	0.009
N1b	33	19 (57.6%)	10 (30.3%)	4 (12.1%)	
N2a	33	19 (57.6)	8 (24.2%)	6 (18.2%)	
N2b	37	11 (29.7%)	12 (32.4%)	14 (37.8%)	
Tumour size					
≤4.5 cm	81	49 (60.5%)	23 (28.4%)	9 (11.1%)	0.024
>4.5 cm	64	28 (43.8%)	18 (28.1%)	18 (28.1%)	
LVI					
Negative	48	34 (70.8%)	11 (22.9%)	3 (6.3%)	0.004
Positive	97	43 (44.3%)	30 (30.9%)	24 (24.7%)	
Tumour location					
Right	20	10 (50%)	7 (35%)	3 (15%)	0.877
Left	66	36 (54.5%)	19 (28.8%)	11 (16.7%)	
Rectum	59	31 (52.5%)	15 (25.4%)	13 (22%)	

AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control; LVI, lymphovascular invasion.

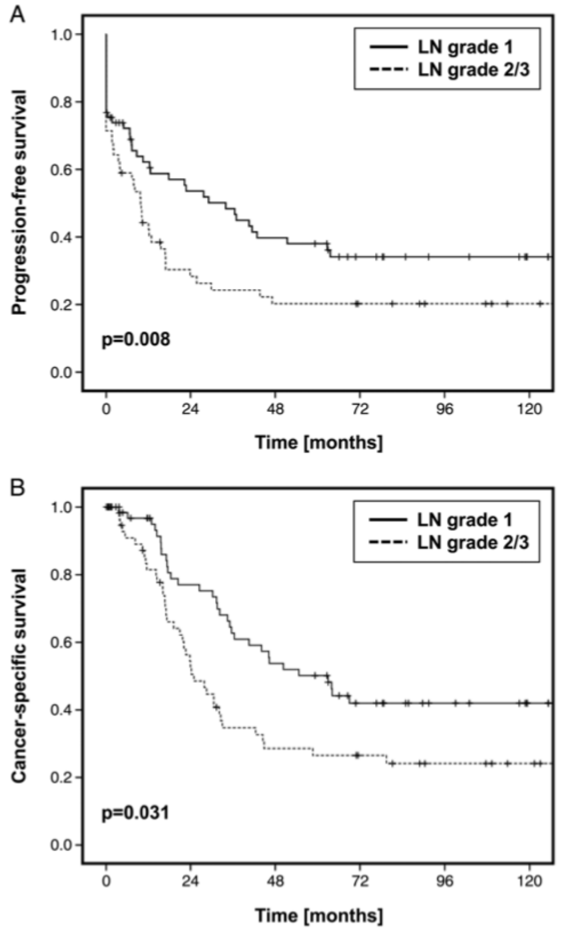

Figure 1 Progression-free (A) p=0.031 and cancer-specific (B) p=0.008 survival of patients with American Joint Committee on Cancer/Union for International Cancer Control stage III colorectal cancer related to lymph node (LN) grade.

on both progression-free (p=0.214) and cancer-specific (p=0.071) survival.

To compare the prognostic yield of lymph node and primary tumour grade, Cox's proportional hazards regression models were applied, which indicated superiority of lymph node grade in prognostication of patients with node-positive cancer (table 3). Finally, lymph node grade proved to be an independent predictor of cancer-specific survival in multivariable analysis including T classification, lymph node grade, LVI as well as patient age and gender (table 4).

DISCUSSION

Patients with AJCC/UICC stage III CRC usually receive adjuvant therapy; but only 30% to 50% develop distant metastasis within 5 years, mainly depending on the extent of regional lymph node spread.[11] Five-year relative survival rates of 90.3%, 70.4% and 12.5% have been calculated for patients with localised, regional and distant disease.[12] The identification of parameters, which are associated with disease progression or, on the contrary, favourable outcome is an important aspect of current cancer research, since these parameters might influence patient management, in particular guide therapeutic decisions.

In CRC, tumour grading appears to be poorly standardised and prone to considerable interobserver variation. Despite these limitations histological grade has repeatedly been demonstrated to be a stage-independent prognostic parameter.[13] Interestingly, tumour grade has so far only been assessed in primary tumour tissues, but not in metastatic lymph node deposits.

According to our data, lymph node grade is significantly associated with different primary tumour characteristics. Survival analysis proved lymph node grade to be a significant novel prognostic parameter, which appeared superior to primary tumour grade. In addition, we were able to identify lymph node grade as independent outcome predictor regarding cancer-specific survival in this cohort. Specifically, low lymph node grade may be used to identify those patients with AJCC/UICC stage III disease, who will experience comparably favourable outcome.

Our study has strengths and limitations. Review pathology was performed independently by two experienced gastrointestinal pathologists. The investigated cohort of node-positive CRCs is small, but it has a comparably long follow-up time. By excluding patients with neoadjuvant chemotherapy, patients with synchronous or metachronous secondary CRC as well as patients with competitive invasive cancers originating from other sites if metastatic deposits were not assessed by histology, we tried to control the homogeneity of the study population. Nevertheless, the patients included in this study underwent surgical therapy by multiple surgeons from both academic and community settings.[14]

In conclusion, lymph node grade emerged as a novel prognosticator in CRC and should be included routinely in the pathology report. Additional studies are warranted to validate this new finding.

Table 3 Cox's proportional hazards regression models comparing the prognostic significance of primary and lymph node grade in AJCC/UICC stage III colorectal cancer

	Unadjusted			Adjusted		
	HR	95% CI	p Value	HR	95% CI	p Value
Progression-free survival						
Primary tumour grade (G1 vs G2/3)	0.71	0.41 to 1.25	0.238	0.84	0.45 to 1.53	0.553
Lymph node grade (G1 vs G2/3)	0.64	0.42 to 0.99	0.043	0.68	0.43 to 1.08	0.105
Cancer-specific survival						
Primary tumour grade (G1 vs G2/3)	0.56	0.29 to 1.06	0.075	0.69	0.34 to 1.39	0.305
Lymph node grade (G1 vs G2/3)	0.53	0.33 to 0.85	0.009	0.60	0.36 to 0.99	0.047

The adjusted analyses include both given parameters.AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control.

Table 4 Cox's proportional hazards regression models of patients with AJCC/UICC stage III colorectal cancer, analysing progression-free and cancer-specific survival

	Progression-free survival						Cancer-specific survival					
	Unadjusted			Adjusted*			Unadjusted			Adjusted*		
	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
Age (>70 years)	1.01	0.66 to 1.55	0.958	1.04	0.67 to 1.63	0.864	1.37	0.86 to 2.17	0.185	1.54	0.95 to 2.49	0.079
Female gender	1.01	0.65 to 1.57	0.953	0.95	0.61 to 1.48	0.810	1.11	0.69 to 1.78	0.669	1.02	0.63 to 1.66	0.937
T3/T4 vs T1/T2	1.49	0.65 to 3.43	0.342	1.44	0.61 to 3.42	0.407	1.62	0.65 to 4.02	0.299	1.51	0.60 to 3.84	0.385
LVI+ vs LVI−	1.44	0.91 to 2.29	0.124	1.19	0.72 to 1.97	0.489	1.5	0.91 to 2.49	0.112	1.16	0.68 to 1.98	0.599
Lymph node grade G1 vs G2/3	0.64	0.41 to 0.98	0.043	0.71	0.44 to 1.12	0.140	0.53	0.33 to 0.85	0.009	0.55	0.33 to 0.90	0.018

*The adjusted analyses include all given parameters.AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control; LVI, lymphovascular invasion.

Take-home messages

- Lymph node grade is significantly associated with N classification, tumour size and the presence of lymphovascular invasion.
- Low lymph node grade is significantly associated with favourable progression-free survival and cancer-specific survival and appears to be superior to low primary tumour grade.
- Multivariable analysis proved lymph node grade to be the only independent predictor of cancer-specific survival.

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REFERENCES

1 Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet* 2014;383:1490–502.
2 Washington MK. Colorectal carcinoma: selected issues in pathologic examination and staging and determination of prognostic factors. *Arch Pathol Lab Med* 2008;32:1600–7.
3 Benson AB, Bekaii-Saab T, Chan E, *et al*. National Comprehensive Cancer Network Localized colon cancer, version 3.2013: featured updates to the NCCN Guidelines. *J Natl Compr Canc Netw* 2013;11:519–28.
4 Resch A, Langner C. Lymph node staging in colorectal cancer: old controversies and recent advances. *World J Gastroenterol* 2013;19:8515–26.
5 Betge J, Schneider NI, Pollheimer MJ, *et al*. Is there a rationale to record lymphatic invasion in node-positive colorectal cancer? *J Clin Pathol* 2012;65:847–50.
6 Moertel CG, Fleming TR, Macdonald JS, *et al*. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *New Engl J Med* 1990;322:352–8.
7 Sobin LH, Gospodarowicz MK, Wittekind C. eds. *TNM classification of malignant tumors*. 7th edn. New York: Wiley-Liss Inc, 2009:100–5.
8 Hamilton SR, Bosman FT, Boffetta P. Carcinoma of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. *World Health Organization classification of tumours of the digestive system*. Lyon: IARC Press, 2010:131–81.
9 Betge J, Langner C. Vascular invasion, perineural invasion, and tumour budding: predictors of outcome in colorectal cancer. *Acta Gastroenterol Belg* 2011;74:516–29.
10 Langner C, Harbaum L, Pollheimer MJ, *et al*. Mucinous differentiation in colorectal cancer—indicator of poor prognosis? *Histopathology* 2012;60:1060–72.
11 Edge SB, Byrd DR, Compton CC, *et al*. eds. *AJCC cancer staging manual*. 7th edn. New York, Dordrecht, Heidelberg, London: Springer, 2010:143–64.
12 Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014;64:104–17.
13 Sagaert X. Prognostic biomarkers in colorectal cancer: where do we stand? *Virchows Arch* 2014;464:379–91.
14 Pollheimer MJ, Kornprat P, Pollheimer VS, *et al*. Clinical significance of pT sub-classification in surgical pathology of colorectal cancer. *Int J Colorectal Dis* 2010;25:187–96.