Postop Cytokines over Time

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Quick Data Summary

time	surgery	il.18__mean	il.18__se	il.18__min	il.18__max	il.2__mean	il.2__se	il.2__min	il.2__max
0	N	58.65000	11.56047	0.0	144.25	36.10000	12.89930	0	150.00
0	Y	74.53125	23.09355	0.0	531.00	70.56250	23.09292	0	527.50
24	N	80.85000	19.10669	0.5	242.50	48.75000	18.28955	0	211.50
24	Y	71.68750	22.98364	0.0	525.50	65.96875	21.96040	0	500.50
48	N	58.00000	11.45688	0.0	147.00	32.55000	11.41460	0	133.25
48	Y	73.62500	22.87577	0.0	525.50	67.03125	21.90395	0	500.50

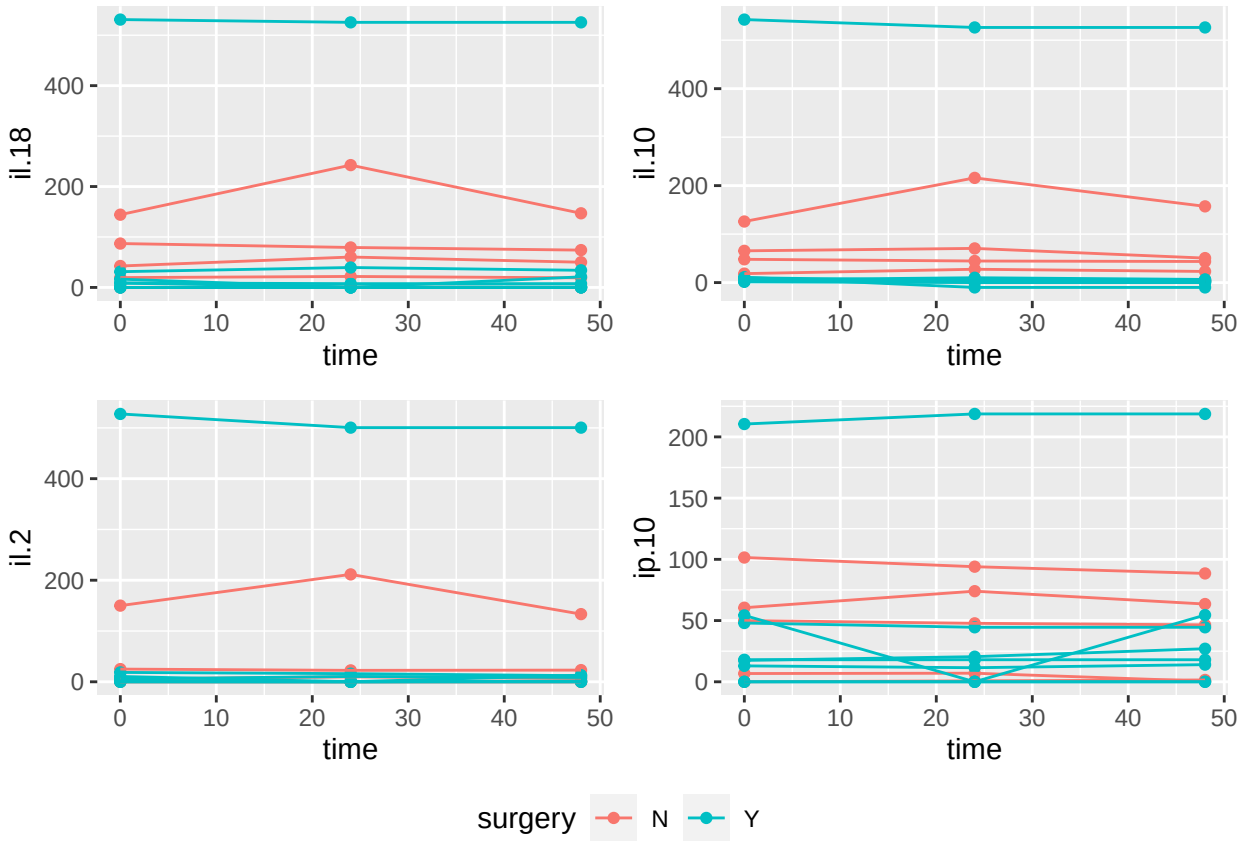
time	surgery	ip.10__mean	ip.10__se	ip.10__min	ip.10__max	il.10__mean	il.10__se	il.10__min	il.10__max
0	N	43.75000	8.329316	0.00	101.50	52.20000	9.590255	3.0	126.00
0	Y	45.15625	8.717269	0.00	210.50	72.56250	23.739247	1.5	542.50
24	N	44.70000	8.155474	0.75	94.00	72.30000	16.803363	3.0	216.00
24	Y	39.15625	9.263337	0.00	218.75	67.56250	23.177960	-10.0	526.25
48	N	40.10000	7.738992	0.50	88.50	55.45000	11.972698	3.0	157.25
48	Y	47.09375	9.002541	0.00	218.75	66.84375	23.212197	-10.0	526.25

time	surgery	temp__mean	temp__se	temp__min	temp__max	wbc__mean	wbc__se	wbc__min	wbc__max
0	N	99.74000	0.0268328	99.6	99.9	5.640000	0.1293058	5.0	6.7
0	Y	99.88750	0.0599246	99.2	100.7	7.487500	0.2467139	4.7	10.0
24	N	99.50000	0.0927362	98.9	100.0	6.660000	0.1245793	5.9	7.3
24	Y	99.81250	0.0751301	98.8	100.5	8.000000	0.2970089	4.3	11.8
48	N	99.72000	0.0433590	99.4	99.9	6.300000	0.1208305	5.4	6.9
48	Y	99.87143	0.0722403	99.3	100.5	7.542857	0.4287188	3.7	12.5

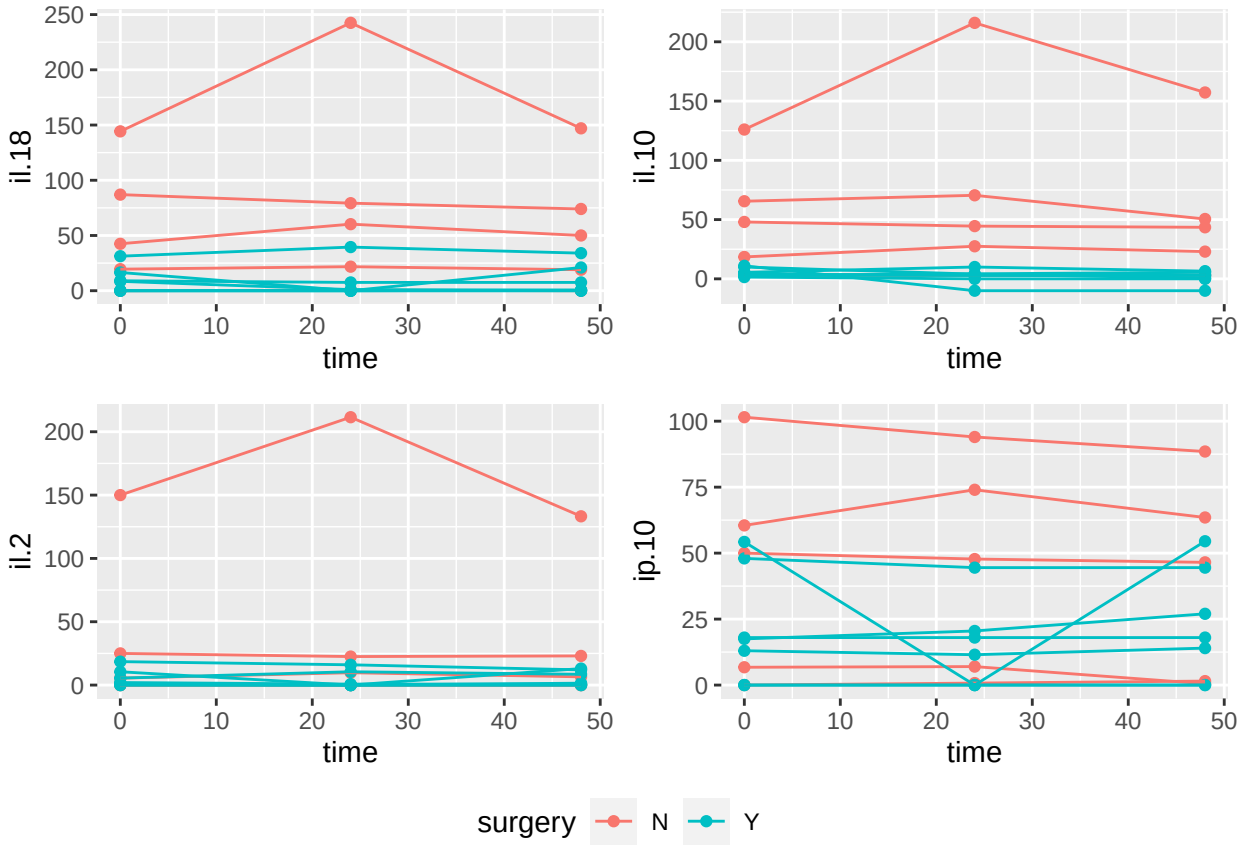
time	surgery	neut__mean	neut__se	neut__min	neut__max	lymph__mean	lymph__se	lymph__min	lymph__max
0	N	2.740000	0.0657267	2.2	3.0	2.700000	0.1523155	1.9	3.9
0	Y	5.075000	0.2185331	3.3	8.1	2.150000	0.1006674	1.2	3.4
24	N	3.200000	0.1240967	2.8	4.3	3.080000	0.1438054	2.3	4.2
24	Y	6.312500	0.2928034	3.3	10.4	1.537500	0.0550720	0.9	2.1
48	N	2.800000	0.0489898	2.5	3.1	3.260000	0.0901110	2.7	3.8
48	Y	5.585714	0.3905716	2.6	9.9	1.728571	0.0731759	1.0	2.5

time	surgery	fibr__mean	fibr__se	fibr__min	fibr__max
0	N	240.0000	10.95445	200	300
0	Y	287.5000	15.58029	100	500
24	N	260.0000	10.95445	200	300
24	Y	325.0000	12.93873	200	500
48	N	240.0000	17.88854	100	300
48	Y	328.5714	19.71616	200	600

Start with the plotting full cytokine data set

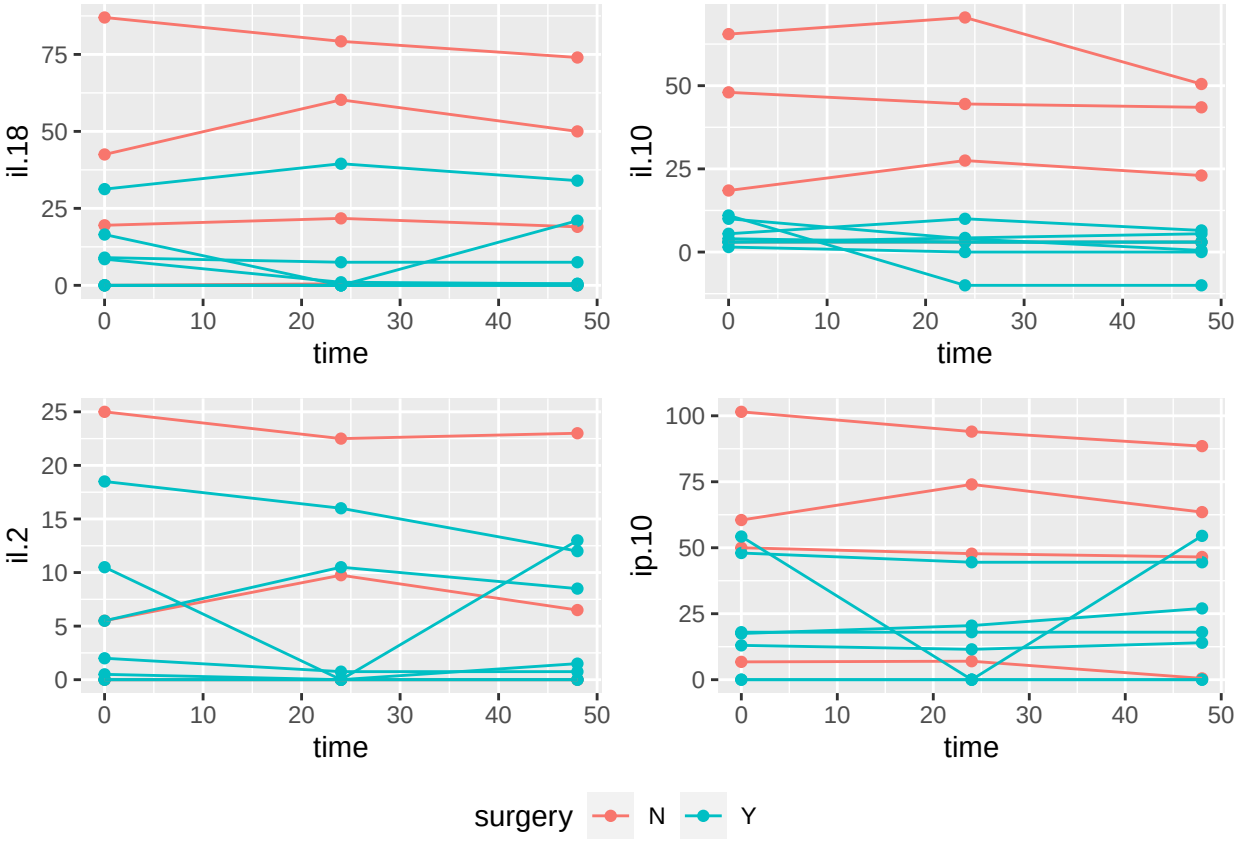


Remove the obvious outlier at the top and recheck plots



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.

```
## 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

## 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

## 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
```

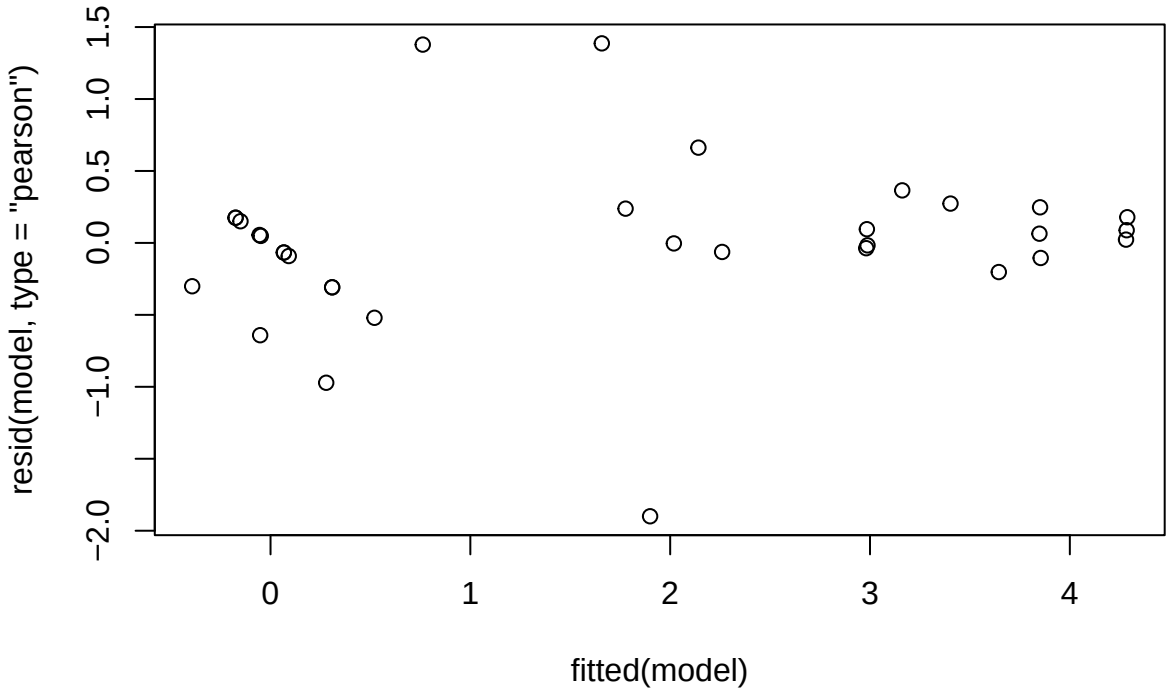
```
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.3705 -0.1377 -0.0506  0.0569  1.8560
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  horse    (Intercept) 633.8    25.17
##  Residual             109.8    10.48
## Number of obs: 33, groups:  horse, 11
##
## 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

## 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
```

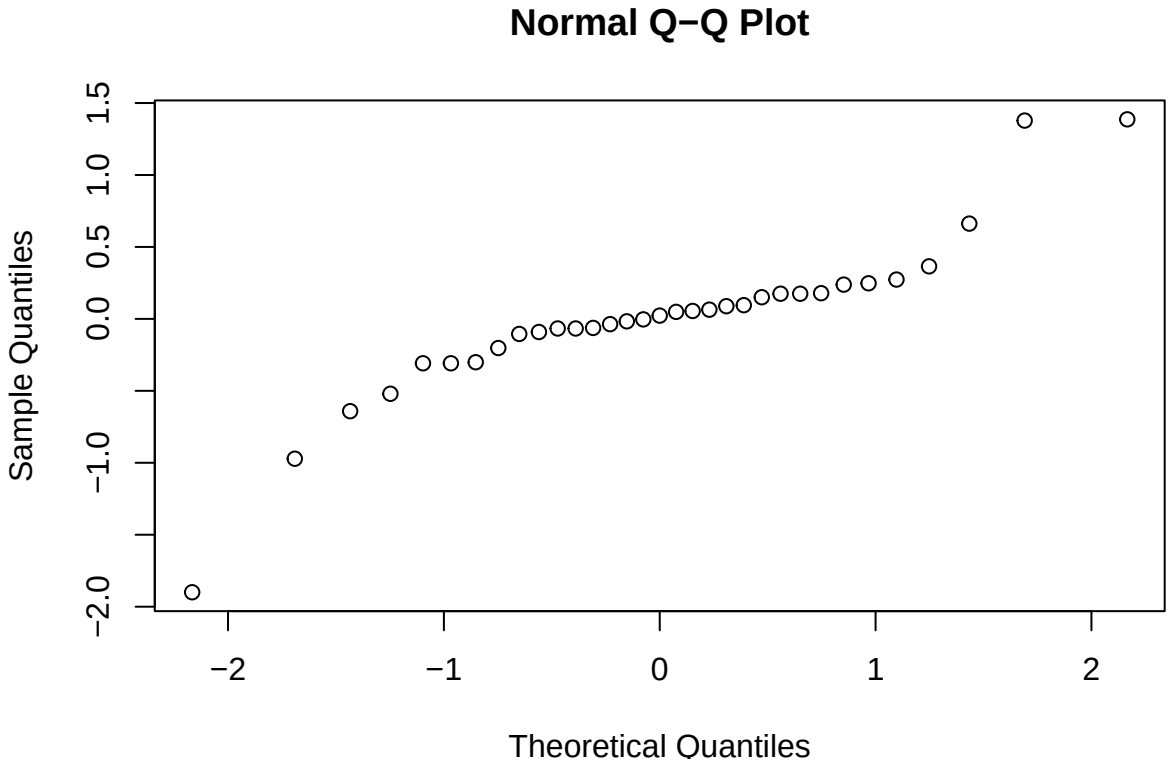
```
## surgeryY    -0.798
## time        -0.234  0.187
## surgeryY:tm  0.187 -0.234 -0.798
```

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

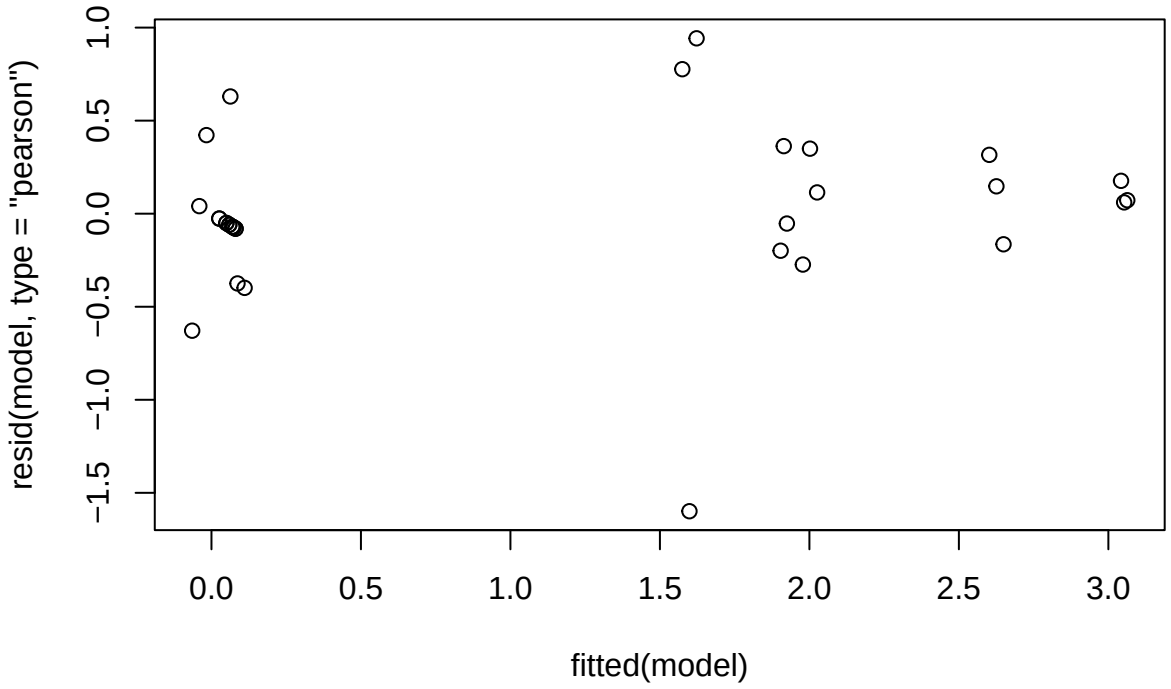
Distribution is not great, so try log transformation of all 4

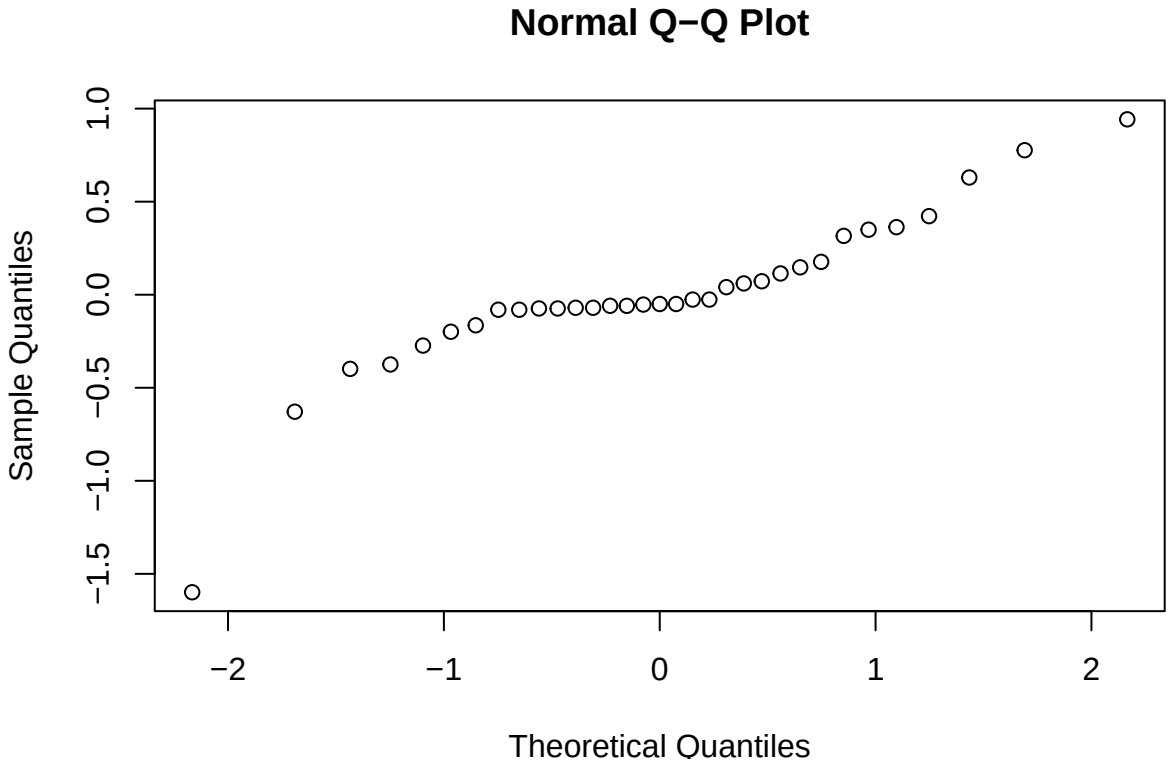
```
## Type III Analysis of Variance Table with Satterthwaite's method
##               Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
## surgery      0.83377 0.83377     1 10.648  1.6594 0.2250
## time         0.30513 0.30513     1 20.000  0.6073 0.4449
## surgery:time 0.28957 0.28957     1 20.000  0.5763 0.4566
```



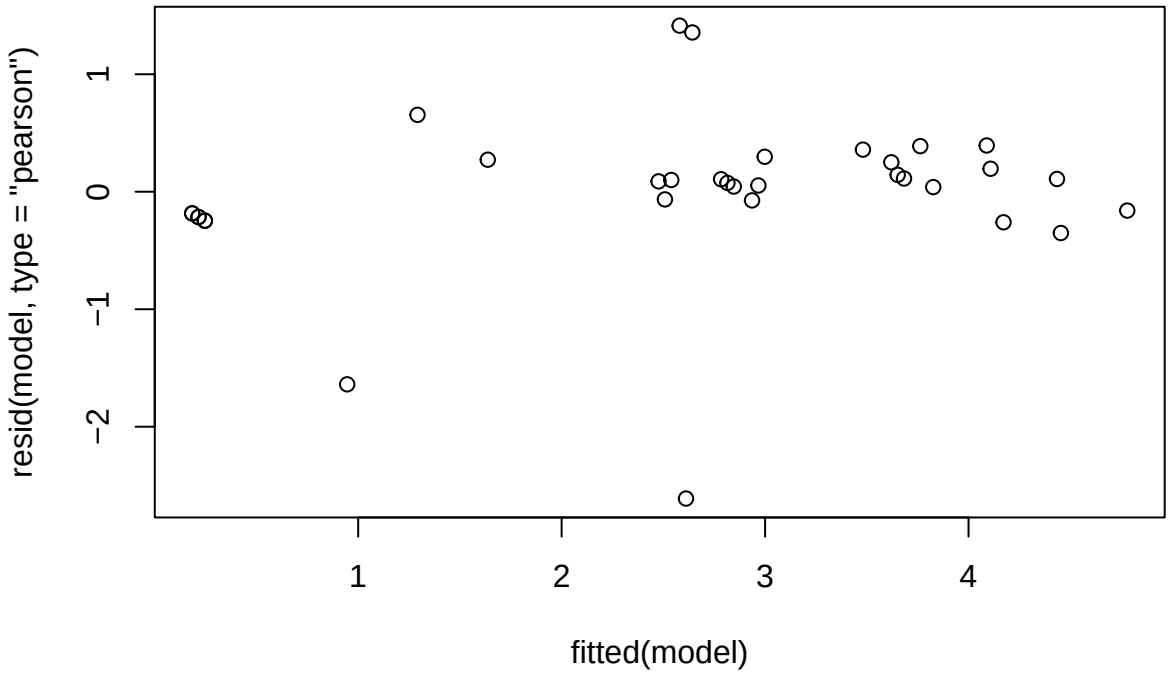


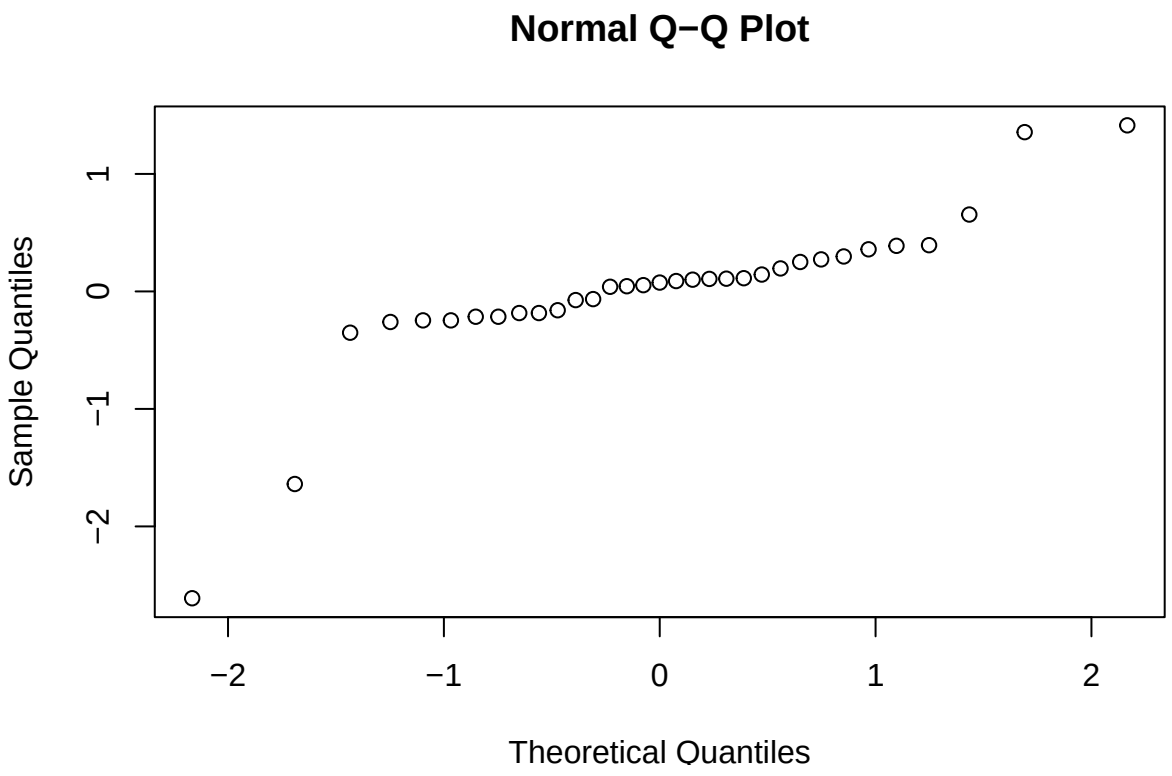

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                Sum Sq  Mean Sq NumDF  DenDF F value Pr(>F)
## surgery      0.055878 0.055878     1 10.511  0.1928 0.6694
## time         0.005990 0.005990     1 20.000  0.0207 0.8871
## surgery:time 0.000912 0.000912     1 20.000  0.0031 0.9558
```



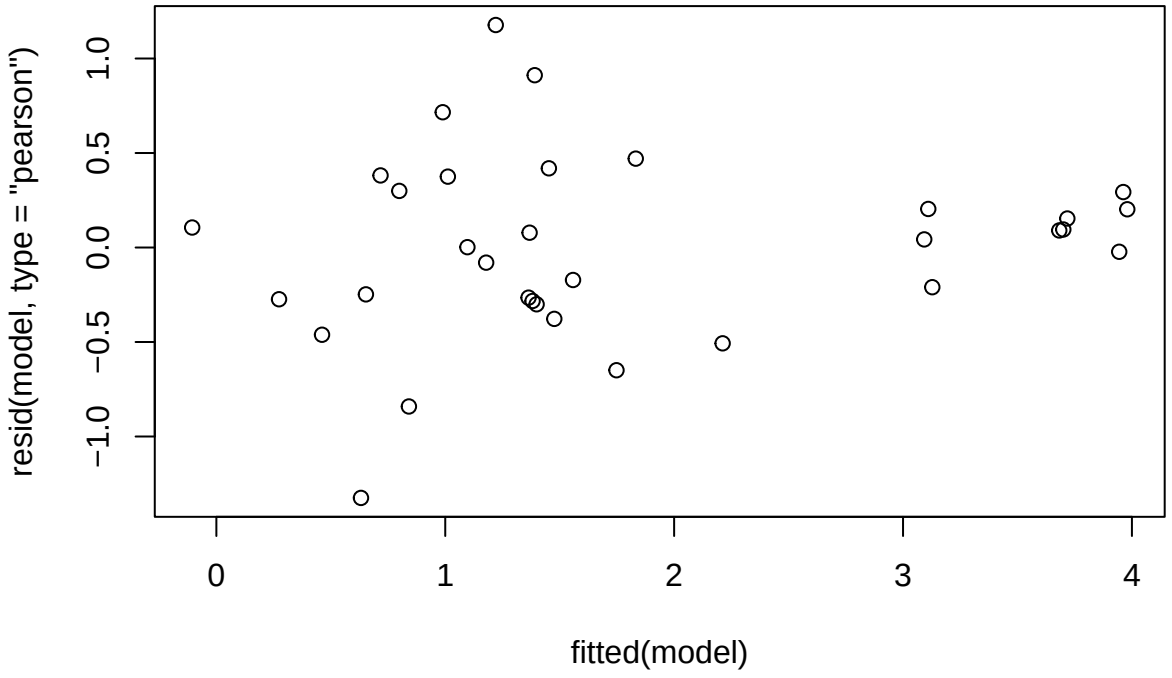


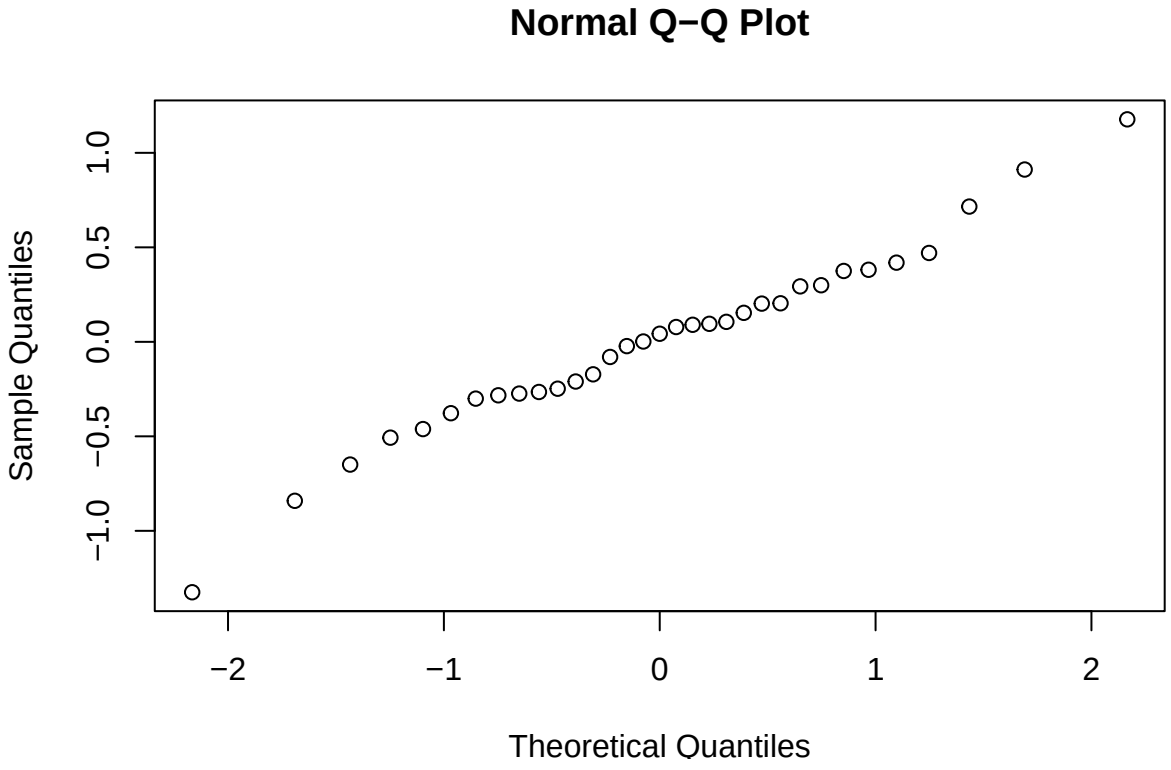

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
## surgery      1.81985 1.81985     1 11.799  2.5257 0.1384
## time         0.50291 0.50291     1 20.000  0.6980 0.4133
## surgery:time 0.72233 0.72233     1 20.000  1.0025 0.3287
```






```
## Type III Analysis of Variance Table with Satterthwaite's method
##               Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## surgery      2.31157 2.31157     1 13.086  6.4099 0.02494 *
## time         0.80294 0.80294     1 20.000  2.2265 0.15126
## surgery:time 0.66692 0.66692     1 20.000  1.8494 0.18899
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



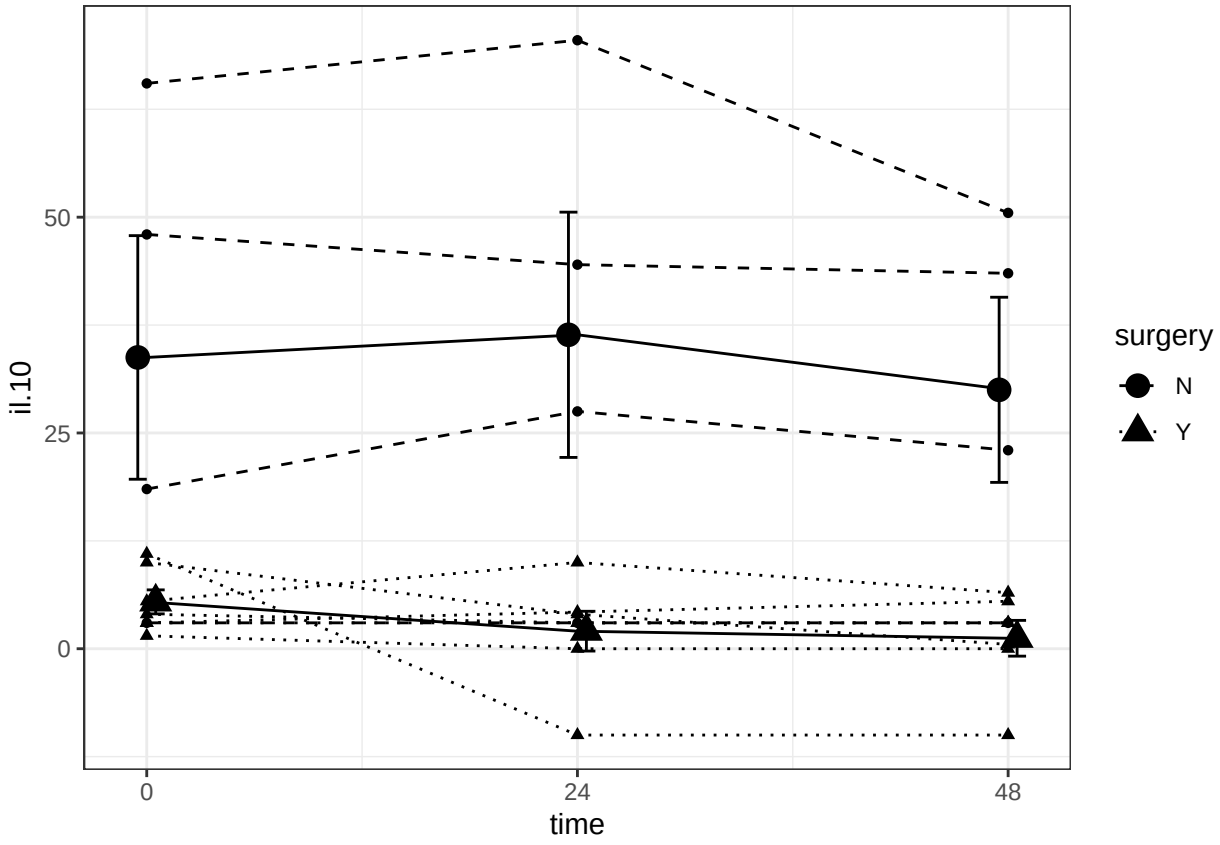


Focus on IL-10 and run contrasts

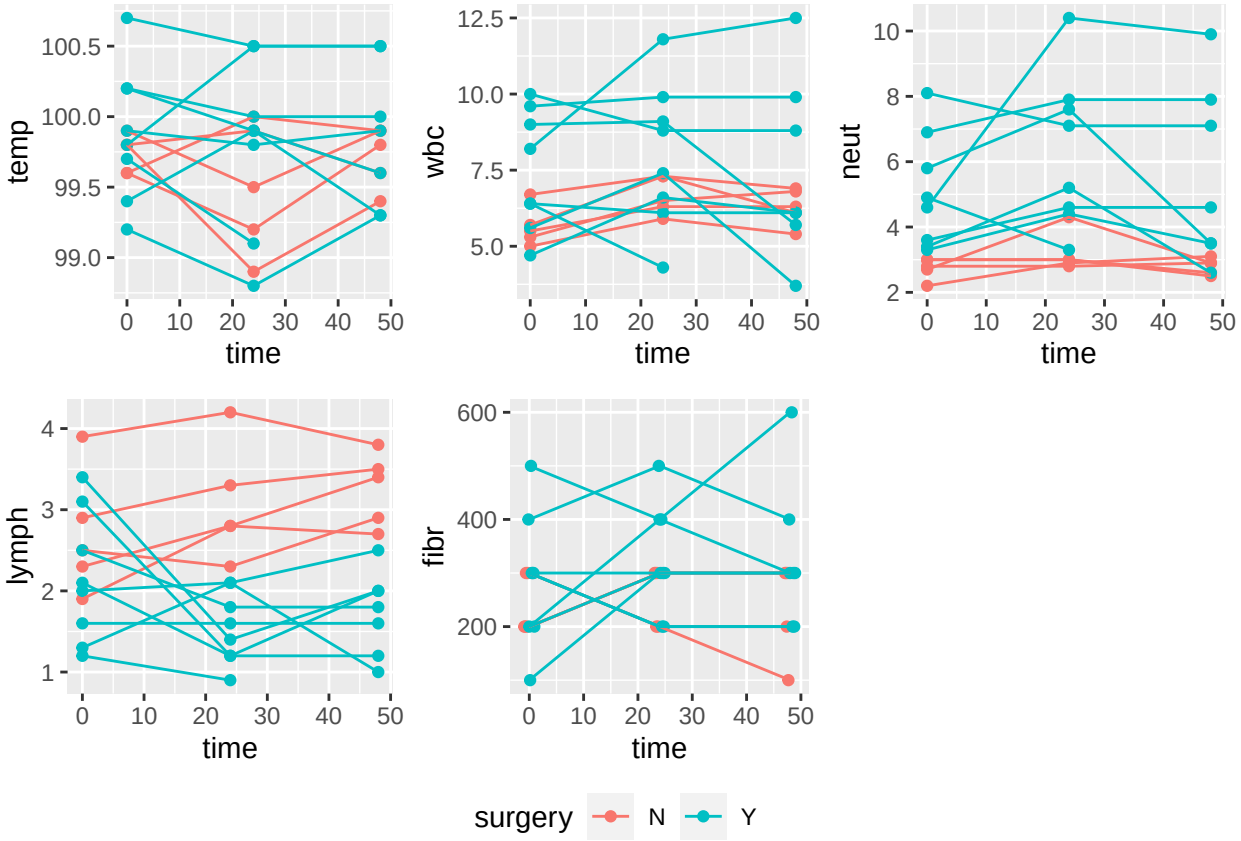
```
## time =  0:
##  contrast estimate    SE df t.ratio p.value
##  Y - N       -1.53 0.654 15  -2.343  0.0334
##
## time = 24:
##  contrast estimate    SE df t.ratio p.value
##  Y - N       -2.07 0.654 15  -3.162  0.0065
##
## time = 48:
##  contrast estimate    SE df t.ratio p.value
##  Y - N       -2.26 0.654 15  -3.449  0.0036
##
## Degrees-of-freedom method: kenward-roger

## surgery = N:
##  contrast        estimate    SE df t.ratio p.value
##  time0 - time24   -0.0986 0.446 18  -0.221  0.9735
##  time0 - time48    0.0352 0.446 18   0.079  0.9966
##  time24 - time48   0.1338 0.446 18   0.300  0.9518
##
## surgery = Y:
##  contrast        estimate    SE df t.ratio p.value
##  time0 - time24    0.4373 0.337 18   1.297  0.4148
##  time0 - time48    0.7591 0.337 18   2.252  0.0893
##  time24 - time48   0.3218 0.337 18   0.954  0.6140
##
```

```
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

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.

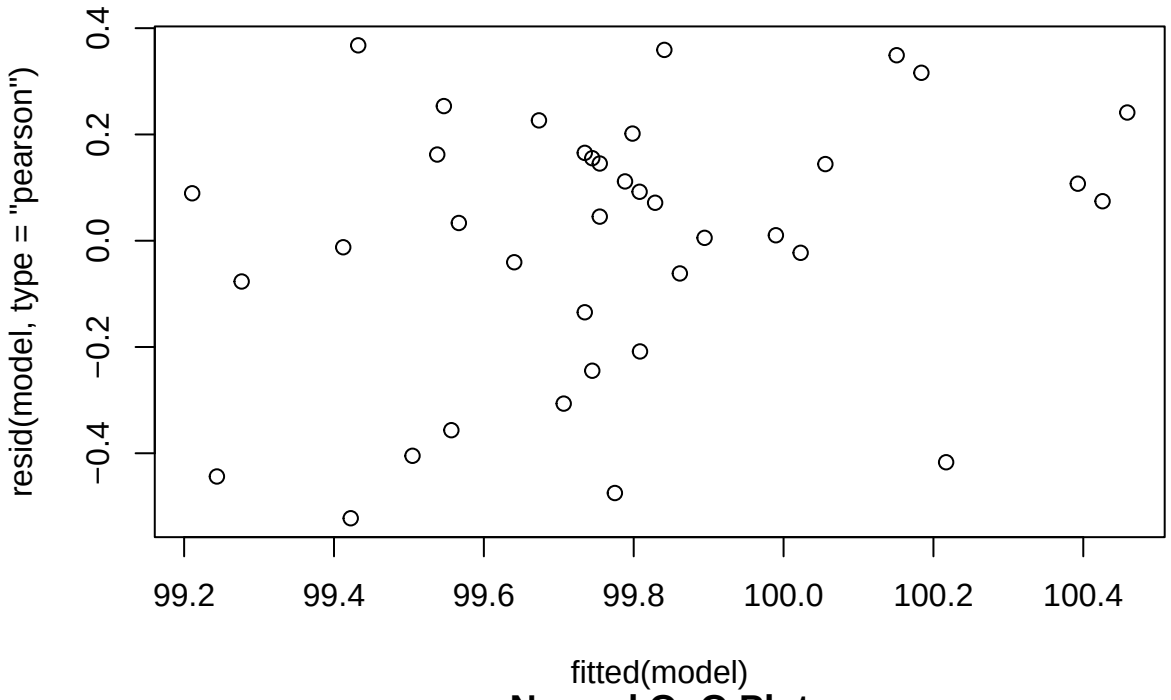


Graph physiologic/CBC variables

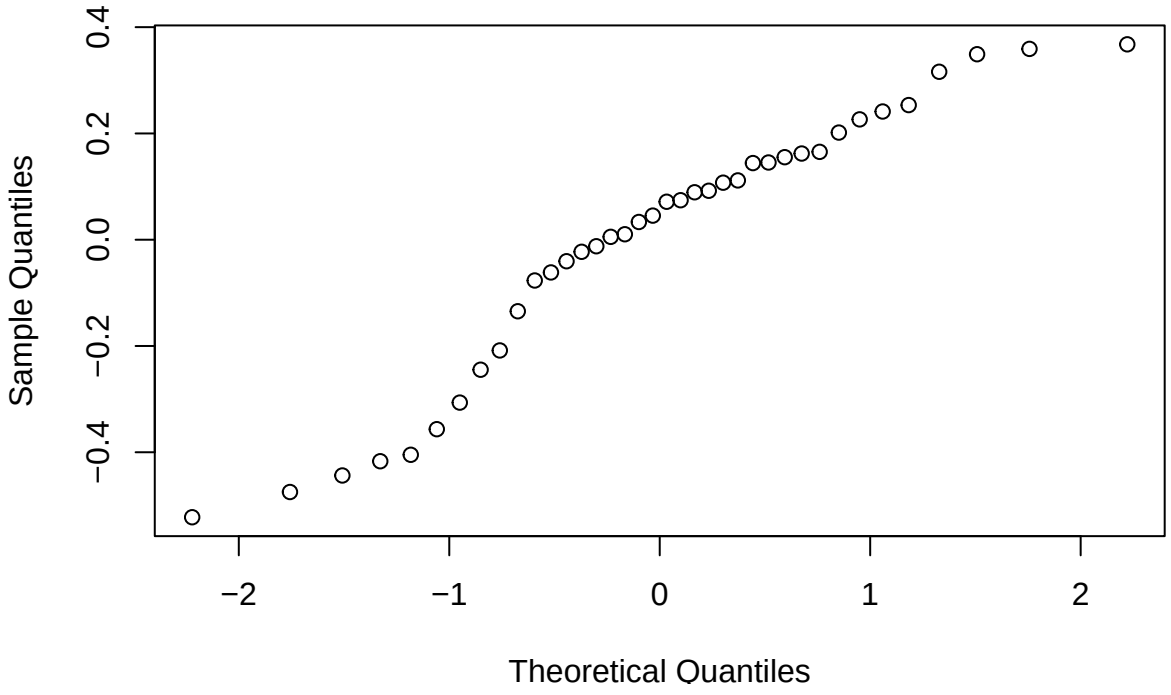


Nothing stands out, so model individually

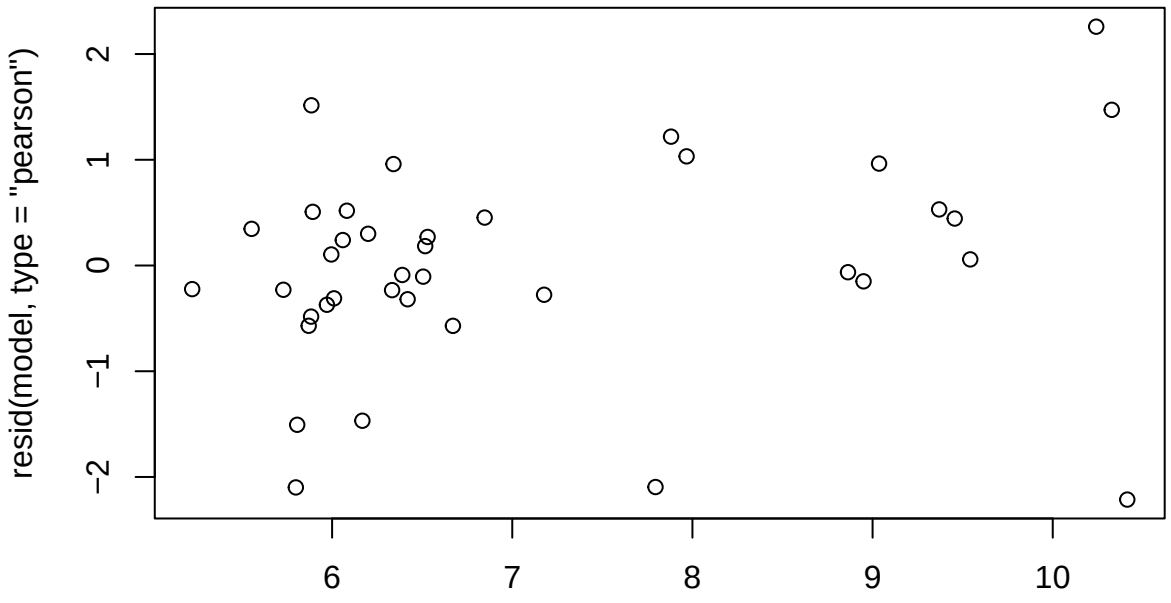
```
## Type III Analysis of Variance Table with Satterthwaite's method
##                 Sum Sq  Mean Sq NumDF  DenDF F value Pr(>F)
## surgery      0.061244 0.061244     1 17.353  0.6825 0.4199
## time         0.010986 0.010986     1 23.082  0.1224 0.7296
## surgery:time 0.003145 0.003145     1 23.082  0.0350 0.8531
```



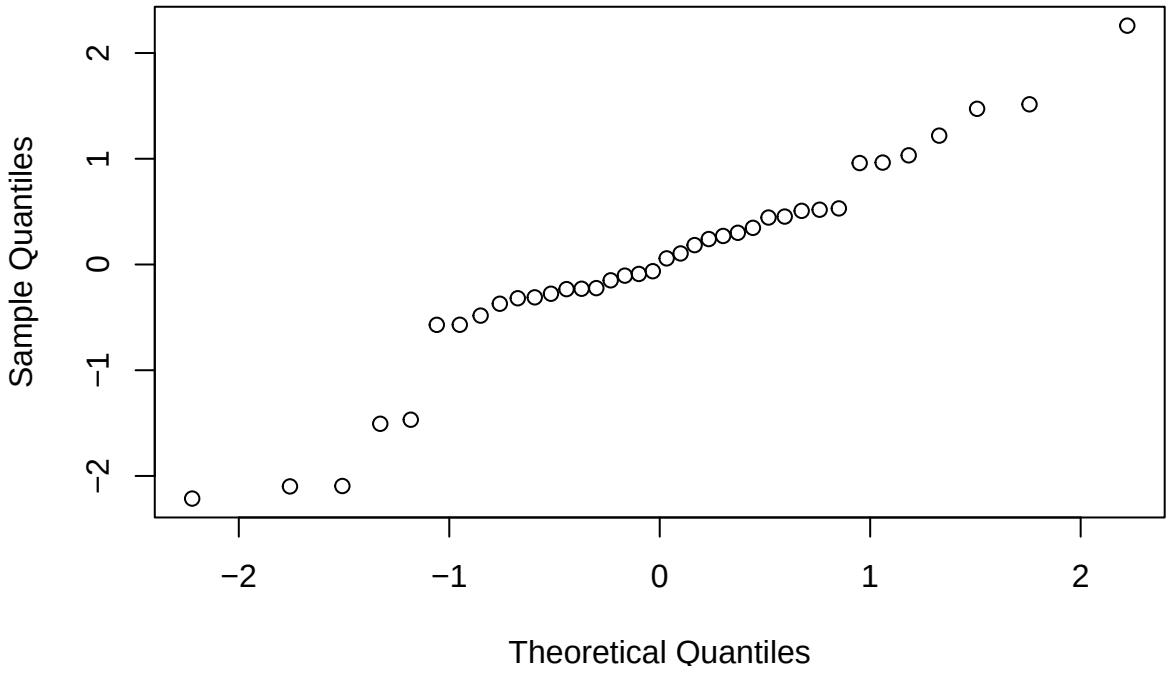
Normal Q−Q Plot



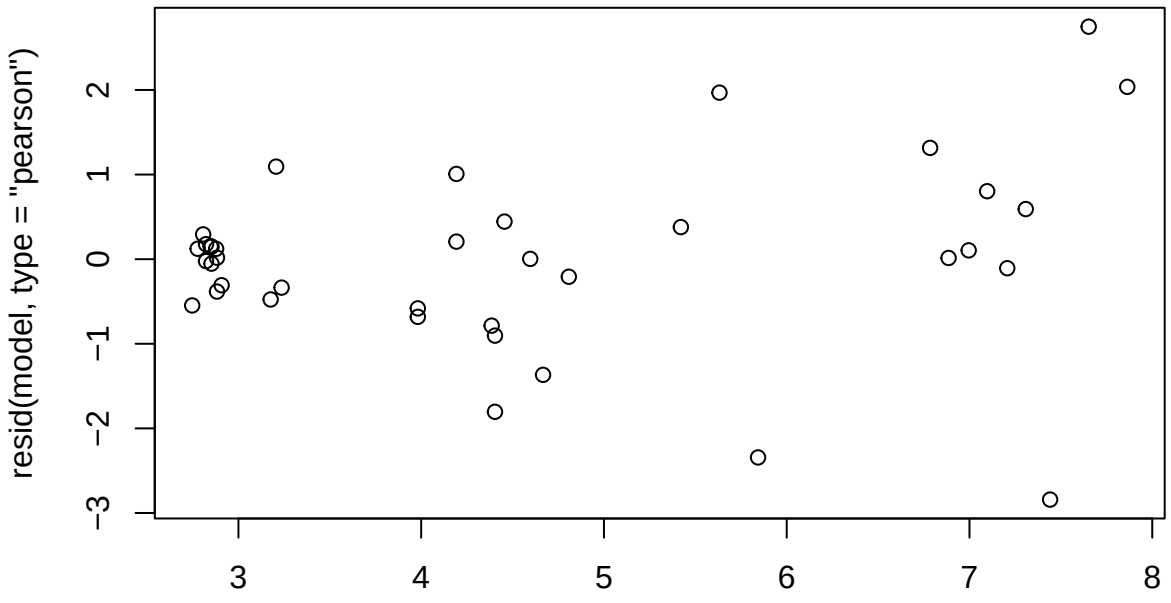

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
## surgery      3.8598  3.8598     1 16.057  2.7186 0.1186
## time         0.3526  0.3526     1 23.009  0.2483 0.6230
## surgery:time 1.0282  1.0282     1 23.009  0.7242 0.4035
```

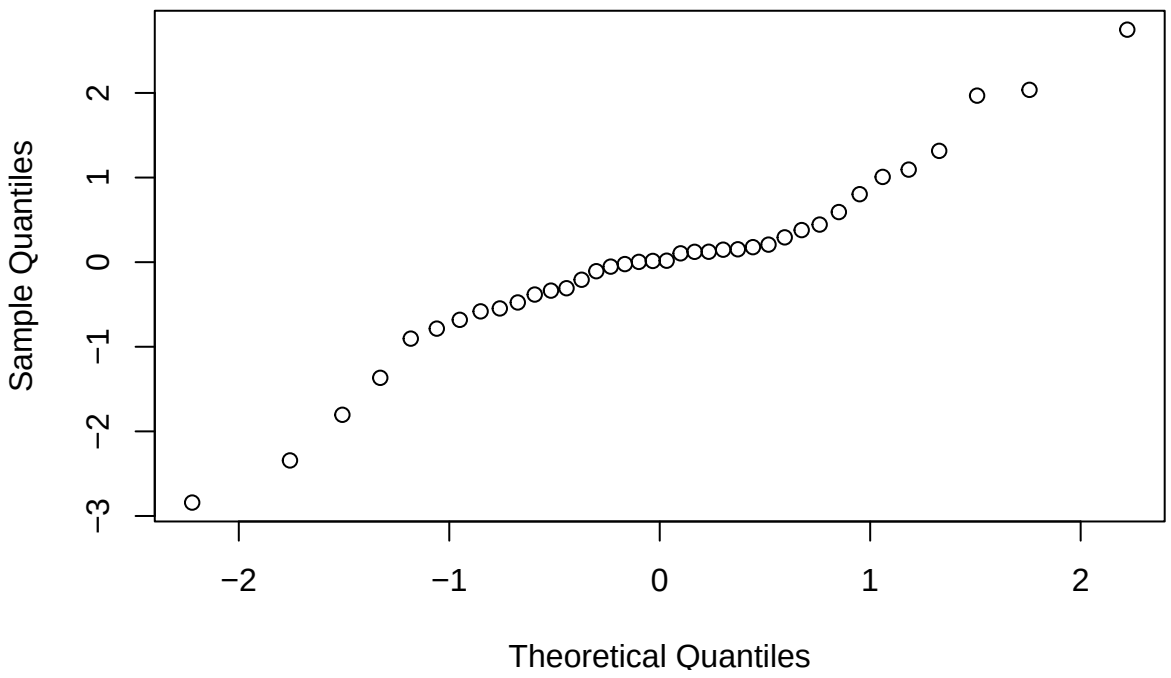


Normal Q−Q Plot

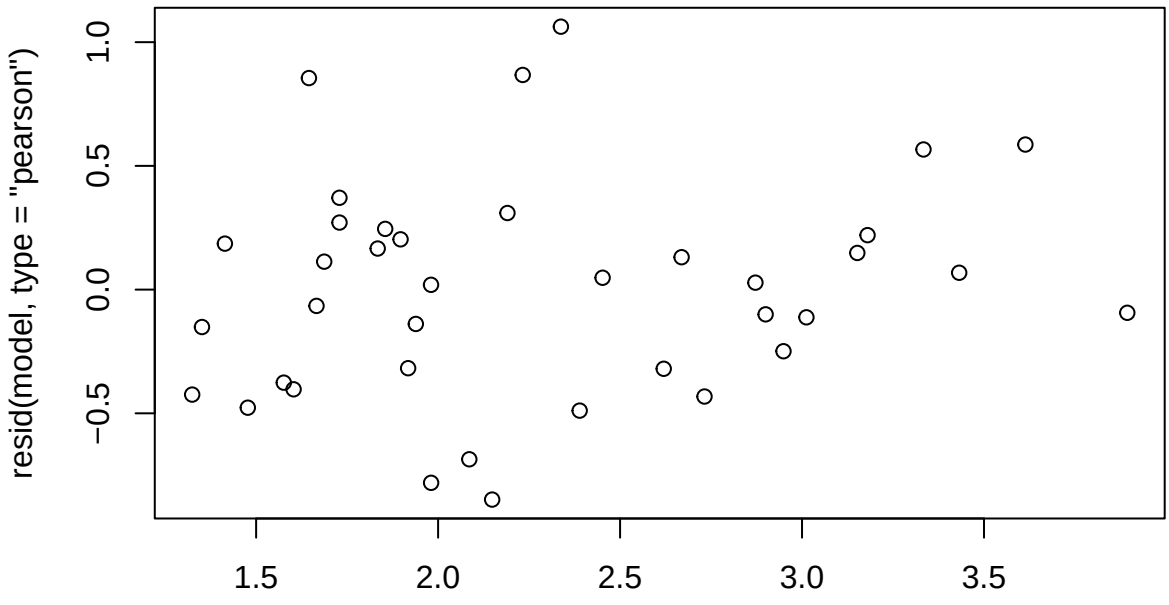



```
## Type III Analysis of Variance Table with Satterthwaite's method
##               Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## surgery      10.5238 10.5238     1 19.012  6.3077 0.02121 *
## time          0.3455  0.3455     1 23.197  0.2071 0.65329
## surgery:time  0.1950  0.1950     1 23.197  0.1168 0.73556
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

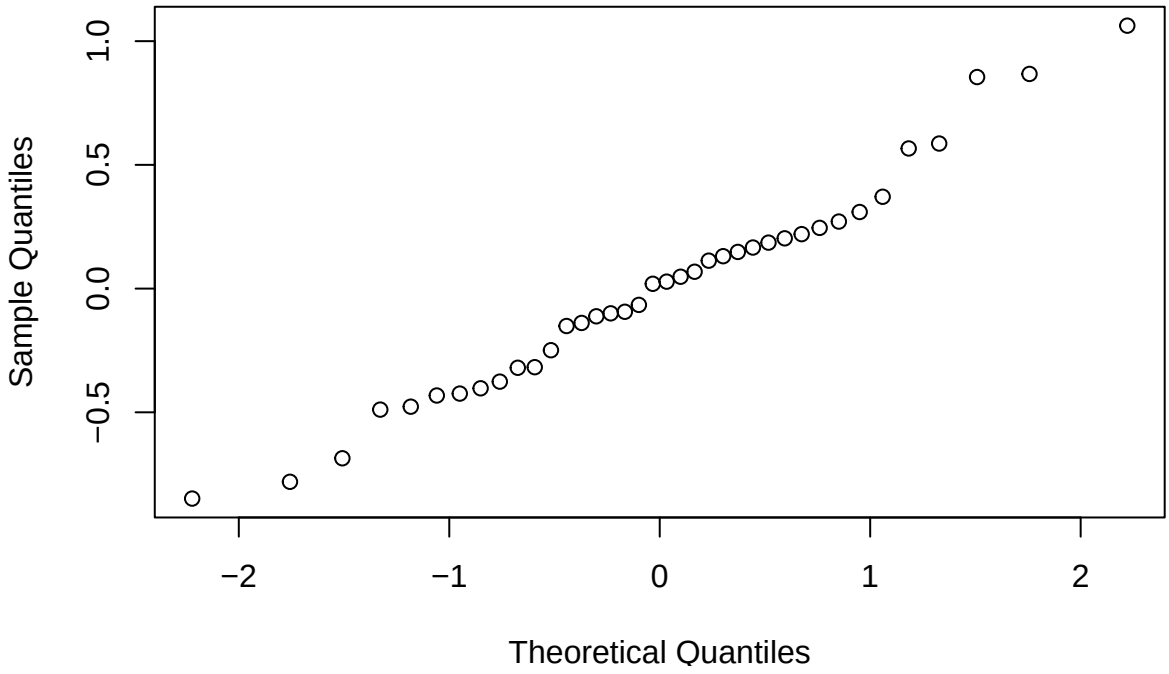




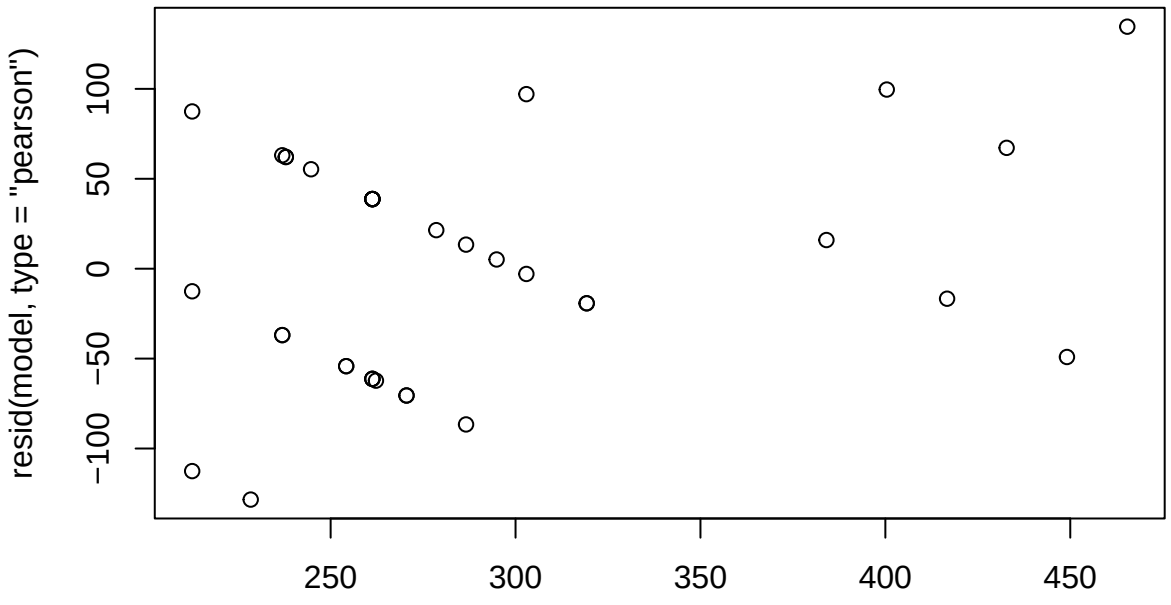

```
## Type III Analysis of Variance Table with Satterthwaite's method
##               Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## surgery      1.08629 1.08629     1 22.283  4.1358 0.05406 .
## time         0.00476 0.00476     1 22.717  0.0181 0.89407
## surgery:time 1.68301 1.68301     1 22.717  6.4077 0.01875 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

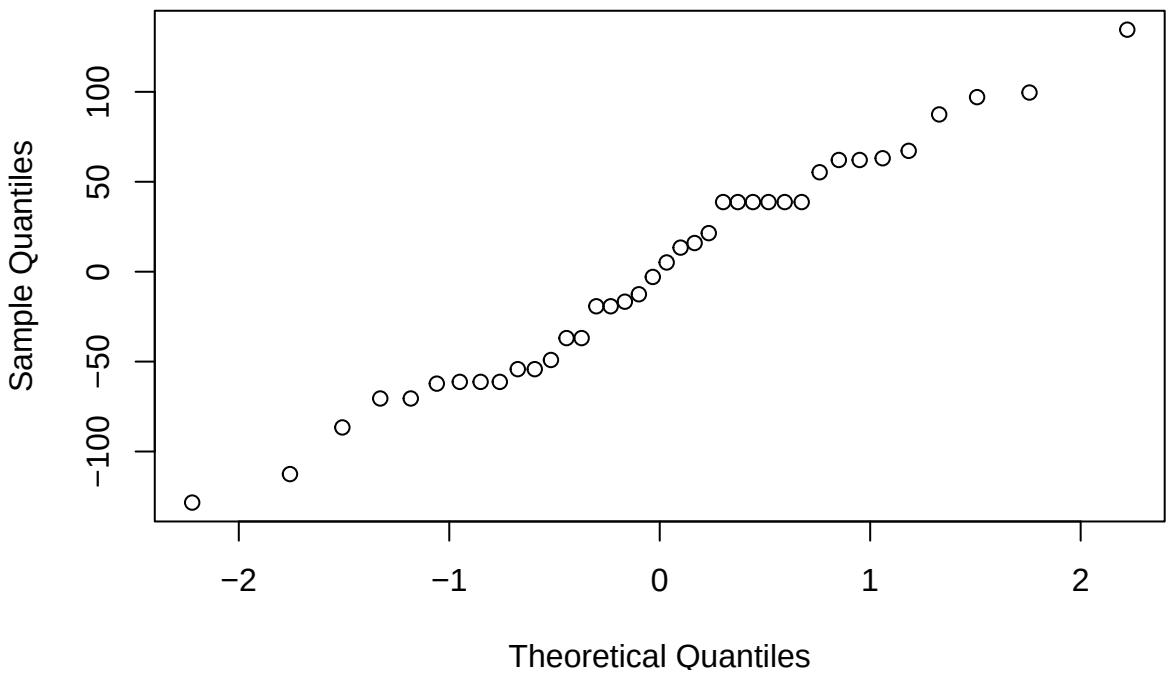


Normal Q−Q Plot




```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
## surgery      4055.2  4055.2     1 19.716  0.7125 0.4087
## time         1582.0  1582.0     1 23.090  0.2780 0.6031
## surgery:time 1582.0  1582.0     1 23.090  0.2780 0.6031
```





Looks like neutrophils and lymphocytes have some significant differences

resids look fine, let's check the contrasts for both

First neutrophils

```
## time =  0:
```

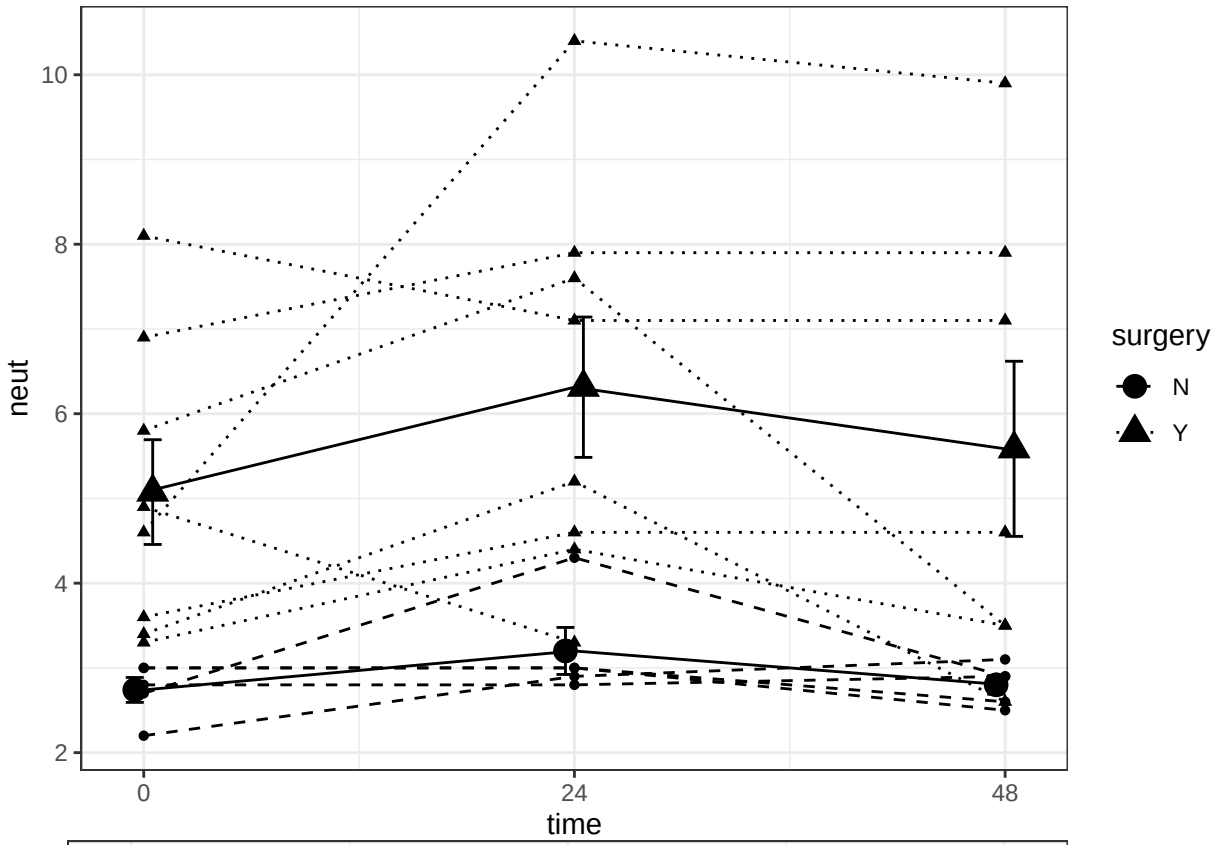
```
##  contrast estimate   SE   df t.ratio p.value
##  Y - N        2.33 1.04 20.2   2.240  0.0365
##
## time = 24:
##  contrast estimate   SE   df t.ratio p.value
##  Y - N        3.11 1.04 20.2   2.985  0.0072
##
## time = 48:
##  contrast estimate   SE   df t.ratio p.value
##  Y - N        2.62 1.06 21.1   2.474  0.0220
##
## Degrees-of-freedom method: kenward-roger

## surgery = N:
##  contrast        estimate    SE   df t.ratio p.value
##  time0 - time24    -0.460 0.776 21.0  -0.593  0.8254
##  time0 - time48    -0.060 0.776 21.0  -0.077  0.9967
##  time24 - time48    0.400 0.776 21.0   0.516  0.8647
##
## surgery = Y:
##  contrast        estimate    SE   df t.ratio p.value
##  time0 - time24    -1.238 0.613 21.0  -2.017  0.1327
##  time0 - time48    -0.349 0.644 21.4  -0.542  0.8514
##  time24 - time48    0.888 0.644 21.4   1.380  0.3688
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

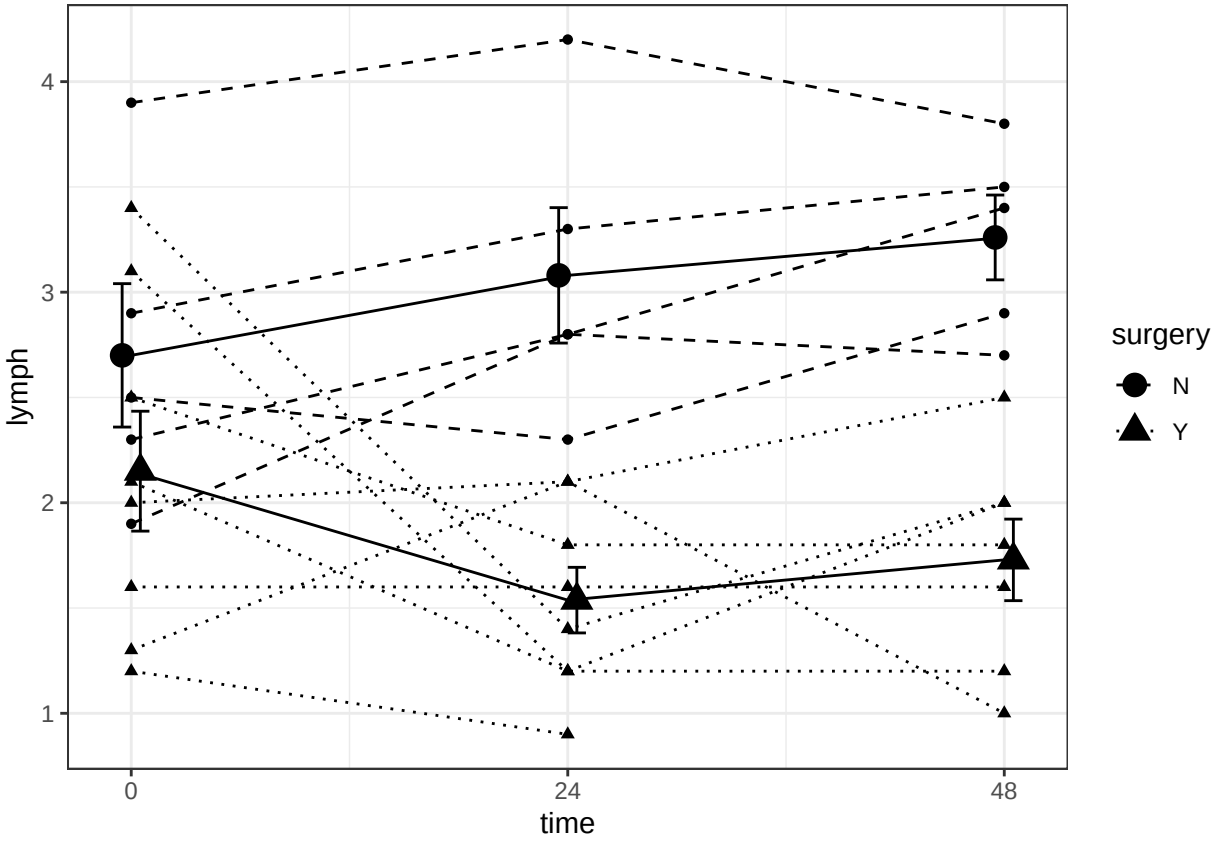
Then lymphocytes

```
## time =  0:
##  contrast estimate    SE   df t.ratio p.value
##  Y - N       -0.55 0.361 25.6  -1.526  0.1394
##
## time = 24:
##  contrast estimate    SE   df t.ratio p.value
##  Y - N       -1.54 0.361 25.6  -4.278  0.0002
##
## time = 48:
##  contrast estimate    SE   df t.ratio p.value
##  Y - N       -1.59 0.369 26.4  -4.312  0.0002
##
## Degrees-of-freedom method: kenward-roger

## surgery = N:
##  contrast        estimate    SE   df t.ratio p.value
##  time0 - time24    -0.380 0.320 21.0  -1.187  0.4738
##  time0 - time48    -0.560 0.320 21.0  -1.749  0.2111
##  time24 - time48   -0.180 0.320 21.0  -0.562  0.8414
##
## surgery = Y:
##  contrast        estimate    SE   df t.ratio p.value
##  time0 - time24     0.613 0.253 21.0   2.420  0.0614
```

```
##  time0 - time48     0.481 0.265 21.6   1.815  0.1883
##  time24 - time48   -0.131 0.265 21.6  -0.495  0.8746
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

And graphically





#

There was a significant effect of surgery on neutrophil counts, which were increased at all time points in the surgery group, but did not change from baseline in either group # Lymphocyte demonstrated a significanntly different change over time (the slopes of the best fit lines are different), with counts decreased at 24 and 48 hours in the surgery group over the non-surgery group, but no differences at baseline