Analysis of risk factors associated with renal function trajectory over time: a comparison of different statistical approaches

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ABSTRACT

Background. The most commonly used methods to investigate risk factors associated with renal function trajectory over time include linear regression on individual glomerular filtration rate (GFR) slopes, linear mixed models and generalized estimating equations (GEEs). The objective of this study was to explain the principles of these three methods and to discuss their advantages and limitations in particular when renal function trajectories are not completely observable due to dropout.

Methods. We generated data from a hypothetical cohort of 200 patients with chronic kidney disease at inclusion and seven subsequent annual measurements of GFR. The data were generated such that both baseline level and slope of GFR over time were associated with baseline albuminuria status. In a second version of the dataset, we assumed that patients systematically dropped out after a GFR measurement of <15 mL/min/1.73 m². Each dataset was analysed with the three methods.

Results. The estimated effects of baseline albuminuria status on GFR slope were similar among the three methods when no patient dropped out. When 32.7% dropped out, standard GEE provided biased estimates of the mean GFR slope in normo-, micro- and macroalbuminuric patients. Linear regression on individual slopes and linear mixed models provided slope estimates of the same magnitude, likely because most patients had at least three GFR measurements. However, the linear mixed model was the only method to provide effect estimates on both slope and baseline level of GFR unaffected by dropout.

Conclusion. This study illustrates that the linear mixed model is the preferred method to investigate risk factors associated with renal function trajectories in studies, where patients may dropout during the study period because of initiation of renal replacement therapy.

Keywords: chronic kidney disease, dropout, generalized estimating equations, glomerular filtration rate, linear mixed model

INTRODUCTION

In studies investigating trajectories of renal function, patients are typically followed over time and their renal function is measured at several time points. Often, these time points differ between patients, and some patients may dropout during the study, i.e. their follow-up is terminated earlier than intended. This can result in substantial between-patient variation in the length of follow-up as well as in the number of renal function measurements. Statistical regression models that investigate risk factors associated with renal function decline should be robust to this heterogeneity of available data.

As described in our recent literature review [1], the most commonly used approaches to investigate changes of renal function over time are (i) linear regression with individual slopes of decline as the outcome variable (see ref. [2, 3]); (ii) linear mixed models (see ref. [4, 5]) and (iii) generalized estimating equations (GEEs) (see ref. [6, 7]). In the statistical literature, it is well
known that linear mixed models have generally a superior performance than linear regression on individual slopes, especially if many patients dropout early [8, 9]. It is also recognized that when the reason of dropout depends on the past observed values of the outcome, e.g. when patients dropout after having reached a low observed value of the glomerular filtration rate (GFR), standard linear mixed models provide more accurate results than standard GEEs. However, to our knowledge, the differences between these methods have mostly been explained and illustrated in statistical text books (see ref. [8]), but never in the nephrology literature.

The objectives of this study were to explain the principles of linear regression on individual slopes, linear mixed models and GEE; to contrast and highlight their advantages and limitations; and to interpret and compare their results when investigating factors associated with renal function trajectory over time. We illustrate this by making use of two hypothetical datasets in which patients’ renal function is followed over time, and where patients may or may not dropout during follow-up. Use of hypothetical, instead of real data, gives us full control of the reason for dropout, and allows us to investigate sensitivity of the results to dropout.

A HYPOTHETICAL CLINICAL COHORT

Data from a hypothetical observational cohort of 200 patients with chronic kidney disease (CKD) were simulated. We assumed that inclusion into the cohort corresponds to the time the patients were referred to the participating renal centre, and that inclusion criteria are any cause and stage of CKD. Patients already on renal replacement therapy (dialysis or transplantation) at the time of referral were not included in our hypothetical study.

For each patient, we first randomly generated values of gender (0 for males and 1 for females), indicators of normo-, micro- and macroalbuminuria at baseline, as well as eight consecutive random annual follow-up visit times (in years). For each patient, we further generated observed values of GFR (in mL/min/1.73 m^2) at baseline and at each subsequent visit. Note that these generated observed values of GFR may virtually be either estimated GFR (eGFR) or measured GFR (mGFR). We assumed that baseline GFR values depended on gender, age and albuminuria status at baseline. For simplicity, we further assumed that the mean trajectory of GFR over time was linear, and that the mean slope of GFR over time depended only on albuminuria status at baseline.

Our first dataset consisted of the full data on the 200 patients without any dropouts. A second dataset consisted of the same 200 patients, but we assumed that replacement therapy was systematically initiated after an observed GFR value of <15 mL/min/1.73 m^2 and that GFR values were no longer available. In other words, the second dataset includes data ‘missing at random’ (MAR) since the reason for dropout is associated only with previously observed values of GFR. This scenario resembles many longitudinal studies on CKD. Note that if the reason for dropout was unrelated to GFR, the data missing after the last observed value of GFR would be ‘missing completely at random’ (MCAR). In contrast, if the reason of dropout was related to ‘unobserved’ values of renal function, the data would be ‘missing not at random’ (MNAR). An extract of the second dataset is summarized in Table 1 and observed GFR trajectories of 20 randomly selected patients from this dataset are shown in Figure 1.

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$^{*}$Patient is the identification number of the patient; visit is the visit number; GFR is the value of the measured glomerular filtration rate in mL/min/1.73 m^2 observed at that visit; age is the age at baseline in years; gender equals 1 for females, 0 for males; normo equals 1 if the patient was normoalbuminuric at baseline, 0 otherwise; micro equals 1 if the patient was microalbuminuric at baseline, 0 otherwise; macro equals 1 if the patient was macroalbuminuric at baseline, 0 otherwise.

FIGURE 1: Example of observed trajectories of GFR (in mL/min/1.73 m^2) of 20 randomly selected hypothetical patients (dataset with dropout).

STATISTICAL METHODS

Linear regression on individual slopes, GEEs and linear mixed models were applied to the two datasets in order to illustrate
their differences in terms of methods and results. The mathematical representation of each model is provided in Appendix, which may be useful for some readers, but is not required to understand the study. Accompanying software code to fit the models presented in this study can be found at http://www.meduniwien.ac.at/user/georg.heinz/renal-function-trajectory.

Method 1: Linear regression on individual slopes

Let us suppose that the objective was to investigate the association between baseline albuminuria status (normo-, micro- or macroalbuminuria) and the slope of GFR over time, i.e. the ‘speed’ of GFR decline. This could be achieved by using a linear regression on individual slopes, which is a two-stage approach.

In the first stage, the slope of GFR over time was estimated for each patient, using a simple linear regression model where the outcome variable was GFR (the GFR values in mL/min/1.73 m² observed at the different time points) and the independent variable was time (the time points in years at which GFRs were measured; see equation in Appendix). This first-stage model is based on the assumption that, for each patient, there is an underlying linear GFR trajectory, and the observed GFR values vary randomly in this patient-specific linear trajectory. The resulting estimated slope, expressed in mL/min/1.73 m²/year, gives a patient’s annual GFR decline. In the second stage, a linear regression model was used where the estimated slopes were used as an outcome variable, and the independent variables were the indicators of micro- and macroalbuminuria at baseline with normoalbuminuria as reference (see equation in Appendix and ref. [10] for a discussion on how to represent qualitative variables in regression models).

The advantage of this two-stage approach is that it is conceptually easy to understand. However, it has several disadvantages. First, it does not allow the investigation of risk factors associated with baseline level of renal function. Such analysis has to be performed separately. Secondly, patients with only one renal function assessment are excluded from the analysis since their slope cannot be estimated, which may decrease the sample size and may induce selection bias [11]. Finally, patient-specific slope estimates may be precise for patients with many repeated measurements, but may be inaccurate when derived from only few measurements. Barbour et al. [2] accounted for this type of heterogeneity by weighting each patient in the second stage by the inverse of the standard error of his/her GFR slope estimate derived in the first stage. However, this weighted version is even more prone to selection bias than the unweighted standard version since it also excludes patients with only two visits. Indeed, the derivation of standard error of the slope requires at least three GFR measures. For this reason, and also because the weighted version is less frequently used, we used the unweighted version only.

Method 2: Generalized estimating equations

Let us suppose that the objective was not only to investigate the association between baseline albuminuria status and the slope of GFR over time, but also to investigate the association between baseline albuminuria status and baseline level of GFR adjusted for gender and age. Indeed, in longitudinal observational studies, when the exposure variable is associated with the slope, it is also often associated with baseline value of the outcome. These objectives can be investigated using a single GEE model or a single linear mixed model (Method 3).

In the linear GEE model, the outcome variable was the GFR observed values measured at the different time points, and the independent variables were micro- and macroalbuminuria indicators at baseline, the time points (in years) at which the respective GFR measurement was obtained, interaction terms between time and micro- and macroalbuminuria indicators, as well as gender and age (in years) as potential confounders (see equation in Appendix). Note that this specific GEE model assumed that baseline albuminuria status is potentially associated with both baseline level and slope of renal function, and that age and gender are potentially associated with the baseline level of GFR only. If gender and age are likely to be associated also with the slope of renal function, then one should add in the model their interaction terms with time (same for Method 3). Moreover, as Method 1, this GEE model also assumes linear trajectories over time. If trajectories are likely not linear, then one should use a more appropriate non-linear function of time in the model (same for Method 3).

To use the GEE approach, one has to a priori specify the structure of the ‘working correlation matrix’. This matrix expresses the correlation between any two of all possible GFR measures in the same patient. Different choices are possible. Choosing an ‘independent’ structure assumes that all repeated measures of the same patient are independent. Choosing an ‘exchangeable’ structure is equivalent to using a linear mixed model with a random intercept only without random slope (see Method 3). Choosing an ‘unstructured’ working correlation matrix allows the estimation of specific correlations between each pair of repeated measures, and is thus more complex to estimate; see e.g. Table 3 in Ravani et al. [12] for an illustration of these different types of working correlation matrix. We used both exchangeable and unstructured working correlation matrices, but since an unstructured correlation matrix may sometimes lead to numerical issues when computing the estimates, we only present the results with the simpler exchangeable structure. However, it is important to note that we used standard error estimates that are robust to wrong choice of the working correlation matrix [13] and can be obtained from most software.

The advantage of GEE is that it remains conceptually simple. The major disadvantage of its standard version is that it is not robust to patients dropping out because of their earlier ‘observed’ GFR values (MAR data). Some methods have been proposed to account for such dropouts in GEE (see ref. [14]), but they are not used commonly and therefore, they were not included here.

Method 3: Linear mixed model

We used a linear mixed model that included exactly the same variables as the GEE model (Method 2), except that it additionally included a random intercept and a random slope (see equation in Appendix). The term mixed model refers to the use of both fixed effects and random effects in the model. The fixed effects (i.e. regression coefficients β in Appendix)
have exactly the same population-averaged interpretation as the regression coefficients in GEE. In contrast, the additional random effects are subject-specific. More specifically, for a given patient, the random intercept quantifies the difference between his/her specific observed baseline value of GFR and the mean GFR baseline value of all patients with the same gender, age and albuminuria status at baseline. The random slope quantifies the difference between the slope of his/her own specific GFR trajectory over time and the mean GFR slope of all patients with the same baseline albuminuria status (same albuminuria status because albuminuria may potentially modify GFR slope in this model).

The correlation between repeated measures of the same patient is accounted for via random effects. Note that standard outputs from the software do not return individual values of random intercept and random slope, but only the estimates of their variance. Larger estimated variances indicate higher variability in baseline GFR and in GFR slopes between patients, respectively. To estimate the parameters of the linear mixed model, one has to assume a specific distribution of random effects. For a continuous outcome like GFR, it is common to assume a normal distribution, which is implemented in most statistical software programs. If one uses both a random intercept and a random slope, as we did, one should further specify whether the random slope may be correlated with the random intercept. A zero correlation implies that individual departures from the mean slope are not associated with individual departures from the intercept. In the present analysis, we allowed for a possible correlation and thus used an ‘unstructured’ covariance matrix of the random effects. Since the correlation was not statistically significant, we present the results of the model with a ‘diagonal’ covariance structure, imposing the restriction that random slope and random intercept are uncorrelated. Note that specifying a ‘diagonal’ structure may also help to solve numerical problems when computing the estimates.

Random effects in mixed models thus allow the characterization of all individual trajectories. This is the reason why mixed models are often called a ‘subject-specific approach’, as opposed to GEE, which is a ‘population-averaged approach’. However, for a continuous outcome like GFR, linear GEE and linear mixed model will lead to the same estimates if (i) one uses a random intercept only in the linear mixed model and an exchangeable working correlation matrix in linear GEE and (ii) if no patients dropout of the study or if the reason for dropout is unrelated to renal function (MCAR data). However, if some patients dropout of the study early because of initiation of renal replacement therapy due to low previously ‘observed’ values of GFR (MAR data), then the linear mixed model will provide much more reliable estimates than standard GEEs [1].

**RESULTS**

Baseline characteristics are presented in Table 2, and the mean follow-up times and the distribution of the number of visits per patient are reported in Table 3 for the two datasets. In the dropout scenario, 65 (32.5%) patients dropped out of the study after having reached an observed GFR measure below 15 mL/min/1.73 m².

When no patient dropped out of the study, all statistical methods gave similar results (Table 4). For example, according to the linear mixed model, GFR decreased on average by 1.76 mL/min/1.73 m² each year in normoalbuminuric patients at baseline. The average GFR decline was 1.76 + 2.84 = 4.6 mL/min/1.73 m²/year in microalbuminuric patients, and was 1.76 + 3.09 = 4.85 mL/min/1.73 m²/year in macroalbuminuric patients. Similar eGFR slopes over time were obtained with linear regression on individual slopes and GEE. After adjustment for age and gender, micro- and macroalbuminuric patients at baseline had a mean baseline GFR lower by 18.5 and 26.6 mL/min/1.73 m² when compared with normoalbuminuric patients at baseline, with the linear mixed model, respectively. Very similar estimated differences were obtained with GEE.

Differences of estimates between the three methods appeared in the dropout scenario (Table 4). In particular, GEE underestimated the mean slope of GFR in the three groups of albuminuria, when compared with the true mean slopes that we simulated in the data (data not shown). The results of GEE even wrongly suggest that the mean slope in macroalbuminuric patients is not significantly different from that in normoalbuminuric patients (mean slope difference of −1.05,
provide satisfactory estimates, if most patients have at least three visits. However, the analyst should prefer the linear mixed model, because it accounts for all information including patients with only one renal function assessment, can provide all estimates in a single-stage calculation, can deal with unequal number of measurements per patient and also allows investigating factors associated with baseline values of renal function.

In this illustrative study, we made several simplistic assumptions. For instance, we simulated a small cohort of patients with only limited baseline characteristics to adjust for and without any interactions between them. However, all the points that we raised apply to larger cohorts as well, and GEE or linear mixed models may include several other potential baseline confounders or effect modifiers. We also simulated only annual observed GFR values, even for patients with severely reduced kidney function, which may diverge from clinical practice. However, all analytical models accounted for all individual-specific times of GFR measurements/estimations, so that these GFR assessments do not need to be taken at equally spaced times. Moreover, we simplified all models by assuming linear mean trajectories of GFR over time. Although linearity is usually assumed, some studies have shown that many patients may actually have a non-linear GFR trajectory [15]. Such non-linear trends of the renal function over time can be accommodated both in the linear mixed model and in GEE. For example, Alves et al. [5] used a spline function of time in a linear mixed model to describe individual non-linear trends of eGFR over time. The linear mixed model may be extended to account for other particularities of the data at hand. For example, if the distribution of the random effects deviates from normality, as it may be the case when the outcome is GFR, it is possible to transform the outcome variable or to use more appropriate distributions of random effects (see e.g. [16]). Finally, we assumed that dropout was only related to

<table>
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<th>Table 4. Estimated effects of baseline albuminuria status on baseline level and slope of GFR (in mL/min/1.73 m²)</th>
<th>Method 1 Linear regression on individual slopes</th>
<th>Method 2 Linear GEE</th>
<th>Method 3 Linear mixed model</th>
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<td>In micro versus normo</td>
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<tr>
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<tr>
<td>No dropout</td>
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<td>−1.05 (−2.42; 0.32)</td>
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95% confidence interval: −2.42, 0.32 mL/min/1.73 m²/year). Linear regression on individual slopes and linear mixed models provided estimates of the same magnitude likely because most patients (98.5%) had at least two visits (Table 3). In other words, excluding the three patients who had only one measure in the linear regression on individual slopes did not affect its results (Table 4).

**CONCLUSION**

Our study illustrates differences and similarities among the results obtained from three different statistical methods commonly used to investigate risk factors of renal function trajectories. More specifically, we compared linear regression on individual slopes, linear mixed models and GEE, all of which can be used for investigating the impact of some baseline characteristics (albuminuria status in our example) on the slope of renal function over time. Our comparison indicates that when no patients dropout of the study, the three methods yield similar results. However, in practice most studies will be prone to dropout of patients for various reasons. Renal replacement therapy initiation and death are the most common causes of dropout when studying renal decline. Because the decision on renal replacement therapy initiation is usually based on the previously observed values of renal function, and thus dropping out of the study for that reason is not completely random, the linear mixed model is the method of choice. Indeed, as illustrated in our study, the results of standard GEE are not robust to such dropout. If GEE is preferred for any reason, the analyst should consider one of its extensions to account for such dropout, such as a weighted version [14]. Linear regression on individual renal function slopes may provide satisfactory estimates, if most patients have at least three visits. However, the analyst should prefer the linear mixed model, because it accounts for all information including patients with only one renal function assessment, can provide all estimates in a single-stage calculation, can deal with unequal number of measurements per patient and also allows investigating factors associated with baseline values of renal function.

In this illustrative study, we made several simplistic assumptions. For instance, we simulated a small cohort of patients with only limited baseline characteristics to adjust for and without any interactions between them. However, all the points that we raised apply to larger cohorts as well, and GEE or linear mixed models may include several other potential baseline confounders or effect modifiers. We also simulated only annual observed GFR values, even for patients with severely reduced kidney function, which may diverge from clinical practice. However, all analytical models accounted for all individual-specific times of GFR measurements/estimations, so that these GFR assessments do not need to be taken at equally spaced times. Moreover, we simplified all models by assuming linear mean trajectories of GFR over time. Although linearity is usually assumed, some studies have shown that many patients may actually have a non-linear GFR trajectory [15]. Such non-linear trends of the renal function over time can be accommodated both in the linear mixed model and in GEE. For example, Alves et al. [5] used a spline function of time in a linear mixed model to describe individual non-linear trends of eGFR over time. The linear mixed model may be extended to account for other particularities of the data at hand. For example, if the distribution of the random effects deviates from normality, as it may be the case when the outcome is GFR, it is possible to transform the outcome variable or to use more appropriate distributions of random effects (see e.g. [16]). Finally, we assumed that dropout was only related to
previous low ‘observed’ GFR values (MAR data). If dropout of the study is not predictable from the observed GFR values, but may depend on ‘unobserved’ ones (MNAR data), then neither standard GEE nor standard mixed models should be used. Instead, a joint modelling approach combining a linear mixed model and a time-to-event model can be used to account for this informative dropout [17]. If the objective is to further identify different subpopulations of trajectories over time, a joint latent class linear mixed model may be used [18].

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST STATEMENT**

None declared.

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**APPENDIX**

**METHOD 1: LINEAR REGRESSION ON INDIVIDUAL SLOPES**

In the first stage, the following linear regression model was estimated for each patient $i$

$$GFR_{ij} = A_i + B_i t_{ij} + e_{ij},$$

where $t_{ij}$ is the time (in years) of visit $j$ for patient $i$; $GFR_{ij}$ is the GFR value of patient $i$ at time $t_{ij}$; $A_i$ is the expected value of GFR at baseline (intercept) for patient $i$; $B_i$ is the slope of GFR for patient $i$, i.e. his/her average change in GFR per year; $A_i + B_i t_{ij}$ gives the expected value of GFR for patient $i$ at time $t_{ij}$; $e_{ij}$ is a random deviation about the expected value of GFR at time $t_{ij}$. All $e_{ij}$ are assumed to be independent and normally distributed with mean zero and the same variance.

In the second stage, all individual estimated slopes ($B_i$) derived from the first stage were used to estimate the following linear regression model:

$$B_i = \gamma_0 + \gamma_1 \text{micro} + \gamma_2 \text{macro} + u_i,$$

where $\gamma_0$ (intercept) is the mean (population-averaged) slope of GFR in normoalbuminuric patients at baseline; $\gamma_1$ is the mean (population-averaged) difference in GFR slopes between micro- and normoalbuminuric patients at baseline; $\gamma_2$ is the mean (population-averaged) difference in GFR slopes between macro- and normoalbuminuric patients at baseline; $u_i$ is a random deviation of patient $i$ about the mean (population-averaged) GFR slope. All $u_i$ are assumed to be independent and normally distributed with mean zero and identical variance.

**METHOD 2: GENERALIZED ESTIMATING EQUATIONS**

In the GEE model, the general equation expressing the expected (i.e. mean or predicted) value of GFR at visit $j$ for a patient $i$ with a given baseline albuminuria status, age and gender is given by

$$E(GFR_{ij}) = \beta_0 + \beta_1 \text{micro} + \beta_2 \text{macro} + \beta_3 t_{ij} + \beta_4 \text{micro} \times t_{ij} + \beta_5 \text{macro} \times t_{ij} + \beta_6 \text{age} + \beta_7 \text{gender},$$
From this equation, it is possible to write the expected GFR value at time $t_{ij}$ for a patient with a given gender, age and albuminuria status at baseline. For example,

For a normoalbuminuric male aged 65 years at baseline:

$$E(GFR_{ij}| \text{micro} = 0, \text{macro} = 0, \text{gender} = 0, \text{age} = 65, t_{ij}) = \beta_0 + \beta_3 t_{ij} + 65\beta_6.$$  

For a microalbuminuric male aged 65 years at baseline:

$$E(GFR_{ij}| \text{micro} = 1, \text{macro} = 0, \text{gender} = 0, \text{age} = 65, t_{ij}) = \beta_0 + \beta_1 + (\beta_3 + \beta_4)t_{ij} + 65\beta_6.$$  

For a macroalbuminuric male aged 65 years at baseline:

$$E(GFR_{ij}| \text{micro} = 0, \text{macro} = 1, \text{gender} = 0, \text{age} = 65, t_{ij}) = \beta_0 + \beta_2 + (\beta_3 + \beta_5)t_{ij} + 65\beta_6.$$  

From these examples, one can deduce the following interpretation of regression coefficients of interest:

- $\beta_1$ is the mean (population-averaged) difference in GFR at baseline ($t_{ij} = 0$) between micro- and normoalbuminuric patients of the same gender and age at baseline.
- $\beta_2$ is the mean (population-averaged) difference in GFR at baseline ($t_{ij} = 0$) between macro- and normoalbuminuric patients of the same gender and age at baseline.
- $\beta_3$ is the mean (population-averaged) slope of GFR in normoalbuminuric patients at baseline.
- $\beta_4$ is the mean (population-averaged) difference in GFR slopes between micro- and normoalbuminuric patients at baseline.
- $\beta_5$ is the mean (population-averaged) difference in GFR slopes between macro- and normoalbuminuric patients at baseline.

**METHOD 3: LINEAR MIXED MODEL**

In the linear mixed model, the general equation expressing the observed value of GFR at visit $j$ for a patient $i$ with a given baseline albuminuria status, age and gender is given by:

$$GFR_{ij} = (\beta_0 + u_{0i}) + \beta_1 \text{micro}_i + \beta_2 \text{macro}_i + (\beta_3 + u_{1i})t_{ij} + \beta_4 \text{micro}_i \times t_{ij} + \beta_5 \text{macro}_i \times t_{ij} + \beta_6 \text{age}_i + \beta_7 \text{gender}_i + e_{ij},$$

where all the fixed effects $\beta$ have the same population-averaged interpretation as in linear GEE (Method 2). $u_{0i}$ is a random deviation of patient $i$ about the population-averaged baseline GFR in patients with identical baseline characteristics. All $u_{0i}$ are assumed to be independent and normally distributed with mean zero and the same variance. $u_{1i}$ is a random deviation of patient $i$ about the population-averaged slope of GFR, i.e. about $\beta_3$ for normoalbuminuric; $\beta_3 + \beta_4$ for microalbuminuric and $\beta_3 + \beta_5$ for macroalbuminuric patients at baseline. All $u_{1i}$ are assumed to be independent and normally distributed with mean zero and with the same variance. They are further assumed to be either independent or dependent of $u_{0i}$ (i.e. using either a diagonal or an unstructured covariance matrix for the random effects). $e_{ij}$ is a random error at time $t_{ij}$. All $e_{ij}$ are assumed to be independent and normally distributed with mean zero and identical variance.

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