CBC Splines

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20 January, 2023, 21:07

```
suppressPackageStartupMessages({
  library(tidyverse)
  library(sjPlot)
  library(lmerTest)
  library(readxl)
  library(xlsx)
  library(kableExtra)
  library(splines)
  library(redres)
})
```

Write-up

Using non-linear mixed effects with splines, we were able to assess the changes in peripheral white blood cell populations. We previously showed that antibiotic (ABX) treated animals had increased neutrophil-to-lymphocyte ratios (NLR) over six months when compared to control (CTRL) animals. In addition to NLR, we looked at the frequency and concentrations of total white blood cells, lymphocytes, neutrophils (PMNs), monocytes, eosinophils, and basophils but none of the aforementioned populations were significantly different between ABX and CTRL animals. For this analysis antibiotic H1N1 (ABX H1N1) animals were compared with control H1N1 (CTRL H1N1), and fecal microbiome transplant (FMT) animals were compared against CTRL animals.

The variables used in the original model were sex, treatment, and age (in days) and no difference was detected in NLR when treatment and age were used. Only when sex was considered did we observe significant changes in NLR– increased NLR in ABX animals. It was first hypothesized that FMT would reverse the changes to NLR observed in ABX animals. Our analysis revealed no difference in FMT and CTRL animals for NLR or other peripheral measures. Our lab further hypothesized that influenza infection would exacerbate changes observed in ABX animals. However, this was not the case, but we did observe that ABX H1N1 animals had reduced lymphocyte populations compared to CTRL H1N1 animals.

One concern is the assumption that FMT reversed changes to NLR in recipient animals because one of the variables that went into the original model included sex. Given the difficulty in acquiring non-human primates, we decided to omit females from future study groups to increase our male sample size. Thus, there is no difference in NLR when considering treatment and age in FMT animals, but the same is true when comparing ABX and CTRL animals. Influenza H1N1 infection did not exacerbate NLR in ABX H1N1 animals, but a decrease in lymphocyte populations was observed. Given that NLR is neutrophils over lymphocytes, we expect that NLR should have increased, but the ratios between CTRL H1N1 and ABX H1N1 are more similar than ABX and CTRL. This result is both perplexing and inconsistent.

The best way to alleviate these discrepancies is to compare FMT H1N1 and CTRL H1N1 animals. Hypothetically, if no difference in lymphocytes is observed in the FMT H1N1 group our hypothesis still has merit albeit we orginally expected to measure differences in NLR. With these data, we can draw a better

conclusion on whether FMT reverses early life microbiome changes to the periphery and if H1N1 severity is reduced with early FMT intervention.

H1N1 Control vs. Antibiotic
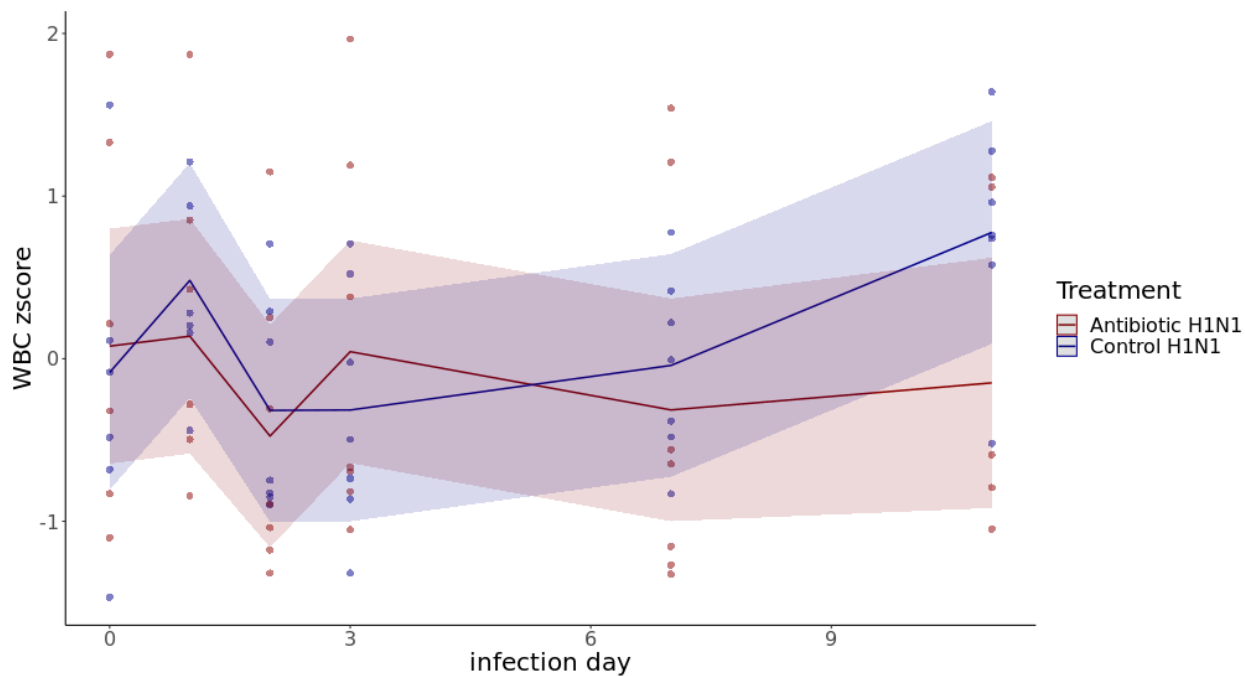
White Blood cells/ul Z-scores

Table 1: Total WBC Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(infection_day, df = 5)	5.060	1.012	5	54.226	2.099	0.079
Treatment	0.047	0.047	1	39.926	0.097	0.757
ns(infection_day, df = 5):Treatment	3.048	0.610	5	54.226	1.265	0.292

Polymononuclear Cells/ul z-scores
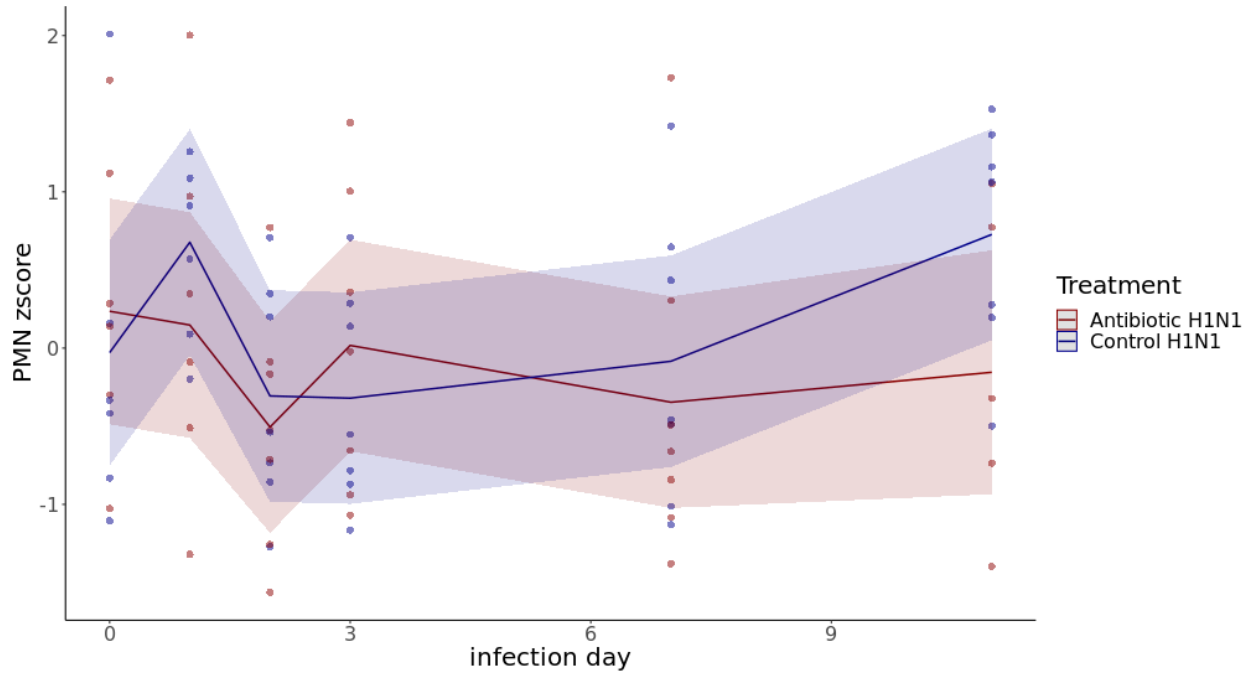
Table 2: PMN Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(infection_day, df = 5)	6.299	1.260	5	54.148	2.073	0.083
Treatment	0.157	0.157	1	53.226	0.258	0.614
ns(infection_day, df = 5):Treatment	3.321	0.664	5	54.148	1.093	0.375

Lymphoyctes/ul z-scores
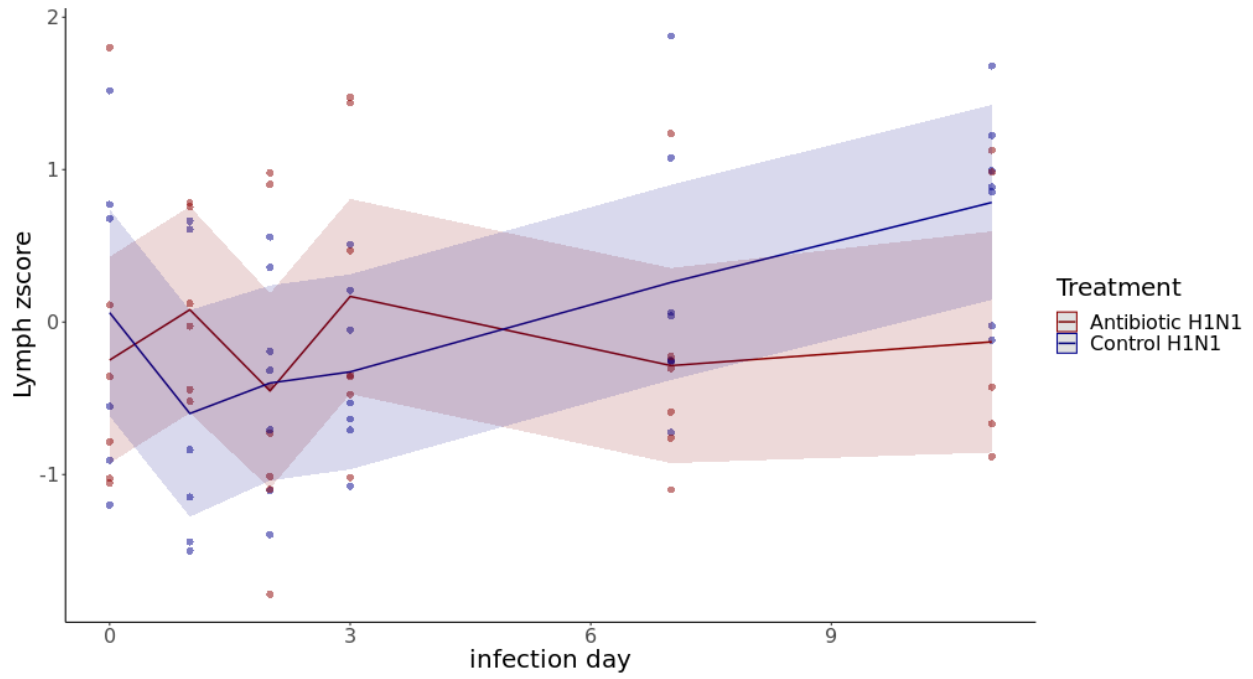
Table 3: Lymphocyte Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(infection_day, df = 5)	3.967	0.793	5	54.783	1.721	0.145
Treatment	0.186	0.186	1	45.138	0.404	0.528
ns(infection_day, df = 5):Treatment	5.754	1.151	5	54.783	2.496	0.042

Monocytes/ul z-scores
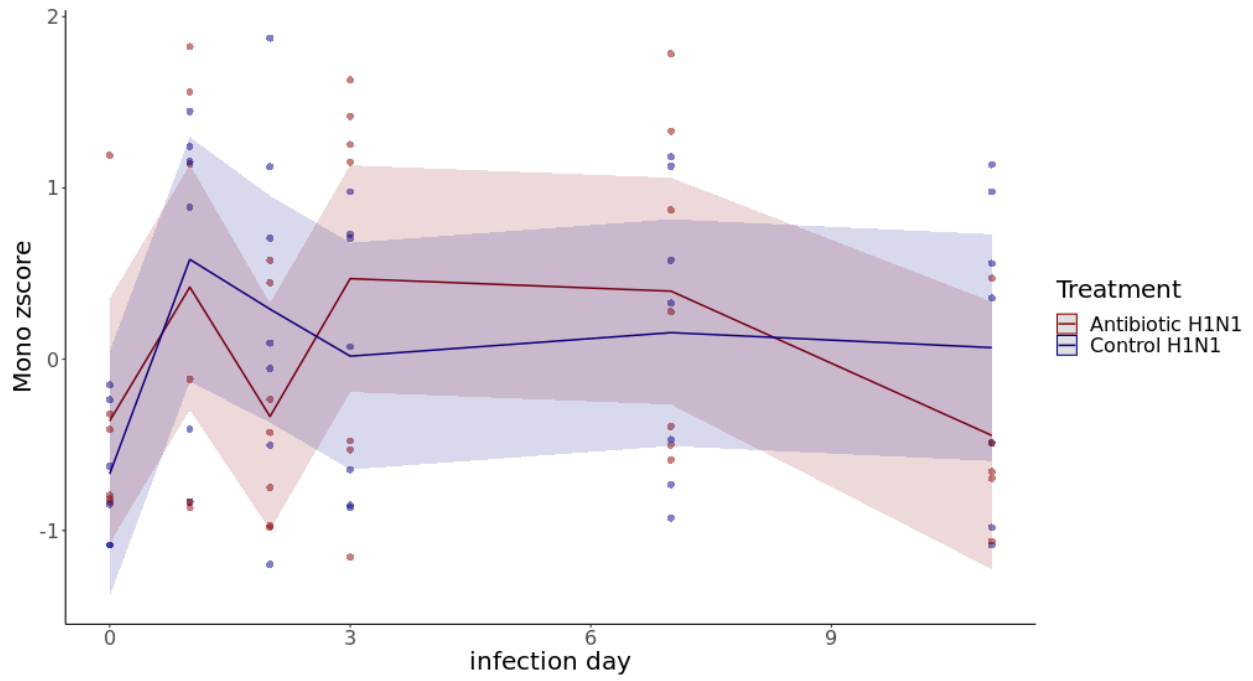
Table 4: Monocyte Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(infection_day, df = 5)	8.259	1.652	5	54.924	2.234	0.064
Treatment	0.267	0.267	1	64.988	0.361	0.550
ns(infection_day, df = 5):Treatment	3.387	0.677	5	54.924	0.916	0.477

Eosinophils/ul z scores
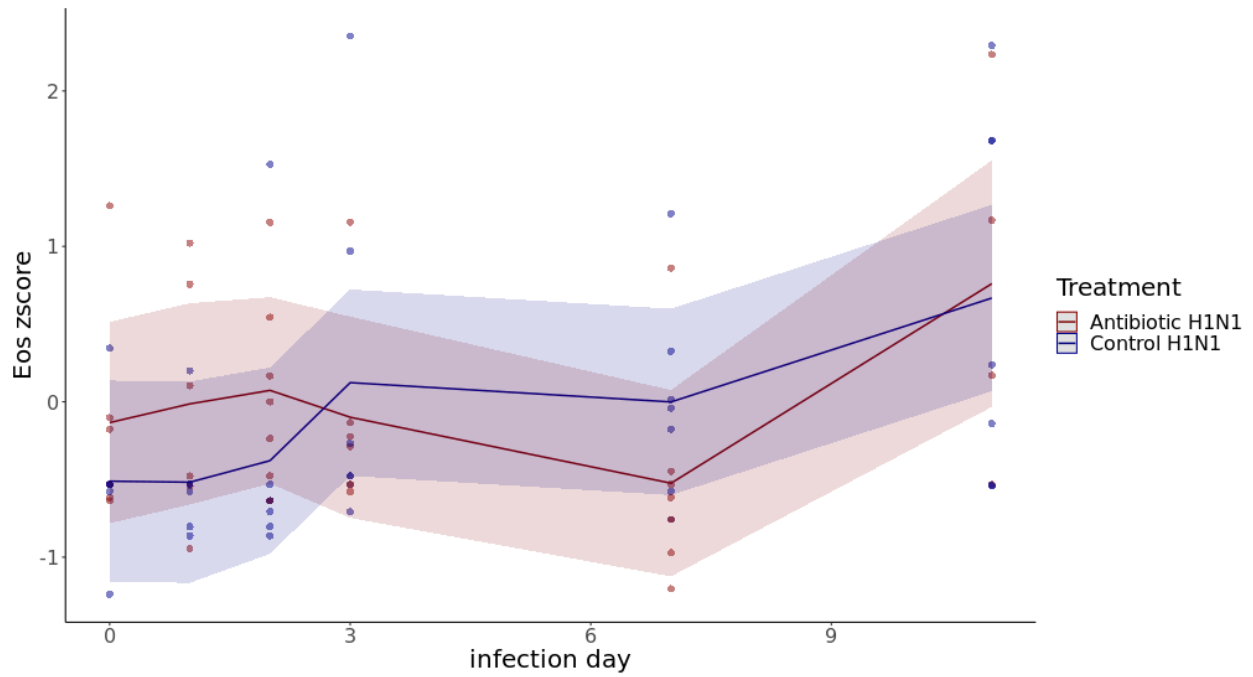
Table 5: Eosinophil Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(infection_day, df = 5)	8.178	1.636	5	64	2.497	0.040
Treatment	0.427	0.427	1	64	0.651	0.423
ns(infection_day, df = 5):Treatment	2.850	0.570	5	64	0.870	0.506

Neutrophil to Lymphocte ratio z scores
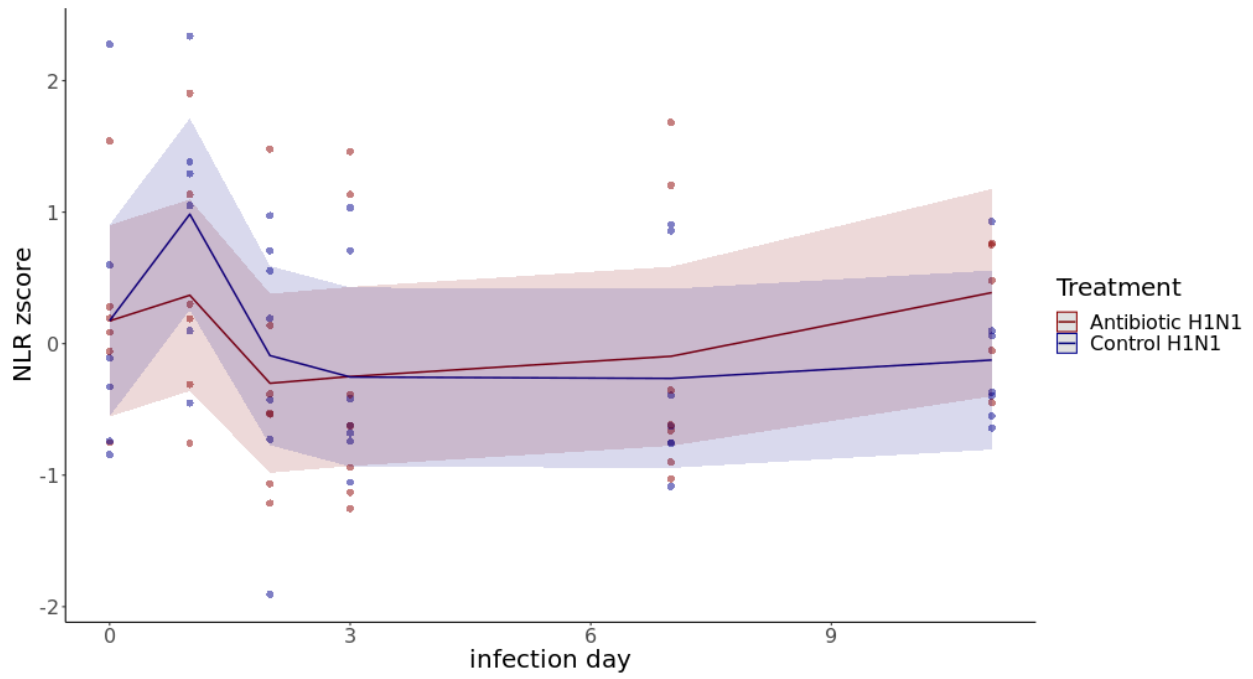
Table 6: Neutrophil to Lymphocyte Ratio Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(infection_day, df = 5)	7.687	1.537	5	54.118	2.417	0.048
Treatment	0.000	0.000	1	55.136	0.000	0.997
ns(infection_day, df = 5):Treatment	2.105	0.421	5	54.118	0.662	0.654

FMT vs. Antibiotic

White Blood cells/ul Z-scores
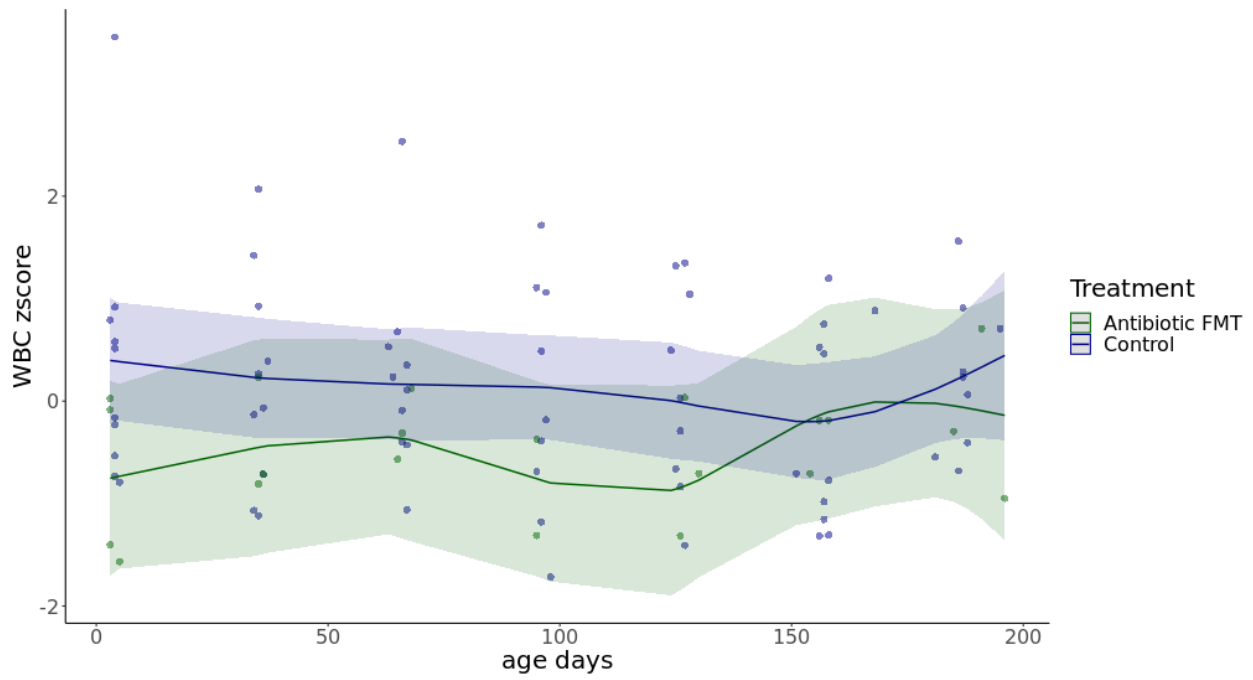
Table 7: Total WBC Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(age_days, df = 5)	1.404	0.281	5	67.766	0.437	0.821
Treatment	2.553	2.553	1	53.714	3.975	0.051
ns(age_days, df = 5):Treatment	2.137	0.427	5	67.766	0.665	0.651

Polymononuclear Cells/ul z-scores
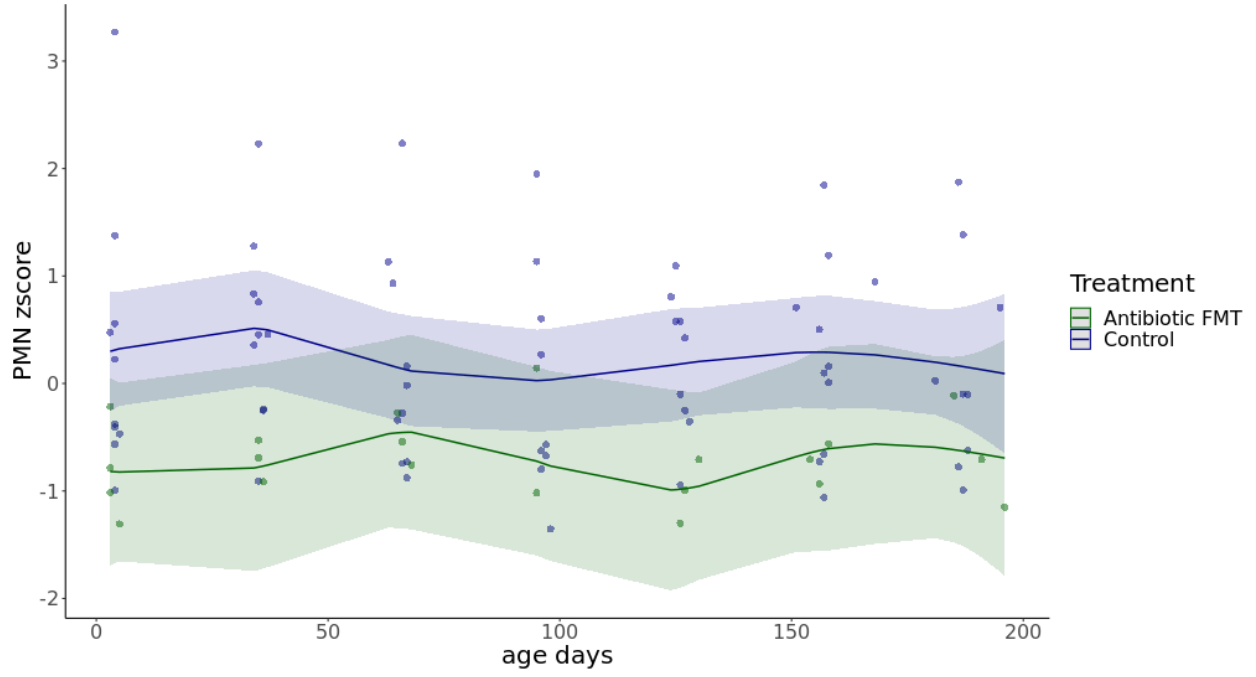
Table 8: PMN Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(age_days, df = 5)	0.513	0.103	5	67.468	0.212	0.956
Treatment	2.169	2.169	1	45.971	4.483	0.040
ns(age_days, df = 5):Treatment	0.868	0.174	5	67.468	0.359	0.875

Lymphoyctes/ul z-scores
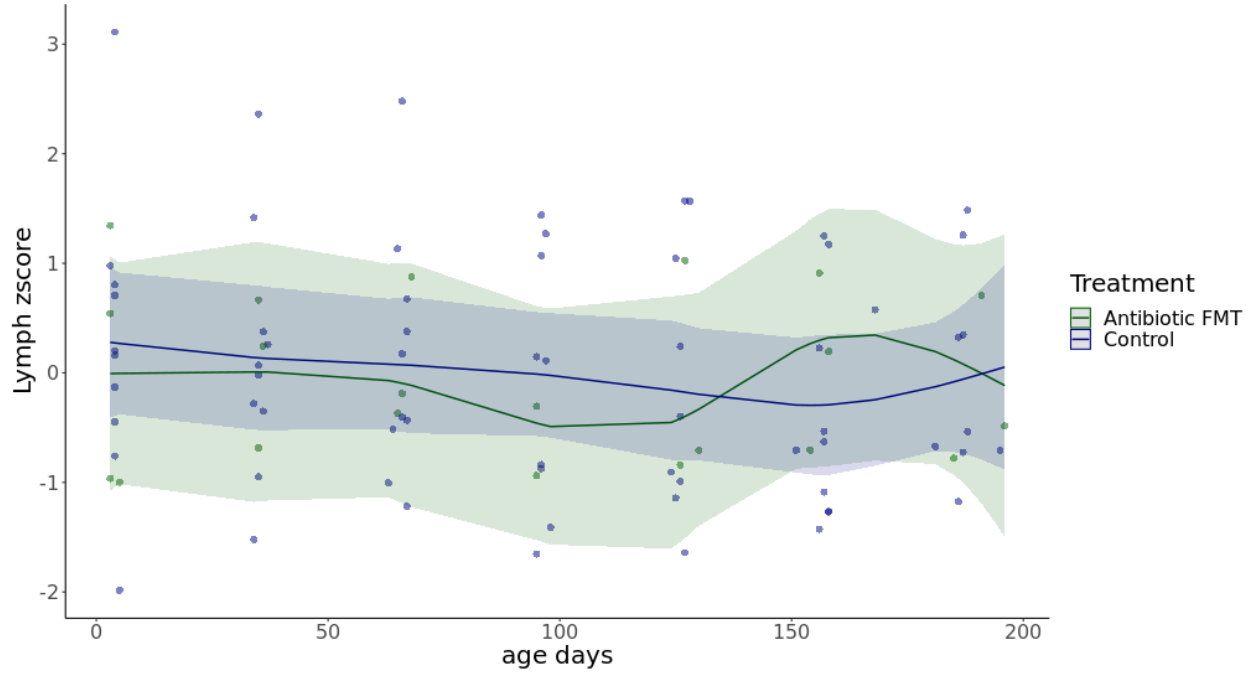
Table 9: Lymphocyte Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(age_days, df = 5)	1.257	0.251	5	67.721	0.300	0.911
Treatment	0.162	0.162	1	56.257	0.193	0.662
ns(age_days, df = 5):Treatment	1.437	0.287	5	67.721	0.343	0.885

Monocytes/ul z-scores
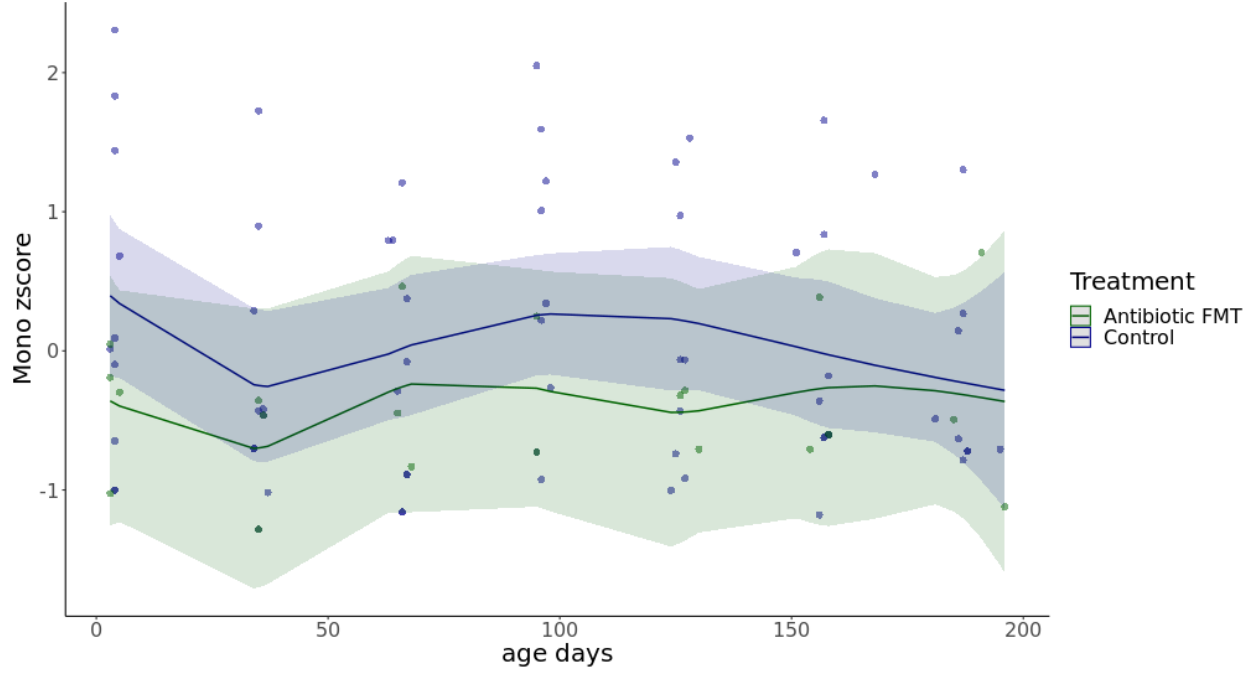
Table 10: Monocyte Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(age_days, df = 5)	1.557	0.311	5	77	0.385	0.858
Treatment	1.554	1.554	1	77	1.921	0.170
ns(age_days, df = 5):Treatment	0.806	0.161	5	77	0.199	0.962

Eosinophils/ul z scores
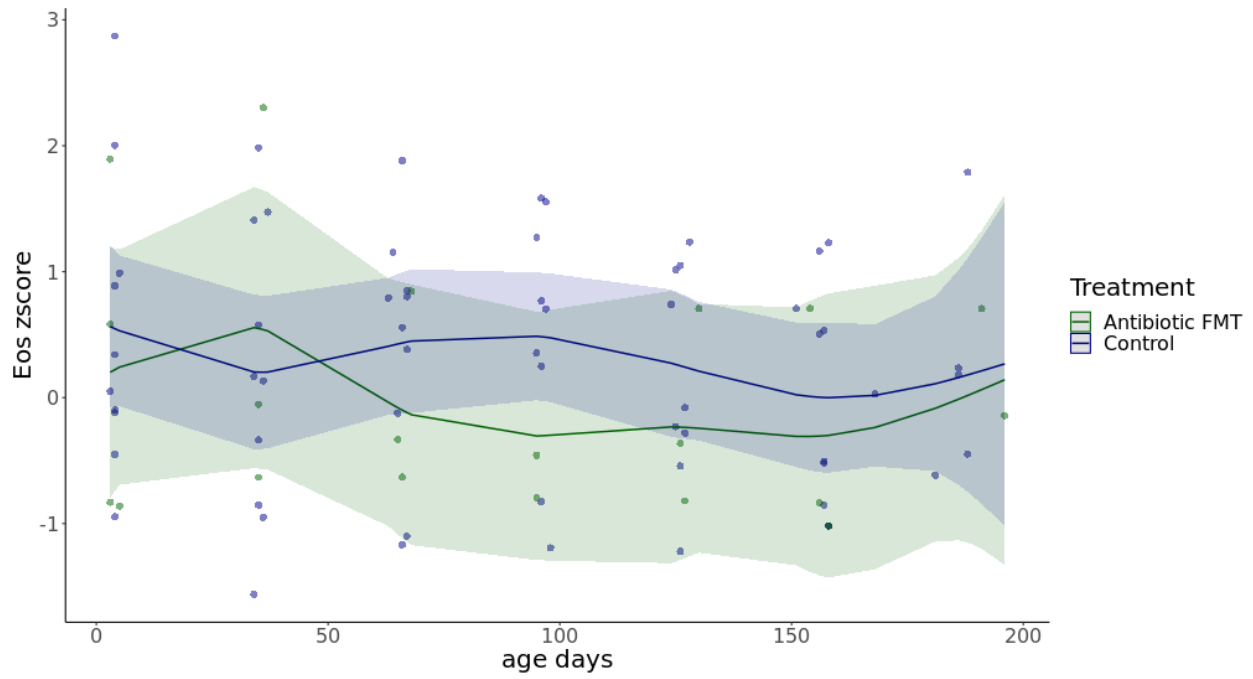
Table 11: Eosinophil Concentration Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(age_days, df = 5)	2.142	0.428	5	64.344	0.502	0.774
Treatment	0.306	0.306	1	66.124	0.359	0.551
ns(age_days, df = 5):Treatment	1.569	0.314	5	64.344	0.368	0.869

Neutrophil to Lymphocte ratio z scores
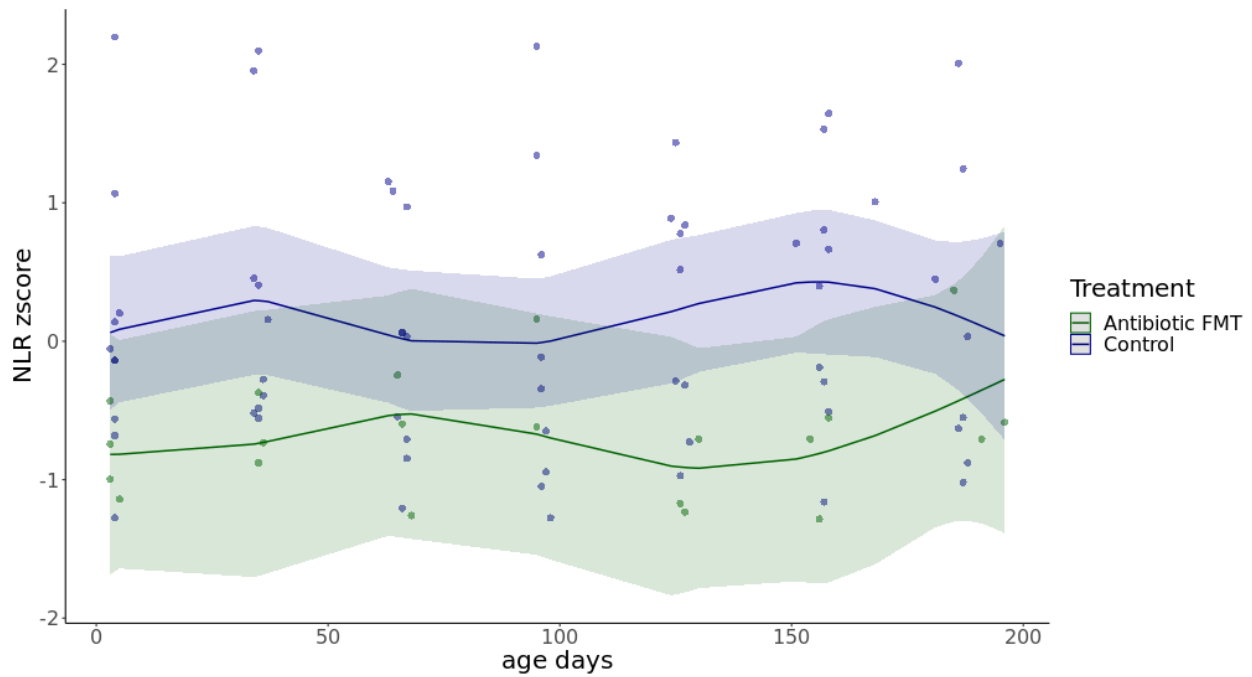
Table 12: Neutrophil to Lymphocyte Ratio Z score

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
ns(age_days, df = 5)	0.443	0.089	5	67.795	0.169	0.973
Treatment	1.461	1.461	1	52.117	2.793	0.101
ns(age_days, df = 5):Treatment	1.207	0.241	5	67.795	0.461	0.804

Session Information

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-conda-linux-gnu (64-bit)
## Running under: Ubuntu 18.04.6 LTS
##
## Matrix products: default
## BLAS/LAPACK: /srv/conda/envs/notebook/lib/libopenblasp-r0.3.21.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=en_US.UTF-8
##  [9] LC_ADDRESS=en_US.UTF-8     LC_TELEPHONE=en_US.UTF-8
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=en_US.UTF-8
##
```

```
## attached base packages:
## [1] splines    stats      graphics   grDevices utils      datasets   methods
## [8] base
##
## other attached packages:
##  [1] redres_0.0.0.9    kableExtra_1.3.4 xlsx_0.6.5       readxl_1.3.1
##  [5] lmerTest_3.1-3    lme4_1.1-27      Matrix_1.3-3     sjPlot_2.8.8
##  [9] forcats_0.5.1     stringr_1.4.0    dplyr_1.0.6      purrr_0.3.4
## [13] readr_1.4.0       tidyr_1.1.3      tibble_3.1.2     ggplot2_3.3.3
## [17] tidyverse_1.3.1
##
## loaded via a namespace (and not attached):
##  [1] nlme_3.1-152       fs_1.5.0          lubridate_1.7.10
##  [4] insight_0.14.0     webshot_0.5.2     httr_1.4.2
##  [7] numDeriv_2016.8-1.1 tools_3.6.3      backports_1.2.1
## [10] DT_0.18            utf8_1.2.1        R6_2.5.0
## [13] sjlabelled_1.1.8   DBI_1.1.1         colorspace_2.0-1
## [16] withr_2.5.0        tidyselect_1.1.1  emmeans_1.6.0
## [19] compiler_3.6.3     performance_0.7.2 cli_3.6.0
## [22] rvest_1.0.0        xml2_1.3.2        labeling_0.4.2
## [25] bayestestR_0.9.0   checkmate_2.1.0   scales_1.1.1
## [28] DEoptimR_1.0-11    robustbase_0.95-0 mvtnorm_1.1-1
## [31] systemfonts_1.0.2  digest_0.6.27     minqa_1.2.4
## [34] rmarkdown_2.20     svglite_2.0.0     qqplotr_0.0.5
## [37] pkgconfig_2.0.3    htmltools_0.5.4   dbplyr_2.1.1
## [40] fastmap_1.1.0      htmlwidgets_1.5.3 rlang_1.0.6
## [43] rstudioapi_0.13    shiny_1.7.4       farver_2.1.0
## [46] generics_0.1.0     jsonlite_1.7.2    magrittr_2.0.1
## [49] parameters_0.13.0  Rcpp_1.0.9        munsell_0.5.0
## [52] fansi_0.4.2        lifecycle_1.0.0   stringi_1.6.2
## [55] yaml_2.2.1         snakecase_0.11.0  brio_1.1.2
## [58] MASS_7.3-54        grid_3.6.3        promises_1.2.0.1
## [61] sjmisc_2.8.7       crayon_1.4.1      lattice_0.20-44
## [64] cowplot_1.1.1      ggeffects_1.1.0   haven_2.4.1
## [67] xlsxjars_0.6.1     sjstats_0.18.1    hms_1.1.0
## [70] knitr_1.33         pillar_1.6.1      boot_1.3-28
## [73] estimability_1.3   effectsize_0.4.4-1 reprex_2.0.0
## [76] glue_1.4.2         evaluate_0.14     modelr_0.1.8
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## [82] testthat_3.1.6     cellranger_1.1.0  gtable_0.3.0
## [85] assertthat_0.2.1   xfun_0.36         mime_0.10
## [88] xtable_1.8-4       broom_0.7.6       later_1.2.0
## [91] viridisLite_0.4.0  rJava_1.0-4       tinytex_0.31
## [94] ellipsis_0.3.2
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