Assessment of mean mtDNA effect on ACR/AMR

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1 Multivariable analysis - logistic regression models

Multivariable analysis was performed separately for ACR, AMR and complications. Each section covers two or three types of modelling: a) logistic regression using all time points (with and/or without random effect for patient) and using information about rejection and complication from previous measurement, b) logistic regression using first timepoint (0-9 or 10-19 post HTx days, depending on the exact measurement time of patients). For a) glmer function from lme4 package version 1.1-35.5 was used when random effect was included, when not lrm function from rms package version 6.8-2 was used. For b) glm function from stats version 4.4.1 was used.

1.1 ACR

First, ACR was modeled using random effect for patient which leaded to singular model (estimated variance-covariance matrices with less than full rank). Multicolinearity was not an issue (performance check), therefore the second model

does not include random effect for patient. The fitted model does not explain a lot of variablity in the data (R2 = 0.139), its prediction ability is moderate C 0.717, and LR test is insignificant. Although, the model has its limitations it is obvious that mean_mtDNA is not a good predictor of ACR - odds ratio 1. Third model uses only 0-9 follow-up time to predict if ACR will happen sometime in future.

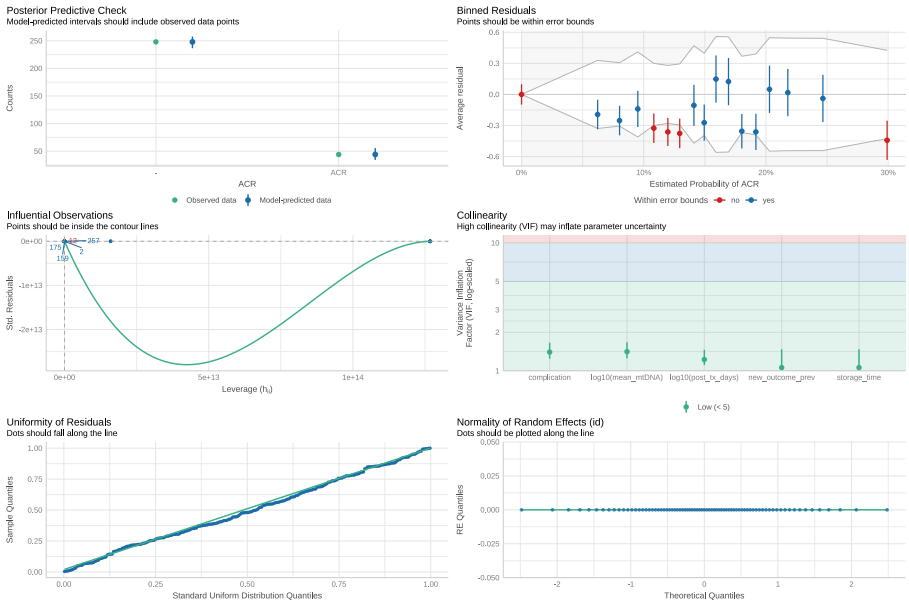
1.1.1 Logistic regression with random effect for patient

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: ACR ~ log10(mean_mtDNA) + storage_time + log10(post_tx_days) +
    new_outcome_prev + complication + (1 | id)
   Data: new_old_combined

      AIC       BIC    logLik -2*log(L)  df.resid
    252.0     285.1    -117.0     234.0       283

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.7347 -0.4671 -0.3844 -0.2717  4.5868

Random effects:
 Groups Name        Variance Std.Dev.
 id     (Intercept) 0        0
Number of obs: 292, groups:  id, 77

Fixed effects:
                            Estimate Std. Error z value Pr(>|z|)
(Intercept)               -4.114e+00  1.869e+00  -2.202   0.0277 *
log10(mean_mtDNA)          7.575e-01  4.904e-01   1.545   0.1224
storage_time              -4.780e-04  2.765e-04  -1.729   0.0838 .
log10(post_tx_days)        3.840e-01  3.366e-01   1.141   0.2539
new_outcome_prev- AMR     -3.399e+01  1.126e+07   0.000   1.0000
new_outcome_prevACR -     -2.601e-01  4.986e-01  -0.522   0.6019
new_outcome_prevACR AMR   -3.032e+01  3.998e+06   0.000   1.0000
complicationcomplication -4.125e-01  6.881e-01  -0.599   0.5489
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) l10(_D strg_t l10(__ n__-AM n__AC- n__ACA
lg10(m_DNA) -0.919
storage_tim -0.316  0.108
lg10(pst__) -0.522  0.206  0.156
```

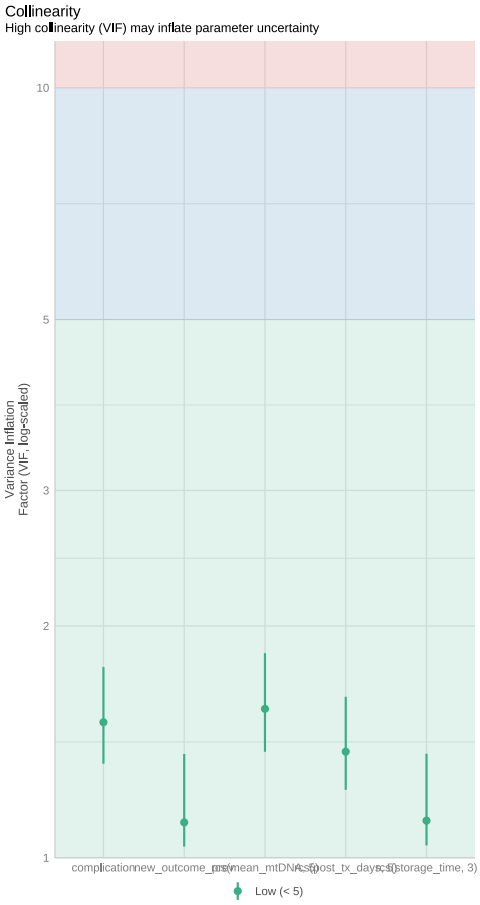
```
nw_tcm_-AMR  0.000  0.000  0.000  0.000
nw_tcm_ACR-  0.064 -0.059  0.081 -0.202  0.000
nw_t_ACRAMR  0.000  0.000  0.000  0.000  0.000  0.000
cmplctncmpl  0.266 -0.441  0.092  0.186  0.000  0.021  0.000
fit warnings:
Some predictor variables are on very different scales: consider rescaling
optimizer (Nelder_Mead) convergence code: 0 (OK)
boundary (singular) fit: see help('isSingular')
```

```
[1] "isSingular"
```

```
[1] TRUE
```



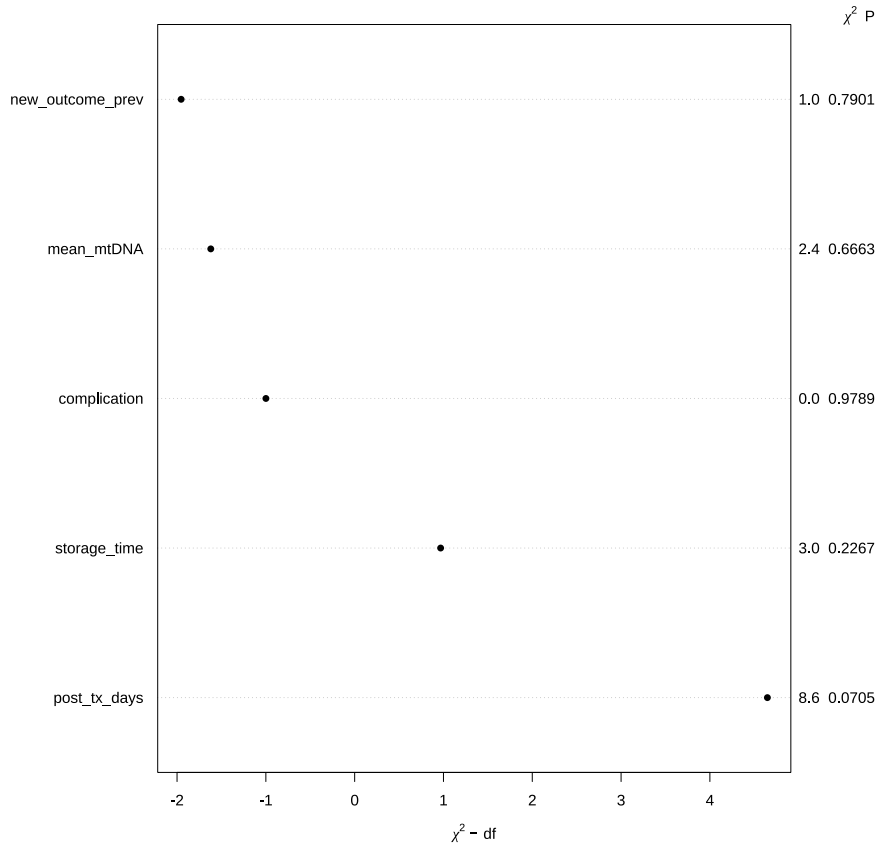
1.1.2 Logistic regression without random effect for patient



	Model Likelihood Ratio Test	Discrimination Indexes	Rank Discrim. Indexes
Obs 292	LR ^{2} 24.12	R^2 0.139	C 0.717
- 248	d.f. 14	$R^2_{14,292}$ 0.034	D_{xy} 0.434
ACR 44	Pr($>^2$) 0.0443	$R^2_{14,112.1}$ 0.086	0.434
max | log L/ | 10		Brier 0.118	$_a$ 0.111

		S.E.	Wald Z	Pr(>|Z|)
Intercept	-4.1367	1.7172	-2.41	0.0160
mean_mtDNA	0.0022	0.0020	1.10	0.2716
mean_mtDNA'	-0.0895	0.1125	-0.80	0.4266

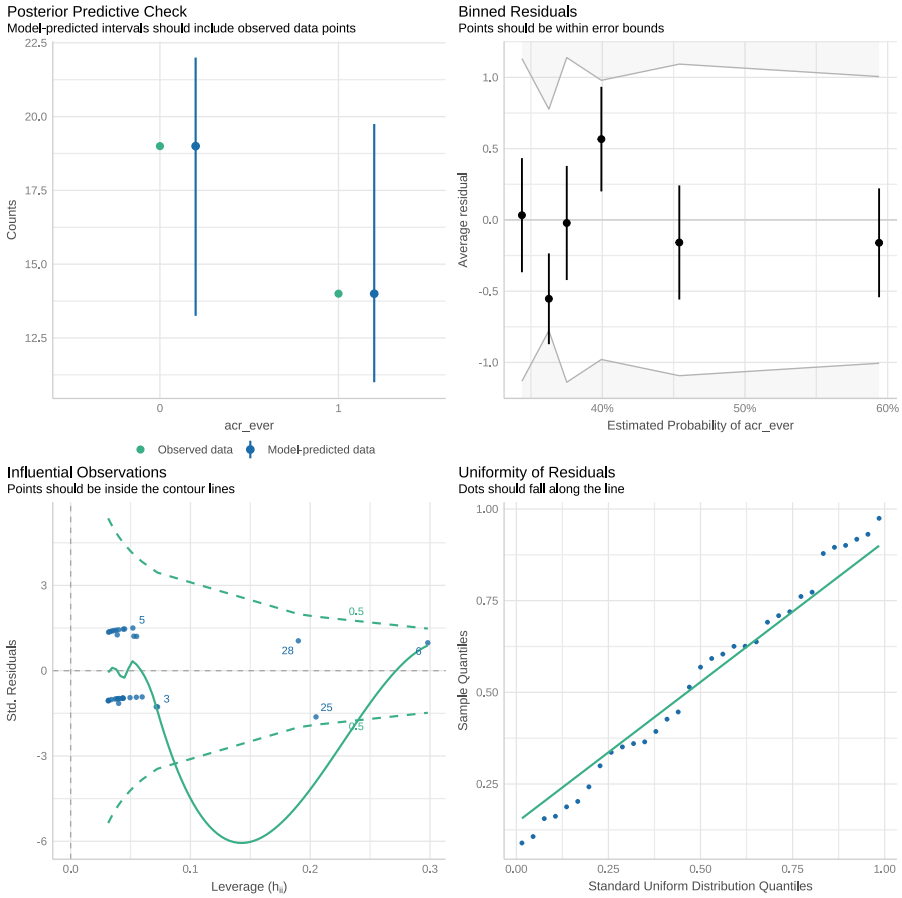
		S.E.	Wald Z	$\Pr(>\|Z\|)$
mean_mtDNA''	0.2199	0.3055	0.72	0.4716
mean_mtDNA'''	-0.1363	0.2140	-0.64	0.5243
storage_time	0.0002	0.0007	0.34	0.7344
storage_time'	-0.0012	0.0012	-1.03	0.3008
post_tx_days	0.0211	0.0345	0.61	0.5409
post_tx_days'	-0.0150	2.0446	-0.01	0.9941
post_tx_days''	-0.1842	3.4522	-0.05	0.9575
post_tx_days'''	0.2459	1.4790	0.17	0.8680
complication=complication	0.0189	0.7155	0.03	0.9789
new_outcome_prev=- AMR	-7.1618	22.1976	-0.32	0.7470
new_outcome_prev=ACR -	-0.4965	0.5173	-0.96	0.3372
new_outcome_prev=ACR AMR	-7.5913	48.5665	-0.16	0.8758



Effects Response: ACR	Low	High	Diff.	Effect	S.E.	Lower 0.95	Upper 0.95
mean_mtDNA	888.9531	2591.812	1702.859	0.1890940	0.4780410	-0.7478492	1.126037e+00
Odds Ratio	888.9531	2591.812	1702.859	1.2081545	NA	0.4733836	3.083413e+00
storage_time	739.5000	1794.000	1054.500	-0.4177622	0.3267705	-1.0582207	2.226963e-01
Odds Ratio	739.5000	1794.000	1054.500	0.6585188	NA	0.3470728	1.249441e+00
post_tx_days	24.0000	184.000	160.000	0.7453813	0.4596409	-0.1554983	1.646261e+00
Odds Ratio	24.0000	184.000	160.000	2.1072447	NA	0.8559886	5.187546e+00
complication complication	1.0000	2.000	NA	0.0189056	0.7155375	-1.3835222	1.421333e+00
Odds Ratio	1.0000	2.000	NA	1.0190854	NA	0.2506940	4.142640e+00
new_outcome_prev AMR	1.0000	2.000	NA	-7.1617734	22.1976488	-50.6683655	3.634482e+01
Odds Ratio	1.0000	2.000	NA	0.0007757	NA	0.0000000	6.086312e+15
new_outcome_prev ACR	1.0000	3.000	NA	-0.4964885	0.5173241	-1.5104251	5.174481e-01
Odds Ratio	1.0000	3.000	NA	0.6086643	NA	0.2208161	1.677741e+00
new_outcome_prev ACR AMR	1.0000	4.000	NA	-7.5913039	48.5665276	-102.7799490	8.759734e+01
Odds Ratio	1.0000	4.000	NA	0.0005048	NA	0.0000000	1.104185e+38

1.1.3 Logistic regression using first timepoint (0-9 post HTx days)

Characteristic	**0** N = 19	**1** N = 14
mean mtDNA	NA	NA
N Non-missing	19	14
Mean (SD)	1,877 (1,502)	2,545 (2,067)
Median (Q1, Q3)	1,256 (1,002, 2,116)	1,696 (1,181, 3,516)
N Missing (% Missing%)	0 (0%)	0 (0%)
complication	NA	NA
-	18 (95%)	12 (86%)
complication	1 (5.3%)	2 (14%)



Characteristic	**OR**	**95% CI**	**p-value**
mean_mtDNA	1.00	1.00, 1.00	0.3

1.2 AMR

Here, omiting of random effect of patients for model using all time point leaded to multicolinearity. Therefore, random effect were included. Resulting model did converged, but with maximum gradient exceeding the tolerance, therefore its interpretation should be cautious. The model did not indicate that mean_mtDNA would have significant effect on AMR rejection (p-value = 0.5). Modeling AMR using only 10-19 follow-up time also did not lead to significant effect of meant mtDNA on AMR.

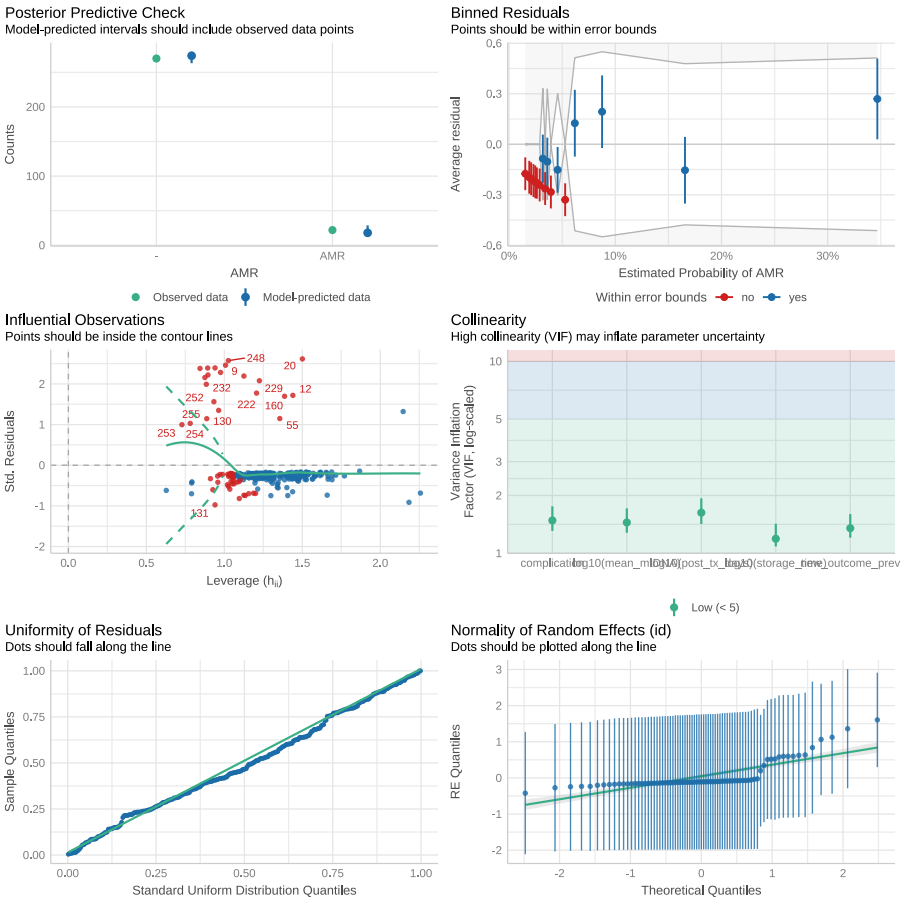
1.2.1 Logistic regression with random effect for patient

```
Generalized linear mixed model fit by maximum likelihood (Laplace
```

Characteristic	**OR**	**95% CI**	**p-value**
log10(mean_mtDNA)	2.11	0.52, 8.59	0.3
log10(post_tx_days)	0.70	0.23, 2.14	0.5
complication	NA	NA	NA
-	NA	NA	NA
complication	1.90	0.33, 10.8	0.5
log10(storage_time)	0.91	0.12, 7.09	>0.9
new_outcome_prev	NA	NA	NA
- -	NA	NA	NA
- AMR	6.62	0.62, 70.2	0.12
ACR -	1.99	0.44, 9.01	0.4
ACR AMR	3.90	0.16, 92.9	0.4

```
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula:
AMR ~ log10(mean_mtDNA) + log10(post_tx_days) + (1 | id) + complication +
    log10(storage_time) + new_outcome_prev
   Data: new_old_combined

      AIC       BIC    logLik -2*log(L)  df.resid
      156       189       -69       138       283

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.7803 -0.2206 -0.1791 -0.1497  5.4343

Random effects:
 Groups Name        Variance Std.Dev.
 id     (Intercept) 1.005    1.003
Number of obs: 292, groups:  id, 77

Fixed effects:
                        Estimate Std. Error z value Pr(>|z|)
(Intercept)             -4.88182    4.81566  -1.014    0.311
log10(mean_mtDNA)        0.74619    0.71631   1.042    0.298
log10(post_tx_days)     -0.35686    0.57142  -0.625    0.532
complicationcomplication  0.64001    0.88913   0.720    0.472
log10(storage_time)     -0.09304    1.04698  -0.089    0.929
new_outcome_prev- AMR    1.89033    1.20482   1.569    0.117
new_outcome_prevACR -    0.68625    0.77163   0.889    0.374
new_outcome_prevACR AMR  1.36102    1.61775   0.841    0.400

Correlation of Fixed Effects:
```

```
            (Intr) l10(_D l10(__ cmplct l10(_) n__-AM n__AC-
lg10(m_DNA) -0.645
lg10(pst__) -0.505  0.156
cmplctncmpl  0.039 -0.433  0.269
lg10(strg_) -0.833  0.193  0.319  0.107
nw_tcm_-AMR  0.234 -0.122 -0.391 -0.048 -0.036
nw_tcm_ACR-  0.003 -0.050 -0.262  0.025  0.036  0.075
nw_t_ACRAMR  0.176 -0.143 -0.189 -0.050 -0.043  0.364  0.042
optimizer (Nelder_Mead) convergence code: 0 (OK)
Model failed to converge with max|grad| = 0.0473664 (tol = 0.002, component 1)
```



1.2.2 Logistic regression using first timepoint (10-19 post HTx days)

```
Call:
glm(formula = amr_ever ~ mean_mtDNA, family = binomial, data = AMR_data)
```

Characteristic	**0** N = 25	**1** N = 5
mean mtDNA	NA	NA
N Non-missing	25	5
Mean (SD)	6,720 (11,778)	8,559 (8,823)
Median (Q1, Q3)	2,026 (1,274, 5,477)	5,901 (1,723, 11,749)
N Missing (% Missing%)	0 (0%)	0 (0%)
complication	NA	NA
-	19 (76%)	3 (60%)
complication	6 (24%)	2 (40%)

```
Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.712e+00  5.895e-01  -2.904  0.00368 **
mean_mtDNA   1.352e-05  4.005e-05   0.338  0.73562
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 27.034  on 29  degrees of freedom
Residual deviance: 26.926  on 28  degrees of freedom
AIC: 30.926

Number of Fisher Scoring iterations: 4
```

Characteristic	**OR**	**95% CI**	**p-value**
mean_mtDNA	1.00	1.00, 1.00	0.7

1.3 Complication

When random effects for patients were included in the model, the mean mtDNA was a significant predictor of the complication occurence. The model without random effects for patient did not lead to multicolinearity, however its AIC was higher than in the previous model.The model explained half of the variability in the data R2 0.608 and had very good prediction C = 0.950. Logistic regression using first timepoint was not possible to perform due to lack of data (most of the complications happened at the beginning of the follow-up).

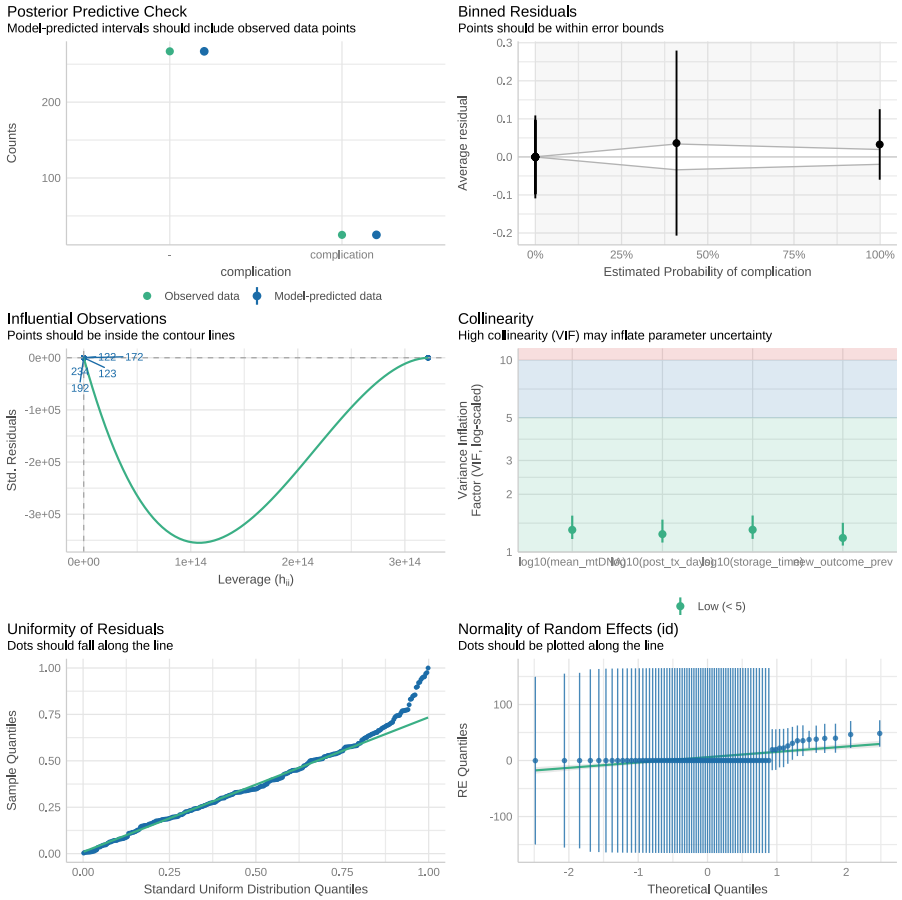
1.3.1 Logistic regression with random effect for patient

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: complication ~ log10(mean_mtDNA) + log10(post_tx_days) + (1 |
```

Characteristic	**OR**	**95% CI**	**p-value**
log10(mean_mtDNA)	8.85	8.81, 8.90	<0.001
log10(post_tx_days)	0.00	0.00, 0.00	<0.001
log10(storage_time)	0.01	0.01, 0.01	<0.001
new_outcome_prev	NA	NA	NA
- -	NA	NA	NA
- AMR	0.00	0.00, 0.00	<0.001
ACR -	3.91	3.89, 3.93	<0.001
ACR AMR	0.16	0.00, 4,452,939,219,215	>0.9

```
   id) + log10(storage_time) + new_outcome_prev
  Data: new_old_combined

       AIC       BIC    logLik -2*log(L)  df.resid
      69.0      98.4     -26.5      53.0       284

Scaled residuals:
      Min        1Q    Median        3Q       Max
-0.072803 -0.000001  0.000000  0.000000  0.103228

Random effects:
 Groups Name        Variance Std.Dev.
 id     (Intercept) 7098     84.25
Number of obs: 292, groups:  id, 77

Fixed effects:
                          Estimate Std. Error    z value Pr(>|z|)
(Intercept)              2.374e+01  2.028e-03  11705.694  < 2e-16 ***
log10(mean_mtDNA)        2.181e+00  2.621e-03    832.151  < 2e-16 ***
log10(post_tx_days)     -3.259e+01  3.231e-03 -10085.797  < 2e-16 ***
log10(storage_time)     -5.101e+00  2.631e-03  -1938.789  < 2e-16 ***
new_outcome_prev- AMR   -6.140e+03  1.024e+03     -5.996 2.02e-09 ***
new_outcome_prevACR -    1.363e+00  2.316e-03    588.495  < 2e-16 ***
new_outcome_prevACR AMR -1.859e+00  1.581e+01     -0.118    0.906
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) l10(_D l10(__ l10(_) n__-AM n__AC-
lg10(m_DNA) 0.189
lg10(pst__) 0.167  0.351
lg10(strg_) 0.189  0.397  0.353
nw_tcm_-AMR 0.000  0.000  0.000  0.000
nw_tcm_ACR- 0.176  0.311  0.275  0.311  0.000
```

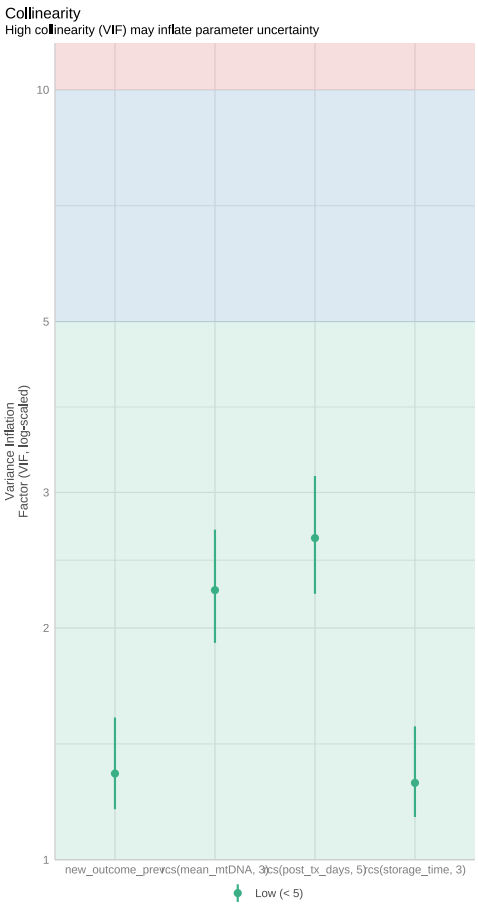
```
nw_t_ACRAMR 0.000  0.000  0.000  0.000  0.000  0.000
optimizer (Nelder_Mead) convergence code: 0 (OK)
Model failed to converge with max|grad| = 0.0585356 (tol = 0.002, component 1)
Model is nearly unidentifiable: large eigenvalue ratio
 - Rescale variables?
```




```
[1] "AIC"
```

```
[1] 68.99982
```

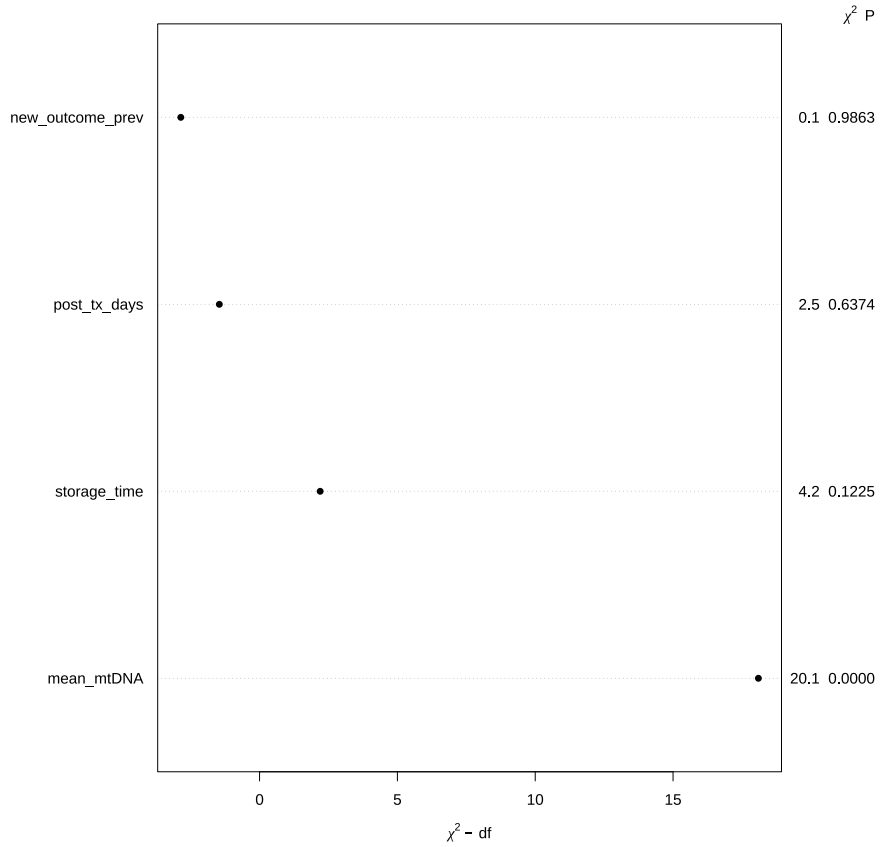
1.3.2 Logistic regression without random effect for patient



	Model Likelihood Ratio Test		Discrimination Indexes		Rank Discrim. Indexes	
Obs	292	LR ^{2}	91.50	R^2	0.608	C	0.950
-	267	d.f.	11	$R^2_{11,292}$	0.241	D_{xy}	0.899
complication	25	Pr($>^2$)	<0.0001	$R^2_{11,68.6}$	0.691		0.900
max | log				Brier	0.039	$_a$	0.141
L/ |	0.5						

		S.E.	Wald Z	Pr($>\|Z\|$)
Intercept	-3.3782	1.7921	-1.89	0.0594
mean_mtDNA	0.0011	0.0007	1.63	0.1025
mean_mtDNA'	-0.0015	0.0016	-0.94	0.3462

		S.E.	Wald Z	Pr(>|Z|)
storage_time	-0.0011	0.0014	-0.80	0.4232
storage_time'	-0.0002	0.0025	-0.08	0.9397
post_tx_days	0.0065	0.0495	0.13	0.8948
post_tx_days'	3.3409	5.6823	0.59	0.5566
post_tx_days''	-12.3562	13.5244	-0.91	0.3609
post_tx_days'''	12.1952	10.3609	1.18	0.2392
new_outcome_prev=- AMR	-7.1111	83.7744	-0.08	0.9324
new_outcome_prev=ACR -	0.0508	1.3041	0.04	0.9690
new_outcome_prev=ACR AMR	0.9141	2.4859	0.37	0.7131




```
[1] "AIC"
```

```
[1] 103.1871
```

Effects Response: ACR	Low	High	Diff.	Effect	S.E.	Lower 0.95	Upper 0.95
mean_mtDNA	888.9531	2591.812	1702.859	1.5157596	0.757099	0.0318728	2.999646e+00
Odds Ratio	888.9531	2591.812	1702.859	4.5528781	NA	1.0323862	2.007844e+01
storage_time	739.5000	1794.000	1054.500	-1.2573614	0.620675	-2.4738621	-4.086060e-02
Odds Ratio	739.5000	1794.000	1054.500	0.2844035	NA	0.0842588	9.599629e-01
post_tx_days	24.0000	184.000	160.000	-47.4141970	32.417284	-110.9509058	1.612251e+01
Odds Ratio	24.0000	184.000	160.000	0.0000000	NA	0.0000000	1.004426e+07
new_outcome_prev AMR	1.0000	2.000	NA	-7.1111335	83.774381	-171.3059036	1.570836e+02
Odds Ratio	1.0000	2.000	NA	0.0008160	NA	0.0000000	1.661715e+68
new_outcome_prev ACR	1.0000	3.000	NA	0.0507574	1.304067	-2.5051659	2.606681e+00
Odds Ratio	1.0000	3.000	NA	1.0520676	NA	0.0816620	1.355399e+01
new_outcome_prev ACR AMR	1.0000	4.000	NA	0.9141486	2.485931	-3.9581861	5.786483e+00
Odds Ratio	1.0000	4.000	NA	2.4946504	NA	0.0190977	3.258650e+02