

Postop Cytokines over Time

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2023-11-08

Quick Data Summary

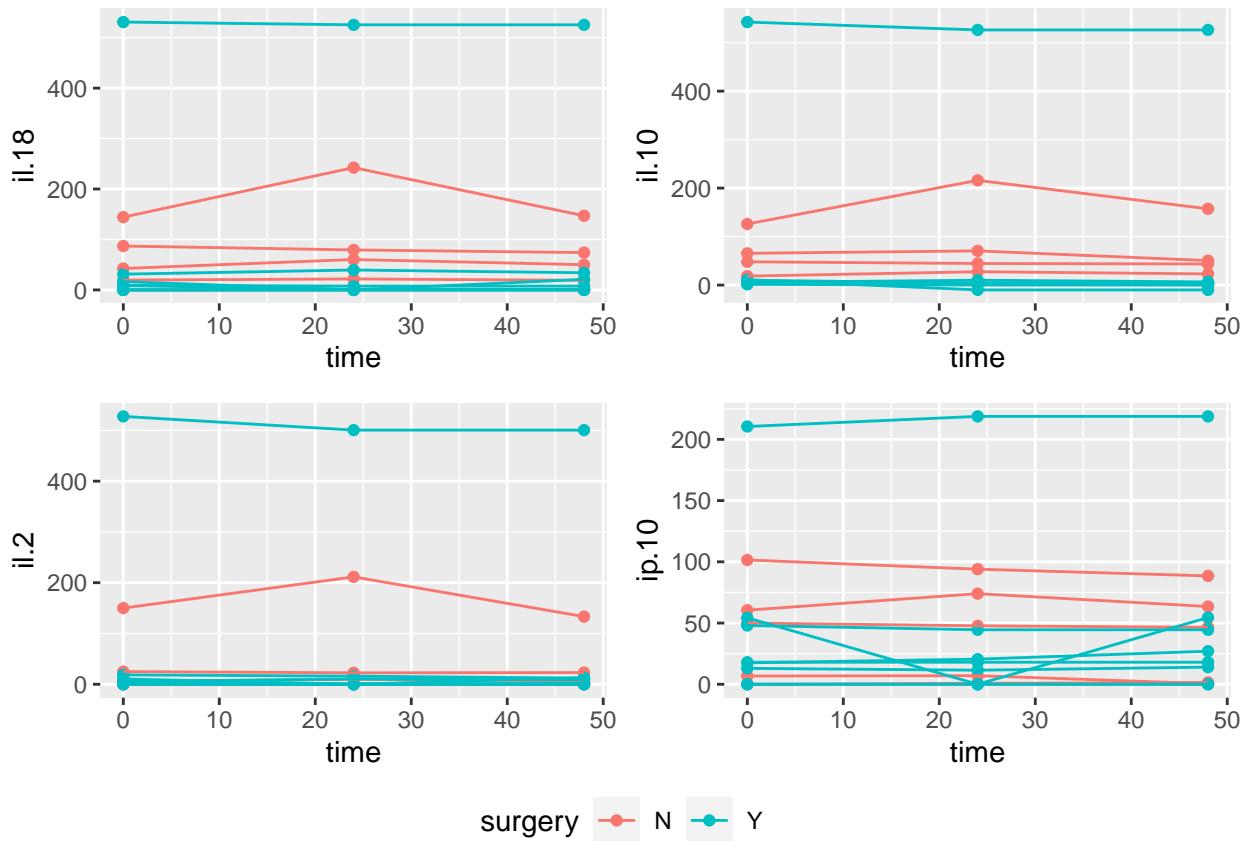
```
data %>% group_by(time, surgery) %>%
  summarise(across(c(il.18, il.2, ip.10, il.10),
                  c(mean = ~mean(.x), sd = ~sd(.x)))) %>%
  kableExtra::kable()
```

```
## `summarise()` has grouped output by 'time'. You can override using the
## `.` argument.
```

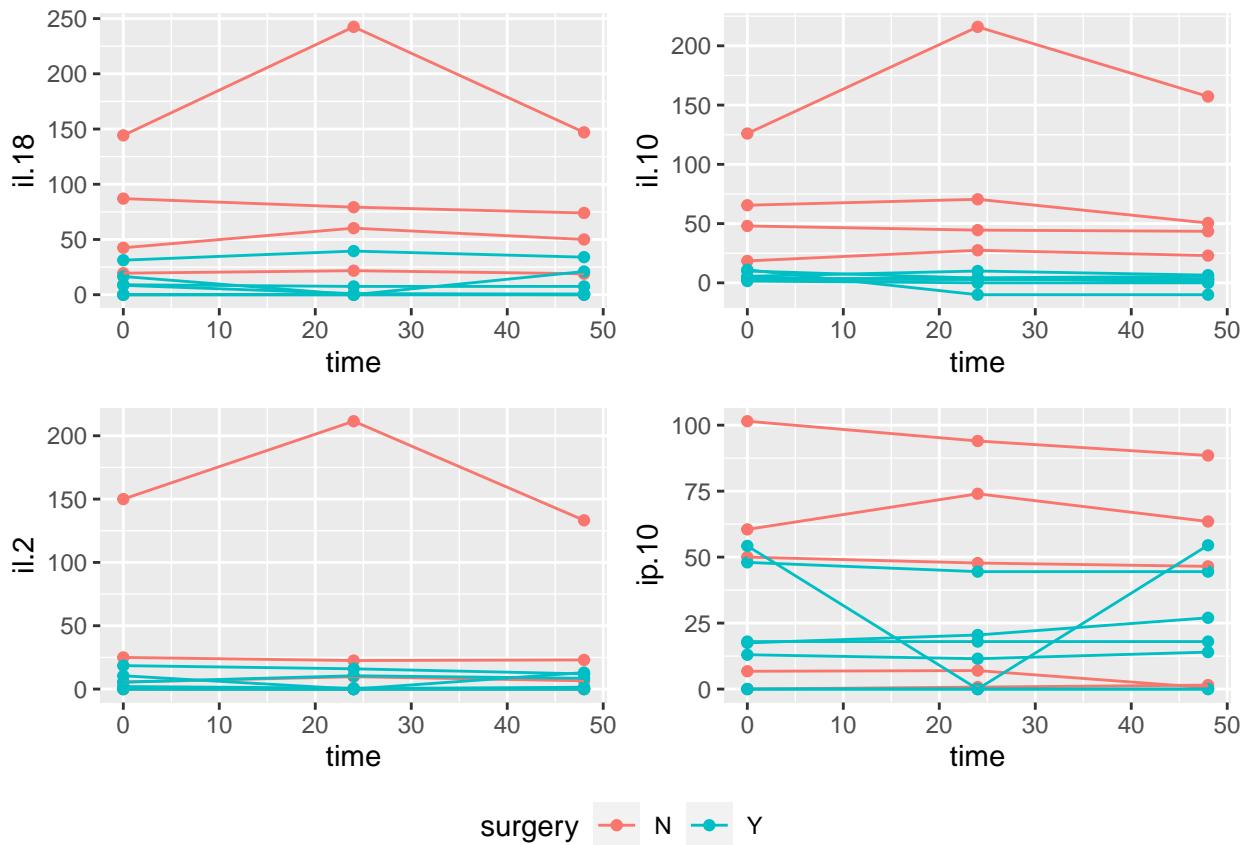
time	surgery	il.18_mean	il.18_sd	il.2_mean	il.2_sd	ip.10_mean	ip.10_sd	il.10_mean	il.10_sd
0	N	58.65000	57.80236	36.10000	64.49651	43.75000	41.64658	52.20000	47.95128
0	Y	74.53125	184.74840	70.56250	184.74334	45.15625	69.73815	72.56250	189.91397
24	N	80.85000	95.53347	48.75000	91.44773	44.70000	40.77737	72.30000	84.01681
24	Y	71.68750	183.86912	65.96875	175.68318	39.15625	74.10670	67.56250	185.42368
48	N	58.00000	57.28438	32.55000	57.07298	40.10000	38.69496	55.45000	59.86349
48	Y	73.62500	183.00620	67.03125	175.23160	47.09375	72.02033	66.84375	185.69757

Nothing is significant, but the plots are interesting

Start with the full data set

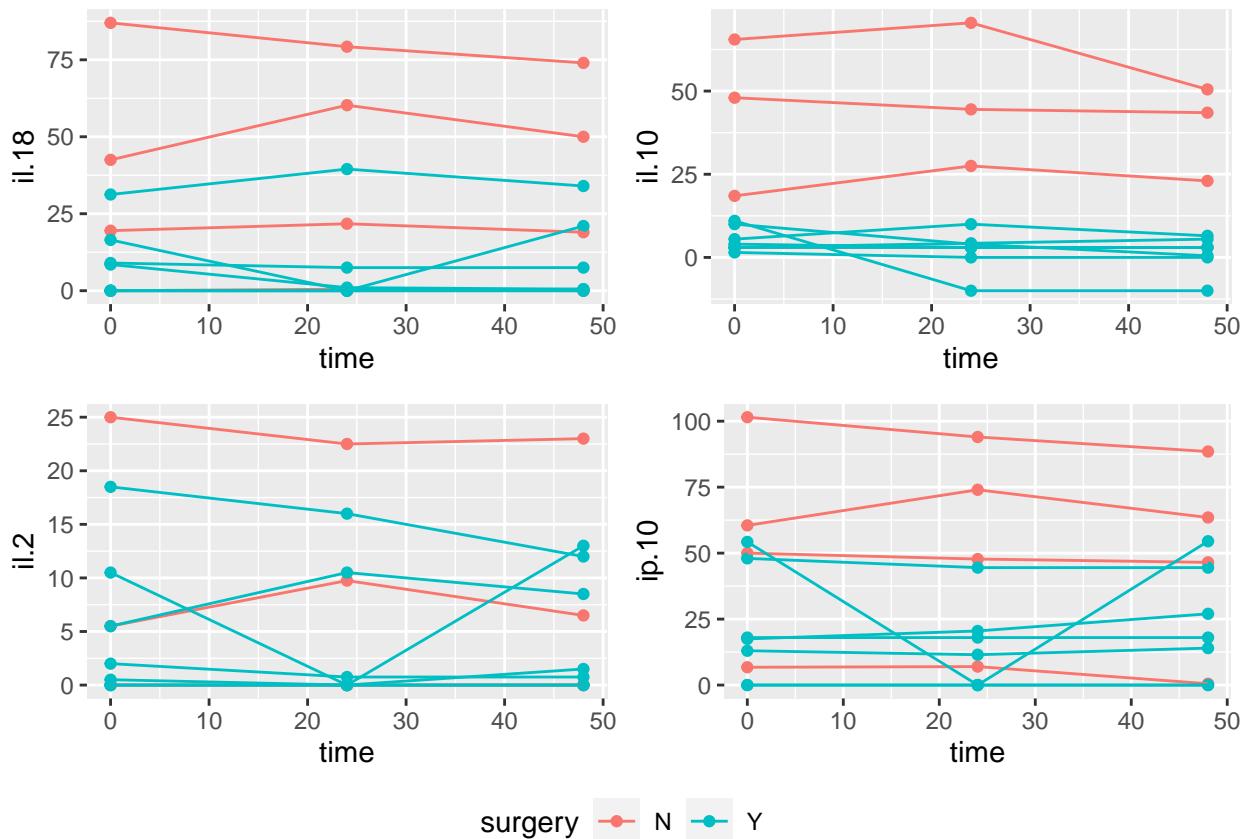


Remove the obvious outlier at the top and recheck plots



The 3 interleukins still demonstrate a single horse as an outlier (although not as bad)

Let's remove that one too



Interindividual distribution looks better now - run models on all 4 cytokines using surgery (Y/N), time, and an interaction term with individual horse as a random effect.

```
model3_il.18 <- lmer(il.18 ~ surgery*time + (1|horse), data = data3)
summary(model3_il.18)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [ 
## lmerModLmerTest]
## Formula: il.18 ~ surgery * time + (1 | horse)
##   Data: data3
##
## REML criterion at convergence: 238.2
##
## Scaled residuals:
##       Min     1Q   Median     3Q    Max 
## -2.33632 -0.12358 -0.05242  0.05784  1.79994
##
## Random effects:
```

```

## Groups      Name      Variance Std.Dev.
## horse      (Intercept) 506.66   22.509
## Residual           28.28    5.318
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 38.56250 11.51332 9.49681 3.349 0.00792 **
## surgeryY    -30.02083 14.43271 9.49681 -2.080 0.06565 .
## time        -0.03125  0.07835 20.00000 -0.399 0.69422
## surgeryY:time  0.02604  0.09821 20.00000  0.265 0.79360
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY time
## surgeryY -0.798
## time      -0.163  0.130
## surgeryY:tm  0.130 -0.163 -0.798

model3_il.2 <- lmer(il.2 ~ surgery*time + (1|horse), data = data3)
summary(model3_il.2)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: il.2 ~ surgery * time + (1 | horse)
## Data: data3
##
## REML criterion at convergence: 192.2
##
## Scaled residuals:
##     Min      1Q Median      3Q     Max
## -2.86970 -0.15346 -0.06627  0.22660  1.99691
##
## Random effects:
## Groups      Name      Variance Std.Dev.
## horse      (Intercept) 62.011   7.875
## Residual           7.234    2.690
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 7.812500 4.124304 10.024759 1.894 0.0874 .
## surgeryY    -2.961310 5.170089 10.024759 -0.573 0.5794
## time        -0.005208  0.039622 20.000000 -0.131 0.8967
## surgeryY:time  0.001488  0.049668 20.000000  0.030 0.9764
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY time
## surgeryY -0.798
## time      -0.231  0.184
## surgeryY:tm  0.184 -0.231 -0.798

```

```
model3_ip.10 <- lmer(ip.10 ~ surgery*time + (1|horse), data = data3)
summary(model3_ip.10)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: ip.10 ~ surgery * time + (1 | horse)
##   Data: data3
##
## REML criterion at convergence: 267.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.3705 -0.1377 -0.0506  0.0569  1.8560
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   horse    (Intercept) 633.8    25.17
##   Residual           109.8    10.48
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 55.8437   13.4654 10.5029  4.147 0.00179 **
## surgeryY    -37.1592   16.8798 10.5029 -2.201 0.05108 .
## time       -0.1029    0.1544 20.0000 -0.666 0.51280
## surgeryY:time  0.1244    0.1935 20.0000  0.643 0.52749
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY time
## surgeryY -0.798
## time      -0.275  0.219
## surgeryY:tm  0.219 -0.275 -0.798
```

```
model3_il.10 <- lmer(il.10 ~ surgery*time + (1|horse), data = data3)
summary(model3_il.10)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: il.10 ~ surgery * time + (1 | horse)
##   Data: data3
##
## REML criterion at convergence: 229.8
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.6778 -0.4067  0.0008  0.3464  2.2560
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   horse    (Intercept) 220.89   14.86
```

```

## Residual           26.73   5.17
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 35.250000  7.796948 10.062127  4.521 0.00109 **
## surgeryY    -30.250000  9.773992 10.062127 -3.095 0.01127 *
## time        -0.078125  0.076168 20.000000 -1.026 0.31728
## surgeryY:time -0.009673  0.095482 20.000000 -0.101 0.92032
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY time
## surgeryY -0.798
## time     -0.234  0.187
## surgeryY:tm 0.187 -0.234 -0.798

```

None of the interactions are significant (no change in the time effect depending on whether surgery or not), so re-run without interaction term

```

model3_il.18 <- lmer(il.18 ~ surgery+time + (1|horse), data = data3)
summary(model3_il.18)

```

```

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: il.18 ~ surgery + time + (1 | horse)
## Data: data3
##
## REML criterion at convergence: 235.5
##
## Scaled residuals:
##      Min     1Q   Median     3Q    Max
## -2.39046 -0.20015 -0.05976  0.10416  1.83912
##
## Random effects:
## Groups   Name       Variance Std.Dev.
## horse    (Intercept) 507.07   22.518
## Residual            27.03    5.199
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 38.16477  11.41270  9.17188  3.344 0.00838 **
## surgeryY   -29.39583  14.23894  9.00000 -2.064 0.06898 .
## time       -0.01468   0.04619 21.00000 -0.318 0.75378
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY
## surgeryY -0.794

```

```

## time      -0.097  0.000

model3_il.2 <- lmer(il.2 ~ surgery+time + (1|horse), data = data3)
summary(model3_il.2)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: il.2 ~ surgery + time + (1 | horse)
##   Data: data3
##
## REML criterion at convergence: 188
##
## Scaled residuals:
##       Min     1Q Median     3Q    Max
## -2.94258 -0.16622 -0.06544  0.22969  2.04904
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   horse     (Intercept) 62.13     7.882
##   Residual            6.89     2.625
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 7.789773  4.052013 9.351898  1.922  0.0855 .
## surgeryY   -2.925595  5.030791 9.000000 -0.582  0.5752
## time       -0.004261  0.023317 21.000000 -0.183  0.8567
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY
## surgeryY -0.790
## time      -0.138  0.000

model3_ip.10 <- lmer(ip.10 ~ surgery+time + (1|horse), data = data3)
summary(model3_ip.10)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: ip.10 ~ surgery + time + (1 | horse)
##   Data: data3
##
## REML criterion at convergence: 266.6
##
## Scaled residuals:
##       Min     1Q Median     3Q    Max
## -3.4211 -0.1254 -0.0594  0.1033  1.9090
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   horse     (Intercept) 634.8     25.19
##   Residual            106.7    10.33

```

```

## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 53.94318   13.13179   9.52524  4.108  0.00235 **
## surgeryY    -34.17262   16.22833   9.00000 -2.106  0.06451 .
## time        -0.02367    0.09178  21.00000 -0.258  0.79896
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY
## surgeryY -0.786
## time      -0.168  0.000

model3_il.10 <- lmer(il.10 ~ surgery+time + (1|horse), data = data3)
summary(model3_il.10)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: il.10 ~ surgery + time + (1 | horse)
## Data: data3
##
## REML criterion at convergence: 226.9
##
## Scaled residuals:
##     Min      1Q Median      3Q     Max
## -1.69999 -0.39998  0.00078  0.33795  2.32994
##
## Random effects:
## Groups   Name       Variance Std.Dev.
## horse    (Intercept) 221.31   14.877
## Residual           25.47    5.047
## Number of obs: 33, groups: horse, 11
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 35.39773   7.65563   9.36481  4.624  0.00112 **
## surgeryY   -30.48214   9.50156   9.00000 -3.208  0.01069 *
## time        -0.08428   0.04484  21.00000 -1.880  0.07409 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) srgryY
## surgeryY -0.790
## time      -0.141  0.000

```

Focus on IL-10, as this is the only one that demonstrated a difference between horses that had surgery and those that didn't.

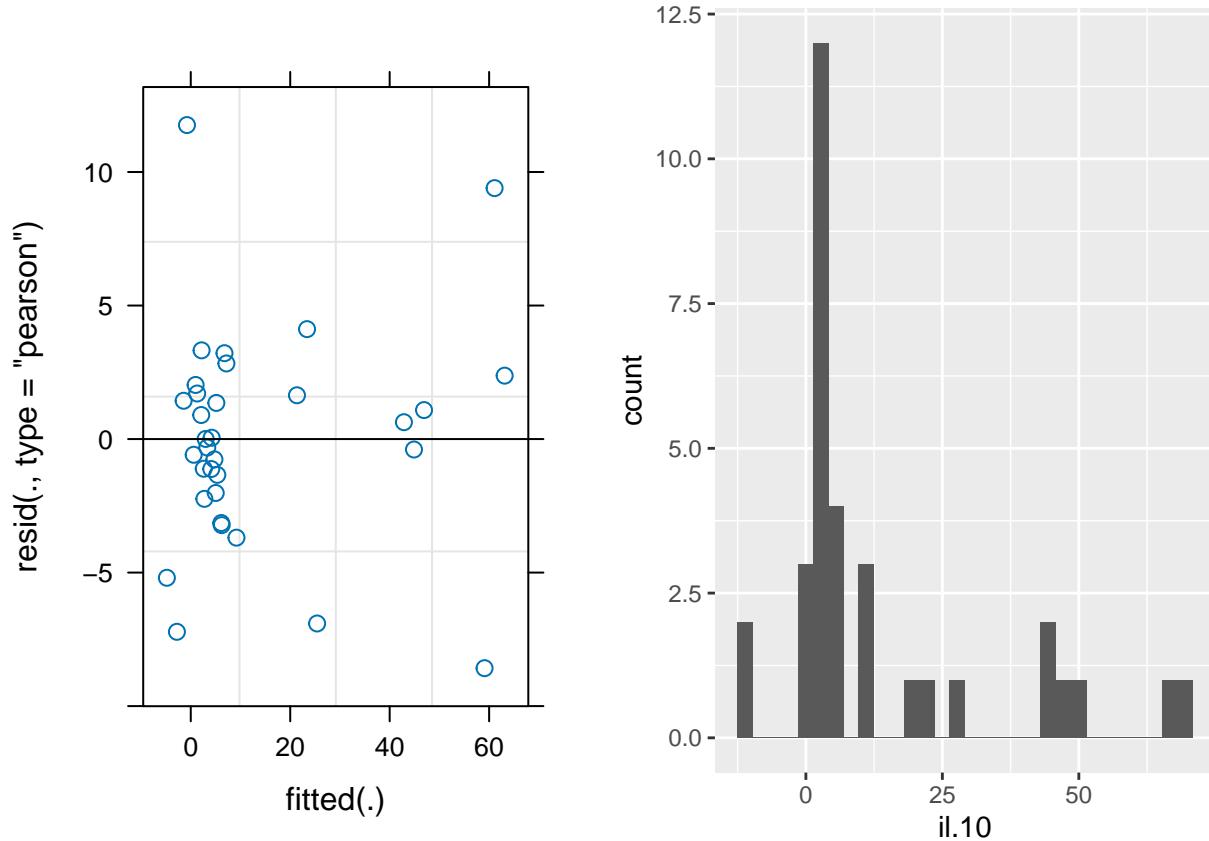
Need to check the distribution of the data

```
shapiro.test(data3$il.10)
```

```
##  
##  Shapiro-Wilk normality test  
##  
## data:  data3$il.10  
## W = 0.76489, p-value = 7.391e-06  
  
plot_norm1 <- plot(model3_il.10)  
plot_norm2 <- ggplot(data = data3, aes(x = il.10)) +  
  geom_histogram()  
plot_norm_1 <- ggarrange(plot_norm1, plot_norm2, nrow = 1)
```

'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.

```
plot_norm_1
```



So not distributed super well - try log transforming without the outlier data

```
data4 <- data3 %>% mutate(log_il.10 = if_else(il.10 <= 0, 0, log(il.10)))
```

```
## Warning: There was 1 warning in 'mutate()' .
## i In argument: 'log_il.10 = if_else(il.10 <= 0, 0, log(il.10))' .
## Caused by warning in 'log()' :
## ! NaNs produced
```

```
model4_il.10 <- lmer(log_il.10 ~ surgery+time + (1|horse), data = data4)
```

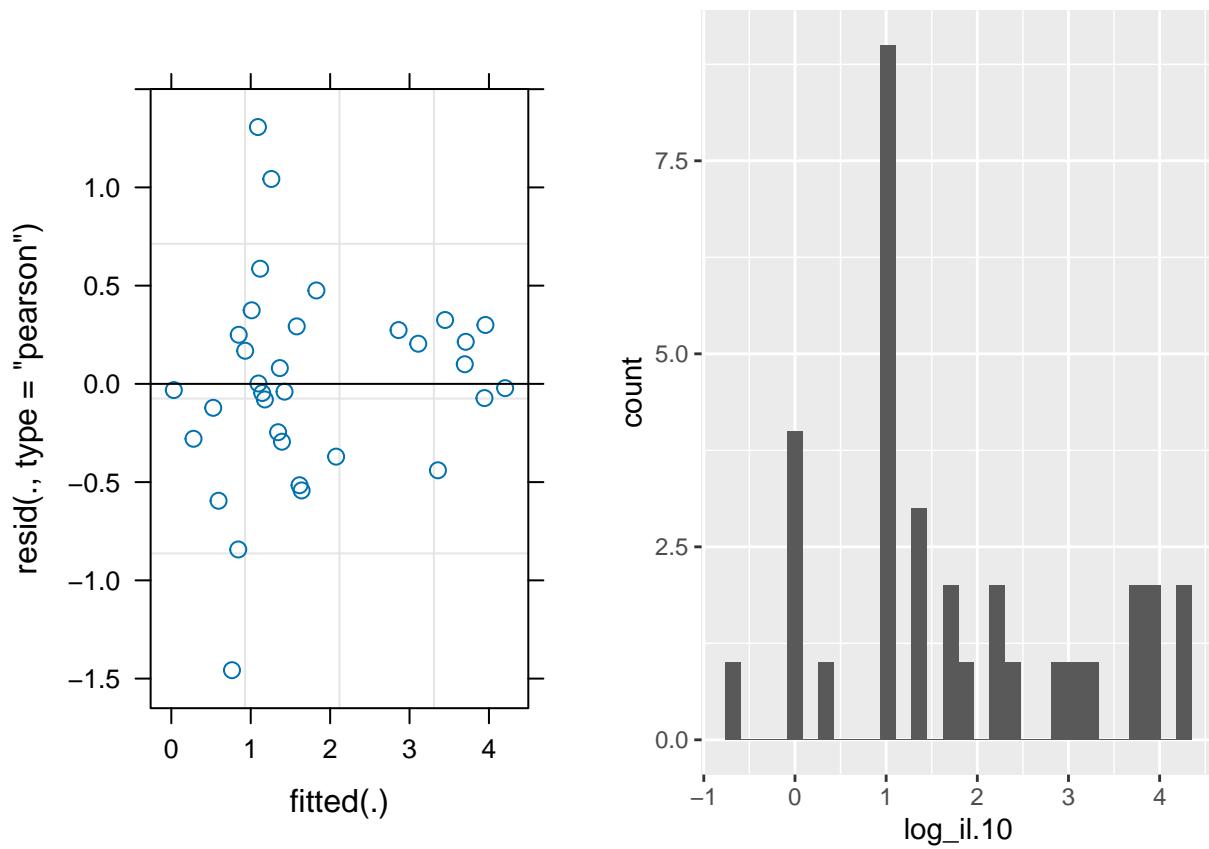
```
shapiro.test(data4$log_il.10)
```

```
##
## Shapiro-Wilk normality test
##
## data: data4$log_il.10
## W = 0.93161, p-value = 0.03889
```

```
plot_norm3 <- plot(model4_il.10)
plot_norm4 <- ggplot(data = data4, aes(x = log_il.10)) +
  geom_histogram()
plot_norm_2 <- ggarrange(plot_norm3, plot_norm4, nrow = 1)
```

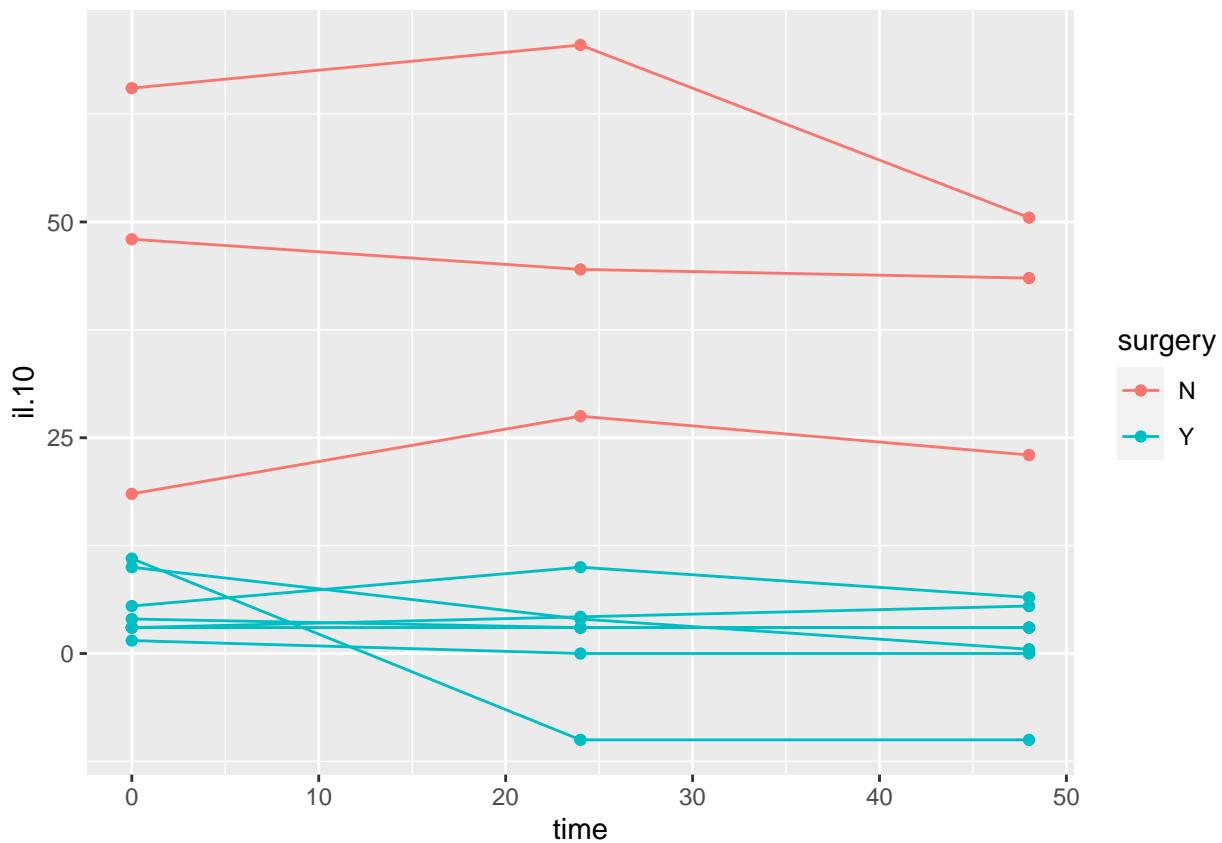
```
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
```

```
plot_norm_2
```



Slightly better - should probably use this transformation for analysis

p2_3



```
anova(model4_il.10)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##          Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## surgery  4.4170  4.4170     1      9 11.7722 0.007496 **
## time     1.3523  1.3523     1     21  3.6041 0.071467 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

IL-10 is significantly different in the first 48 hours when comparing horses that had surgery (lower) to those that didn't (higher), although there is no significant change over time in these 2 groups.