

SAVEMORE analysis

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1. Data overview

- Exposure: anakinra vs. placebo.
- Survival outcome: 28-day and 90-day survival.
- Mediator: IL6 on days 1, 4 and 7.

2. Descriptives

2.1. Table 1

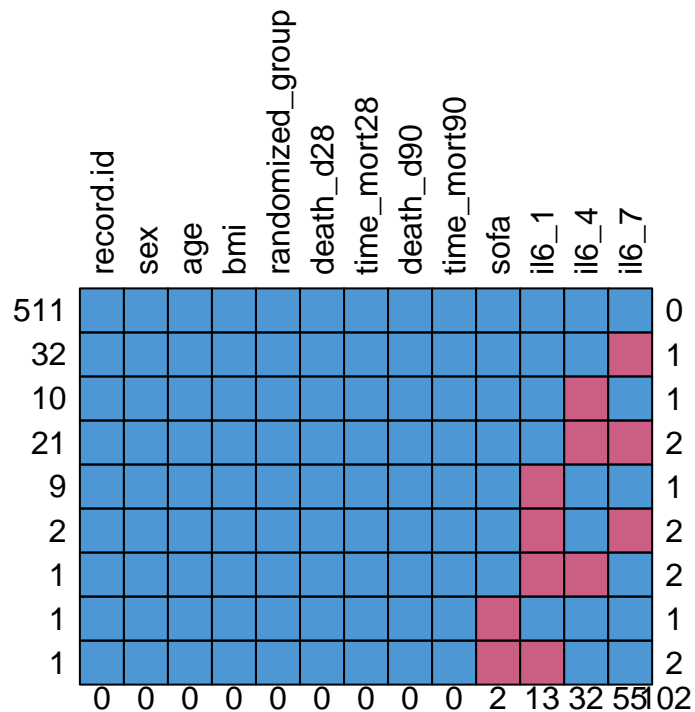
##		Stratified by randomized_group	
##		Placebo	Anakinra
##	n	187	401
##	death_d28 = 1 (%)	13 (7.0)	13 (3.2)
##	death_d90 = 1 (%)	19 (10.2)	22 (5.5)
##	il6_1 (mean (SD))	1.26 (0.57)	1.20 (0.54)
##	il6_4 (mean (SD))	1.05 (0.59)	0.91 (0.55)
##	il6_7 (mean (SD))	0.99 (0.63)	0.80 (0.57)
##	sex = male (%)	106 (56.7)	235 (58.6)
##	age (mean (SD))	61.53 (11.33)	62.09 (12.43)

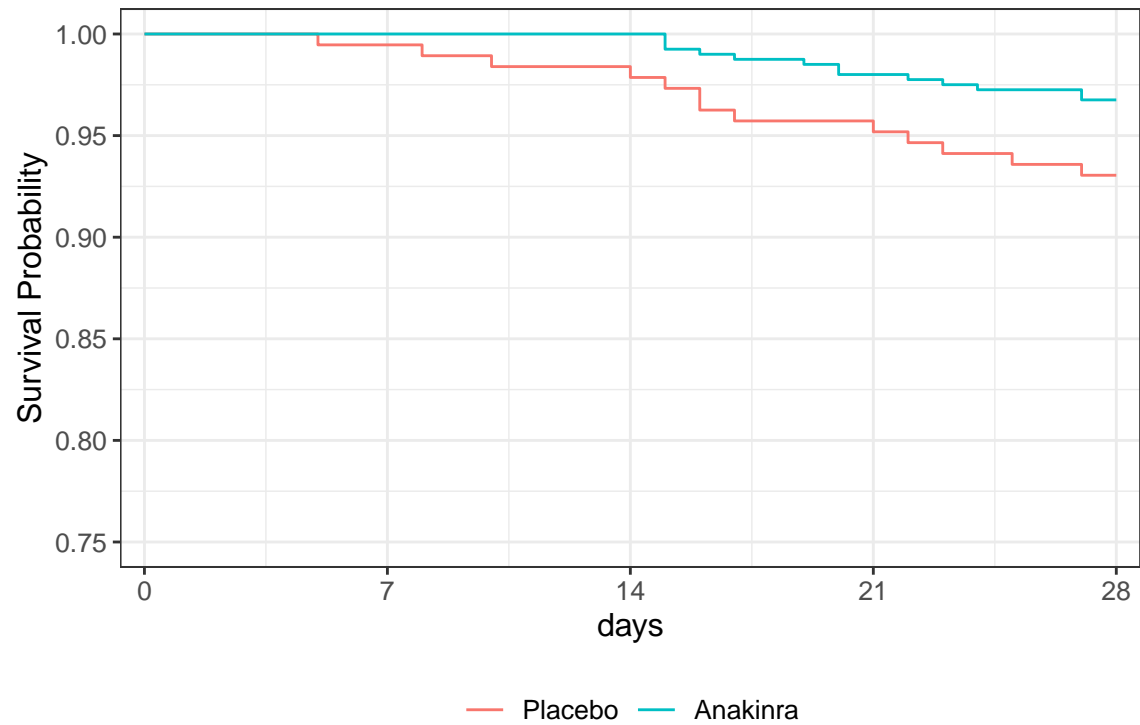
```
##   bmi (mean (SD))   29.73 (5.49)  29.40 (5.53)
##   sofa (mean (SD))   2.46 (1.15)   2.38 (1.11)
```

2.2. Missingness Of 593 patients, 5 have no IL6 biomarker measures. They are not included in any of the analyses.

For the 588 subjects who do have IL6 measures, this is the pattern of missingness:

```
missing_savemore <- mice::md.pattern(savemore_wide, rotate.names = TRUE, plot = TRUE)
```



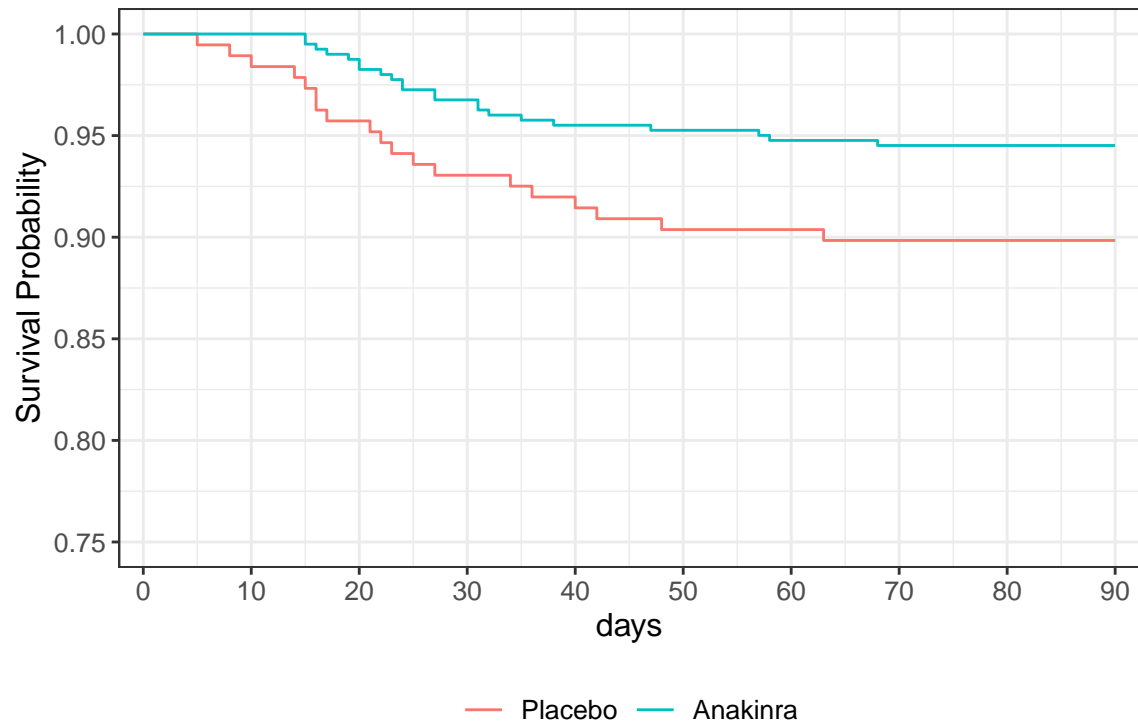


2.3. 28-day survival

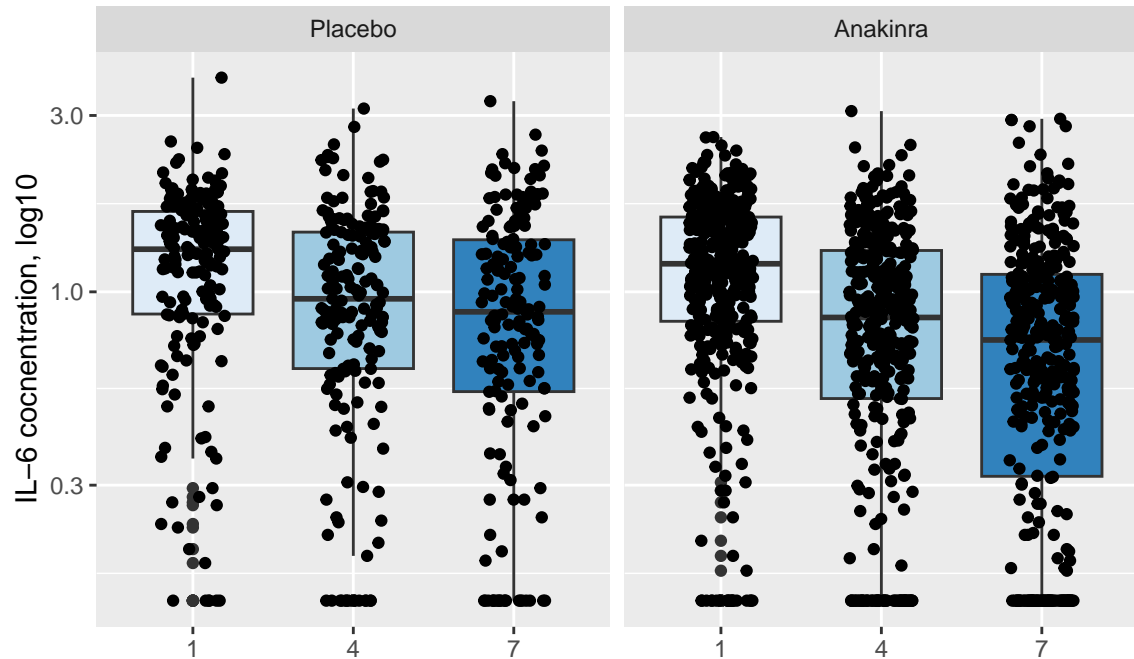
2.4. 90-day survival

```
class(savemore_surv$death_d90) <- "integer"

survfit2(Surv(time_mort90, death_d90) ~ randomized_group, data = savemore_surv) %>%
  ggsurvfit() +
  scale_ggsurvfit(x_scales= list(breaks = c(0, 10, 20, 30, 40, 50, 60, 70, 80, 90)))+
  ylim(c(.75, 1))+
  xlab("days")
```



IL-6 concentration on days 1, 4 and 7



2.5. IL-6 over time

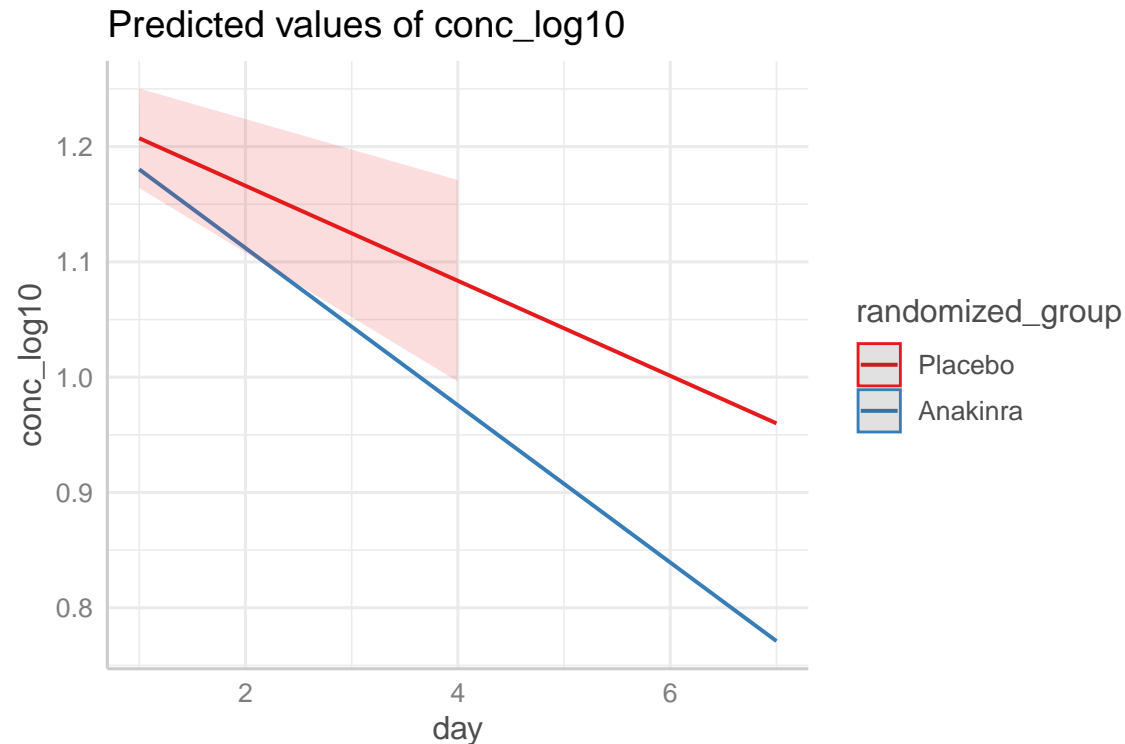
3. Models

```
# fit linear mixed model
lmeFit.savemore <-lme(conc_log10~ day:randomized_group +day,
                    random = ~ day| record.id, data = savemore_long,
                    control = lmeControl(opt = "optim"),
                    na.action = na.omit)
summary(lmeFit.savemore)
```

3.1. Linear-mixed model for IL-6 over time

```
## Linear mixed-effects model fit by REML
##   Data: savemore_long
##       AIC      BIC    logLik
##  2663.727 2701.633 -1324.863
##
## Random effects:
## Formula: ~day | record.id
## Structure: General positive-definite, Log-Cholesky parametrization
##           StdDev      Corr
## (Intercept) 0.34478060 (Intr)
## day          0.05371349 -0.28
## Residual     0.42544483
##
## Fixed effects:  conc_log10 ~ day:randomized_group + day
##               Value Std.Error   DF t-value p-value
## (Intercept)      1.2484091 0.024396881 1074  51.17085  0e+00
## day            -0.0412061 0.007195113 1074  -5.72695  0e+00
## day:randomized_groupAnakinra -0.0269798 0.007669486 1074  -3.51780  5e-04
## Correlation:
##               (Intr) day
## day            -0.467
## day:randomized_groupAnakinra  0.004 -0.736
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -2.54974446 -0.58093507 -0.06755401  0.53782487  4.71086242
##
## Number of Observations: 1664
## Number of Groups: 588
##
## Approximate 95% confidence intervals
##
## Fixed effects:
##               lower      est.      upper
## (Intercept)      1.20053819  1.24840915  1.29628010
## day            -0.05532416 -0.04120608 -0.02708801
## day:randomized_groupAnakinra -0.04202863 -0.02697975 -0.01193088
##
library(sjPlot)
library(sjmisc)
theme_set(theme_sjplot())

plot_model(lmeFit.savemore, type = "int", terms = c("randomized_group", "day"))
```



```
# save the interaction estimate
a_res <- get_int(lmefit.savemore, "randomized_groupAnakinra")

saveRDS(a_res, "SAVEMORE_beta_est.rds")
```

3.2. Cox proportional hazards models For 28-day and 90-day survival.

```
# Fit cox proportional hazard model
coxfit.savemore28 <- coxph(Surv(time_mort28, death_d28) ~ randomized_group, data = savemore_surv, x = TRUE)

summary(coxfits.savemore28)
```

3.2.1. 28-day survival

```
## Call:
## coxph(formula = Surv(time_mort28, death_d28) ~ randomized_group,
##       data = savemore_surv, x = TRUE)
##
## n= 588, number of events= 26
##
##               coef exp(coef) se(coef)      z Pr(>|z|)
## randomized_groupAnakinra -0.7883   0.4546   0.3922 -2.01   0.0445 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##               exp(coef) exp(-coef) lower .95 upper .95
## randomized_groupAnakinra  0.4546      2.2   0.2108   0.9807
##
## Concordance= 0.595 (se = 0.049 )
```

```
## Likelihood ratio test= 3.94 on 1 df, p=0.05
## Wald test = 4.04 on 1 df, p=0.04
## Score (logrank) test = 4.25 on 1 df, p=0.04
confint(coxfit.savemore28) %>% exp() %>% round(3)
```

```
## 2.5 % 97.5 %
## randomized_groupAnakinra 0.211 0.981
```

```
# Fit cox proportional hazard model
```

```
coxfit.savemore90 <- coxph(Surv(time_mort90, death_d90) ~ randomized_group, data = savemore_surv, x = TRUE)
```

```
summary(coxfit.savemore90)
```

3.2.2. 90-day survival

```
## Call:
## coxph(formula = Surv(time_mort90, death_d90) ~ randomized_group,
## data = savemore_surv, x = TRUE)
##
## n= 588, number of events= 41
##
##               coef exp(coef) se(coef)      z Pr(>|z|)
## randomized_groupAnakinra -0.6491    0.5225    0.3132 -2.073  0.0382 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##               exp(coef) exp(-coef) lower .95 upper .95
## randomized_groupAnakinra    0.5225      1.914    0.2828    0.9654
##
## Concordance= 0.577 (se = 0.039 )
## Likelihood ratio test= 4.16 on 1 df, p=0.04
## Wald test = 4.3 on 1 df, p=0.04
## Score (logrank) test = 4.45 on 1 df, p=0.03
confint(coxfit.savemore90) %>% exp() %>% round(3)
```

```
## 2.5 % 97.5 %
## randomized_groupAnakinra 0.283 0.965
```

3.3. Joint models Using 28- and 90-day survival as endpoints.

```
set.seed(15)
# Fit joint model for 28 day survival
jointfit.savemore_28 <- JMBayes2::jm(coxfit.savemore28, lmeFit.savemore, time_var = "day", n_chains = 4)

# save results
saveRDS(jointfit.savemore_28, "jointfit.savemore_28.rds")

# Fit joint model for 90 day survival
jointfit.savemore_90 <- JMBayes2::jm(coxfit.savemore90, lmeFit.savemore, time_var = "day", n_chains = 4)

saveRDS(jointfit.savemore_90, "jointfit.savemore_90.rds")
```

3.3.1. 28-day endpoint

```
##
## Call:
## JMBayes2::jm(Surv_object = coxfit.savemore28, Mixed_objects = lmefit.savemore,
##   time_var = "day", n_chains = 2, n_iter = 50000L, n_burnin = 5000L,
##   n_thin = 5)
##
## Data Descriptives:
## Number of Groups: 592          Number of events: 26 (4.4%)
## Number of Observations:
##   conc_log10: 1661
##
##               DIC      WAIC      LPML
## marginal      2907.000 2971.415 -1550.877
## conditional 1460.744 2731.342 -21749.354
##
## Random-effects covariance matrix:
##
##      StdDev  Corr
## (Intr) 0.3143 (Intr)
## day    0.0415 0.0869
##
## Survival Outcome:
##               Mean StDev   2.5% 97.5%      P  Rhat
## randomized_groupAnakinra 0.2423 0.7734 -1.2396 1.8143 0.7589 1.0001
## value(conc_log10)        3.0819 0.9965  1.4228 5.3825 0.0000 1.0314
##
## Longitudinal Outcome: conc_log10 (family = gaussian, link = identity)
##               Mean StDev   2.5% 97.5%      P  Rhat
## (Intercept)  1.1850 0.0212  1.1427 1.2265 0.0000 1.0038
## day          -0.0409 0.0077 -0.0560 -0.0258 0.0000 1.0017
## d:_A         -0.0260 0.0085 -0.0431 -0.0094 0.0016 1.0001
## sigma        0.4344 0.0109  0.4133  0.4563 0.0000 1.0011
##
## MCMC summary:
## chains: 2
## iterations per chain: 50000
## burn-in per chain: 5000
## thinning: 5
## time: 12.1 min
```

3.3.2. 90-day endpoint

```
##
## Call:
## JMBayes2::jm(Surv_object = coxfit.savemore90, Mixed_objects = lmefit.savemore,
##   time_var = "day", n_chains = 2, n_iter = 50000L, n_burnin = 5000L,
##   n_thin = 5)
##
## Data Descriptives:
## Number of Groups: 592          Number of events: 41 (6.9%)
## Number of Observations:
##   conc_log10: 1661
##
```



```
##               DIC               WAIC               LPML
## marginal      3311.347 6.021164e+03 -4.206220e+03
## conditional 3447233.293 6.678743e+16 -6.634874e+10
##
## Random-effects covariance matrix:
##
##      StdDev   Corr
## (Intr) 0.3224 (Intr)
## day    0.0667 -0.1341
##
## Survival Outcome:
##               Mean   StDev   2.5%  97.5%      P   Rhat
## randomized_groupAnakinra -0.1051 0.9359 -2.1706 1.6944 0.9277 1.0621
## value(conc_log10)        1.9649 0.5242  0.9024 2.7771 0.0000 1.3804
##
## Longitudinal Outcome: conc_log10 (family = gaussian, link = identity)
##               Mean   StDev   2.5%  97.5%      P   Rhat
## (Intercept)  1.1629 0.0295  1.1044  1.2164 0.0000 1.3484
## day          -0.0308 0.0136 -0.0535 -0.0030 0.0254 1.4917
## d:_A         -0.0290 0.0105 -0.0506 -0.0094 0.0030 1.0976
## sigma        0.4282 0.0113  0.4063  0.4508 0.0000 1.0032
##
## MCMC summary:
## chains: 2
## iterations per chain: 50000
## burn-in per chain: 5000
## thinning: 5
## time: 5 min
```

4. Results

4.1. Indirect, direct, and total effects Of anakinra through IL6 on the hazard of death.

```
res28 <- get_effects(jointfit.savemore_28, coxfit.savemore28,
  "randomized_groupAnakinra") %>% cbind(endpoint =c("28-day endpoint"))
res28
```

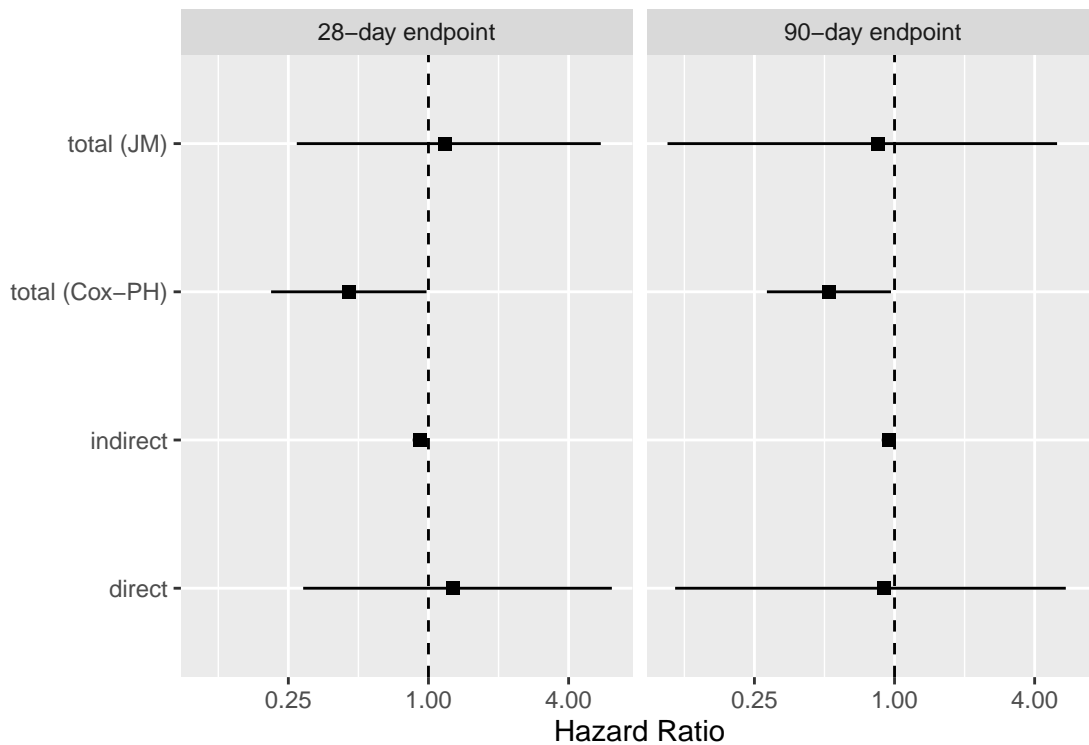
```
##           effect           est  CI_lower  CI_upper      endpoint
## 1           direct  0.24229995 -1.2395534  1.81427247 28-day endpoint
## 2           indirect -0.08003438 -0.1567002 -0.02451719 28-day endpoint
## 3 total (Cox-PH) -0.78825892 -1.5570289 -0.01948896 28-day endpoint
## 4           total (JM) 0.16226557 -1.3027106  1.70492160 28-day endpoint
```

```
res90 <- get_effects(jointfit.savemore_90, coxfit.savemore90,
  "randomized_groupAnakinra") %>% cbind(endpoint =c("90-day endpoint"))
res90
```

```
##           effect           est  CI_lower  CI_upper      endpoint
## 1           direct -0.10505573 -2.170580  1.69444173 90-day endpoint
## 2           indirect -0.05689723 -0.126946 -0.01364629 90-day endpoint
## 3 total (Cox-PH) -0.64909520 -1.262941 -0.03524972 90-day endpoint
## 4           total (JM) -0.16195296 -2.246248  1.60819108 90-day endpoint
```

```
res <- rbind(res28, res90)
saveRDS(res, "savemore_res.rds")
```

```
res %>%
  ggplot(aes(y = effect)) +
  theme_grey() +
  geom_point(aes(x=exp(est)), shape=15, size=2) +
  geom_linerange(aes(xmin=exp(CI_lower), xmax=exp(CI_upper))) +
  geom_vline(xintercept = 1, linetype="dashed") +
  labs(x="Hazard Ratio", y="")+
  scale_x_continuous(trans = "log2") +
  facet_grid(~endpoint)
```



4.2. Association parameter Hazard ratio estimate and 95% CI for the association parameter α for a one unit increase (at any time point) of IL-6 and the hazard of death over 28 days.

```
# save association estimates
```

```
alpha_28 <- get_alpha(jointfit.savemore_28, "28-day endpoint")
alpha_90 <- get_alpha(jointfit.savemore_90, "90-day endpoint")
```

```
alpha_est <- rbind(alpha_28, alpha_90)
```

```
saveRDS(alpha_est, "SAVEMORE_alpha_est.rds")
```

4.4. Conclusions

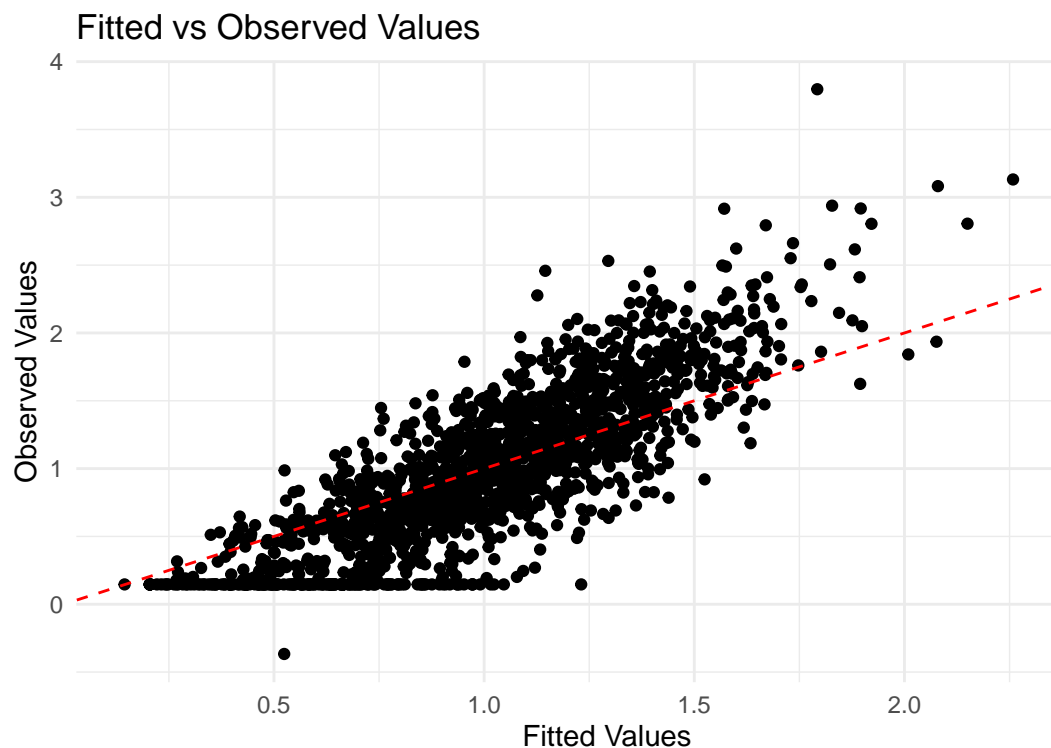
- From lme and JM we can conclude that there is an effect of anakinra over time on IL-6.
- From joint model we conclude 1) that there is no direct effect of anakinra on survival when controlling

for IL-6, 2) there is an association between IL-6 and survival, and (3) some of the effect of anakinra is mediated by IL-6.

5. Model checks

```
# get fitted values
fitted_values<- fitted(lmefit.savemore)
savemore_long <- savemore_long %>% drop_na(conc_log10)

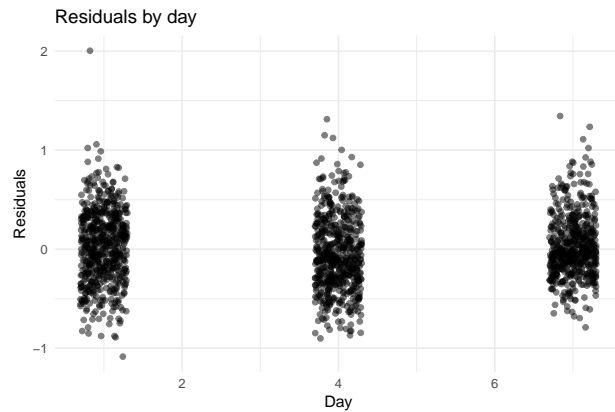
# plot observed vs fitted values
ggplot(data = savemore_long, aes(x = fitted_values, y = conc_log10)) +
  geom_point() +
  geom_abline(slope = 1, intercept = 0, linetype = "dashed", color = "red") + # Line of perfect fit
  labs(x = "Fitted Values", y = "Observed Values") +
  ggtitle("Fitted vs Observed Values") +
  theme_minimal()
```



5.1. Longitudinal submodel

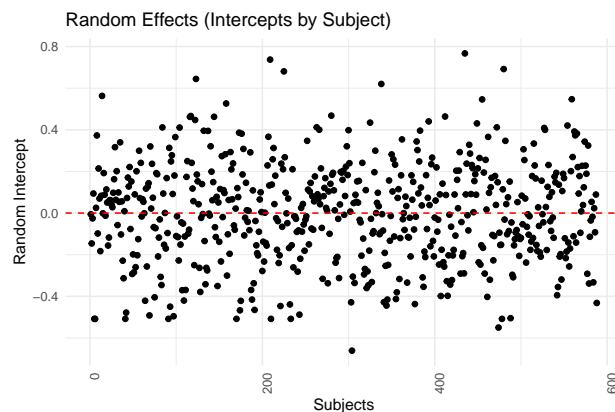
```
# get residuals
residuals_values <- resid(lmefit.savemore)

# plot residuals vs time
ggplot(savemore_long, aes(x = day, y = residuals_values)) +
  geom_jitter(width = 0.3, alpha = 0.5) +
  labs(x = "Day", y = "Residuals") +
  ggtitle("Residuals by day") +
  theme_minimal()
```

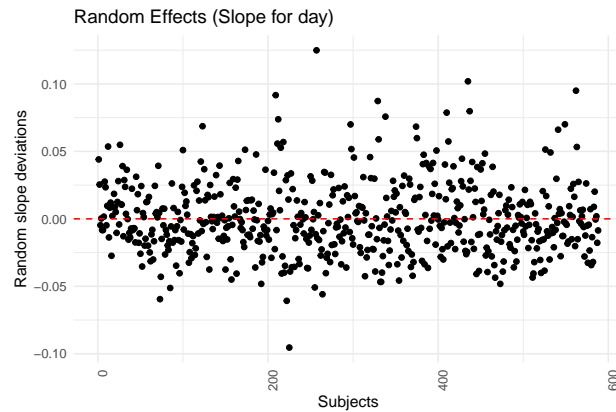


```
# get random effects
random_effects <- ranef(lmefit.savemore)

#plot random effects
ggplot(random_effects, aes(x = c(1:nrow(savemore_surv)), y = `(Intercept)`)) +
  geom_point() +
  geom_hline(yintercept = 0, linetype = "dashed", color = "red") +
  labs(x = "Subjects", y = "Random Intercept") +
  ggtitle("Random Effects (Intercepts by Subject)") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```

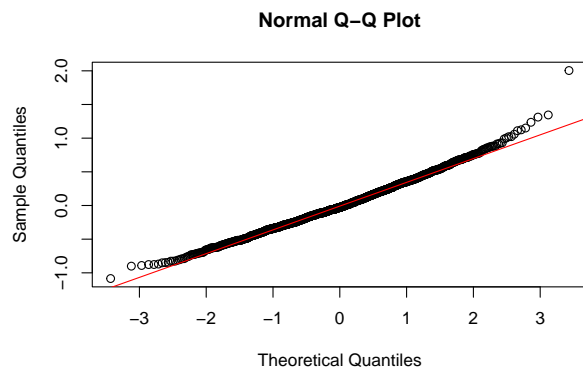


```
#plot random effects
ggplot(random_effects, aes(x = c(1:nrow(savemore_surv)), y = `day`)) +
  geom_point() +
  geom_hline(yintercept = 0, linetype = "dashed", color = "red") +
  labs(x = "Subjects", y = "Random slope deviations") +
  ggtitle("Random Effects (Slope for day)") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```

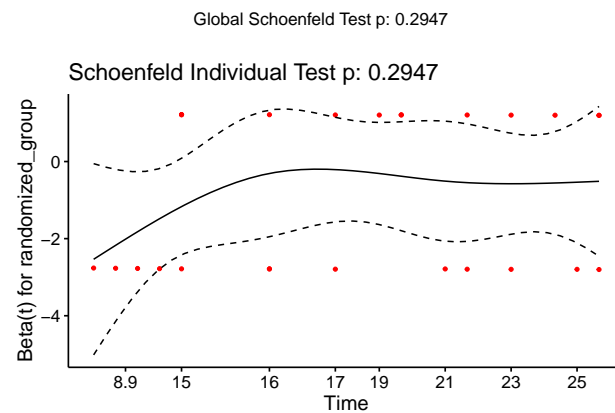


```
# qq plot for residuals
qqnorm(resid(lmefit.savemore))

qqline(resid(lmefit.savemore), col = "red")
```

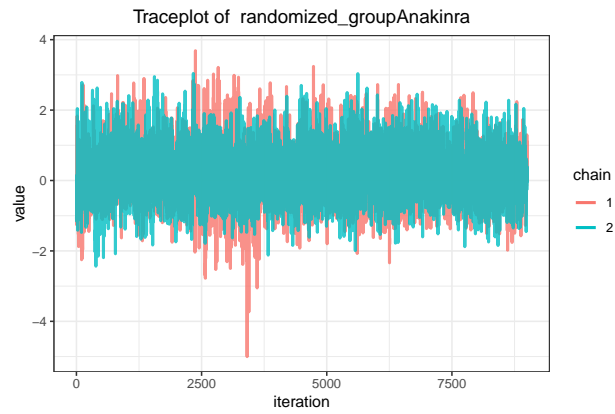


```
test.ph <- cox.zph(coxfit.savemore28)
survminer::ggcoxzph(test.ph)
```



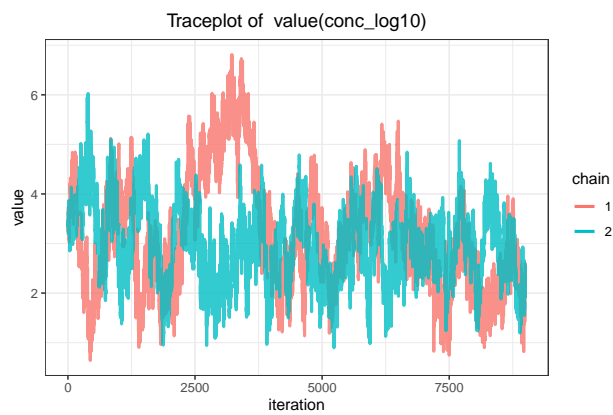
5.2 Survival submodel

```
ggtraceplot(jointfit.savemore_28, "gammas")
```

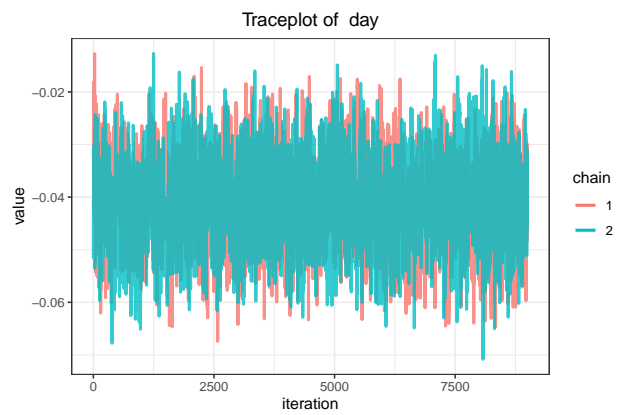
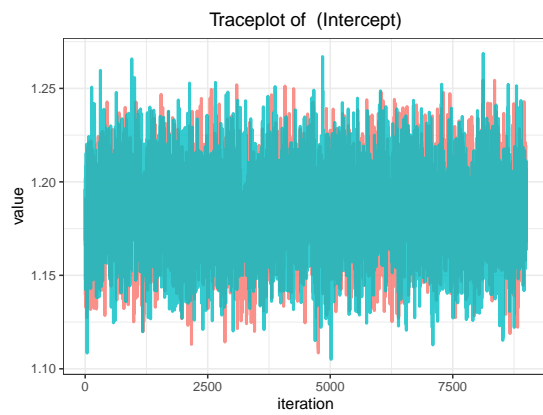


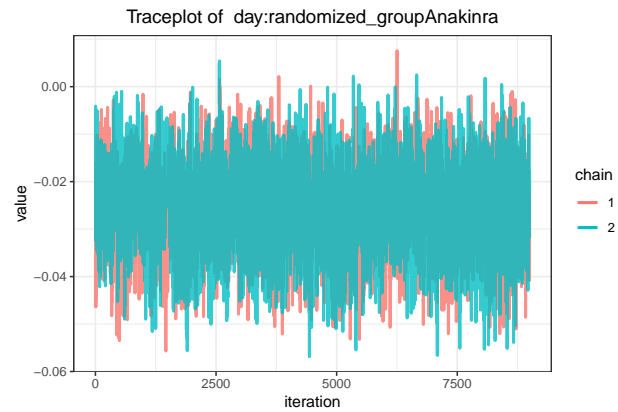
5.1. 28-day endpoint

```
ggtraceplot(jointfit.savemore_28, "alphas")
```

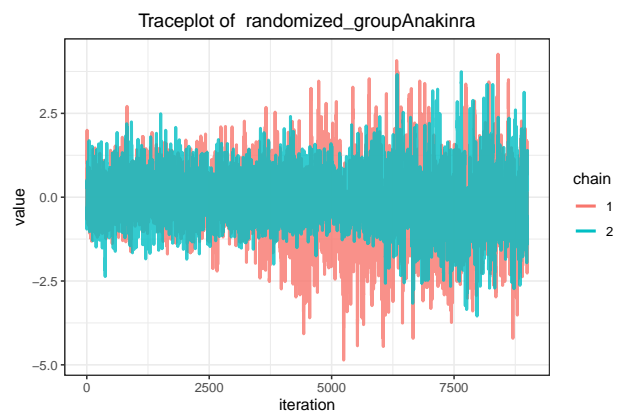


```
ggtraceplot(jointfit.savemore_28, "betas")
```



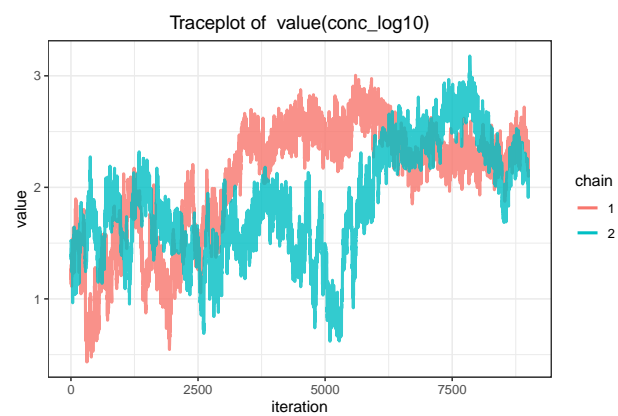


```
ggtraceplot(jointfit.savemore_90, "gammas")
```



5.2. 90-day endpoint

```
ggtraceplot(jointfit.savemore_90, "alphas")
```



```
ggtraceplot(jointfit.savemore_90, "betas")
```

