Technical Report - Analysis

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Introduction

The paper shows that although colon cancer is a significant cause of mortality worldwide, stage III patients benefit from adjuvant therapy. This technical report analyzes the effectiveness of different therapy approaches with three groups: observation only, levamisole alone, or fluorouracil plus levamisole. Using the 'colon' dataset from the R survival package, we employed multiple biostatistical models to evaluate the outcomes while accounting for the confounders.

Besides, this analysis selects the best model from an adjusted cox model, an unadjusted cox model, and a cox model with LASSO to provide robust evidence of treatment efficacy and identify key prognostic factors that influence survival outcomes.

Methods

Data Preparation

The analysis utilized data from stage III colon cancer patients, with a dataset containing 16 variables. Patients were randomized to one of three treatment arms: observation (control), levamisole monotherapy, or combination therapy with fluorouracil plus levamisole. In the data preparation, for our primary interest, we first filtered the dataset to include only death events (etype = 2). Then for clinical validity, the number of positive lymph nodes was recorded from nodes = 0 to nodes = 1. Finally, we excluded observations with missing data to ensure the reliability.

RStudio: Notebook Output

A tibble: 20 × 4

Variable	Obs	Lev	Lev+5FU
<chr>	<chr>	<chr>	<chr>
Male_n	161	164	135
Male_Percent	52.79	55.78	46.71
Female_n	144	130	154
Female_Percent	47.21	44.22	53.29
Age_Mean_SD	59.47 (12.05)	60.09 (11.74)	59.9 (11.99)
Obstruct_Yes_Percent	20.33	19.73	17.65
Perfor_Yes_Percent	2.95	3.4	2.77
Adhere_Yes_Percent	14.75	15.31	13.15
Nodes_Mean_SD	3.84 (3.75)	3.71 (3.6)	3.44 (3.23)
Time_Mean_SD	1599.68 (855.81)	1625.09 (894.23)	1804.52 (858.38)
Differ_Well_Percent	8.85	12.59	9
Differ_Moderate_Percent	74.1	73.13	73.36
Differ_Poor_Percent	17.05	14.29	17.65
Extent_Submucosa_Percent	2.3	1.02	3.11
Extent_Muscle_Percent	11.8	11.9	10.73
Extent_Serosa_Percent	80.33	83.67	82.7
Extent_Contiguous_Percent	5.57	3.4	3.46
Surg_Short_Percent	70.49	74.15	75.09
Surg_Long_Percent	29.51	25.85	24.91
Node4_Yes_Percent	27.54	27.55	24.22

20 rows

Figure 1: Baseline Characteristics of Stage III Colon Cancer Patients by Treatment Arm

Statistical Analysis Structure

We implemented the analysis in 5 steps with three cox proportional hazards models:

1. Plot Kaplan-Meier survival curves stratified by variables and apply log-rank tests to compare survival distributions.
2. Apply two cox proportional hazards models: an unadjusted model and an adjusted for confounders model.
3. Apply LASSO penalized regression cox proportional hazards model to identify key variables.
4. Compare models using statistical methods such as AIC/BIC criteria, Harrell's C-index for discrimination, and calibration.
5. Select the final model based on the best fit, discrimination, and calibration.

These structured steps adhere precisely to Part 1. However, one small deviation is that while the initial Kaplan-Meier plan focused primarily on treatment (rx) effects, we included other relevant variables to provide more insights. Moreover, for adjusted cox models, we will also stratify by several confounders to avoid violations. These deviations would help us to select the best model.

Results

Kaplan-Meier Survival Curves

Firstly, performing stratified Kaplan-Meier analyses is a good choice for key variables selection based on their impact on survival outcomes. These variables below were chosen because they represent essential factors:

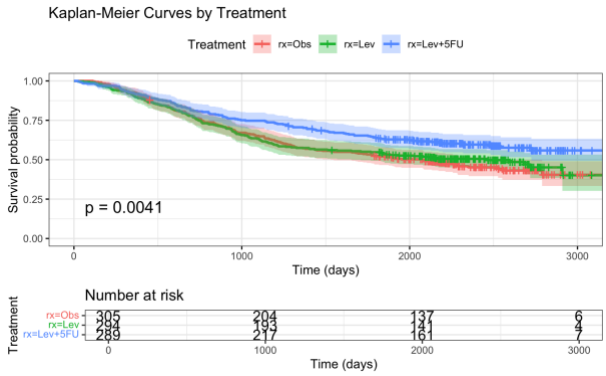

Figure 2: Kaplan-Meier Curves by Treatment (p = 0.0041)

- The combination therapy (Lev+5FU) showed good survival compared to observation and levamisole alone.
- At 3000 days, the Lev+5FU group maintained approximately 60% survival compared to 40% in other groups.

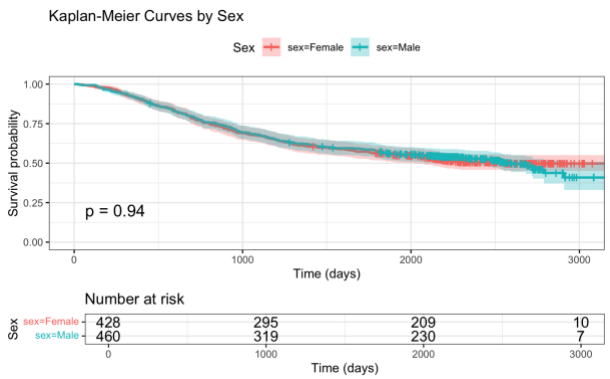

Figure 3: Kaplan-Meier Curves by Sex (p = 0.94)

- No significant difference in survival between males and females.
- Curves remained closely overlapped throughout the follow-up period.

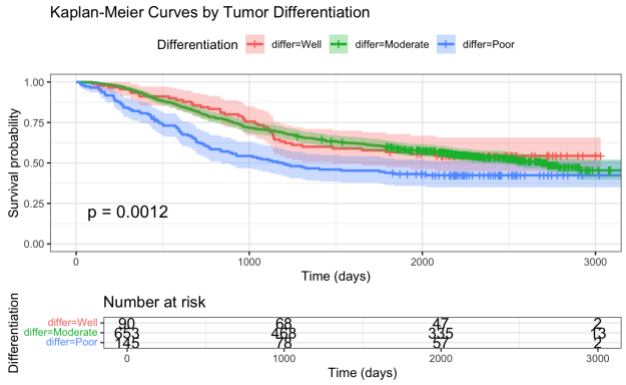

Figure 4: Kaplan-Meier Curves by Tumor Differentiation (p = 0.0012)

- Well had better survival compared to moderate and poor.
- Poor had consistently worse outcomes.

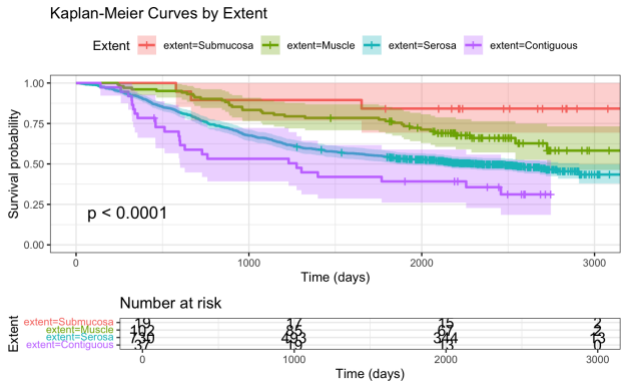

Figure 5: Kaplan-Meier Curves by Extent of Local Spread (p < 0.0001)

- Clear hierarchical pattern: submucosa (best survival) > muscle > serosa > contiguous spread (worst survival).

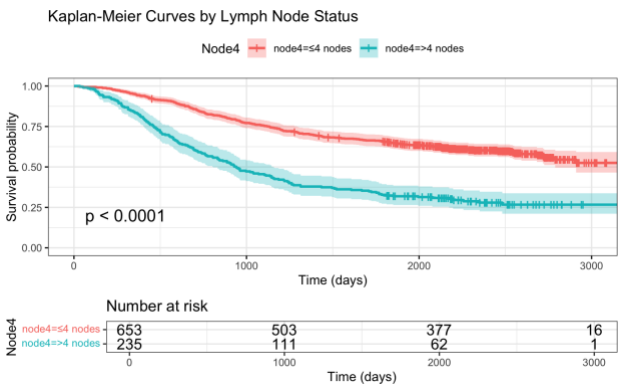

Figure 6: Kaplan-Meier Curves by Lymph Node Status ($p < 0.0001$)

- Patients with ⩾4 positive nodes showed worse survival.
- The difference was apparent early and maintained throughout follow-up.

These analyses highlight three important prognostic factors that strongly influence survival: extent of local spread, lymph node involvement, and tumor differentiation (all $p < 0.01$). Moreover, the treatment effect ($p = 0.0041$) supports the primary hypothesis regarding the benefit of combination therapy.

Log-Rank Tests

1. Treatment ($\chi^2 = 11.02$, $p = 0.004$):

- Significantly different survival across treatment groups.
- The Lev+5FU group had fewer observed deaths (117) than expected (149).

2. Sex ($\chi^2 = 0.006$, $p = 0.936$) has same conclusion as above.

3. Tumor Differentiation ($\chi^2 = 13.44$, $p = 0.001$) has same conclusion as above.

4. Extent of Spread ($\chi^2 = 24.23$, $p < 0.0001$):

- Strong association with survival.
- Submucosa had better survival (3 vs. 12 expected deaths), but contiguous spread had worse outcomes (24 vs. 14.1 expected deaths).

5. Lymph Node Status ($\chi^2 = 99.45$, $p < 0.0001$):

- The strongest predictor of survival.
- Patients with >4 nodes had worse survival.

These numerical results confirm the prognostic importance of treatment choice, tumor characteristics, and lymph node while showing no gender differences.

1 - Unadjusted Cox Proportional Hazards Model Using rx

1. Treatment Effects:

- Lev+5FU showed significant survival outcomes (HR = 0.68, 95% CI: 0.54-0.86, p = 0.002).
- Levamisole alone had minimal effect (HR = 0.94, 95% CI: 0.75-1.17, p = 0.580).

2. Model Validation:

- All three tests (likelihood ratio, Wald, and score) were consistent (p = 0.003-0.004).
- Moderate discrimination (concordance = 0.537).

3. Proportional Hazards Assumption:

- Schoenfeld residual plot shows relatively flat curves around zero.
- The global test (p = 0.36) confirms that the proportional hazards assumption is met.
- No significant time-varying effects were detected.

These numerical outputs demonstrate that the combination therapy (Lev+5FU) maintains its crucial effect for unadjusted cox model.

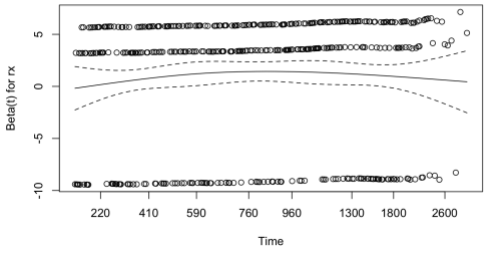

Figure 7: Treatment Variable (rx) Over Time for Proportional Hazards Assumption

This Schoenfeld Residuals Plot shows the smoothed estimate as a solid line, the 95% confidence interval as dashed lines, and the points as individual residuals.

2 - Adjusted Cox Proportional Hazards Model

For all confounders:

1. Treatment Effects (vs. observation):

- Lev+5FU maintained significant benefit (HR = 0.69, 95% CI: 0.54-0.87, p = 0.002).
- Levamisole alone remained non-significant (HR = 0.95, 95% CI: 0.76-1.18, p = 0.628).

2. Strongest Prognostic Factors:

- Number of positive nodes (⩾4 nodes): (HR = 1.96, 95% CI: 1.48-2.59, p < 0.001).
- Extent of spread (contiguous): (HR = 3.87, 95% CI: 1.15-13.02, p = 0.029).

- Obstruction: (HR = 1.31, 95% CI: 1.03-1.65, p = 0.027).

3. Non-significant Factors:

- Sex (p = 0.903).
- Age (p = 0.069).
- Tumor differentiation (p > 0.39).
- Perforation (p = 0.969).

These numerical outputs demonstrate that even after adjusting for all confounders, the combination therapy (Lev+5FU) maintains its crucial effect.

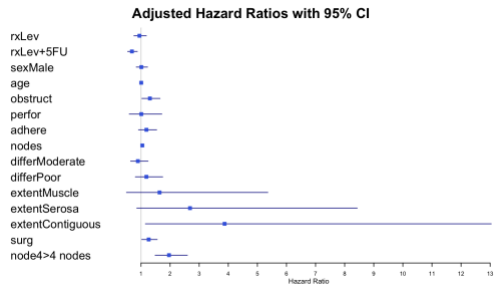

Figure 8: Adjusted Hazard Ratios with 95% Confidence Intervals for Prognostic Factors

Nevertheless, this adjusted cox model for all confounders has PH assumption violations:

- differ (p = 0.00022): Strong violation.
- obstruct (p = 0.01032): Clear violation.
- node4 (p = 0.03145): Moderate violation.

Therefore, we stratified by differ for this adjusted cox model:

- AIC decreased from 5422.9 to 4754.4.
- The likelihood ratio test was highly significant (p < 2.2e-16).
- However, obstruct still violates the PH assumption (p = 0.0076).

Then, provide the adjusted cox model with both differ and obstruct for stratification:

- AIC decreased from 4754.4 to 4307.9.
- Treatment with Levamisole+5FU reduced mortality risk by 29% (HR=0.71, 95% CI: 0.56-0.90) compared to observation.
- Each additional lymph node increased mortality risk by 5% (HR=1.05, 95% CI: 1.02-1.08).
- Having >4 positive nodes increased mortality risk by 93% (HR=1.93, 95% CI: 1.46-2.56).
- Contiguous increased risk by 4.2 (HR=4.23, 95% CI: 1.26-14.22).

- Age and surgical time showed modest but significant associations.

The adjusted cox model with confounders stratified by differ and obstruct demonstrated good discrimination (concordance=0.68) and met the proportional hazards assumption (global test p=0.077).

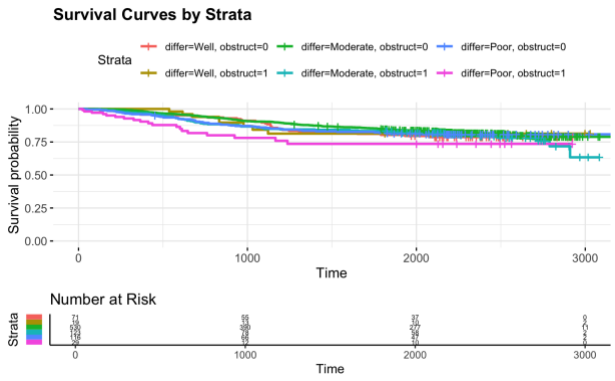

Figure 9: Adjusted Survival Curves Stratified by Tumor Differentiation and Obstruction Status

3 - LASSO Penalized Regression for Cox Model

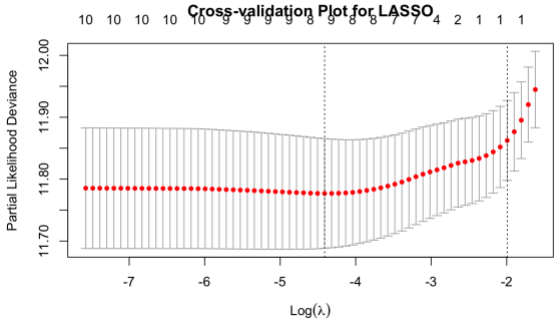

Figure 10: Cross-Validation Plot

In the plot, there are two lambda values:

- Minimum lambda (λmin) = 0.012.
- 1 Standard Error lambda (λ1se) = 0.1362.

In addition, LASSO identified the important predictors with the strongest effects from up to down:

- Contiguous extent (0.695).
- Serosa extent (0.381).
- Number of nodes (0.300).
- Levamisole+5FU treatment (-0.258).
- Time from surgery to registration (0.205).

Thus, the LASSO cox model retained the number of nodes as a significant predictor (HR = 1.096, 95% CI: 1.077-1.115, $p < 0.001$), suggesting that for each additional involved node, the hazard of death increases by about 10%.

Additionally, the LASSO cox model shows reasonable discrimination with a concordance index of 0.637, indicating moderate predictive ability. And the significant likelihood ratio test ($p < 0.001$) confirms the statistical significance.

Model Comparison

The adjusted cox model with stratifications also called as the Full model shows the best performance across all metrics:

- Lowest AIC (4307.85).
- Lowest BIC (4356.62).
- Highest C-index (0.683).
- The greatest improvement in discrimination (31.5% vs. unadjusted cox model).
- Likelihood Ratio Tests: Full model shows substantial improvement over LASSO ($\chi^2 = 1181.1$, df = 11, $p < 2.2e-16$) and unadjusted models ($\chi^2 = 1240.6$, df = 10, $p < 2.2e-16$).

LASSO model has more parsimonious (1 parameter) with moderate improvement:

- C-index of 0.637 (similar to the Full model).
- 21.6% improvement in discrimination vs. unadjusted cox model.
- Higher AIC (5466.98) and BIC (5471.04) than the full model.

Final Model Selection

Therefore, the Full model is the optimal model because:

- Best performance across all criteria (AIC, BIC) and best discrimination ability (C-index = 0.683).
- Significant improvement in model fit over both LASSO and unadjusted models.
- The significant improvement in model performance justifies the added complexity (12 parameters).

While the LASSO model is also great, the Full model substantially improves fit, discrimination, and calibration, which support it as the final model.

Discussion

In comparison with original paper, this technical report analysis found:

- The Lev+5FU group showed a 29% reduction in mortality risk (HR=0.71, 95% CI: 0.56-0.90) compared to observation, which is close to the data from the original paper, which showed that fluorouracil plus levamisole reduced the death rate by 33% (P = 0.0007).

- Levamisole alone remained non-significant (HR = 0.95, 95% CI: 0.76-1.18, p = 0.628), and the original paper also reported that levamisole alone had a minimal effect (2% reduction in recurrence, 6% reduction in death rate).

While this analysis used different statistical approaches, the core findings are consistent - both showed that fluorouracil plus levamisole provided significant survival outcomes while levamisole alone had minimal effect. Moreover, both analyses proved similar prognostic factors that are significant predictors, such as lymph nodes and the extent of the tumor.

In addition, the main difference lies in the methods section—this report employed LASSO and two Cox models with stratification and chose the Full model as the final model. However, these models weren't commonly used in clinical analysis in 1995. In summary, other contents aligned well with the original findings except for these models.

Appendix

- colon.Rmd – R code with numerical results and plots for this technical report.