# Reproducibility of Colon Tumor Grade and Relationship to Recurrence in the Context of Clinical, Pathologic, and Genomic Tumor Features in 504 Stage II Colon Cancer Patients Treated With Surgery Alone at the Cleveland Clinic

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## Background

- High tumor grade is included in practice guidelines as a marker of higher recurrence risk in stage II colon cancer.[1]
- Published studies of the prognostic power of tumor grade in colon cancer have reported variable findings.
  - Recent large studies, including QUASAR (n=711),[2] PETACC-3 (n=420),[3] and studies from the NSABP and Cleveland Clinic (n=1,007),[4] have consistently found that high tumor grade is not associated with higher recurrence risk in stage II colon cancer.
- An added challenge is the existence of multiple systems for colon tumor grading, without a standardized approach.[5]
  - Standardized, reproducible assays are needed for decision-making in clinical practice.
  - The 12-gene colon cancer Recurrence Score® assay, as an example, is a standardized, clinically validated assay which has been analytically validated for reproducibility and precision.[6]
- There is little data regarding tumor grade inter-reader reproducibility.[7-9]

## Study Objectives

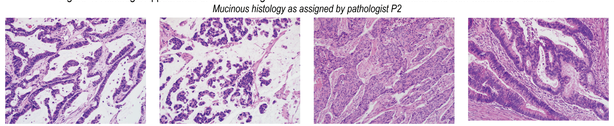
- Characterize the agreement of two methods for tumor grading and association with recurrence in the context of:
  - clinical and pathological covariates such as mismatch-repair (MMR), mucinous histology and tumor location.
  - the 12-gene colon cancer Recurrence Score (RS) assay, previously validated in stage II colon cancer patients from QUASAR.[2]

## Study Design and Methods

- Tumors from 504 stage II colon cancer patients treated with surgery alone at the Cleveland Clinic were graded independently by two academic gastrointestinal pathologists (P1 and P2) who employed the grading methods used in their colon cancer clinical practice.
- For both pathologists, grade was defined by percent tumor with gland-like structures: well (>95%), moderately (50-95%) and poorly (<50%) differentiated.
  - P1 used a three-tier system while P2 used a two-tier system with well and moderately differentiated tumors defined as low grade and poorly differentiated as high grade; for analysis purposes, well and moderately differentiated tumors by P1 were defined as low grade and poorly differentiated tumors were defined as high grade.
  - All mucinous tumors were considered high grade by P2 but not by P1.
- MMR status was assessed by immunohistochemistry (IHC) for hMLH1 and hMSH2 using two 5 µm sections on glass slides.
  - The IHC testing was conducted by the Cleveland Clinic Foundation Department of Pathology using antibody clones MSH2 (FE-11) and MLH-1 (G168-15) from Biocare Medical (2940 Camino Diablo, Suite 300 Walnut Creek, CA 94597).
- Gene expression was quantitated by RT-PCR from 30 µm of manually microdissected, fixed, paraffin-embedded primary colon cancer tissue to obtain the 12-gene RS.

Figure 1: Histologic Appearance of Low and High Grade Colon Tumors with Mucinous and Non-Mucinous Features

*Mucinous histology as assigned by pathologist P2*



Colon Carcinoma, Low Grade, Mucinous Histology | Colon Carcinoma, High Grade, Mucinous Histology | Colon Carcinoma, Low Grade, Non-Mucinous Histology | Colon Carcinoma, High Grade, Non-Mucinous Histology

## Analysis Methods

- Associations between grade and MMR status, tumor location and mucinous histology were assessed using chi-square tests.
- Association between grade and risk of recurrence was assessed using:
  - univariate Cox proportional hazards regression[10]
  - multivariate Cox regression including grade and the 12-gene RS
  - multivariate Cox regression including RS, MMR status, tumor location and mucinous histology
  - definition of the endpoint: time (in years) from surgery to first colon cancer recurrence or death due to recurrence of colon cancer
- Using the two-tier scheme, agreement between grade assessments by two pathologists was assessed using Cohen's kappa statistic.[11]

## Results

Figure 2. Distribution of Tumor Grade

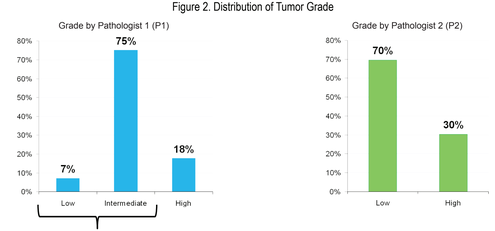



Figure 3. Distribution of Pathologic Markers: P1 - High vs. Low Grade

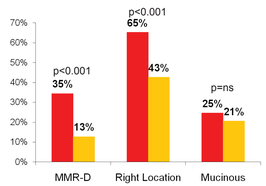
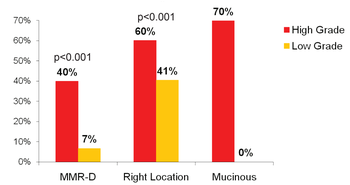



Figure 4. Distribution of Pathologic Markers: P2 - High vs. Low Grade




*Y-axis: percentage of patients with high (or low) grade, as indicated, with the tumor characteristics shown on the x-axis. Mucinous histology was assessed by P2 only.*

- High grade tumors were more likely to be MMR-deficient and right-sided compared to low grade tumors for both P1 and P2.
- The proportion of mucinous tumors was similar for high and low grade by P1 (P2 assigned all mucinous tumors to high grade).

### *Association of Grade with Recurrence Risk in Univariate Analyses*

Table 1: Univariate Analyses of Grade

| Grade | HR | HR 95% CI | P value |
|---|---|---|---|
| P1 Grade: High vs. Low | 0.78 | (0.40, 1.53) | 0.46 |
| P2 Grade: High vs. Low | 0.63 | (0.36, 1.12) | 0.099 |

- In univariate analyses, P1 grade was not associated with risk of recurrence, while P2 high grade trended to be associated with lower recurrence.
- Difference in HRs appeared to be relatively small and confidence limits overlapped substantially.

### *Association of Grade with Recurrence Risk in Conjunction with Pathologic Markers*

Table 2: Multivariate Analysis of P1 Grade and Other Pathologic Markers

| Variable | HR | HR 95% CI | P value |
|---|---|---|---|
| P1 Grade: High vs. Low | 0.84 | (0.42, 1.68) | 0.62 |
| MMR-D vs. MMR-P | 0.59 | (0.26, 1.34) | 0.18 |
| Mucinous Tumor | 0.70 | (0.36, 1.40) | 0.30 |
| Tumor Location (Right vs. Other) | 1.24 | (0.75, 2.06) | 0.40 |

Table 3: Multivariate Analysis of P2 Grade and Other Pathologic Markers

| Variable | HR | HR 95% CI | P value |
|---|---|---|---|
| P2 Grade: High vs. Low | 0.72 | (0.25, 2.12) | 0.54 |
| MMR-D vs. MMR-P | 0.62 | (0.26, 1.46) | 0.26 |
| Mucinous Tumor | 0.93 | (0.29, 3.02) | 0.91 |
| Tumor Location (Right vs. Other) | 1.25 | (0.75, 2.06) | 0.39 |

- Neither grade by P1 or P2 was associated with risk of recurrence after controlling for MMR status, tumor location, and mucinous histology or by controlling for MMR status and tumor location only (results not shown).

### *Association of Grade with Recurrence Risk in Conjunction with 12-gene Recurrence Score*

Table 4: Multivariate Analysis of P1 Grade and RS

| Grade | HR | HR 95% CI | P value |
|---|---|---|---|
| P1 Grade: High vs. Low | 0.71 | (0.36, 1.39) | 0.30 |
| RS per 25 units | 2.59 | (1.64, 4.07) | <0.001 |

Table 5: Multivariate Analysis of P2 Grade and RS

| Grade | HR | HR 95% CI | P value |
|---|---|---|---|
| P2 Grade: High vs. Low | 0.46 | (0.26, 0.84) | 0.007 |
| RS per 25 units | 2.80 | (1.81, 4.34) | <0.001 |

- In multivariate analyses including grade and RS, P1 grade was not associated with risk of recurrence while P2 high grade appeared to be associated with lower recurrence.
- No evidence of interaction between either P1 or P2 grade and RS was observed (both p>0.26).
- Confidence limits for HRs for P1 and P2 grade overlapped substantially.

### *Association of Grade with Recurrence Risk in Conjunction with Recurrence Score and Pathologic Markers*

Table 6: Multivariate Analysis of P1 Grade, Other Pathologic Markers and RS

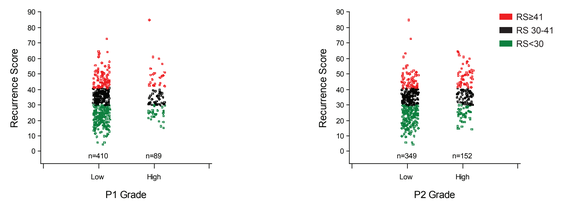
| Variable | HR | HR 95% CI | P value |
|---|---|---|---|
| P1 Grade: High vs. Low | 0.72 | (0.36, 1.44) | 0.34 |
| MMR-D vs. MMR-P | 0.72 | (0.31, 1.64) | 0.41 |
| Mucinous Tumor | 0.44 | (0.21, 0.90) | 0.015 |
| Tumor Location (Right vs. Other) | 1.21 | (0.73, 2.00) | 0.46 |
| RS per 25 units | 2.96 | (1.85, 4.73) | <0.001 |

Table 7: Multivariate Analysis of P2 Grade, Other Pathologic Markers and RS

| Variable | HR | HR 95% CI | P value |
|---|---|---|---|
| P2 Grade: High vs. Low | 0.64 | (0.22, 1.87) | 0.39 |
| MMR-D vs. MMR-P | 0.76 | (0.32, 1.79) | 0.52 |
| Mucinous Tumor | 0.65 | (0.20, 2.16) | 0.49 |
| Tumor Location (Right vs. Other) | 1.21 | (0.73, 2.00) | 0.46 |
| RS per 25 units | 2.86 | (1.81, 4.50) | <0.001 |

- Neither grade by P1 or P2 was associated with risk of recurrence after controlling for RS, MMR status, tumor location and mucinous histology.
- In a multivariate model controlling for RS, MMR status, and tumor location only, high grade by P2 was associated with lower risk of recurrence (HR=0.47, 95% CI 0.24-0.89, p=0.015) while P1 grade was not associated with recurrence risk (p=0.36).

Figure 5. Distribution of RS Values by Tumor Grade




- A wide range of RS values was observed for high and low grade tumors by either pathologist, including a substantial proportion of patients with high Recurrence Score disease (RS ≥ 41) with either high or low grade tumors.
- Individual RS values cannot be predicted from tumor grade.

Table 8: Agreement Between Two Pathologists

All patients

| | P1 Grade: Low | P1 Grade: High | P1 Grade: Total |
|---|---|---|---|
| P2 Grade: Low | 315 | 34 | 349 |
| P2 Grade: High | 98 | 55 | 153 |
| P2 Grade: Total | 413 | 89 | 502 |

Kappa=0.30, 95% CI (0.21, 0.39)

No mucinous tumors

| | P1 Grade: Low | P1 Grade: High | P1 Grade: Total |
|---|---|---|---|
| P2 Grade: Low | 315 | 34 | 349 |
| P2 Grade: High | 13 | 33 | 46 |
| P2 Grade: Total | 328 | 67 | 395 |

Kappa=0.52, 95% CI (0.40, 0.64)

- Tumor Grade: Using the two-tier scheme, agreement between the two pathologists was low in all patients and moderate if mucinous tumors were excluded.

## Strengths and Limitations

Strengths
- A large dataset of stage II colon cancer patients treated with surgery alone
- Central grade assessments by two academic pathologists with specialization in GI cancers
- 12-gene Recurrence Score assessment using established, reproducible RT-PCR platform
- IHC testing for MMR status was performed centrally by a single laboratory (Cleveland Clinic Department of Pathology)

Limitations
- Exploratory analysis in studies designed for development of the RS
- Method of grading is confounded with pathologist effect
  - Different grading of mucinous tumors by P1 and P2

## Conclusions

- High tumor grade was not found to be a marker of higher recurrence risk in stage II colon cancer by either of two pathologists using their methods used for clinical practice.
- Contrary to conventional expectations, but consistent with other reported studies, high grade was associated in some circumstances with a lower risk of recurrence in stage II colon cancer.
- For the stage II colon cancer patient, recurrence risk should be assessed using T stage, MMR status, and RS, the three key predictors of recurrence risk in stage II colon cancer, as reported in the QUASAR validation study.
- Inter-pathologist agreement on colon tumor grade is modest overall in this study, and moderate after excluding mucinous cases, even with central expert review.

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