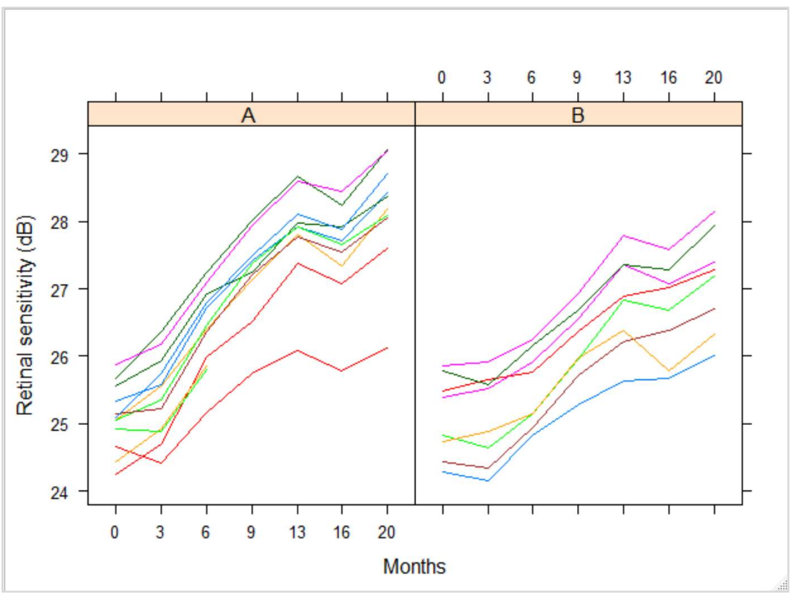
Exercise 3 Long-term follow-up of chronic central serous chorioretinopathy after successful treatment with photodynamic therapy or micropulse laser

Answer parts a to parts d, but choose one from parts e1, e2, one from f1, f2, and one from i1, i2. So, e.g. e1, f2, i2 is fine, but e.g. e1, f1, f2, i1 is not because then f2 will not be graded.

A prospective long-term follow-up study has been set up to study the long-term effects of half-dose photodynamic therapy (PDT) and high-density subthreshold micropluse laser (HSML) in patients with chronic central serous chorioretinopathy (cCSC). The investigators followed up 20 patients: 12 (group A) received PDT, and 8 (group B) received HSML. The level of the retinal sensitivity on microperimetry (continuous variable) and presence of subretinal fluid (binary variable) was measured 8 months after initiation of treatment (day 0) and at 3, 6, 9, 13, 16 and 20 months after inclusion in the study.

Due to side-effects on month 6, 2 patients underwent the therapy again and thus no data are available for them after month 6.

The data collected for Retinal Sensitivity are shown in the following plots:



The proportion of patients with presence of subretinal fluid over time and per group is:

Month	A	B
0	0.67	0.5
3	0.67	0.5
6	1	0.75
9	1	1
13	1	1
16	1	1
20	1	1

The investigators started the analyses with the continuous outcome, namely the retinal sensitivity. They fitted two models: Model A and Model B. For the mean part they assumed the same saturated model for both models. For the random part, they considered two different options. The results from both models are given in the output in Appendix I below at the end of this exercise. The variable names which appear in the output are:

- ID: patient number,
- Treat: group A = PDT, B = HSML,
- Y: retinal sensitivity (numeric),
- Fluid: presence of fluid: 0 (no); 1 (yes),
- Month: month indicator 0, 3, 6, 9, 13, 16 and 20 (i.e. factor).
- MonthC: month since inclusion in the study 0, 3, 6, 9, 13, 16 and 20 (i.e. numeric).

Study the output of Model A and Model B and answer the following questions:

a1 What is the difference between Model A and Model B in terms of the correlation structure assumed?

Solution:

Model A assumes an unstructured covariance matrix, i.e. all pairwise correlation are different. Model B assumes compound symmetry, i.e. constant correlation in time.

a2 Which test(s) can be used to test which of these 2 models fits best on the data? Based on the output of these two models, compute the test statistic(s) and specify the asymptotic distribution under the null hypothesis including if relevant degrees of freedom.

Solution:

We can you use the Likelihood ratio test.

```
anova(model.1, model.2)
        Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model.1     1 42 62.04156 178.4103  10.97922
model.2     2 22 86.56600 147.5211 -21.28300 1 vs 2 64.52444  <.0001
```

LRT = 2log(L_A)-2log(L_0) \sim \chisq_20.

a3 Which test(s) can be used for the hypothesis that the mean protein profiles are the same between group A and group B? Give the name(s) of the test(s) and the asymptotic distribution (with number of degrees of freedom if relevant) under the null hypothesis.

Solution:

We can use the LRT, which follows chisq with 7dfs.

We may also use the Wald test which follows again chisq with 7dfs.

We may also use the F test which follows F with numerator dfs 8 and denominator dfs which can be estimated from the data using Kenward-Roger, Satterthwaite etc.

A linear mixed effects model has been fitted on the same data assuming for the fixed effects part, linear evolutions in time that differ in the two groups and a random intercepts term to model the within patient correlations.

Study the output in Appendix II of Model C and reply to the following questions:

b1 What is the estimated variance of Y at Month 9?

Solution:

Var(y_{i9})=Var(b_{i0+\epsilon_{i9}) = Var(b_{i0}) + Var(\epsilon_{i0}) = 0.6231968^2+ 0.3577317^2=0.5163462.

b2 What is the estimated correlation between Y on Month 0 with Y on Month 9?

Solution:

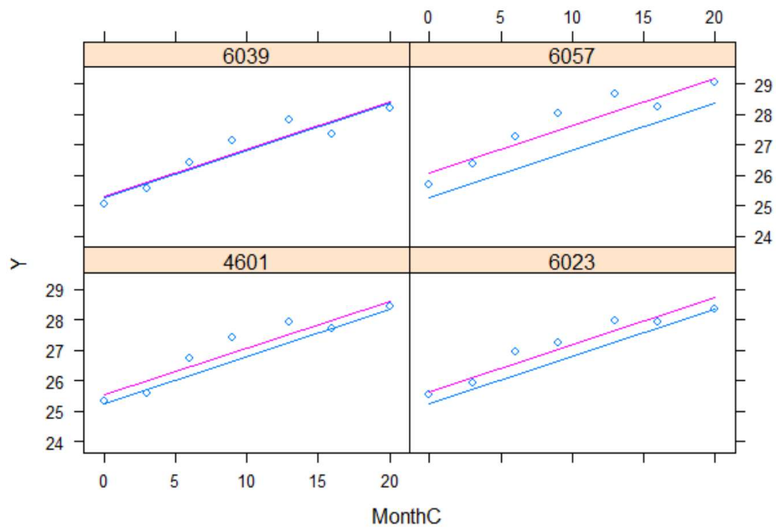
Cov (y_{i0}, y_{i9}) =Cov(b_{i0}+epsilon_{i0}, b_{i0}+\epsilon_{i9}) = Var(b_{i0})=0.6231968^2. For the correlation divide with the product of the corresponding stds i.e. corr= 0.6231968^2/(0.5163462)= 0.7521586.

b3 Let y_{ij} retinal sensitivity of patient i (i = 1,…, 20) at month j (j = 0, …, 20) and b_i the random intercepts term. What is the estimate of $var(y_{i9}|b_i)$?

Solution:

$$var(y_{i9}|b_i) = \sigma^2 = 0.3577317^2$$

c3 From the fitted model (Model C) two type of predictions can be derived: the marginal and the individualized predictions. See for the relevant output Appendix II. These predictions are shown in the figure below, where the blue lines correspond to the marginal predictions and the pink lines correspond to the individualized predictions. Based on the output of this model compute for patient "6057" his predicted values both marginal and individualized at month 9. Note that patient "6057" was assigned to treatment group B.



Solution:

i. *Marginal is: 26.7. This is computed using only the fixed effects part at month 9 and treatme nt 0.*
ii. *Individualized is: 27.5. This is computed using only the fixed effects part at month 9 and tre atment 0 and the estimated random effect of this patient.*

Make either d1 or d2, but not both.

d1 To fill in the missing values for the 2 patients, the unconditional mean imputation has been used. Discuss the consequences that this has on the inference using Model C above.

Solution:

The unconditional mean imputation means that the data from 6 – 20 month are filled in with the mean of the data available from the other patients at each visit. This approach is only valid under MCAR. In our case, the mechanism which applies is MAR and thus the unconditional mean imputation induces bi as in the estimation of the effects of interest. Besides, the sampling variability of the value we impute is not taken into account, even though this is not the most important concern.

d2 To fill in the missing values for the 2 patients, the conditional mean imputation has been used. Discuss the consequences that this has on the inference using Model C above.

Solution:

The conditional mean imputation means that the data from 6 – 20 month are filled in once based on th e conditional distribution of the last visit given the rest. The parameters in this conditional distribution are estimated from the completers. This mean that this practice is valid under MAR, so the inference o f Model A will not be biased but the precision will be overestimated. The sampling variability of the va lue we impute is not taken into account.

.... **from the next four questions, answer one from e1, e2, one from f1, f2, not more** ....

The researchers proceeded further with the analysis of the binary outcome i.e. the presence of Retinal sensitivity. They have used the GEE approach, where:

- for the mean part they assumed linear evolutions in time that differ in the two groups and
- for the correlation they used an AR1 correlation matrix.

Study the output in Appendix III of the GEE-1 and answer the following questions:

Make either e1 or e2, but not both.

e1 What is the estimated odds ratio for the presence of retinal fluid between group B and A at Months 16? Give an interpretation.

Solution:

Let the logistic regression be written as:
*Log-odds = beta_0+beta_1*Months +beta_2*Treat + beta_3*Months *Treat*
*Then the requested log-odds ratio is: beta_2+16*beta_3*
*and thus the odds ratio is exp(beta_2+16*beta_3). Namely, exp(-0.8313-16*0.0391)= 0.233. It means that the odds of retinal fluid for patients in group B at Month 16 are by 77% lower than that of patient s in group A.*

e2 What is the estimated log-odds ratio for the presence of retinal fluid between group B and A at month 1? Report a 95% confidence interval. Is it statistically significant at significance level 5%?

Solution:

Log-odds ratio = beta_2 + beta_3 = -0.8313-0.0391=-0.8704.
*To construct the 95% CI we need first the s.e (beta_2 + beta_3) = sqrt(var(beta_2 + beta_3)). Then v ar(beta_2 + beta_3) = var(beta_2)+var(beta_3)+2*cov(beta_2, beta_3) = 1.029+ 0.005+ 2*(-0.058) = 0.918.*
s.e (beta_2 + beta_3) =0.958
*and the 95%CI is: (-0.87-1.96*0.958; -0.87+1.96*0.958)=(-2.748; 1.001). The requested is not statist ically significant at 5%.*

f1 The researchers want to test the hypothesis that the log odds profiles are the same for the two treatments using the multivariate Wald test.

(i) Give the form of the contrast matrix needed to test this hypothesis.
(ii) What is the asymptotic distribution of the Wald statistic under the null hypothesis in this case?

Solution:

(i) *The contrast matrix is: L=[0 0 1 0; 0 0 0 1].*
(ii) *W ~ chi-squared distribution with 2 degrees of freedom.*

f2 The data are analysed again using the GEE approach with the same mean structure as in GEE-1 (i.e. linear evolutions in time that differ in the two groups) but the exchangeable correlation matrix has been to capture the within patient correlation. The output is given in Appendix III in the part GEE-2. Which of the two GEEs you would prefer in the specific dataset analysed here? Motivate your answer.

Solution:

In the GEE approach the correlation may be treated as nuisance provided that the mean model has been correctly specified and the sandwich approach is used to estimate the std errors. Both of them are valid, however the AR1 is more realistic in the longitudinal studies setting because we expect the correlation to decrease in time and not stay constant as the exchangeable correlation matrix assumes.

g Is the inference derived using the GEE approach valid given that two patients have missing values? Explain why.

Solution:

The inference is not valid because the mechanism that generates the missing data is MAR.

The researchers analysed the same binary outcome i.e. the presence of Retinal sensitivity using the mixed effects logistic regression where the fixed effects part is the same as the mean part of the GEE approach.

Study the output in Appendix III of Model C and reply to the following questions:

h Give the expressions for the mixed effects logistic regression. Carefully state all the model assumptions. Introduce your own notation and explain your notation.

Solution:

Log(\frac{\pi_{ij}}{1-\pi_{ij}}) = \beta_0 + \beta_1 MonthC_{ij} + \beta_2 Treat_i + \beta_3 MonthC_{ij} Treat_i + b_i, where b_i \sim N(0, \sigma^2_b).*

\pi_{ij} denotes the probability of presence of fluid for patient i with i = 0, \ldots, 20 at month j with j \in (0, 20).

b_i is the subject specific random effect for which we assume it follows normal and \sigma^2_b is its variance.

MonthC_{ij} benotes the month at which the measurement for patient i are collected and Treat_i is the treatment indicator.

Make either h1 or h2, but not both.

i1 What is the interpretation of the coefficient of the term "`MonthC`"?

Solution:

The change in the log-odds of presence of fluid in the placebo group given a certain patient with a cert ain unobserved liability b_i.

i2 What is the interpretation of the coefficient of the term "`MonthC`" in the output with the margina l coefficients?

Solution:

The change in the log-odds of presence of fluid in the placebo group overall the patients.

Model C presented in Appendix C is now extended to model D, where only a different random effects structure is used. The fixed effects remain unchanged. Study the output in Appendix IV of Model D and reply to the following questions:

j State the assumptions for the random effects part.

Solution:

Here we assume a random intercepts b_{i0} and a random slopes b_{i1} part.

b_i = [b_{i0}, b_{i1}] \sim N_2(0, D) where D is a symmetric 2 by 2 covariance matrix with elements d_{11}; d_{12}= d_{21} and d_22 denoting the random intercepts variance, the covariance between the random intercepts and random slopes and the random slopes variance .

k To test if Model C is equivalent to Model D, state the null and alternative hypothesis you need to test using the notation you introduced in questions h and j? Which test(s) can be used in this case? Give the asymptotic null distribution including degrees of freedom if necessary.

Solution:

H_0: d_{12}= d_{22} =0 vs H_0: d_{12} not 0 or d_{22} not 0. This hypothesis can be tested using the LRT and the asymptotic null is a mixture with 1 and 2 degrees of freedom.

Even though not expected to reply this: Due to the small sample size the bootstrap approach should be preferred here.

Output for Exercise 3:

Long-term follow-up of chronic central serous chorioretinopathy after successful treatment with photodynamic therapy or micropulse laser

Appendix I

Model A

```
model.1 <- gls(Y ~ Month * Treat,
               data = data.c,
               correlation = corSymm(form = ~ 1 | ID),
               weights = varIdent(form = ~ 1 | Month),
               na.action = na.exclude, method = "REML")
summary(model.1)
```

```
Generalized least squares fit by REML
  Model: Y ~ Month * Treat
  Data: data.c
  AIC BIC logLik
   62 178     11

Correlation Structure: General
 Formula: ~1 | ID
 Parameter estimate(s):
 Correlation:
  1     2     3     4     5     6
2 0.929
3 0.885 0.962
4 0.860 0.938 0.966
5 0.824 0.912 0.955 0.977
6 0.828 0.888 0.951 0.948 0.966
7 0.810 0.891 0.955 0.959 0.976 0.982
Variance function:
 Structure: Different standard deviations per stratum
 Formula: ~1 | Month
 Parameter estimates:
   0    3    6    9   13   16   20
1.00 1.18 1.11 1.16 1.35 1.37 1.52

Coefficients:
               Value Std.Error t-value p-value
(Intercept)    25.09    0.1543   162.6  0.0000
Month3          0.31    0.0690     4.6  0.0000
Month6          1.31    0.0797    16.5  0.0000
Month9          2.01    0.0935    21.5  0.0000
Month13         2.60    0.1218    21.4  0.0000
Month16         2.33    0.1232    18.9  0.0000
Month20         2.92    0.1454    20.1  0.0000
TreatB          0.01    0.2440     0.1  0.9596
Month3:TreatB  -0.33    0.1090    -3.0  0.0031
Month6:TreatB  -0.90    0.1260    -7.1  0.0000
Month9:TreatB  -0.93    0.1457    -6.4  0.0000
Month13:TreatB -0.90    0.1896    -4.7  0.0000
Month16:TreatB -0.75    0.1918    -3.9  0.0002
Month20:TreatB -0.90    0.2271    -4.0  0.0001


Residual standard error: 0.535
Degrees of freedom: 132 total; 118 residual
```

Model B

```
model.2 <- gls(Y ~ Month*Treat,
               data = data.c,
               correlation = corCompSymm(form = ~ MonthC | ID),
               weights = varIdent(form = ~ 1 | Month),
               na.action = na.exclude, method = "REML")
summary(model.2)
```

```
Generalized least squares fit by REML
  Model: Y ~ Month * Treat
  Data: data.c
     AIC      BIC  logLik
  86.566 147.5211 -21.283

Correlation Structure: Compound symmetry
 Formula: ~MonthC | ID
 Parameter estimate(s):
      Rho
0.9206797
Variance function:
 Structure: Different standard deviations per stratum
 Formula: ~1 | Month
 Parameter estimates:
       0        3        6        9       13       16       20
1.000000 1.125754 1.039353 1.074911 1.249763 1.274157 1.417293

Coefficients:
                   Value  Std.Error   t-value p-value
(Intercept)    25.088140 0.16208949 154.77956  0.0000
Month3          0.314394 0.07146740   4.39913  0.0000
Month6          1.314576 0.06612620  19.87981  0.0000
Month9          2.025350 0.07256211  27.91196  0.0000
Month13         2.622481 0.08780577  29.86684  0.0000
Month16         2.358649 0.09045054  26.07667  0.0000
Month20         2.948194 0.10766037  27.38421  0.0000
TreatB          0.012399 0.25628599   0.04838  0.9615
Month3:TreatB  -0.328705 0.11299988  -2.90889  0.0043
Month6:TreatB  -0.896922 0.10455470  -8.57849  0.0000
Month9:TreatB  -0.948918 0.11048397  -8.58874  0.0000
Month13:TreatB -0.915252 0.13409596  -6.82535  0.0000
Month16:TreatB -0.775272 0.13823667  -5.60829  0.0000
Month20:TreatB -0.926509 0.16526983  -5.60604  0.0000

Standardized residuals:
       Min         Q1        Med         Q3        Max
-2.4043424 -0.6397051  0.1652227  0.7726158  1.5216951

Residual standard error: 0.5614945
Degrees of freedom: 132 total; 118 residual
```

Appendix II

Model C

```
library(nlme)
model.2 <- lme(Y ~ MonthC*Treat, random = ~1|ID,
               data = data.c, method = "REML")
summary(model.2)

Linear mixed-effects model fit by REML
 Data: data.c
  AIC BIC logLik
  191 208  -89.3

Random effects:
 Formula: ~1 | ID
        (Intercept) Residual
StdDev:       0.623    0.358

Fixed effects: Y ~ MonthC * Treat
              Value Std.Error  DF t-value p-value
(Intercept)   25.26    0.1928 110   131.0   0.000
MonthC         0.15    0.0064 110    24.3   0.000
TreatB        -0.27    0.3045  18    -0.9   0.382
MonthC:TreatB -0.04    0.0096 110    -4.3   0.000
 Correlation:
              (Intr) MonthC TreatB
MonthC        -0.282
TreatB        -0.633  0.178
MonthC:TreatB  0.187 -0.663 -0.287

Standardized Within-Group Residuals:
   Min     Q1    Med     Q3    Max
-2.725 -0.642 -0.054  0.633  1.856

Number of Observations: 132
Number of Groups: 20
```

```
ranef(model.2)
     (Intercept)
4601      0.2644
6021      0.3710
6023      0.3769
6025      0.2690
6039      0.0426
6045     -0.1692
6049     -0.5129
6053     -0.9084
6055      0.8175
6057      0.8319
6061     -1.2553
6069     -0.4472
6075      0.0980
6077      0.0174
6081      0.3603
6497      0.8180
6499      0.5801
6525     -0.5023
6527     -0.5873
8533     -0.4644
```

Appendix III

GEE-1

```
library(geepack)
data.c.new <- data.c[order(data.c$ID, data.c$DayC), ]
library(geepack)
data.c.new <- data.c[order(data.c$ID, data.c$MonthC), ]
gee1 <- geeglm(Fluid ~ MonthC * Treat,
                  data = data.c.new,
                  id = ID, family = binomial("logit"),
                  corstr = "ar1")
summary(gee1)

Call:
geeglm(formula = Fluid ~ MonthC * Treat, family = binomial("logit"),
    data = data.c.new, id = ID, corstr = "ar1")

 Coefficients:
              Estimate Std.err   Wald Pr(>|W|)
(Intercept)     0.5156  0.6409   0.65     0.42
MonthC          0.3427  0.0323 112.32   <2e-16 ***
TreatB         -0.8313  1.0143   0.67     0.41
MonthC:TreatB  -0.0391  0.0690   0.32     0.57
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation structure = ar1
Estimated Scale Parameters:

            Estimate Std.err
(Intercept)    0.473    0.12
  Link = identity

Estimated Correlation Parameters:
      Estimate Std.err
alpha    0.435  0.0822
Number of clusters:   20  Maximum cluster size: 7


round(vcov(gee1), 3)
              (Intercept) MonthC TreatB MonthC:TreatB
(Intercept)         0.411 -0.021 -0.411         0.021
MonthC             -0.021  0.001  0.021        -0.001
TreatB             -0.411  0.021  1.029        -0.058
MonthC:TreatB       0.021 -0.001 -0.058         0.005
```

GEE-2

```
gee2 <- geeglm(Fluid ~ MonthC * Treat,
               data = data.c.new,
               id = ID, family = binomial("logit"),
               corstr = "exchangeable")
summary(gee2)

Call:
geeglm(formula = Fluid ~ MonthC * Treat, family = binomial("logit"),
    data = data.c.new, id = ID, corstr = "exchangeable")

 Coefficients:
              Estimate Std.err  Wald Pr(>|W|)
(Intercept)     0.3759  0.6692  0.32     0.57
MonthC          0.3695  0.0460 64.50    1e-15 ***
TreatB         -0.9608  1.0814  0.79     0.37
MonthC:TreatB  -0.0298  0.0854  0.12     0.73
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation structure = exchangeable
Estimated Scale Parameters:

            Estimate Std.err
(Intercept)    0.463  0.0874
  Link = identity

Estimated Correlation Parameters:
      Estimate Std.err
alpha     0.19   0.065
Number of clusters:   20  Maximum cluster size: 7
```

Appendix IV

MODEL C

```
library(GLMMadaptive)
model.1 <- mixed_model(Fluid ~ MonthC * Treat,
                       data = data.c.new,
                       random = ~ 1 | ID,
                       family = binomial())
summary(model.1)

Call:
mixed_model(fixed = Fluid ~ MonthC * Treat, random = ~1 | ID,
    data = data.c.new, family = binomial(),)

Data Descriptives:
Number of Observations: 132
Number of Groups: 20

Model:
 family: binomial
 link: logit

Fit statistics:
 log.Lik  AIC  BIC
     -26 61.9 66.9

Random effects covariance matrix:
            StdDev
(Intercept)   5.24

Fixed effects:
              Estimate Std.Err z-value p-value
(Intercept)      0.209   0.699  0.2996    0.76
MonthC           1.017   0.408  2.4909    0.01
TreatB          -0.110   0.740 -0.1491    0.88
MonthC:TreatB   -0.039   0.405 -0.0962    0.92

Integration:
method: adaptive Gauss-Hermite quadrature rule
quadrature points: 11

Optimization:
method: hybrid EM and quasi-Newton
converged: TRUE


marg <- marginal_coefs(model.1, std_errors = TRUE)
marg
              Estimate Std.Err z-value p-value
(Intercept)    -0.3850   0.611 -0.6300     0.5
MonthC          0.4318   0.279  1.5499     0.1
TreatB         -0.0956   0.679 -0.1409     0.9
MonthC:TreatB  -0.0113   0.309 -0.0367     1.0
```

MODEL D

```
model.2 <- mixed_model(Fluid ~ MonthC * Treat,
                       data = data.c.new,
                       random = ~ MonthC | ID,
                       family = binomial(),
                       penalized = TRUE)
summary(model.2)

Call:
mixed_model(fixed = Fluid ~ MonthC * Treat, random = ~MonthC |
    ID, data = data.c.new, family = binomial(), penalized = TRUE)

Data Descriptives:
Number of Observations: 132
Number of Groups: 20

Model:
 family: binomial
 link: logit

Fit statistics:
 log.Lik   AIC   BIC
   -22.7 59.4 66.3

Random effects covariance matrix:
             StdDev    Corr
(Intercept)  9.2971
MonthC       0.8246 -0.9941

Fixed effects:
              Estimate Std.Err z-value p-value
(Intercept)     0.1854   0.752   0.246    0.81
MonthC          0.9845   0.389   2.533    0.01
TreatB         -0.0961   0.756  -0.127    0.90
MonthC:TreatB  -0.2111   0.352  -0.600    0.55

Integration:
method: adaptive Gauss-Hermite quadrature rule
quadrature points: 11

Optimization:
method: hybrid EM and quasi-Newton
converged: TRUE
```