Association between Bilirubin and Survival in Primary Biliary Cirrhosis

Memorial Sloan Kettering Cancer Center

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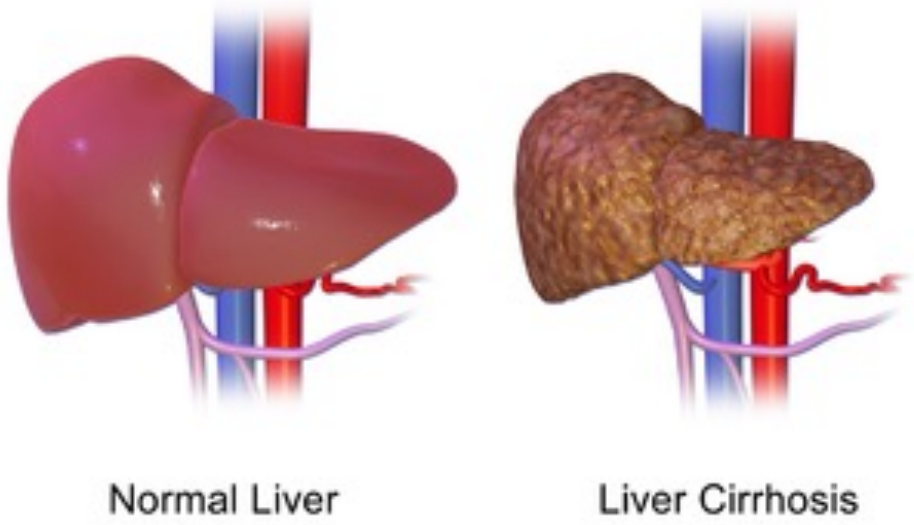
Primary Biliary Cirrhosis (PBC)



(Source: Bruce Blaus, Wikimedia Commons)

- Immune system attacks liver
- Damage bile ducts
- Lead to liver fibrosis and cirrhosis
- Possibly lead to liver cancer

- Relatively rare disease (1/3000)
- Common in women

Symptoms:

- Jaundice
- Fatigue
- Loss of appetite
- No symptoms

Treatment:

- Medications
- Liver transplantation

Study data - PBC Clinical Trial

MAYO CLINIC

- Follow-up for 10 years
- 312 patients: 154 in placebo group

  158 in treatment group
- Clinical factors: age, drug, sex
- Longitudinal biomarkers: Repeatedly measured

at 6 months, one year, and annually thereafter

e.g: **serum bilirubin**, albumin, prothrombin, platelets, etc.

- Outcome: alive/transplanted vs died

Research objectives

1. Measure the association between bilirubin and overall survival among PBC patients

Motivation:

- High level of bilirubin causes yellowing of the skin
- Help personalize patient care
- Better adjust medication for patients
- Allocate healthcare resources efficiently

Research objectives

1. Measure the association between bilirubin and overall survival among PBC patients

2. Use three different statistical approaches in survival analysis

3. Compare the results from three models

Survival Analysis

- Study of **time-to-event/censored data**
- Outcome variables:
  - Time: $y_i = min(T_i, C_i)$

  Event: death, tumor recurrence, etc.

  - Event indicator: δ_i

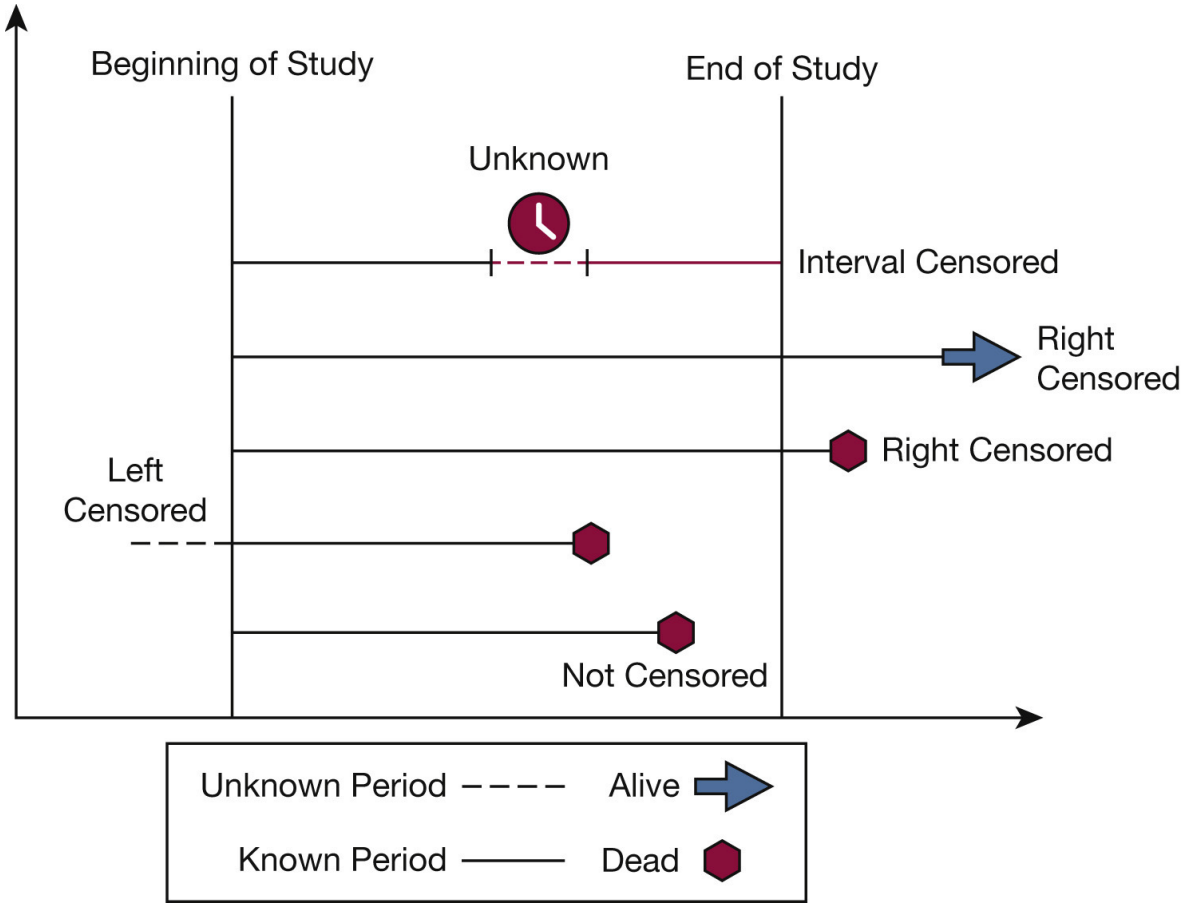
  1 if event observed; 0 otherwise

id	years	status
1	1.09	dead
2	14.15	alive
3	2.78	dead
4	5.27	dead
5	4.12	transplanted
6	6.85	dead
7	6.84	alive
8	6.75	dead
9	6.57	dead
10	0.14	dead

Survival Analysis

- Censoring:
  - Loss to follow-up
  - Withdrawal from study
  - Event not observed

- Motivation for Cox PH Model



Source: Dey, Tanujit, Anish Mukherjee, and Sounak Chakraborty. *Chest* 158.1 (2020).

Hazard Function

- Formula: $h(t) = \lim_{dt \to 0} \frac{\Pr(t \le T^* \le t+dt \mid T^* \ge t)}{dt}$

  Instantaneous risk for event occurrence in time interval $[t, t + dt)$

- Cumulative hazard function: $\mathrm{H}(t) = \int_0^t h(s)ds$

  Accumulated risk up to time t

Survival Function

- Formula: $S(t) = \Pr(T^* > t) = \int_t^\infty f(s)ds$

$$S(t) = \exp\{-\mathrm{H}(t)\} = \exp\{-\int_0^t h(s)ds\}$$

- Kaplan-Meier Approach:

$$\hat{S}(t) = \hat{S}(t-1) \times \Pr(T^* > t \,|T^* > t\ - 1)$$

$$= \prod_{i:\, t_i \le t} (T^* > t \,|T^* > t\ - 1)$$

$$= \prod_{i:\, t_i \le t} \frac{r_i - d_i}{r_i}$$

d_i: number of events at t_i

r_i: number of subjects at risk at time t_i

Cox Proportional Hazards Model

- Formula: $h_i(t \mid w_i) = h_0(t) \exp\{\gamma^T w_i\}$
- Baseline function $h_0(t)$ unspecified
- Hazard Ratio (HR): $\frac{h_i(t \mid w_i)}{h_k(t \mid w_k)} = \exp\{\gamma^T (w_i - w_k)\}$
- HR: Constant over time → Proportionality Assumption

  Assessing Technique: Goodness-of-fit test

Methods

Cox Proportional Hazard Model

Time-Dependent Cox Model

Joint Model

Cox PH Model

- Measure the association between baseline level of biomarker and survival

! Problem: Can only handle baseline bilirubin

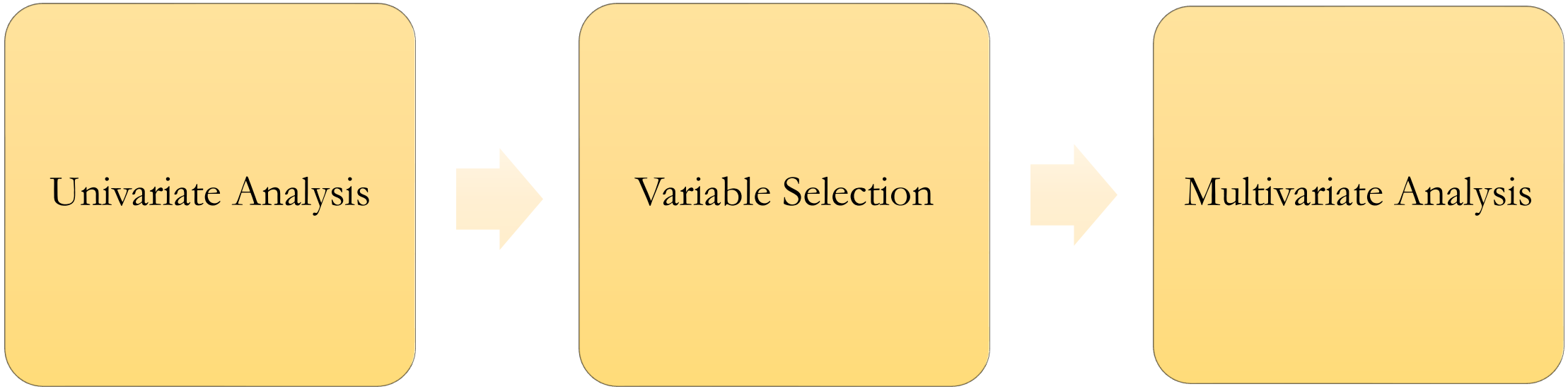
$$h(t \mid w_i) = h_0(\mathrm{t}) \exp\{\boldsymbol{\gamma^T w_i}\}$$

Cox PH Model	Time-Dependent Cox Model
• Measure the association between <u>baseline</u> level of biomarker and survival	• Measure the association between <u>current</u> level of biomarker and survival • Account for progression of biomarker over time
! Problem: Can only handle baseline bilirubin	! Problem: Assume bilirubin is exogenous
$h(t \mid w_i) = h_0(\mathrm{t}) \exp\{\boldsymbol{\gamma^T w_i}\}$	$h(t \mid Y_i(t), w_i)$ $= h_0(\mathrm{t}) \exp\{\boldsymbol{\gamma^T w_i} + \alpha \boldsymbol{y_i}(\mathrm{t})\}$

Cox PH Model	Time-Dependent Cox Model	Joint Model
• Measure the association between <u>baseline</u> level of biomarker and survival	• Measure the association between <u>current</u> level of biomarker and survival • Account for progression of biomarker over time	• Measure the association between <u>current</u> level of biomarker and survival • Account for progression of biomarker over time • Account for measurement error
! Problem: Can only handle baseline bilirubin	! Problem: Assume bilirubin is exogenous	<u>Longitudinal sub-model:</u> $y_i(t) = \boldsymbol{m_i(t)} + \varepsilon_i(t)$ $\varepsilon_i(t) \sim \mathcal{N}(0, \sigma^2 I_{n_i})$
$h(t \mid w_i) = h_0(\mathrm{t}) \exp\{\boldsymbol{\gamma^T w_i}\}$	$h(t \mid Y_i(t), w_i)$ $= \mathrm{h_0(t)} \exp\{\boldsymbol{\gamma^T w_i} + \alpha \boldsymbol{y_i(t)}\}$	<u>Survival sub-model:</u> $h(t \mid M_i(t), w_i)$ $= h_0(\mathrm{t}) \exp\{\boldsymbol{\gamma^T w_i} + \alpha \boldsymbol{m_i(t)}\}$

Methodology

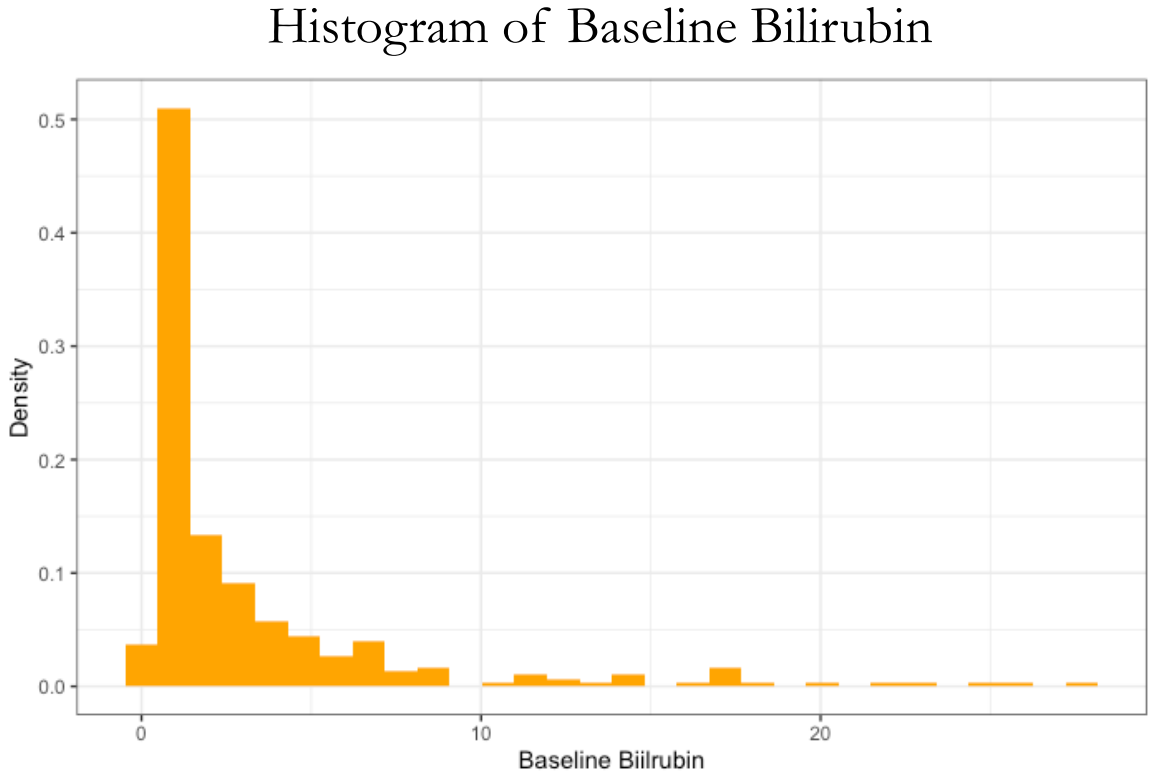
Each model follows this procedure:



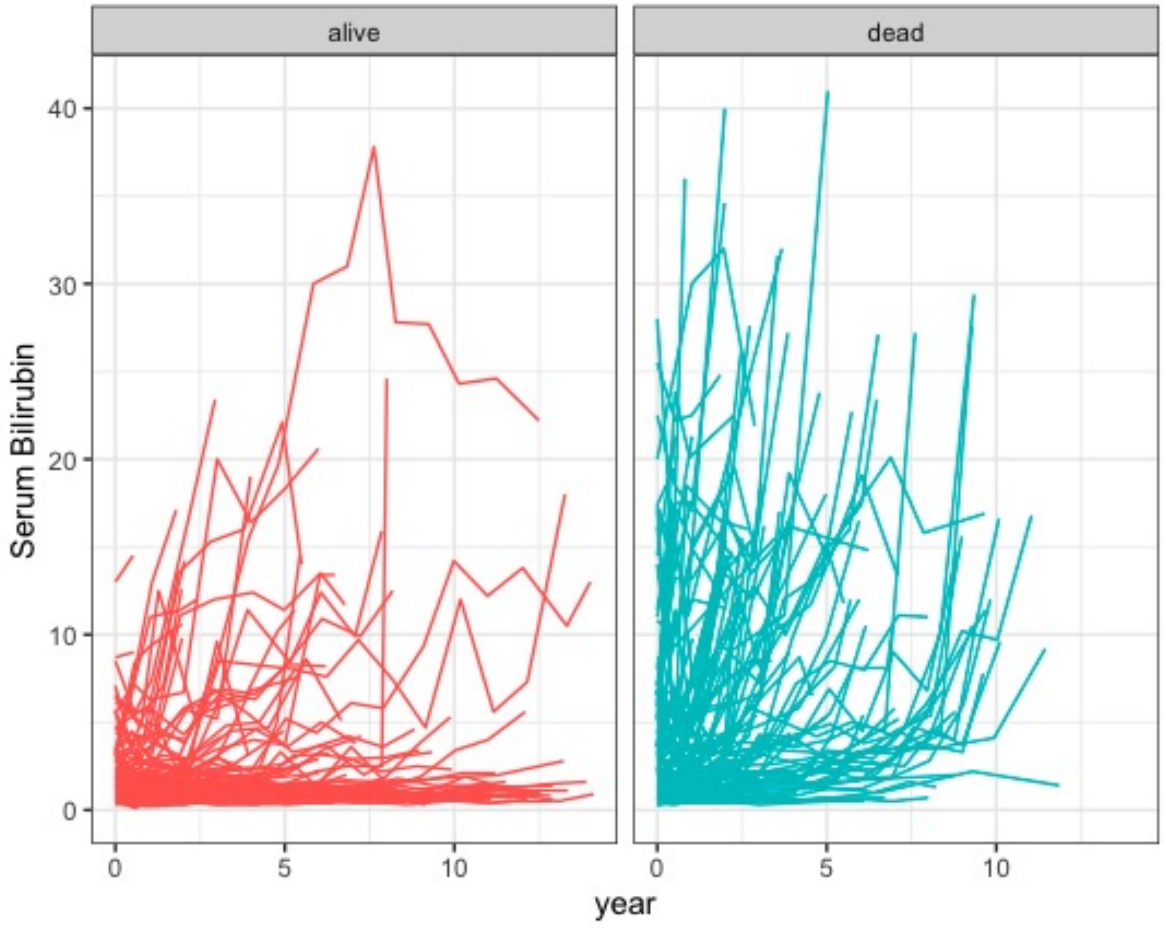
Summary Statistics

- 51% in treatment group
- Median age: 50 (IQR: 42 – 57)
- Sex: 88.5% females
- Median baseline bilirubin: 1.4 (IQR: 0.8 – 3.4)
- Median follow-up duration: 6.3 years
- Patients: 172 alive/transplanted

  140 died

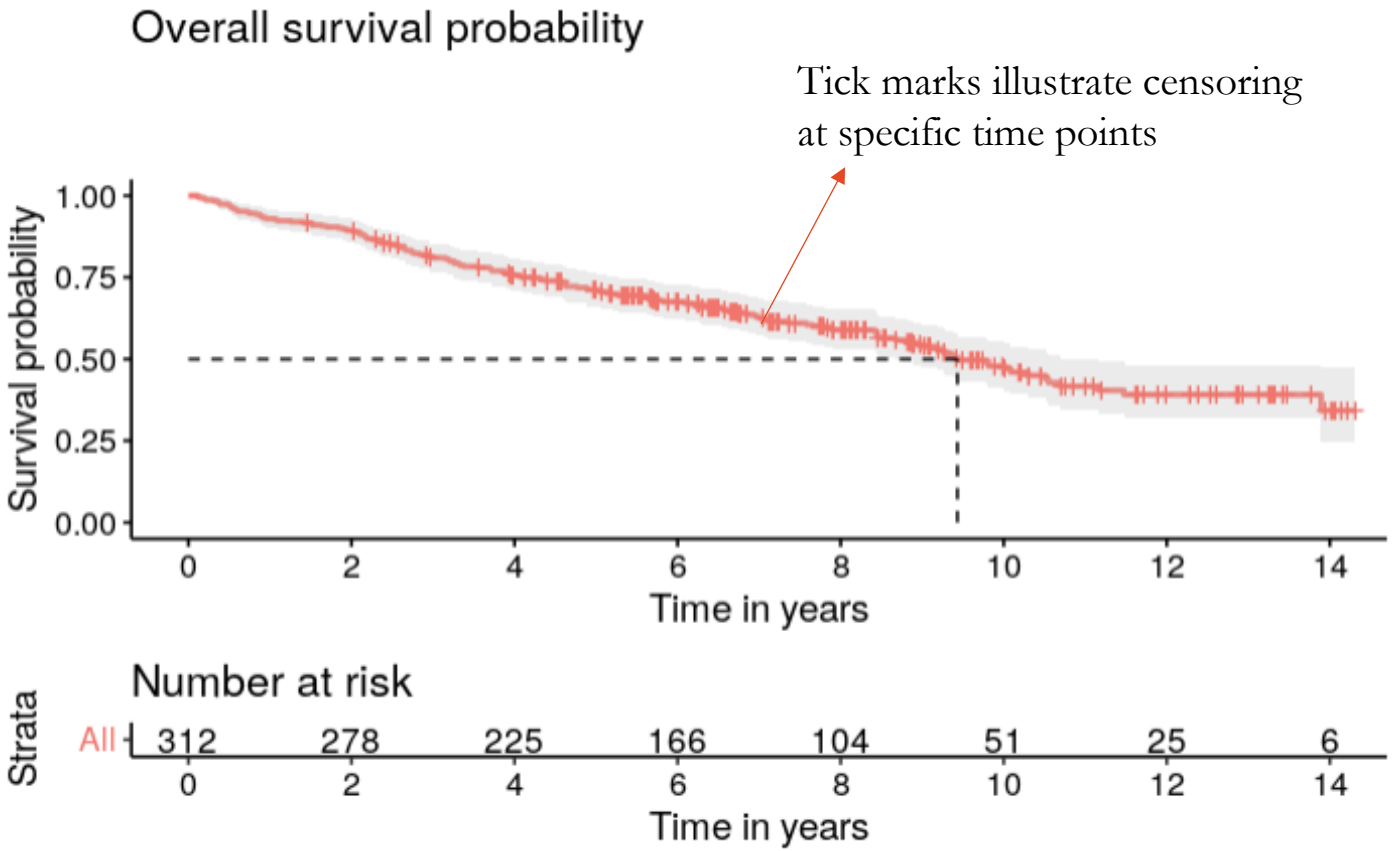


Spaghetti plot of longitudinal bilirubin



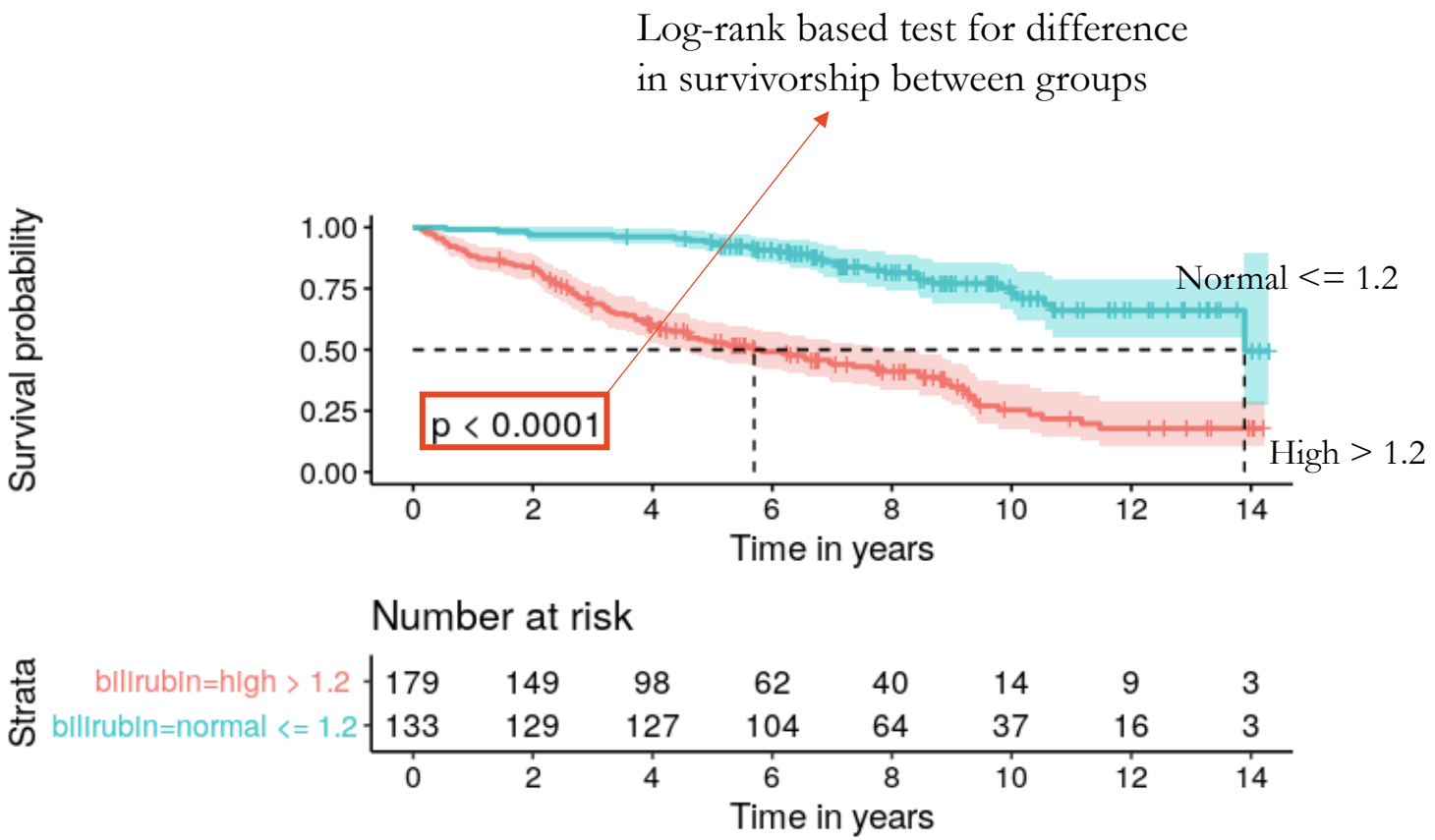
- Median follow-up duration: 6.3 years
- Patients who died seemed to have higher level of bilirubin

Kaplan-Meier Curve



- Survival decreases over time
- Median survival time: 9.5 years
- After 6 years: 166 patients at risk

Baseline bilirubin



- Clinical cutoff: 1.2 mg/dl
- Normal baseline bilirubin
- High baseline bilirubin

Univariate Analysis for Cox Models

Clinical Factor	Hazard Ratio	95% Confidence Interval	p-value
Drug	1.00	(0.72 – 1.39)	>0.9
Age	1.05	(1.03 – 1.06)	<0.001
Sex: male	Ref		
female	0.52	(0.34 – 0.80)	0.005

Interpretation:

- There's no treatment effect on the survival
- One-year increase in the baseline age is associated with a 5% increase in the hazard of death
- Female patients have a 48% lower hazard of death than male patients

Univariate Analysis for Cox Models

Biomarker	Hazard Ratio	95% Confidence Interval	p-value
Albumin	0.19	(0.13 – 0.28)	<0.001
Alkaline	1.00	(1.00 – 1.00)	0.094
SGOT	1.01	(1.00 – 1.01)	<0.001
Platelets	1.00	(0.99 – 1.00)	<0.001
Prothrombin	2.12	(1.81 – 2.48)	<0.001
Ascites *(No/Yes)*	7.58	(4.78 – 12.0)	<0.001
Hepatomegaly *(No/Yes)*	3.06	(2.14 – 4.38)	<0.001
Spiders *(No/Yes)*	2.42	(1.72 – 3.42)	<0.001
Edema*: No edema*	ref		<0.001
Edema no diuretics	1.63	(1.04 – 2.55)	
Edema diuretics	10.9	(6.61 – 18.0)	
Histologic: *1*	ref		<0.001
2	6.39	(0.86 – 47.5)	
3	9.66	(1.33 – 70.1)	
4	24.0	(3.33 – 174)	

Univariate Analysis – Serum Bilirubin

Univariate Analysis - Serum Bilirubin			
Model	**Hazard Ratio**	**95% Confidence Interval**	**p-value**
Model 1: Cox PH	1.16	(1.13 – 1.19)	<0.001
Model 2: Time-Dependent Cox	1.16	(1.14 – 1.18)	<0.001
Model 3: Joint Model	1.83	(1.66 - 2.02)	<0.0001

Interpretation:

- Model 1(**baseline bilirubin**): One unit increase in the baseline bilirubin is associated with 16% increase in the death risk
- Model 2 (**longitudinal bilirubin**): One unit increase in the longitudinal bilirubin is associated with 16% increase in the death risk
- Model 3 (**longitudinal bilirubin & measurement error**): One unit increase in the longitudinal bilirubin is associated with 83% increase in the death risk

Multivariate Analysis – Serum Bilirubin

Multivariate Analysis - Serum Bilirubin			
Model	**Hazard Ratio**	**95% Confidence Interval**	**p-value**
Model 1: Cox PH	1.11	(1.06 – 1.15)	<0.001
Model 2: Time-Dependent Cox	1.20	(1.17 – 1.22)	<0.001
Model 3: Joint Model	1.82	(1.64 - 2.03)	<0.0001

High bilirubin worsens survival

Model Adjustment:

- Model 1: bilirubin, albumin, age, edema, histologic, SGOT, prothrombin
- Model 2: bilirubin, albumin, age, edema, histologic
- Model 3: bilirubin, albumin, age, edema

Multivariate Analysis – Proportionality

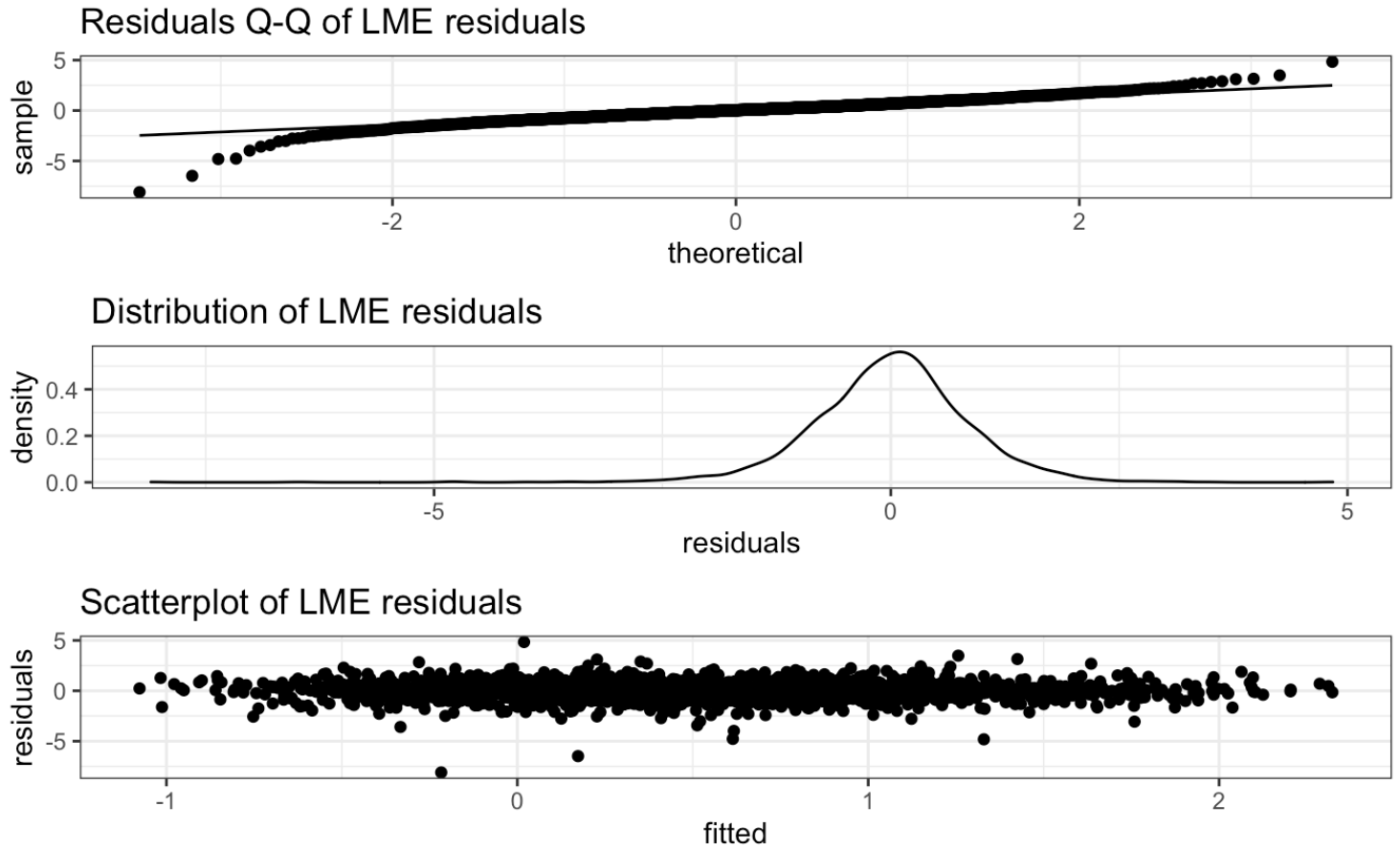
Cox PH Model			
Characteristics	**Chisq**	**df**	**P-value**
Bilirubin	6.305	1	0.012
Albumin	3.043	1	0.081
Age	0.195	1	0.659
Edema	4.208	2	0.122
Histologic	6.603	3	0.086
SGOT	0.405	1	0.525
Prothrombin	1.482	1	0.223
GLOBAL	20.873	10	0.022

Survival Sub-model of Joint Model			
Characteristics	**Chisq**	**df**	**P-value**
Albumin	2.279	1	0.131
Age	0.204	1	0.651
Edema	4.848	2	0.089
GLOBAL	6.394	4	0.172

H_0: proportionality is met

H_A: proportionality is violated

Diagnostic plots for LME



Conclusions

1. Association between bilirubin and survival from Multivariate Analysis:

   Cox PH: HR = 1.11, Time-Dependent Cox: HR = 1.20, Joint Model: HR = 1.82

2. Difference in model outputs:
   - Cox PH: baseline values of bilirubin
   - Time-Dependent Cox: progression of bilirubin
   - Joint Model: progression of bilirubin & measurement error

Conclusions

1. Association between bilirubin and survival from Multivariate Analysis:

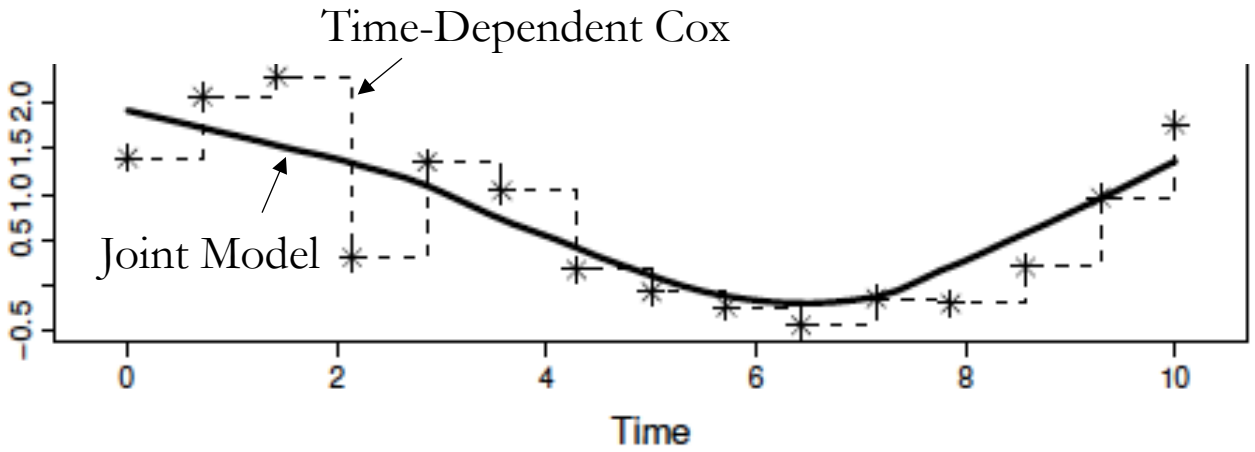
   Cox PH: HR = 1.11, Time-Dependent Cox: HR = 1.20, Joint Model: HR = 1.82

2. Difference in model outputs:
   - Cox PH: baseline values of bilirubin
   - Time-Dependent Cox: progression of bilirubin
   - **Joint Model: progression of bilirubin & measurement error**

Discussion

Advantages of Joint Model:

- Smooth longitudinal trajectory
- Reduce potential bias



Source: Joint Models (Rizopoulos, 2012)

Disadvantages of Joint Model:

- Computational expense
- Large sample size

→ Time-Dependent Cox Model

Trade-off: Biased estimate of HR

Discussion

Model Adjustment:

- Model 1: bilirubin, albumin, age, edema, histologic, SGOT, prothrombin
- Model 2: bilirubin, albumin, age, edema, histologic
- Model 3: bilirubin, albumin, age, edema

Explanation:

- Baseline SGOT and prothrombin not informative to the association in Model 2
- Baseline SGOT and prothrombin significant in the longitudinal generating process of bilirubin in Model 3

*Model 1: Cox PH; Model 2: Time-Dependent Cox; Model 3: Survival sub-model

Limitations

- PBC Clinical Data: real dataset

  → Cannot evaluate model performances with the true HR

- Survival Sub-model of Joint Model:

  Baseline hazard is piecewise-constant with six knots

  More knots allow more flexibility

  ! Computational demand

Future Work

- Simulation with predetermined censoring and hazard rate to estimate the bias
- Variable selection with BIC or AIC
- Multiple imputation method for missing data
- Competing Risk Analysis (alive vs transplanted)
- Joint Model for more than one longitudinal biomarker

Acknowledgements

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- QSURE faculties: Drs. Kay See Tan and Margaret Du
- Math/Stat Department, MHC
- Biostat/Epi Department, MSKCC

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Selected References

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Thank you!

Any question?

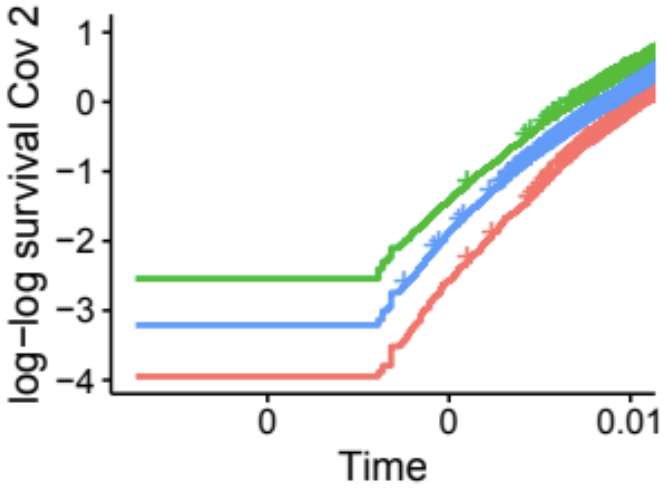
Supplementary slides

Assessing Proportionality

- Log-log survival curves
- Time-covariate interaction
- Goodness-of-fit test

Graphics with survival curves

- Log-log transformation: $\ln\{-\ln(S(t))\}$
- If the assumption is met, the curves are equally spaced
- For continuous covariates, stratify them into groups depending on their distribution



Time-covariate interaction

- X_i is suspicious of having time-varying effect, the added term is $X_i f(t)$
- Function of time $f(t)$ can be linear, logarithmic, exponential, etc.
- If $X_i f(t)$ is statistically significant, proportionality is violated

Goodness-of-fit test

- Formula for Schoenfeld residuals of suspiciously time-varying covariate A:

$$Schoenfeld\ residual\ A = Observed\ A - Weighted\ Average\ A$$

- If Schoenfeld residuals are correlated with failure times, proportionality is violated

Missingness mechanism

- Missing not at random (MNAR)

  Depend on both observed and unobserved data

- Missing completely at random (MCAR)

  Depend on unobserved data

- Missing at random (MAR)

  Not depend on observed or unobserved data

Short data format

id <fctr>	years <dbl>	status <fctr>	drug <fctr>	age <dbl>
1	1.0951703	dead	D-penicil	58.76684
2	14.1523382	alive	D-penicil	56.44782
3	2.7707809	dead	D-penicil	70.07447
4	5.2705071	dead	D-penicil	54.74209
5	4.1205782	transplanted	placebo	38.10645
6	6.8530281	dead	placebo	66.26054
7	6.8475523	alive	placebo	55.53609
8	6.7517249	dead	placebo	53.05826
9	6.5710218	dead	D-penicil	42.50904
10	0.1396342	dead	placebo	70.56182

Counting Process format

id <fctr>	years <dbl>	status <fctr>	drug <fctr>	age <dbl>
1	1.0951703	dead	D-penicil	58.76684
1	1.0951703	dead	D-penicil	58.76684
2	14.1523382	alive	D-penicil	56.44782
2	14.1523382	alive	D-penicil	56.44782
2	14.1523382	alive	D-penicil	56.44782
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2	14.1523382	alive	D-penicil	56.44782

Results from Multivariate Analysis

Subsets of significant covariates from variable selection for Cox PH Model									
	Multivariate Cox PH Model			Multivariate Time-Dependent Cox Model			Survival Submodel of Joint Model		
Characteristic	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
bilirubin	**1.11**	**(1.06-1.15)**	**<0.001**	**1.20**	**(1.17-1.23)**	**<0.001**	**1.81**	**(1.60-2.04)**	**<0.0001**
albumin	0.52	(0.32-0.85)	0.008	0.59	(0.36-0.97)	0.036	0.67	(0.41-1.08)	0.1011
age	1.04	(1.03-1.06)	<0.001	1.06	(1.05-1.08)	<0.001	1.05	(1.03-1.07)	<0.0001
edema			0.019			0.003			0.0003
no edema	ref			ref			ref		
edema no diuretics	1.04	(0.65-1.67)		1.18	(0.72-1.93)		1.97	(1.18-3.30)	
edema diuretics	2.37	(1.33-4.22)		3.09	(1.66-5.72)		3.11	(1.64-5.89)	
histologic			0.014			<0.001			0.3422
1	ref			ref			ref		
2	4.49	(0.60-33.8)		1.97	(0.26-14.9)		1.72	(0.26-11.26)	
3	5.79	(0.79-42.5)		4.48	(0.61-33.0)		2.02	(0.31-13.00)	
4	8.04	(1.09-59.5)		6.69	(0.90-49.4)		2.58	(0.40-16.70)	
SGOT	1.00	(1.00-1.01)	0.012	1.00	(0.99-1.00)	0.4	1.00	(0.99-1.00)	0.9569
prothrombin	1.46	(1.20-1.78)	<0.001	1.11	(0.91-1.36)	0.3	1.22	(0.99-1.51)	0.623

Results from Multivariate Analysis

Subsets of significant covariates from variable selection for Time-Dependent Cox Model									
	Multivariate Cox PH Model			Multivariate Time-Dependent Cox Model			Survival Submodel of Joint Model		
Characteristic	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
bilirubin	**1.15**	**(1.11-1.18)**	**<0.001**	**1.20**	**(1.17-1.22)**	**<0.001**	**1.84**	**(1.65-2.06)**	**<0.0001**
albumin	0.50	(0.31-0.81)	0.005	0.62	(0.38-1.01)	0.005	0.60	(0.37-0.97)	0.0364
age	1.04	(1.02-1.06)	<0.001	1.07	(1.05-1.09)	<0.001	1.05	(1.03-1.07)	<0.0001
edema			0.002			0.001			<0.0001
no edema	ref			ref			ref		
edema no diuretics	0.97	(0.60-1.55)		1.26	(0.78-2.04)		2.16	(1.30-3.59)	
edema diuretics	2.92	(1.62-5.27)		3.31	(1.80-6.09)		3.63	(1.96-6.70)	
histologic			<0.001			<0.001			0.0878
1	ref			ref			ref		
2	4.63	(0.62-34.5)		1.85	(0.24-14.0)		0.92	(0.21-4.12)	
3	6.39	(0.88-46.7)		4.35	(0.59-32.0)		1.23	(0.28-5.29)	
4	10.9	(1.49-80.1)		7.09	(0.96-52.3)		1.70	(0.39-7.38)	

Results from Multivariate Analysis

Subsets of significant covariates from variable selection for Joint Model									
	Multivariate Cox PH Model			Multivariate Cox PH Model			Survival Submodel of Joint Model		
Characteristic	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
bilirubin	**1.14**	**(1.11-1.17)**	**<0.001**	**1.19**	**(1.16-1.21)**	**<0.001**	**1.82**	**(1.64-2.03)**	**<0.0001**
albumin	0.37	(0.23-0.58)	<0.001	0.47	(0.29-0.75)	0.002	0.52	(0.33-0.82)	0.054
age	1.04	(1.03-1.06)	<0.001	1.07	(1.05-1.09)	<0.001	1.05	(1.04-1.07)	<0.0001
edema			<0.001			<0.001			<0.0001
no edema	ref			ref			ref		
edema no diuretics	1.01	(0.63-1.62)		1.32	(0.83-2.10)		2.05	(1.25-3.36)	
edema diuretics	3.32	(1.83-6.01)		4.16	(2.25-7.70)		3.84	(2.09-7.06)	

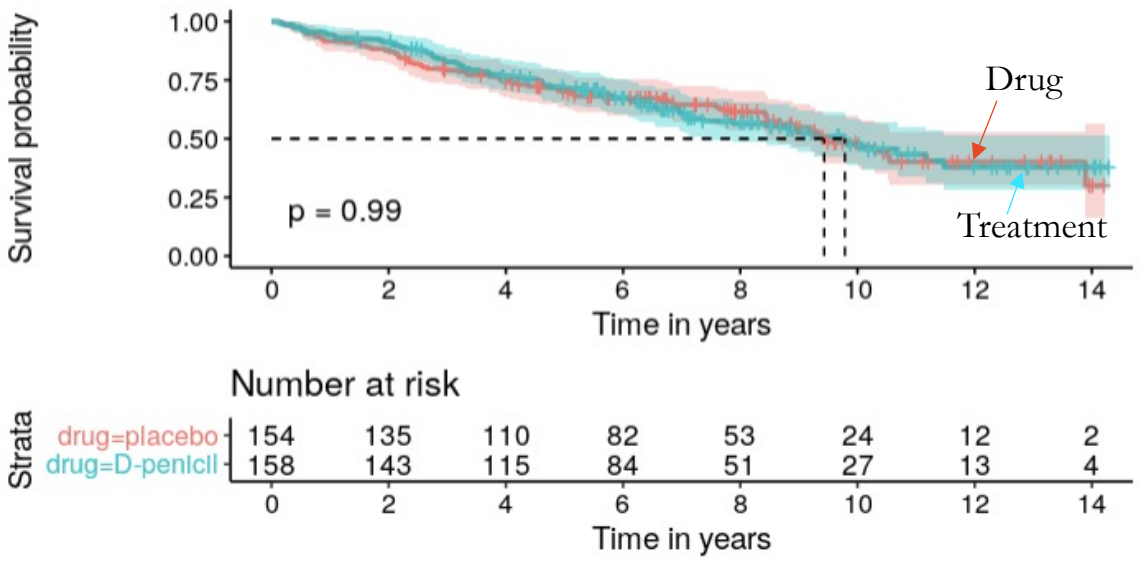
Results from Multivariable Analysis

Multivariable Cox PH Model				Multivariable Cox PH Model			Survival Submodel of Joint Model		
Characteristic	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
bilirubin	1.11	(1.06-1.15)	<0.001	1.20	(1.17-1.23)	<0.001	1.81	(1.60-2.04)	<0.0001
bilirubin	1.15	(1.11-1.18)	<0.001	1.20	(1.17-1.22)	<0.001	1.84	(1.65-2.06)	<0.0001
bilirubin	1.14	(1.11-1.17)	<0.001	1.19	(1.16-1.21)	<0.001	1.82	(1.64-2.03)	<0.0001

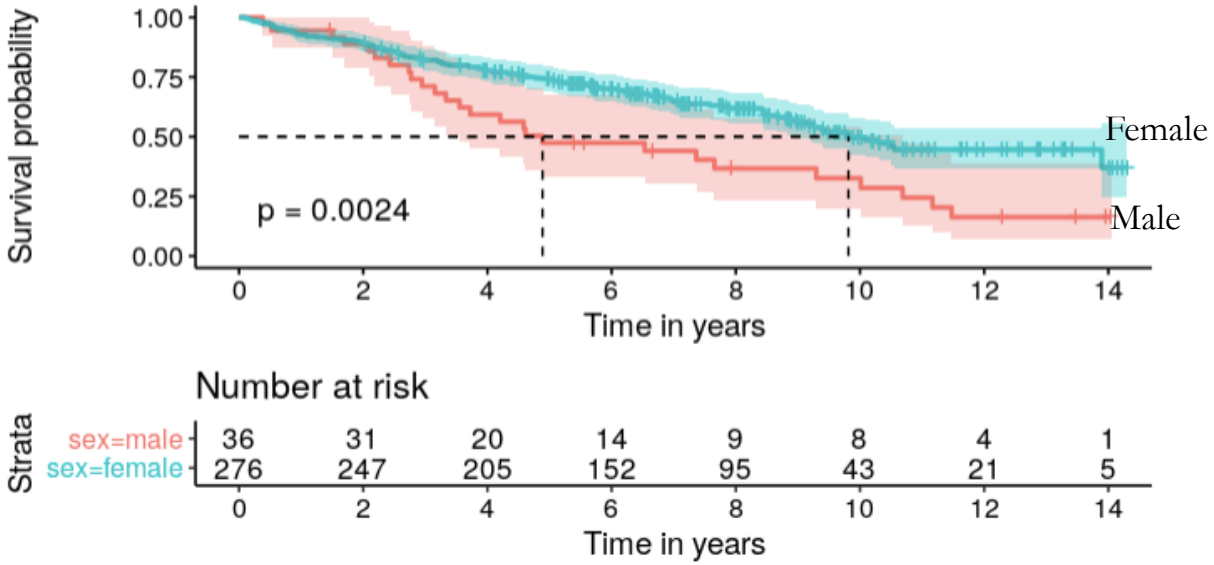
Multivariable Analysis - Serum Bilirubin

Model	Hazard Ratios	95% Confidence Interval	p-value
Model 1: Cox PH	1.11	(1.06 – 1.15)	<0.001
Model 2: Time-Dependent Cox	1.20	(1.17 – 1.22)	<0.001
Model 3: Joint Model	1.82	(1.64 - 2.03)	<0.0001

Treatment effect

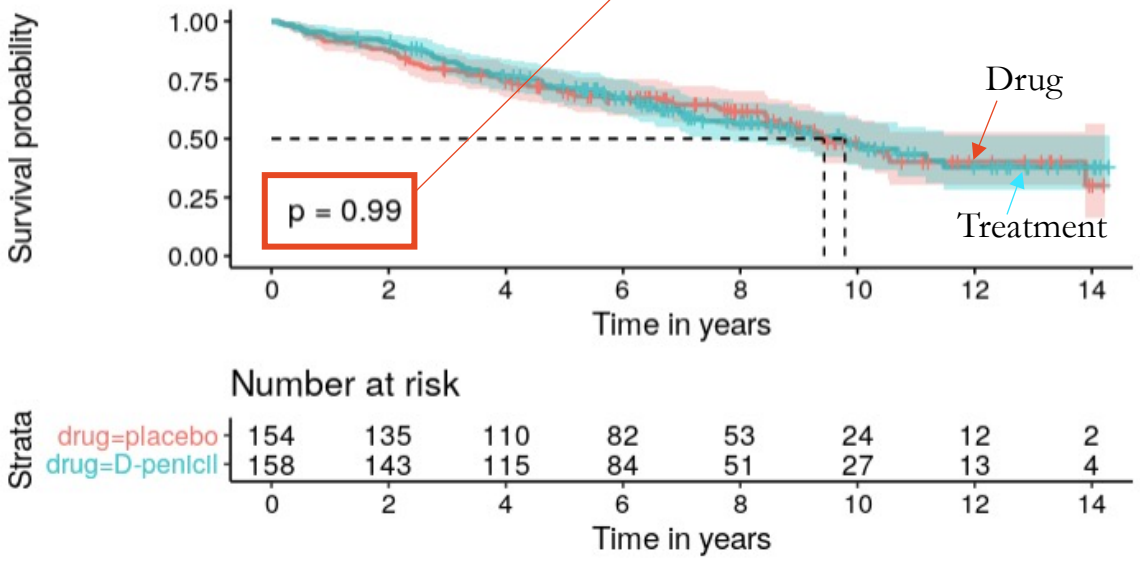


Sex



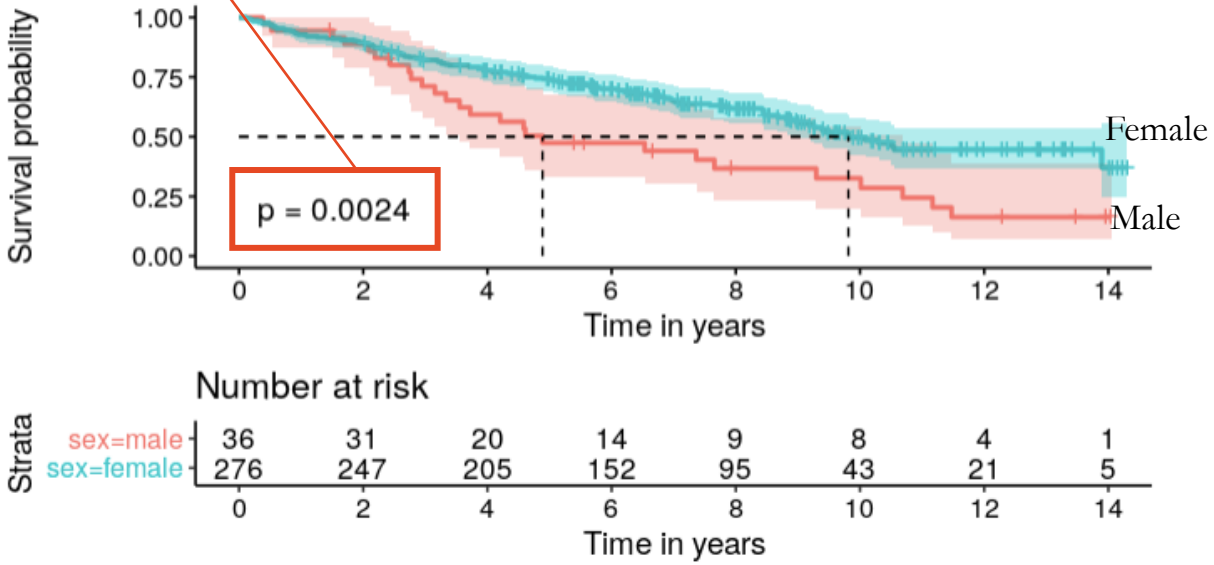
Log-rank based test for difference in survivorship between groups

Treatment effect



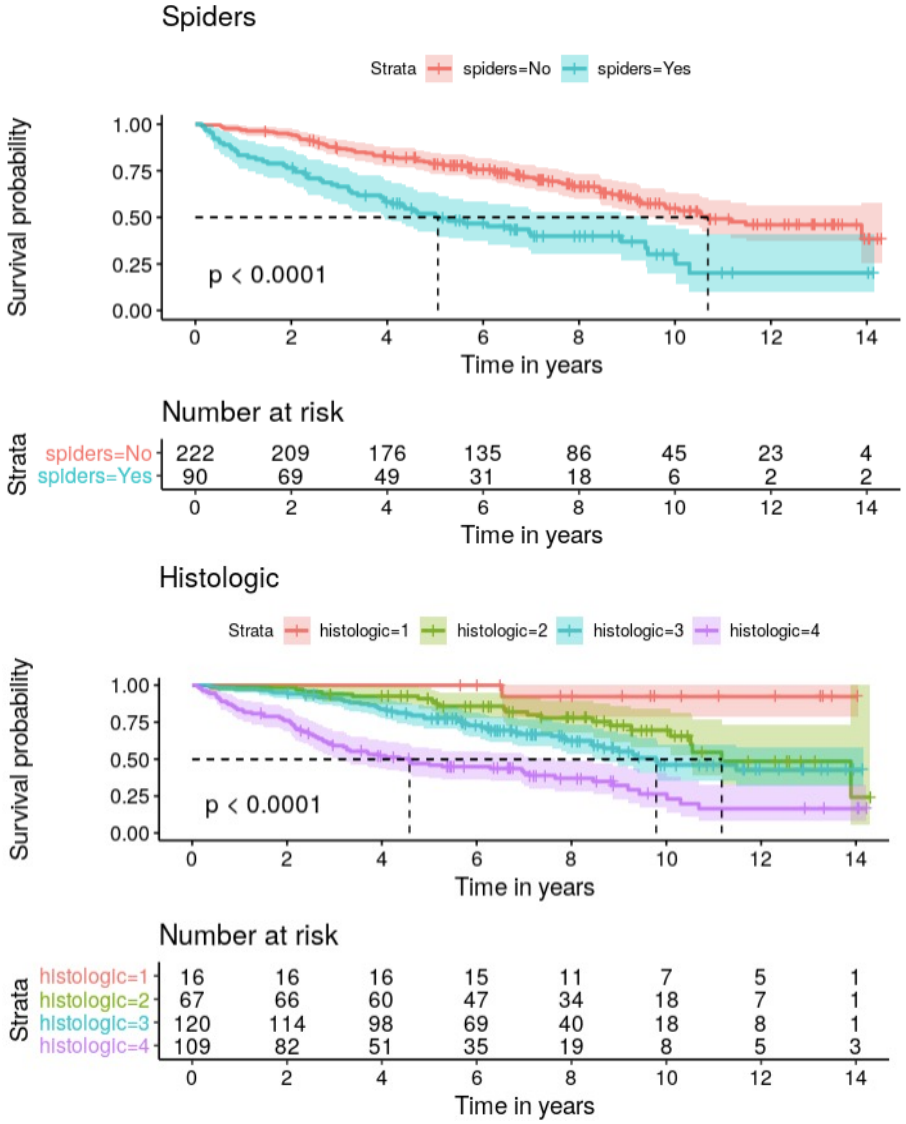
Drug is not statistically significant

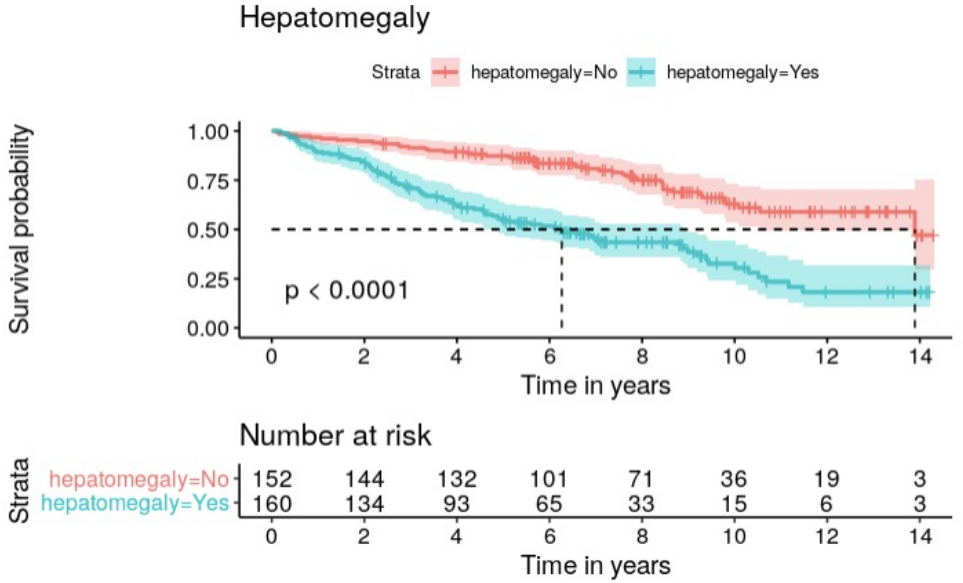
Sex

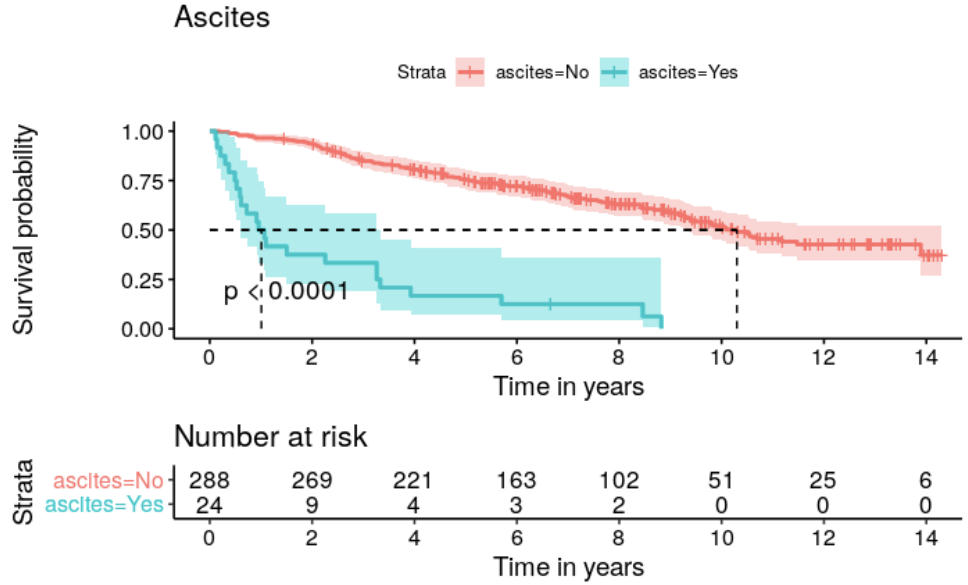


Sex is statistically significant

Kaplan-Meier Curves







Joint Model

- **Longitudinal sub-model**: Linear Mixed-Effect model
  - Response: Longitudinal bilirubin
  - Predictors: sex and baseline covariates for ascites, hepatomegaly, spiders, albumin, alkaline, SGOT, and prothrombin
  - Random Effect: visit-time | id

  Bilirubin varies among patients and changes dynamically within individuals

- **Survival sub-model**: Cox PH model
  - Response: survival
  - Covariates: baseline values for albumin, edema, and age

Empirical CDF

- Formula:

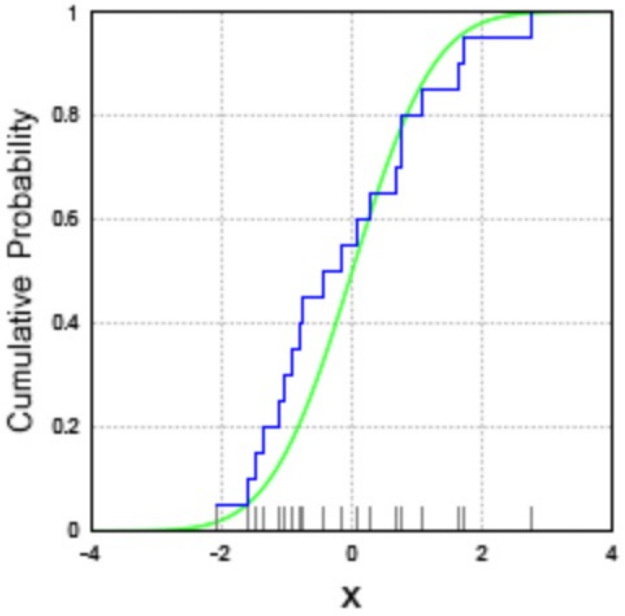
  $\hat{F}_n(t) = \frac{1}{n}\sum_{i=1}^{n} I(X_i \leq t)$

- Indicator $I(X_i \leq t)$ is a

Bernoulli R.V. with $p = F(t)$

- Without censoring:

  $\hat{F}_n(t) = 1 - S(t)$



Source: Wikipedia

Competing Risks Framework

- Cause-Specific Hazards:

$$\lambda_{ik}^{\#}(t) = \lim_{dt \to 0} \frac{\Pr(t \leq T^* \leq t + dt,\ \Delta_i = k \mid T^* \geq t)}{dt}$$

- Sub-distribution Hazards:

$$\lambda_{ik}^{F}(t) = \lim_{dt \to 0} \frac{\Pr(t \leq T^* \leq t + dt, \Delta_i = k \mid T^* \geq t \cup T^* < t, \Delta_i \neq k)}{dt}$$

Log-rank based test

- Nonparametric test for the difference in survival between two or more groups
- Formula:

$$\text{Log-rank statistic} = \sum_{i=1}^{G} \frac{(E_i - O_i)^2}{Var\,(E_i)}$$

- Note: E_i, O_i are the expected and observed number of events
- Under null hypothesis, log-rank statistic approximately follows χ^2_{G-1} distribution