Survival Analysis Using R

Antonio, Fabio Di Narzo

Program

1. Model Based, Classic Statistical Inference and Regression Analysis
2. Nonparametric methods for Survival Analysis
3. Semi-parametric regression
4. Semi-parametric regression: model building and diagnostics
5. Advanced topics

Intro

References

- Modeling Survival Data; Therneau, T., Grambsch, P.
- Modeling Survival Data: Extending the Cox Model. Springer-Verlag, 2000
- Survival Analysis: A Self-Learning Text, Third edition (Hardcover) by David G. Kleinbaum, Mitchel Klein
- Applied Survival Analysis Using R (Use R!) (Paperback) by Dirk F. Moore

Prerequisites

Classic Statistical Inference, Statistical Modeling

RStudio up and running

Being able to write and run an R script

Some extra R packages installed:
asaur, tidyverse, maxLik, survivalROC, glmnet

Objectives

At the end of this course you should be able to perform statistical inference on survival data:

- estimate survival, parametrically or non-parametrically
- compare 2 or more groups
- make predictions

using the R statistical software

Definition: Survival Analysis

- Survival Analysis is the study of survival times and the factors that influence them
- Survival times, aka 'times to failure', have some distinguishing features:
  - non negative
  - the information is often only partially recorded (censoring)
- Aims:
  - Summarize and *interpret* survival/time to failure data
  - Make statistical *predictions*

Some Example Applications

The following examples are all taken from past students projects:

- Clinical trials : life expectancy of cancer patients by clinical outlook and treatment options
- Criminal Recidivism : risk of returning to prison by different follow-up policies
- Phone contract termination : risk by demographics and contract type
- Corruption in [country] : risk of corruption indictment by political party and region
- Unemployment Insurance claims : duration of unemployment by demographic and geographic factors
- Breast feeding behaviors : duration of breast feeding by ethnic, social and clinical background
- Roman Emperors reigns : risk of violent death by historical period, dinasty
- Heroin addiction : risk of relapse of heroin addicts by different treatment options
- Reliability of grid power lines : risk of failure by technology and geographic location

Model-Based, Parametric Statistical Inference

Case Study

We'll go through typical Data Analysis steps:

1. Problem definition and scoping
2. Probabilistic modeling: the Data Generating Process
3. Model estimation and statistical inference
4. Results interpretation and conclusions

Case Studies

- Duration of hospitalization
- Mortality by COVID-19
- Smoking behavior of DSTI students

Probabilistic description of survival data

Probabilistic description of Duration Data

- Support: $0 \leq t < \infty$

The distribution can be specified thorugh one the following:

- Survival function:
$$S(t) = \Pr(T > t)$$
- Cumulative Distribution Function (CDF):
$$F(t) = \Pr(T \leq t) = 1 - \Pr(T > t) = 1 - S(t)$$
- Probability Density Function (PDF):
$$f(t) = F'(t) = -S'(t)$$

Hazard Function

A meaningful quantity linked to a survival distribution is the Hazard function:

$$h(t) = \lim_{\delta \to 0} \frac{\Pr(t < T < t + \delta | T > t)}{\delta}$$

and the derived Cumulative Hazard function:

$$H(t) = \int_0^t h(u)du$$

- Note:
$$h(t) = \frac{f(t)}{S(t)}$$
$$S(t) = exp(-H(t))$$

Mean and median survival time

- Mean survival:
$$\mu = E(T) = \int_0^\infty tf(t)dt = \int_0^\infty S(t)dt$$
Note: μ is only defined if $S(\text{inf}) = 0$.
- Median survival
$$t : S(t) = 0.5$$

Recap (1/2)

For $t \in [0, \infty)$:

- Survival function: $S(t) = \Pr(T > t)$, *right continuous*
- Cumulative Distribution Function (CDF): $F(t) = \Pr(T \leq t)$
- Probability Density Function (PDF): $f(t) = F'(t)$
- Hazard function: $h(t) = \lim_{\delta \to 0} \frac{\Pr(t < T < t + \delta | T > t)}{\delta}$
- Cumulative Hazard function: $H(t) = \int_0^t h(u)du$

Recap (2/2)

Some relationships allow us to switch from one quantity to another:

$$f(t) = F'(t)$$

$$h(t) = \frac{f(t)}{S(t)}$$

$$H(t) = \int_0^t h(u)du$$

$$S(t) = exp(-H(t))$$

$$F(t) = 1 - S(t)$$

$$E(T) = \mu = \int_0^\infty tf(t)dt = \int_0^\infty S(t)dt$$

$$\text{median}(t) = \{t : S(t) = 0.5\} = S^{-1}(0.5)$$

Survival distribution: Exponential

- constant hazard: $h(t) = \lambda$
- cumulative hazard: $H(t) = \int_0^t \lambda du = \lambda \int_0^t du = \lambda t$
- mean: $\int_0^\infty e^{-\lambda t} dt = 1/\lambda$
- Exercise: can you determine:
  - Survival function
  - PDF
  - Median

Survival distribution: Exponential (cont.)

- Survival function: $e^{-\lambda t}$
- PDF: $f(t) = -S'(t) = \lambda e^{-\lambda t}$
- Median: $0.5 = e^{-\lambda t} \implies t_{\text{med}} = ln(2)/\lambda$

Nonparametric methods for censored data

Case study: animal testing mimicking human stress

- one area of pharmacological research focuses on alleviating stress
- drug development requires (animal) testing
- in principle, all that's needed is an animal with a readout that can proxy what would be stress levels in human
- there are several well known blood markers for stress
- here we'll see one *behavioral* marker

Case study: Novel Object Interaction (NOI) in rats: the experiment

For each of 5 rats:

1. put it in the box with a new object and start the timer
2. visually follow the rat for 120s
3. take note of when the first interaction happens (physically touching the new object)

Case study: Novel Object Interaction (NOI) in rats: the data

After running the experiment, our (precious) data table looks like this:

rat ID	time	status
rat1	55	0
rat2	50	1
rat3	70	1
rat4	120	0
rat5	110	1

where we adopted the convention:

$$\text{status} = \begin{cases} 0 & \text{the experiment was somehow interrupted: no interaction} \\ 1 & \text{an interaction actually happened at that time point} \end{cases}$$

Can we estimate the survival function from these data?

We can start by ordering the table by time:

i	rat ID	time	status
1	rat2	50	1
2	rat1	55	0
3	rat3	70	1
4	rat5	110	1
5	rat4	120	0

$$\hat{S}_?(t) = P(\widehat{T > t}) = ?$$

Say $t = 10$. What would be a reasonable estimate of $S(t)$?

$$0 \leq t < 50: \quad \widehat{S}_?(t) = 1.0$$

What happened at $t = 50$? One of the experimental subjects experienced the event. To be precise, 1 out of 5 partecipating subjects experienced the event:

$$t = 50: \quad \widehat{S}_?(t) = 1 - 1/5 = 4/5$$

Thinking about it, as no events happen between $t = 50$ and $t = 70$, we can actually write:

$$50 \leq t < 70: \quad \widehat{S}_?(t) = 4/5 = 0.8$$

$$\widehat{S}_?(70) = ?$$

Let's add one more utility column to our sorted table:

i	rat ID	time	status	n
1	rat2	50	1	5
2	rat1	55	0	4
3	rat3	70	1	3
4	rat5	110	1	2
5	rat4	120	0	1

with $n =$ number of subjects still under observation at that time point

We can write:

$$\begin{aligned} P(T > 70) &= P((T > 70) \cap (T > 50)) \\ &= P(T > 70 | T > 50) \times P(T > 50) \end{aligned}$$

$$\hat{S}_?(70) = \left(1 - \frac{1}{3}\right) \times \frac{4}{5} \simeq 0.533$$

Again, as nothing happens between $t = 70$ and $t = 110$, we can write:

$$70 \leq t < 110 : \quad \widehat{S}_?(t) = \frac{2}{3} \times \frac{4}{5} \simeq 0.533$$

similarly for the next event:

$$110 \leq t < 120 : \quad \widehat{S}_?(t) = \frac{1}{2} \times \frac{2}{3} \times \frac{4}{5} \simeq 0.267$$

We can summarize our calculations in this table:

i	j	rat ID	time	status	n	q	1-q	S
1	1	rat2	50	1	5	1/5	4/5	$4/5 = 0.8$
3	2	rat3	70	1	3	1/3	2/3	$2/3 \times 4/5 = 8/15 \simeq 0.533$
4	3	rat5	110	1	2	1/2	1/2	$1/2 \times 8/15 = 4/15 \simeq 0.267$

Congrats! We just re-discovered the Kaplan-Meier estimator

Notation

- T: time to failure
- U: time to censoring
- δ: I[T < U]
- observed data: $(\min(T, U), \delta)$

Kaplan-Meier Estimator (KM)

$$\hat{S}_{KM}(t) = \prod_{t_i \leq t} (1 - \hat{q}_i) = \prod_{t_i \leq t} \left(1 - \frac{d_i}{n_i}\right)$$

- n_i: # subjects at risk at time t_i
- d_i: # subjects failing at time t_i

Kaplan-Meier estimation in R

The KM estimator can be obtained with the `survival::survfit` function:

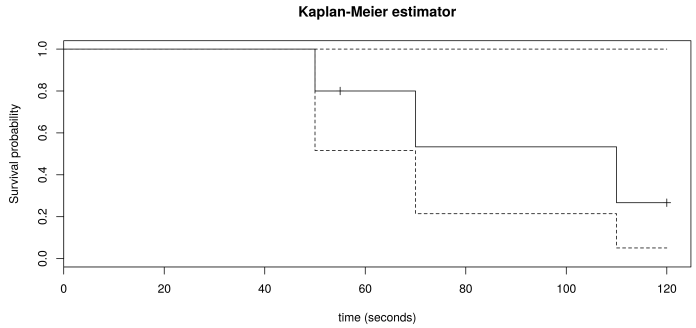
```
library(survival)
dat <- data.frame(ratID = paste0("rat", 1:5),
                  time = c(55, 50, 70, 120, 110),
                  status = c(0, 1, 1, 0, 1))
fit.KM <- survfit(Surv(time, status) ~ 1, data = dat)
summary(fit.KM)
```

```
Call: survfit(formula = Surv(time, status) ~ 1, data = dat)

time n.risk n.event survival std.err lower 95% CI upper 95% CI
  50      5       1    0.800   0.179       0.5161            1
  70      3       1    0.533   0.248       0.2142            1
 110      2       1    0.267   0.226       0.0507            1
```


Kaplan-Meier estimation in R (cont.)

```
plot(fit.KM, mark.time = TRUE,
     main = "Kaplan-Meier estimator",
     ylab = "Survival probability",
     xlab = "time (seconds)")
```



Question: what is the median survival time?

```
fit.KM
```

```
Call: survfit(formula = Surv(time, status) ~ 1, data = dat)

n  events  median 0.95LCL 0.95UCL
5       3     110      70      NA
```

Refined definition of median survival time:

- maximum time t such that $S(t) \geq 0.5$

Note: there's no upper bound as the upper bound SC never goes below 0.5

Censoring

- censoring is a main feature of survival data
- it happens when starting or ending events are not precisely observed
- in this course, we will focus on right censoring: the time to failure for some samples is only known to *exceed* a particular value
- censoring might happen because:
  - the event of interest did not happen by the end of the study
    - e.g., we turn on 100 lightbulbs for 30 days, and we record burnout times; for lightbulbs still on after 30 days, we can only say that the *survival time* was > 30 days
  - the sample drops out from the study from unrelated causes
    - e.g., in a clinical trial, 200 subjects might be administered a new drug and their prognosis followed for 10 years; in these 10 years, some of the 200 subjects might move to a different city, die of unrelated causes, or just plain decide to stop partecipating in the study

An alternative approach

Back to our data table:

i	j	rat ID	time	status	n	q
1	1	rat2	50	1	5	1/5
3	2	rat3	70	1	3	1/3
4	3	rat5	110	1	2	1/2

The q_js can be seen as empirical estimates of instantaneous risks at the times t_j
They can be *cumulated*, to get a corresponding empirical cumulated risk:

$$\hat{H}_j = \sum_{i=1}^{j} q_i$$

From those, an estimator of survival could be:

$$\hat{S}_?(t_j) = e^{-\hat{H}_j}$$

An alternative approach (cont.)

Here is how the calculation looks like:

i	j	rat ID	time	status	n	q	H	S
1	1	rat2	50	1	5	1/5	1/5	$e^{-1/5} \simeq 0.819$
3	2	rat3	70	1	3	1/3	8/15	$e^{-8/15} \simeq 0.587$
4	3	rat5	110	1	2	1/2	31/30	$e^{-31/30} \simeq 0.356$

We just computed the Nelson-AAlen estimator of survival

Nelson-AAlen estimator: definition

- AKA Fleming-Harrington estimator
- based on the relationship between cumulative hazard and survival function

$$\hat{H}_{NA}(t) = \sum_{t_i \leq t} \frac{d_i}{n_i}$$

$$\hat{S}_{NA}(t) = e^{-\hat{H}_{NA}(t)}$$

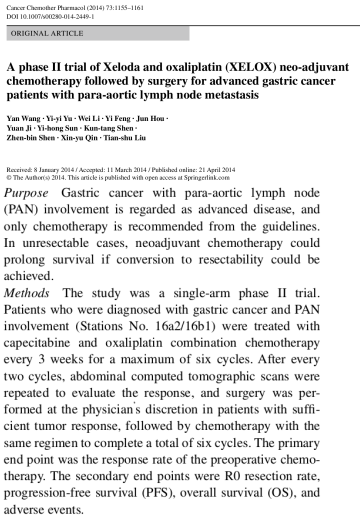
Nelson-AAlen estimation in R

```r
fit.NA <- survfit(Surv(time, status) ~ 1, data = dat, type = "fh")
summary(fit.NA)
```

```
Call: survfit(formula = Surv(time, status) ~ 1, data = dat, type = "fh")

time n.risk n.event survival std.err lower 95% CI upper 95% CI
  50      5       1    0.819   0.183       0.5282            1
  70      3       1    0.587   0.273       0.2356            1
 110      2       1    0.356   0.301       0.0677            1
```

Case study: the XELOX trial



Cancer Chemother Pharmacol (2014) 73:1155–1161
DOI 10.1007/s00280-014-2449-1

ORIGINAL ARTICLE

A phase II trial of Xeloda and oxaliplatin (XELOX) neo-adjuvant chemotherapy followed by surgery for advanced gastric cancer patients with para-aortic lymph node metastasis

Yan Wang · Yi-yi Yu · Wei Li · Yi Feng · Jun Hou · Yuan Ji · Yi-hong Sun · Kun-tang Shen · Zhen-bin Shen · Xin-yu Qin · Tian-shu Liu

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Purpose Gastric cancer with para-aortic lymph node (PAN) involvement is regarded as advanced disease, and only chemotherapy is recommended from the guidelines. In unresectable cases, neoadjuvant chemotherapy could prolong survival if conversion to resectability could be achieved.

Methods The study was a single-arm phase II trial. Patients who were diagnosed with gastric cancer and PAN involvement (Stations No. 16a2/16b1) were treated with capecitabine and oxaliplatin combination chemotherapy every 3 weeks for a maximum of six cycles. After every two cycles, abdominal computed tomographic scans were repeated to evaluate the response, and surgery was performed at the physician's discretion in patients with sufficient tumor response, followed by chemotherapy with the same regimen to complete a total of six cycles. The primary end point was the response rate of the preoperative chemotherapy. The secondary end points were R0 resection rate, progression-free survival (PFS), overall survival (OS), and adverse events.

To load the PFS data in R:

```
library(asaur)
dat <- gastricXelox
```


Case study: questions

- Express the Progress-Free Survival (PFS) times in *months*
- Estimate and plot the survival function using the KM and NA methods
- What's the median survival (and CI) according to the two methods?

Case study: R code

```
library(survival)
library(asaur)

dat <- gastricXelox
dat$months <- with(dat, timeWeeks * 7 / 30.5)
dat$S <- with(dat, Surv(months, delta))
fit.KM <- survfit(S ~ 1, data = dat, type = "kaplan-meier",
                  conf.type = "log-log")
fit.NA <- survfit(S ~ 1, data = dat, type = "fleming-harrington",
                  conf.type = "log-log")

plot(fit.KM)
plot(fit.NA)
```


Case study: median follow up time

A quality metric for a trial is the *median follow up time*:

```
dat$delta.followup <- 1 - dat$delta
survfit(Surv(months, delta.followup) ~ 1, type = "k",
        conf.type = "log-log")
```

```
   n  events  median 0.95LCL 0.95UCL
48.0    16.0    27.5    13.5    42.9
```

Comparing two groups

Back to our 5 rats:

rat ID	time	status	group
rat1	55	0	0
rat2	50	1	1
rat3	70	1	0
rat4	120	0	1
rat5	110	1	1

As it turns out, they were belonging to 2 different experimental groups: group 1, which was sleep deprived, and group 0, which followed a natural sleep pattern.
Is there evidence of a different stress level between the two (precious, though tiny) groups?

Comparing Survival between 2 samples

Null hypothesis:

$$H_0 : S_1(t) = S_0(t)$$

- $S_1(t)$: Survival Distribution in group 1 (e.g. *treated*)
- $S_0(t)$: Survival Distribution in group 0 (e.g. *control*)

The logrank test

For each failure time t_i, we build the following table:

	Control	Treatment	Total
Failures	d_{0i}	d_{1i}	d_i
Non-failures	$n_{0i} - d_{0i}$	$n_{1i} - d_{1i}$	$n_i - d_i$
Total	n_{0i}	n_{1i}	n_i

Under the assumption of independence of the two groups, conditional on the margins, d_{0i} follows the hypergeometric distribution:

$$E(d_{0i}|n_i, d_i, n_{0i}, n_{1i}) = n_{0i} d_i / n_i$$

$$\text{Var}(d_{0i}|n_i, d_i, n_{0i}, n_{1i}) = \frac{n_{0i} n_{1i} d_i (n_i - d_i)}{n_i^2 (n_i - 1)}$$

The logrank test (cont.)

Summing over all time points t_i:

$$U_0 = \sum_i (d_{0i} - e_{0i})$$

with variance:

$$\text{Var}(U_0) = \sum_i \text{Var}(d_{0i}) = V_0$$

Finally, the logrank test:

$$\frac{U_0^2}{V_0} \sim \chi_1^2$$

The logrank test in R

Using the `survival::survdiff` function:

```
dat <- data.frame(ratID = paste0("rat", 1:5),
                  time = c(55, 50, 70, 120, 110),
                  status = c(0, 1, 1, 0, 1),
                  group = c(0, 1, 0, 1, 1))

survdiff(Surv(time, status) ~ group, data = dat)
```

```
Call:
survdiff(formula = Surv(time, status) ~ group, data = dat)

N Observed Expected (O-E)^2/E (O-E)^2/V
group=0 2        1    0.733    0.0970     0.154
group=1 3        2    2.267    0.0314     0.154

Chisq= 0.2  on 1 degrees of freedom, p= 0.7
```


The Fleming-Harrington test

A weighted variation on the logrank test:

$$U_0(w) = \sum w_i(d_{0i} - e_{0i})$$

$$\text{Var}(U_0) = \sum w_i^2 v_{0i} = V_0(w)$$

with:

$$w_i = N(\hat{S}_{KM}(t_i))^\rho$$

- $\rho = 0$: logrank test
- $\rho = 1$: aka Prentice modification of the Gehan-Wilcoxon test: higher weights on *earlier* survival times

Case study: the pancreatic dataset

```
library(asaur)
dat <- pancreatic
head(dat)
```

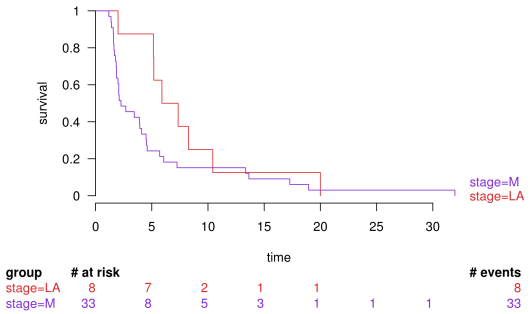
```
  stage    onstudy progression      death
1     M 12/16/2005    2/2/2006 10/19/2006
2     M   1/6/2006   2/26/2006  4/19/2006
3    LA   2/3/2006    8/2/2006  1/19/2007
4     M  3/30/2006           .  5/11/2006
5    LA  4/27/2006   3/11/2007  5/29/2007
6     M   5/7/2006   6/25/2006 10/11/2006
```


Case study: preparing the data for analysis

```
fmt <- "%m/%d/%Y"
dat <- within(dat, {
  onstudy <- as.Date(as.character(onstudy), format = fmt)
  progression <- as.Date(as.character(progression), format = fmt)
  death <- as.Date(as.character(death), format = fmt)
  OS <- death - onstudy
  PFS <- pmin(progression - onstudy, OS)
  PFS[is.na(PFS)] <- OS[is.na(PFS)]
  PFS <- Surv(as.numeric(PFS / 30.5))
  OS <- Surv(as.numeric(OS / 30.5))
})
```

Case study: estimating survival by stage

```
surv.KM <- survfit(PFS ~ stage, data = dat)
plot(surv.KM)
```



Case study: comparing survival by stage

```
survdiff(PFS ~ stage, data = dat)
```

```
             N Observed Expected (O-E)^2/E (O-E)^2/V
stage=LA     8        8     12.3      1.49      2.25
stage=M     33       33     28.7      0.64      2.25

 Chisq= 2.2  on 1 degrees of freedom, p= 0.134
```

```
survdiff(PFS ~ stage, data = dat, rho = 1)
```

```
             N Observed Expected (O-E)^2/E (O-E)^2/V
stage=LA     8     2.34     5.88     2.128      4.71
stage=M     33    18.76    15.22     0.822      4.71

 Chisq= 4.7  on 1 degrees of freedom, p= 0.0299
```


Exercises

- What's the median *Overall Survival* of a patient with Locally Advanced (LA) pancreatic cancer? And that of a patient with Metastatic (M) cancer?
- Can you provide confidence intervals for your estimates?
- Do the two stages experience significantly different survival?
- What's the probability (and 95% CI) of surviving more than a year within each group?

Solutions

- median OS + CIs: fit formula OS $\sim$ stage, then `summary(fit)`
- plot the curves for qualitative assessment, `survdiff(OS ~ stage)` for logrank test
- `summary(fit, time = 12)` will give survival and CIs at 12 months

Stratified tests

- Sometimes we want to compare survival between 2 groups *controlling* for potentially confounding factors, e.g.:
  - gender
  - age group
  - hospital
  - ...
- When this factor is categorical, we can use a stratified logrank test

$$X^2 = \frac{\left(\sum_{g=1}^{G} U_{0g}\right)^2}{\sum_{g=1}^{G} V_{0g}^2}$$

distributed as a χ_1^2

Case study: the pharmacoSmoking dataset

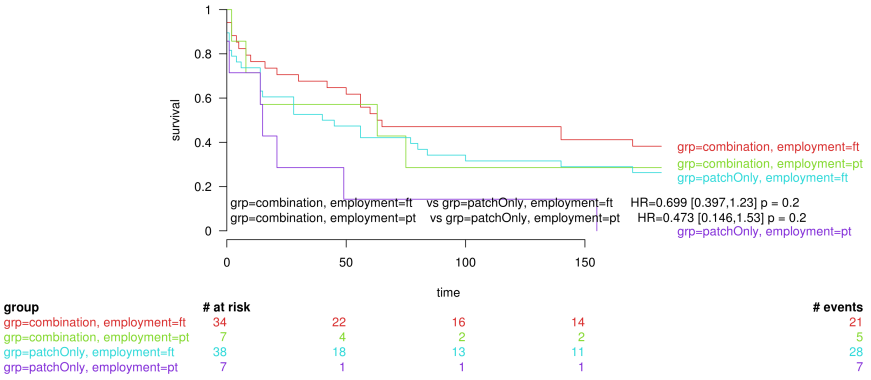
```
dat <- pharmacoSmoking

survdiff(Surv(ttr, relapse) ~ grp, data = dat)
#                  N Observed Expected (O-E)^2/E (O-E)^2/V
#grp=combination 61       37     49.9      3.36      8.03
#grp=patchOnly   64       52     39.1      4.29      8.03
#
# Chisq= 8  on 1 degrees of freedom, p= 0.00461

table(dat$AgeGroup2)
#21-49   50+
#   66    59

survdiff(Surv(ttr, relapse) ~ grp + strata(ageGroup2), data = dat)
#                  N Observed Expected (O-E)^2/E (O-E)^2/V
#grp=combination 61       37     49.1      2.99      7.03
#grp=patchOnly   64       52     39.9      3.68      7.03
#
# Chisq= 7  on 1 degrees of freedom, p= 0.008
```


Exercises

- Assess the significance of the treatment stratifying by employment status
- Can you estimate survival in the 4 groups:
  - grp=combination/pathOnly x employment=ft/pt
- Assess the efficacy of the treatment combination therapy separately within patients working full time (*ft*) and part-time (*pt*)

Exercises: solution

```
survdiff(Surv(ttr, relapse) ~ grp + strata(employment), data = dat
    )

#                  N Observed Expected (O-E)^2/E (O-E)^2/V
#grp=combination 61       37     50.3      3.50      8.58
#grp=patchOnly   64       52     38.7      4.54      8.58
#
# Chisq= 8.6  on 1 degrees of freedom, p= 0.00339
```



Recap

- 1 sample inference: KM, HF (`survival::survfit`)
- 2 samples comparison: logrank test + weighted variations (`survival::survdiff`)

Cox Proportional Hazards Model

Proportional hazards model

- We saw methods for comparing 2 groups
- A more general approach is needed for comparing multiple groups, assessing the effect of continuous factor, and, in general, performing regression analysis
- Meet the Cox Proportional Hazards model:

$$h_i(t) = h_0(t)\exp(\boldsymbol{x}_i'\boldsymbol{\beta})$$

- $h_i(t)$: hazard for subject i at time t
- h_0: baseline hazard function
- $\boldsymbol{x}_i$: vector of covariates for subject i
- $\boldsymbol{\beta}$: vector of effects of each covariate on risk

Proportional hazards model (cont.)

- Given an observed dataset $\{t_i, \delta_i, \boldsymbol{x}_i : i = 1, \ldots, n\}$, one can estimate $\boldsymbol{\beta}$ without having to specify the baseline hazard h_0
- The CPH model is thus called *semi-parametric*
- As failure times are generally *censored*, we cannot compute the classic likelihood, but rather the so called *Partial Likelihood*, which properly takes into account censoring times similarly to how it's done in the KM estimator
- We'll call $\hat{\beta}$ the estimator which maximizes the Partial Likelihood for a given dataset

CPH: Partial Likelihood

- Failure times $t_j : t_1 \leq t_2 \leq \ldots \leq t_j \leq \ldots \leq t_D$
- At time t_j, subject $i(j)$ fails, with hazard $h_i(t_j) = h_0(t_j)\exp(\boldsymbol{x}_{i(j)}\boldsymbol{\beta})$
- At failure time t_j, R_j subjects at risk
- Partial Likelihood:

$$l(\beta) = \prod_{j=1}^{D} \frac{h_0(t_j)\exp(\boldsymbol{x}_{i(j)}\boldsymbol{\beta})}{\sum_{k \in R_j} h_0(t_j)\exp(\boldsymbol{x}_k\boldsymbol{\beta})}$$
$$= \prod_{j=1}^{D} \frac{\exp(\boldsymbol{x}_{i(j)}\boldsymbol{\beta})}{\sum_{k \in R_j} \exp(\boldsymbol{x}_k\boldsymbol{\beta})}$$

Comparing 2 groups

Our beloved rats:

rat ID	time	status	group
rat1	55	0	0
rat2	50	1	1
rat3	70	1	0
rat4	120	0	1
rat5	110	1	1

$$h_i(t) = h_0(t) \cdot \exp(\beta \times x_i)$$

$$x_i = \begin{cases} 0 & \text{rat was not under stress} \\ 1 & \text{rat was under stress} \end{cases}$$

$\exp(\beta)$ = ratio of risk of NOI in a stressed vs not stressed rat

Comparing 2 groups (cont.)

```r
library(survival)
dat <- data.frame(
  ratID = paste0("rat", 1:5),
  time = c(55, 50, 70, 120, 110),
  failure = c(0, 1, 1, 0, 1),
  group = c(0, 1, 0, 1, 1))
fit <- coxph(Surv(time, failure) ~ group, data = d)
summary(fit)
```


Comparing 2 groups (cont.)

```
coxph(formula = Surv(time, failure) ~ x, data = dat)

n= 5, number of events= 3

      coef exp(coef) se(coef)      z Pr(>|z|)
x -0.5493    0.5774   1.4179 -0.387    0.698

  exp(coef) exp(-coef) lower .95 upper .95
x    0.5774      1.732   0.03585     9.297

Concordance= 0.5  (se = 0.202 )
Rsquare= 0.029   (max possible= 0.743 )
Likelihood ratio test= 0.15  on 1 df,   p=0.7
Wald test            = 0.15  on 1 df,   p=0.7
Score (logrank) test = 0.15  on 1 df,   p=0.7
```

Comparing 2 groups: model interpretation

$$h_i(t) = h_0(t)\exp(x_i\beta)$$

$$x_i = \begin{cases} 0 & \text{sample } i \text{ is a control} \\ 1 & \text{sample } i \text{ is treated} \end{cases}$$

$$\hat{\beta} = -0.549 \pm 1.418 \times 1.96$$

What's the risk of a sleep deprived (treated) rat compared to a control?

$$\frac{h_{x=1}(t)}{h_{x=0}(t)} = \frac{h_0(t)\exp(1 \times \hat{\beta})}{h_0(t)\exp(0 \times \hat{\beta})} = \exp((1-0)\hat{\beta})$$
$$= \exp(\hat{\beta}) = 0.577$$

Model interpretation

- in general, $\exp(\beta)$ is the hazard ratio associated with one unit increase of the regressor
- for 0/1 binary variables, it is e.g. a comparison between the group $x = 1$ and the group $x = 0$ (treated vs control, male vs female, *etc.*)
- more generally, x can be *continuous* (e.g., age of the subject)

Continuous covariates

```
d <- data.frame(patient = 1:6,
                time = c(6, 7, 10, 15, 19, 25),
                censored = c(1, 0, 1, 1, 0, 1),
                age = c(67, 62, 34, 41, 46, 28))
fit <- coxph(Surv(time, censored) ~ age, data = d)
```

Questions:

- is the effect of age on risk significant?
- what's the HR for a 1 year increase of age?
- what's the HR for a 10 years increase of age?

Continuous covariates (cont.)

```
  n= 6, number of events= 4

       coef exp(coef) se(coef)     z Pr(>|z|)
age 0.07606   1.07903  0.07316 1.04     0.298

    exp(coef) exp(-coef) lower .95 upper .95
age     1.079     0.9268    0.9349     1.245

Concordance= 0.7  (se = 0.22 )
Rsquare= 0.209   (max possible= 0.76 )
Likelihood ratio test= 1.41  on 1 df,   p=0.2356
Wald test            = 1.08  on 1 df,   p=0.2985
Score (logrank) test = 1.33  on 1 df,   p=0.2482
```

```
exp(0.076 * 10)
# [1] 2.138276
```

Multiple covariates

```
library(asaur)
dat <- pharmacoSmoking
names(pharmacoSmoking)
```

```
 [1] "id"             "ttr"            "relapse"        "grp"
 [5] "age"            "gender"         "race"           "
    employment"
 [9] "yearsSmoking"   "levelSmoking"   "ageGroup2"      "ageGroup4
    "
[13] "priorAttempts"  "longestNoSmoke"
```


Multiple covariates (cont.)

```
library(survival)
fit <- coxph(Surv(ttr, relapse) ~ grp + age + gender +
    priorAttempts,
             data = dat)
summary(fit)
```


Multiple covariates (cont.)

```
  n= 125, number of events= 89

                   coef  exp(coef)   se(coef)      z Pr(>|z|)
grppatchOnly   0.5656340  1.7605636  0.2181634  2.593  0.00952 **
age           -0.0220948  0.9781475  0.0097572 -2.264  0.02355 *
genderMale    -0.1215514  0.8855455  0.2334349 -0.521  0.60257
priorAttempts  0.0002078  1.0002079  0.0010898  0.191  0.84876
---

               exp(coef) exp(-coef) lower .95 upper .95
grppatchOnly      1.7606     0.5680     1.1480     2.700
age               0.9781     1.0223     0.9596     0.997
genderMale        0.8855     1.1292     0.5604     1.399
priorAttempts     1.0002     0.9998     0.9981     1.002

Concordance= 0.623  (se = 0.034 )
Rsquare= 0.107   (max possible= 0.998 )
```


Multiple covariates: interpretation

What's the risk of relapse in subjects treated with patch only, compared to subjects with combination therapy, *all other covariates being the same*?

$$\begin{aligned}
&\frac{h(t|grpPO = 1, age = X, genderMale = Y, priorAttempts = Z)}{h(t|grpPO = 0, age = X, genderMale = Y, priorAttempts = Z)} \\
&= \frac{\exp(1\beta_1 + X\beta_2 + Y\beta_3 + Z\beta_4)}{\exp(0\beta_1 + X\beta_2 + Y\beta_3 + Z\beta_4)} \\
&= \exp[(1-0)\beta_1 + (X-X)\beta_2 + (Y-Y)\beta_3 + (Z-Z)\beta_4] \\
&= \exp(\beta_1) \\
&= \exp(0.5656) = 1.7606
\end{aligned} \tag{1}$$

Note: reordering categorical variables in R

```
dat$grp <- relevel(dat$grp, ref = "patchOnly")
update(fit)
```

```
                    coef exp(coef)  se(coef)      z      p
grpcombination -0.565634  0.568000  0.218163 -2.59 0.0095
age            -0.022095  0.978147  0.009757 -2.26 0.0235
genderMale     -0.121551  0.885546  0.233435 -0.52 0.6026
priorAttempts   0.000208  1.000208  0.001090  0.19 0.8488
```


Predicting Survival

$$\hat{h}_0(t_i) = d_i / \sum_{j \in R_i} \exp(\boldsymbol{x}_j \hat{\boldsymbol{\beta}})$$

$$\hat{H}_0(t) = \sum h_0(t_j), \quad t_j \leq t$$

$$\hat{S}_0(t) = \exp(-\hat{H}_0(t))$$

$$\hat{S}(t|\boldsymbol{x}) = [S_0(t)]^{\exp(x\hat{\beta})}$$

Cfr. R function `survival::survfit.coxph`

Predicting Survival: Exercise

```
d <- data.frame(patient = 1:6,
                time = c(6, 7, 10, 15, 19, 25),
                censored = c(1, 0, 1, 1, 0, 1),
                age = c(67, 62, 34, 41, 46, 28))
```

Predict and plot survival curves at age 20, 50 and 70

Predicting Survival: Exercise (cont.)

```
fit <- coxph(Surv(time, censored) ~ age, data = d)
pred <- survfit(fit, newdata = data.frame(age = c(20, 40, 60)))
plot(pred, col = 1:3)
```

Recap

- when no censoring, classical statistical methods (MLE and friends)
- with right-censored data:
  - 1 sample inference: KM, HF (`survival::survfit`)
  - 2 samples comparison: logrank test + weighted variations (`survival::survdiff`)
  - continuous factors and/or multiple covariates: Cox regression (`survival::coxph`, `survival::survfit`)

Model building

- comparing nested models
- comparing non-nested models
- assessing goodness of fit
- checking model assumptions

Comparing models

We will consider the following models for the `pharmacoSmoking` dataset:

- M0: *no covariates* (hint: ~ 1)
- MA: ageGroup4
- MB: employment
- MC: ageGroup4 + employment

Both models `MA` and `MB` are nested into model `MC`, however `MA` and `MB` are not nested into eachother.
Exercise: fit the 3 models in R and store them in the variables `M0`, `MA`, `MB` and `MC`. We will be comparing these models

An aside: the Likelihood Ratio Test

$$Y_i \sim f(\theta), \quad i = 1, \dots, n \quad \theta \in \Theta$$
$$H_0 : \theta \in \Theta_0$$
$$H_1 : \theta \notin \Theta_0$$

A very important test statistic for H_0 is the LRT:

$$\text{LRT}_n = -2\ln \frac{\sup\{L(\theta; \mathbf{y}) : \theta \in \Theta_0\}}{\sup\{L(\theta; \mathbf{y}) : \theta \in \Theta\}}$$

An aside: Wilks theorem (1938)

Under H_0, $n \to \infty$:

$$\text{LRT}_n \to^p_{n\to\infty} \chi^2_p$$

where p is the difference in dimensionality between Θ_0 and Θ.

Note: a necessary condition for the Theorem to hold is that Θ_0 is in the *interior* of Θ (i.e., Θ_0 should not be on the boundaries of Θ).

Nested models: (partial) Likelihood Ratio Test (LRT)

```
anova(MA, MC)
```

```
Analysis of Deviance Table
 Cox model: response is  Surv(ttr, relapse)
 Model 1: ~ ageGroup4
 Model 2: ~ ageGroup4 + employment
   loglik  Chisq Df P(>|Chi|)
1 -380.04
2 -377.76 4.5666  2     0.1019
```


Non-nested models: AIC

$$\text{AIC} = -2\text{logLik}(\hat{\beta}) + 2 \cdot k \qquad (2)$$

The *smaller* the *better*

```
fits <- list(M0 = M0, MA = MA, MB = MB, MC = MC)
sapply(fits, AIC)
##       MA       MB       MC
## 766.0860 774.2464 765.5194
```


Step-wise model selection based on AIC

```
Mfull <- coxph(Surv(ttr, relapse) ~ grp + gender + race +
                 employment + yearsSmoking + levelSmoking +
                 ageGroup4 + priorAttempts + longestNoSmoke,
               data = dat)
MAIC <- step(Mfull)
```

Step-wise model selection based on AIC (cont.)

First step:

```
Start:  AIC=770.2
Surv(ttr, relapse) ~ grp + gender + race + employment +
    yearsSmoking +
    levelSmoking + ageGroup4 + priorAttempts + longestNoSmoke

                 Df    AIC
- race            3 766.98
- yearsSmoking    1 768.20
- gender          1 768.20
- priorAttempts   1 768.24
- levelSmoking    1 768.47
- longestNoSmoke  1 769.04
<none>              770.20
- employment      2 772.45
- ageGroup4       3 774.11
- grp             1 776.80
```

Check `?step` for further options

Predictive power: Concordance Index

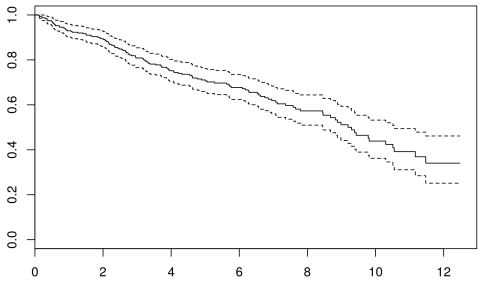
- Harrell's Concordance Index: fraction of pairs of patients whose survival times are correctly ordered by the model-fitted hazard
- the higher, the better
- in R, output of `summary(fit.coxph)`

Predictive power: AUC

- A common measure of predictive power are the *ROC curve* (False Positive rate vs True Positive rate) and the associated *AUC*
- Their computation for survival data is complicated by the presence of censoring
- One can however estimate *time-dependent ROC curves* via Kaplan-Meier or Nearest Neighbor methods of Heagerty, Lumley & Pepe (Biometrics, Vol 56 No 2, 2000, PP 337-344)
- Conveniently implemented in the `survivalROC` R package

AUC (cont.)

```
library(survival)
library(survivalROC)

?mayo
plot(survfit(Surv(time / 365.25, censor) ~ 1, data = mayo))

```



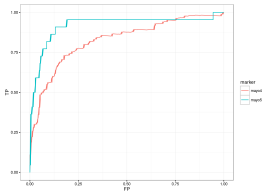
AUC (cont.)

```
ROC.4 <- survivalROC(Stime = mayo$time,
status = mayo$censor,
marker = mayo$mayoscore4,
predict.time = 365.25 * 5,
method="KM")

ROC.5 <- survivalROC(Stime = mayo$time,
status = mayo$censor,
marker = mayo$mayoscore5,
predict.time = 365.25,
method = "KM")

ROC <- list(mayo4 = ROC.4, mayo5 = ROC.5)

sapply(ROC, "[[", "AUC")
##      mayo4      mayo5
## 0.8257006 0.9180251

```


AUC (cont.)

We can plot the ROC curves using e.g. ggplot:

```
      dfl <- lapply(ROC, function(x) with(x,
    data.frame(FP, TP)))
      for(nm in names(dfl)) {
      dfl[[ nm ]]$marker <- nm
      }
      dat <- do.call(rbind, dfl)

      library(ggplot2)
      ggplot(dat, aes(FP, TP, color = marker))
     +
      geom_line() +
      theme_bw(base_size = 9)

```



AUC: exercise

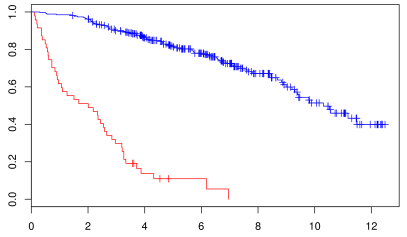
Lets select a cutoff for mayoscore5 with FP = 10%:

```
cutoff <- with(ROC$mayo5, min(cut.values[FP <= 0.1]))
## 7.511961

```

Question: can you compare the survival of patients with low vs high risk according to our chosen cutoff of `mayoscore5`?

AUC: solution

```
mayo$prediction <- ifelse(mayo$mayoscore5 <=
cutoff, "low_risk", "high_risk")

plot(survfit(Surv(time/365, censor) ~ prediction, data = mayo),
col = c("red", "blue"))

```



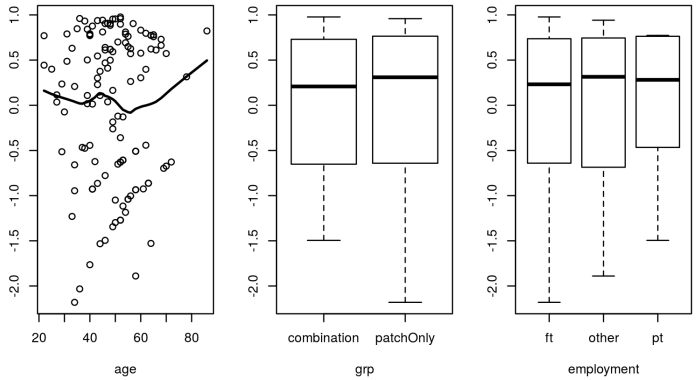
Martingale Residuals

- A model was built and estimated, but how well are we fitting the data?
- In linear regression, we can look at patterns in the model *residuals* (observed value − model predicion)
- For Cox regression, we have *martingale residuals*
  - they sum to 1
  - each is distributed between $-\infty$ and $+1$
  - each has an expected value of 0
  - their sum of squares is not an indicator of goodness of fit
  - patterns might suggest alternative functional forms for continuous covariates
- In R, we use `residuals(fit, type = 'martingale')`, from the `survival` package

Martingale Residuals in R

```r
library(survival)
library(asaur) ## dataset

data(pharmacoSmoking)
dat <- pharmacoSmoking
fit <- coxph(Surv(ttr, relapse) ~ grp + age + employment, data =
    dat)
dat$residual <- residuals(fit, type = 'martingale')

with(dat, {

  plot(age, residual)
  lines(lowess(age, residual), lwd = 2)

  plot(residual ~ grp)

  plot(residual ~ employment)

})
```


Martingale Residuals in R (cont.)

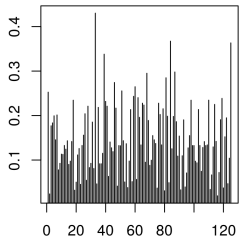


Case deletion residuals

- some samples might have a large impact on the final estimates
- we don't like it, as possibly all of our results (in extremne cases) might be driven by a single sample!
- such influential samples can be identified by estimating $\boldsymbol{\beta}$ twice: once with all the samples, and once without a specific sample i, and measuring the difference in $\boldsymbol{\beta}$
- in R, `residuals(fit, type = 'dfbetas')`

Case deletion residuals in R

```r
dfbetas <- residuals(fit, type = 'dfbetas')
dat$dfbetas <- sqrt(rowSums(dfbetas^2))

plot(dat$dfbetas, type = 'h')
abline(h = 0)
```



Proportionality of hazards

- one key assumption of the Cox model is the proportionality of hazards
- if we are comparing 2 groups:

$$S_1(t) = [S_0(t)]^{\exp(\beta)}$$

- by taking the log of both sides:

$$\log(S_1(t)) = \exp(\beta) \cdot \log[S_0(t)]$$

- finally, we can negate both sides and take a logarithm again:

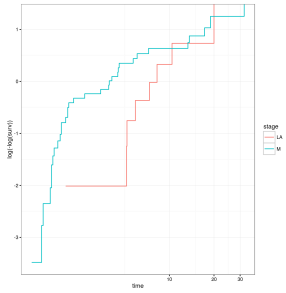
$$\log(-\log(S_1(t))) = \beta + \log(-\log(S_0)(t))$$

- in this scale ($g(u) = \log(-\log(u))$), S_0 and S_1 should be parallel

Proportionality of hazards: complementary log-log plot

Recall the 'pancreatic' dataset from the logrank test chapter:

```r
library(plyr)
dat <- pancreatic
surv <- ddply(dat, .(stage),
    function(x) {
  fit <- survfit(PFS ~ 1, data =
    x)
  data.frame(time = fit$time,
             surv = fit$surv)
})

ggplot(surv,
       aes(x = time,
           y = log(-log(surv)),
           color = stage)) +
  geom_step() +
  coord_trans(x = "log")
```



Schoenfeld Residuals

```r
dat <- pancreatic
residual.sch <- cox.zph(fit)

fit <- coxph(PFS ~ stage, data = dat)
residual.sch <- cox.zph(fit)
##            rho chisq      p
## stageM  -0.328  3.86 0.0496

plot(residual.sch)
```

Dealing with non-proportionality of the risks

- does it really matter?
- stratification
- truncation

Stratified Cox Regression

- Remember the Cox model:

$$h_i(t) = h_0(t)\exp(\mathbf{x}_i\beta) \quad \forall i$$

  where *all samples* share the same baseline hazard $h_0(t)$
- We can somewhat relax this assumption, and allow for 2 (or more) separate baseline hazards in different strata of the samples
- Stratified Cox model, strata A and B:

$$h_i(t) = \begin{cases} h_A(t)\exp(\mathbf{x}_i\beta) & i \in A \\ h_B(t)\exp(\mathbf{x}_i\beta) & i \in B \end{cases}$$

- Question: how is this different from just modeling A and B separately?
- Analyze the `asaur::pharmacoSmoking` dataset, stratifying by employment type

Truncation

- Proportionality of hazards might hold for a shorter, initial time span
- If so, we can restrict the analysis to a properly defined, initial time period
- How, in practice?
- Introduce a new, truncated time variable:

$$t' = \begin{cases} t & t \leq \text{threshold} \\ \text{threshold} & t > \text{threshold} \end{cases}$$

$$\delta' = \begin{cases} \delta & t \leq \text{threshold} \\ 0 & t > \text{threshold} \end{cases}$$

- R session: analyze the `asaur::pancreatic2` dataset, truncating the analysis to the first 6 months

Where to go from here

- regression analysis
- Cox regression
  - time-dependent covariates
  - time-dependent coefficients
  - competing risks
  - left censoring
  - multiple events
- parametric models for censored duration data

Penalized regression

Penalized regression

- generally speaking, if we have n observations, we can only estimate a model with *at most* $p = n$ parameters
- if we have many features p, with $p \gg n$, we can apply more general *machine learning* techniques (features selection, random forest, ...)
- we are still able though to fit Cox models using penalized regression

Elastic Net Cox model

- Remember the partial likelihood from the Cox model:

$$l(\beta) = \prod_{j=1}^{D} \frac{\exp(x'_{i(j)}\beta)}{\sum_{k \in R_j} \exp(x'_k \beta)}$$

When $p > n$, the β which maximizes it goes to $+\infty$

- we thus introduce the following elastic net costraint on β:

$$\alpha \sum |\beta_i| + (1 - \alpha) \sum \beta_i^2 \leq c$$

for some pre-specified value of c, and some pre-set weight α

Elastic Net Cox model (cont.)

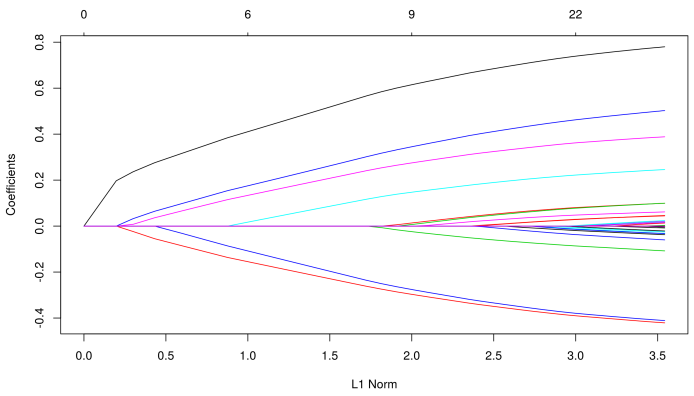
- a suitable value of c can be selected by e.g. cross-validation
- for $\alpha = 1$, we have the special case of the *lasso* penalty
- there is a *very fast* implementation available in the R package `glmnet`, by the same authors of the method: Jerome Friedman, Trevor Hastie and Rob Tibshirani

References: Simon, N., Friedman, J., Hastie, T., Tibshirani, R. (2011) Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent, Journal of Statistical Software, Vol. 39(5) 1-13

Penalized Cox regression in R

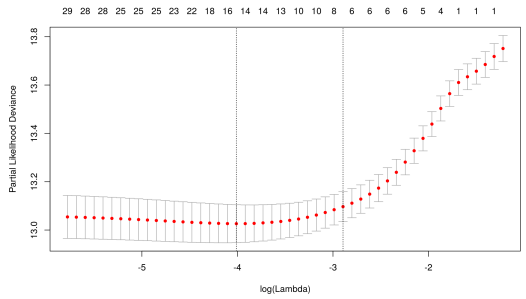
```r
library(survival)
library(glmnet)

set.seed(1234)

N <- 1000 # sample size
p <- 30 # num. features
nzc <- p/3 # num. 'true' predictors

x <- matrix(rnorm(N * p), nrow = N, ncol = p)
beta <- rnorm(nzc)
linear_predictor <- x[, seq_len(nzc)] %*% beta / 3

hazard <- exp(linear_predictor)

y_time <- rexp(N, rate = hazard)
y_cens <- rbinom(n = N, prob = 0.3, size = 1)
y <- Surv(y_time, 1 - y_cens)

fit <- glmnet(x, y, family="cox")
plot(fit)
```


Penalized Cox regression in R (cont.)



Selecting the optimal penalization parameter via cross validation

```r
set.seed(1234)

fit.cv10 <- cv.glmnet(x, y, family = "cox")
plot(fit.cv10)
```



Cross validation results

```r
str(fit.cv10)
```

```
List of 10
 $ lambda    : num [1:50] 0.295 0.269 0.245 0.223 0.203 ...
 $ cvm       : num [1:50] 13.8 13.7 13.7 13.7 13.6 ...
 $ cvsd      : num [1:50] 0.0541 0.0533 0.0533 0.0534 0.0534 ...
 $ cvup      : num [1:50] 13.8 13.8 13.7 13.7 13.7 ...
 $ cvlo      : num [1:50] 13.7 13.7 13.6 13.6 13.6 ...
 $ nzero     : Named int [1:50] 0 1 1 1 1 1 4 4 5 5 ...
  ..- attr(*, "names")= chr [1:50] "s0" "s1" "s2" "s3" ...
...
 $ lambda.min: num 0.0181
 $ lambda.1se: num 0.0553
```

Predictions

```
coef(fit.cv10, s = "lambda.1se")
##                1
## V1    0.58428498
## V2    .
## V3    .
## V4    0.31709716
## V5    0.12829144
## V6    0.25333876
## V7    .
## V8   -0.27412086
## ...

predict(fit.cv10,
        newx = x[1:5, ],
        s = "lambda.1se")
##               1
## [1,] -1.3542387
## [2,]  0.1777181
## [3,]  0.7534189
## [4,] -0.6364879
## [5,]  0.6758198
```

```
b <- coef(fit.cv10, s = "lambda.1
    se")
b.i <- which(b!=0)
bnz <- b[b.i]
y0 <- x[1:5, b.i, drop = FALSE] %
    *% bnz
```


Case Study: a survival microarray dataset

- `LymphomaData.rda`
  - `x`: gene expression matrix: 7399 genes $\times$ 240 samples
  - `time`: survival times
  - `status`: censoring status: 1 = observed, 0 = censored
- Use glmnet to fit a Cox model to find a predictor of survival based on gene expression
- Split the data into a training set, where you develop the model, and a testing set, where the model performance is assessed

Case Study: Validation of Colon Cancer Biomarkers

Case study

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ARTICLE

Test of Four Colon Cancer Risk-Scores in Formalin Fixed Paraffin Embedded Microarray Gene Expression Data

Antonio F. Di Narzo, Sabine Tejpar, Simona Rossi, Pu Yan, Vlad Popovici, Pratyaksha Wirapati, Eva Budinska, Tao Xie, Heather Estrella, Adam Pavlicek, Mao Mao, Eric Martin, Weinrich Scott, Fred T. Bosman, Arnaud Roth, Mauro Delorenzi

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Correspondence to: Mauro Delorenzi, PhD, SIB Swiss Institute of Bioinformatics, and University Lausanne, Office 2021, Génopode-UNIL, Quartier Sorge, CH-1015 Lausanne, Switzerland (e-mail: mauro.delorenzi@unil.ch).

Risk biomarkers for CRC

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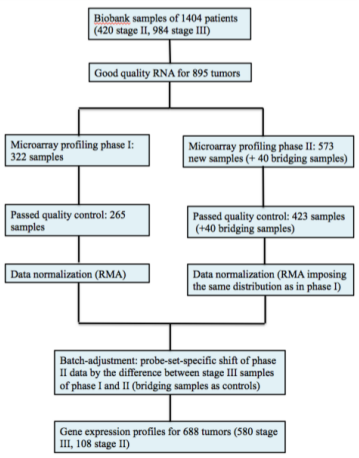
Table 1. Description of the four risk scores analyzed*

	Risk scores			
Abbreviation	**GHS**	**VDS**	**MDA**	**ALM**
Developer	Genomic Health	Veridex	MD Anderson	ALMAC diagnostics
Type of assay	Q-RT-PCR	microarray and Q-RT-PCR	microarray	microarray
Type of tissue	FFPE	fresh frozen and FFPE	fresh frozen	FFPE
Main publication	O'Connell et al. 2010.	Jiang et al. 2008.	Oh et al. 2011.	Kennedy et al. 2011.
Total number of features	7	7	114 (86 genes)	634 (482 genes)
Features used (genes)	7	6	85 (85 genes)	634 (identical platform)

* ALM = the scoring system proposed by Almac researchers; GHS = scoring system proposed by Genomic Health researchers; FFPE = formalin fixed paraffin embedded; MDA = scoring system proposed by researchers from MD Anderson Cancer Center; Q-RT-PCR = quantitative real-time PCR (Q-RT-PCR); VDS = scoring system proposed by Veridex researchers.

Study Design



Prognostic Value

Table 2. Cox models estimates

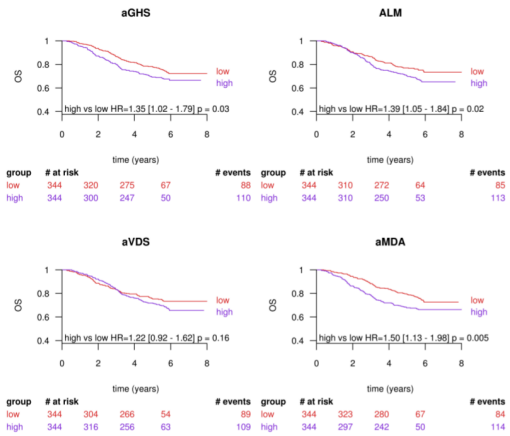
		Univariate*		Multivariable*†	
Outcome	**Marker**	**HR (95% CI)**	***P*‡**	**HR (95% CI)**	***P*‡**
RFS					
	aGHS	1.33 (1.13 to 1.56)	<.001	1.30 (1.11 to 1.53)	.001
	aVDS	1.29 (1.10 to 1.52)	.002	1.27 (1.07 to 1.51)	.007
	aMDA	1.10 (0.93 to 1.30)	.26	1.13 (0.93 to 1.37)	.22
	ALM	1.31 (1.13 to 1.53)	<.001	1.20 (1.02 to 1.40)	.03
	CS4§	1.56 (1.33 to 1.84)	<.001	1.45 (1.23 to 1.71)	<.001
SAR					
	aGHS	1.16 (0.95 to 1.43)	.14	1.16 (0.92 to 1.46)	.20
	aVDS	0.90 (0.72 to 1.13)	.38	0.84 (0.66 to 1.08)	.17
	aMDA	1.81 (1.45 to 2.27)	<.001	1.89 (1.46 to 2.46)	<.001
	ALM	1.19 (0.97 to 1.47)	.10	1.10 (0.88 to 1.36)	.40
	CS4§	1.46 (1.18 to 1.82)	<.001	1.33 (1.05 to 1.67)	.017
OS					
	aGHS	1.36 (1.13 to 1.64)	.001	1.34 (1.10 to 1.62)	.003
	aVDS	1.24 (1.03 to 1.50)	.02	1.21 (0.99 to 1.48)	.07
	aMDA	1.31 (1.08 to 1.58)	.006	1.37 (1.09 to 1.71)	.007
	ALM	1.38 (1.16 to 1.65)	<.001	1.22 (1.02 to 1.47)	.03
	CS4§	1.74 (1.44 to 2.10)	<.001	1.57 (1.29 to 1.91)	<.001

* Cox proportional hazards regression models were used to estimate hazard ratios for one interquartile range variation of the continuous risk scores; no stratification was applied; adjustment by treatment was applied only in the multivariable models. aGHS = microarray-based approximation of the scoring system proposed by Genomic Health researchers; ALM = the scoring system proposed by Almac researchers; aMDA = approximation of the scoring system proposed by researchers from MD Anderson Cancer Center; aVDS = approximation of the scoring system proposed by Veridex researchers; CI = confidence interval; CS4 = the scoring system obtained by combining the four existing systems; HR = hazard ratio; OS = overall survival; RFS = relapse-free survival; SAR = survival after relapse.

† Each multivariable model included one gene expression risk score and the following variables: age, gender, TNM staging (T-stage, N-stage) (27), grade, location (right = proximal, left = distal), treatment arm, presence of lymphovascular invasion, and microsatellite instability (MSI) status.

‡ Shown are single-test *P* values. The statistical significance cutoff by the Bonferroni principle (considering three tests) is at 0.05/3 = 0.0167.

Progonstic Value: OS



Prognostic Value: 3 years survival

Table 3. Three-year survival

Marker	Risk group	RFS % (95% CI) *	SAR % (95% CI) *	OS % (95% CI) *
aGHS	Whole cohort (N = 688)	66.9 (63.5 to 70.5)	34.4 (28.7 to 41.2)	83.4 (80.6 to 86.2)
	low	69.6 (64.9 to 74.7)	40.7 (32.4 to 51.2)	86.5 (83.0 to 90.2)
	high	64.2 (59.4 to 69.5)	28.5 (21.3 to 38.0)	80.2 (76.1 to 84.5)
aVDS	low	70.9 (66.2 to 75.8)	30.0 (21.8 to 41.1)	83.4 (79.5 to 87.4)
	high	63.0 (58.1 to 68.3)	37.6 (30.2 to 46.7)	83.4 (79.5 to 87.4)
aMDA	low	69.1 (64.3 to 74.1)	49.8 (41.2 to 60.1)	88.3 (84.9 to 91.8)
	high	64.8 (60.0 to 70.1)	19.9 (13.6 to 28.9)	78.5 (74.2 to 82.9)
ALM	low	70.8 (66.1 to 75.8)	36.8 (28.3 to 47.8)	86.6 (83.0 to 90.2)
	high	63.1 (58.2 to 68.4)	32.4 (25.1 to 41.6)	80.2 (76.1 to 84.5)
CS4	low	70.5 (65.8 to 75.5)	41.8 (33.1 to 52.9)	87.4 (84.0 to 91.0)
	high	63.4 (58.5 to 68.7)	28.7 (21.8 to 37.8)	79.3 (75.2 to 83.7)

* Estimated proportions of three-year survival (percentage) by the Kaplan-Meier method with 95% confidence intervals for the whole cohort and for risk groups defined by splitting the cohort at the median of each risk score into equally sized subgroups. aGHS = microarray-based approximation of the scoring system proposed by Genomic Health researchers; ALM = the scoring system proposed by Almac researchers; aMDA = approximation of the scoring system proposed by researchers from MD Anderson Cancer Center; aVDS = approximation of the scoring system proposed by Veridex researchers; CI = confidence interval; CS4 = the scoring system obtained by combining the four existing systems; OS = overall survival; RFS = relapse-free survival; SAR = survival after relapse.

Prognostic Value: concordance

Table 4. Concordance by risk score and endpoint groups*

Scoring method	Risk score subgroup	Actual survival group: Poor	Good	Rest
aGHS				
	Q1	46 (26.7%)	116 (67.4%)	10 (5.8%)
	Q2	58 (33.7%)	103 (59.9%)	11 (6.4%)
	Q3	54 (31.4%)	105 (61.0%)	13 (7.5%)
	Q4	69 (40.1%)	84 (48.8%)	19 (11.1%)
aVDS				
	Q1	40 (23.3%)	117 (68.0%)	15 (8.7%)
	Q2	60 (34.9%)	101 (58.7%)	11 (6.4%)
	Q3	63 (36.6%)	96 (55.8%)	13 (7.5%)
	Q4	64 (37.2%)	94 (54.7%)	14 (8.2%)
aMDA				
	Q1	51 (29.7%)	109 (63.4%)	12 (7.0%)
	Q2	55 (32.0%)	100 (58.1%)	17 (9.9%)
	Q3	62 (36.0%)	100 (58.1%)	10 (5.9%)
	Q4	59 (34.3%)	99 (57.6%)	14 (8.1%)
ALM				
	Q1	50 (29.1%)	110 (64.0%)	12 (7.0%)
	Q2	50 (29.1%)	109 (63.4%)	13 (7.6%)
	Q3	54 (31.4%)	103 (59.9%)	15 (8.7%)
	Q4	73 (42.4%)	86 (50.0%)	13 (7.5%)
CS4				
	Q1	36 (20.9%)	123 (71.5%)	13 (7.6%)
	Q2	65 (37.8%)	100 (58.1%)	7 (4.1%)
	Q3	57 (33.1%)	100 (58.1%)	15 (8.7%)
	Q4	69 (40.1%)	85 (49.4%)	18 (10.4%)

Improvement over standard clinical indicators: AUC

Table 5. Time-dependent receiver operating characteristic curves, area under curve (time = 3 years) by endpoint and risk score

Endpoint	Marker	AUC (ref. model) *	AUC gain*	*Pt*
RFS		0.6723		
	aGHS		0.0136	.04
	aVDS		0.0185	.009
	aMDA		0.0085	.17
	ALM		0.0089	.16
	CS4		0.0222	.0008
SAR		0.6406		
	aGHS		0.0192	.11
	aVDS		-0.0001	.79
	aMDA		0.0838	.0001
	ALM		0.0053	.54
	CS4		0.0443	.005
OS		0.6918		
	aGHS		0.0187	.005
	aVDS		0.0135	.03
	aMDA		0.0243	.001
	ALM		0.0140	.02
	CS4		0.0403	.0001

* Area under curve (AUC) for predicting survival status at three years was computed by risk scoring methods and endpoint. A reference model was fitted using the predictor variables N-stage, T-stage, and MSI status. The AUC gain was computed by adding the gene expression risk score to the predictor variables in the model. aGHS = microarray-based approximation of the scoring

Improvement over standard clinical indicators: CI

Supplementary Table 3. Concordance Index gains for risk scores by endpoint.

Endpoint	marker	concordance index*		
		clinical only†	difference	p-value‡
RFS		0.6432		
	aGHS		0.0115	**0.015**
	aVDS		0.0154	**0.001**
	aMDA		0.0070	0.10
	ALM		0.0092	0.04
	CS4		0.0229	**0.0001**
SAR		0.5930		
	aGHS		0.0069	0.35
	aVDS		0.0089	0.23
	aMDA		0.0615	**0.0001**
	ALM		0.0022	0.69
	CS4		0.0201	**0.016**
OS		0.6620		
	aGHS		0.0144	**0.003**
	aVDS		0.0108	0.017
	aMDA		0.0147	**0.004**
	ALM		0.0095	0.03
	CS4		0.0270	**0.0001**