

Analysis of the relationship between Mycophenolic acid (MPA) exposure and anemia using three approaches: logistic regression based on Generalized Linear Mixed Models (GLMM) or on Generalized Estimating Equations (GEE) and Markov mixed-effects model.

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BACKGROUND AND OBJECTIVES

Mycophenolic acid (MPA) is an immunosuppressant widely used in transplantation, anemia is one of its most important adverse effect and could lead to inobservance. However, the association between MPA exposure and anemia is under debate. The goal of this study was to evaluate the influence of MPA exposure on the occurrence of anemia in a one year longitudinal study using 3 modeling approaches: i) a regression logistic with fixed and random effects (GLMM), ii) a regression logistic using the generalized estimating equations (GEE) and iii) a Markov mixed effects model.

METHODS

- Data from adult kidney transplant recipients enrolled in the randomized clinical trial APOMYGRE (NCT0019967) were reanalyzed in this study. In summary, the APOMYGRE study was a 12-month, randomized, prospective multicenter (11 centers) trial (NCT0019967) which compared MMF dosing based on target MPA AUC (concentration controlled arm –CC–) and fixed dose (FD) MMF in de novo adult renal transplant recipients. All patients received mycophenolate mofetil (i.e., the prodrug of MPA) associated with cyclosporine [1].
- The association between MPA AUC at visit t-1 and anemia at visit t was studied using either: GLMM, GEE or Markov mixed-effect models.
- GLMM and GEE models were fitted in R using lme4 [3] and geepack [4] packages respectively. Markov models were fitted in NONMEM® using the Laplacian method
- Effect of covariates (donor and recipient ages, gender, dosing strategy (CC vs FD), hemoglobin level at month 1) was investigated.
- Odds ratios (OR) and 95% confidence interval (CI) of OR were calculated for the 3 models. For the Markov model, the 95% CI of OR were based on the 2,5th and 97,5th percentiles of parameter estimates obtained from 500 bootstrap samples.
- The Markov model was evaluated by performing posterior predictive checks (PPC) using 200 simulated datasets.

RESULTS

PATIENT CHARACTERISTICS

Number of kidney transplant patients	130
Scheduled visits after transplantation	W1, W2, M1, M3, M6, M12
Anemia (events/ patients)	42 / 32
Recipient age (mean ± SD)	49,8 ± 13,7 years
Donor age (mean ± SD)	47,4 ± 14,3 years
Hb level at M1 (mean ± SD)	11,5 ± 1,4 g/dl
Gender (M/F)	84/ 46
Dosing strategy (FD/CC)	65/ 65

Table 1: Patients and study data. W: week, M: month, SD: standard deviation, Hb: hemoglobin, M: male, F: female, FD: fixed dose; CC: concentration-controlled.

- One hundred and thirty kidney transplant patients included in the APOMYGRE trial treated with MPA were analyzed in this study
- Both MPA area under the curve (AUC) previously estimated using a bayesian estimator based on a 3 point sampling strategy [2] (2,5th-97,5th percentiles: 13-73 mg.h/L) and the Hb level were collected at different visits post-graft (weeks 1 and 2, months 1,3,6 and 12).
- Anemia was defined as categorical data: 1 for having anemia (i.e. hemoglobin <10 g/dl), and 0 for not having anemia. Seven patients have repeated anemia events.
- MPA AUC values were considered at the last observed visit carried forward (e.g. MPA AUC at W1 to study the onset of anemia at W2)

MODELING PROCESS: GEE, GLMM and MARKOV MIXED-EFFECTS MODELS

- The following equation form was used for the 3 models:

$$\text{Log}(\text{Pi}/1-\text{Pi}) = \alpha + \beta_1x_{i1} + \beta_2x_{i2} + \dots + \beta_kx_{ik} + \eta$$

With **Pi**: probability of having anemia in individual *i*;
α: intercept, and **η**: random effect around intercept for subject *i*;
x_{ik}: explanatory variables which could be time dependent or time independent, and *k* is the number of explanatory variables;
β_k: regression coefficient associated to the *k*th explanatory variable.

- The random effect Eta was estimated for the GLMM and the Markov model, which are *subject-specific* models but not for the GEE model which is a *population-averaged* model.
- For the GEE model, 3 different correlation structures were tested to take into account the dependencies between the repeated observations: *exchangeable*, *autoregressive* and *unstructured* working correlation matrix. The unstructured correlation structure which is more adapted for unbalanced data (*i.e measurements at the same times for all individuals, but may be unequally spaced*) was selected.
- Time-dependent MPA AUC values and Hb levels at M1 were significant predictors of anemia. For the GLMM model, time was also included as fixed and random effects in the model. The obtained ORs (=exp(β)) associated with a one unit increase of AUC [95% CI] were: 1,027 [1,001-1,053], p=0,044 for the GLMM model; 1,016 [1,002-1,031], p=0,029 for the GEE model and 1,020 [1,003-1,444] for the Markov mixed-effects model.
- For the Markov model, 4 different transitions were estimated. The PPC showed that, for the transition to anemia (transition 1), the observed number of transitions was within the 95% prediction interval (Figure 1),

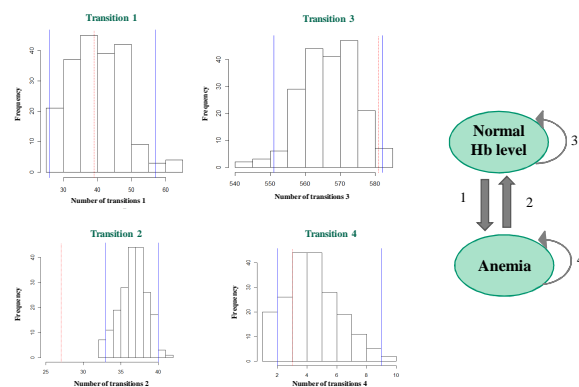


Figure 1: PPC plots of the Markov mixed-effects model. Histograms represent the number of the different simulated transitions (transition 1 to 4). The dashed red lines represent the observed number of transitions in the analysed data set, and the solid blue lines represent the 2.5th and 97.5th percentiles of the simulated number of transitions.

DISCUSSION - CONCLUSION

- The 3 models showed a significant MPA exposure-anemia relationship; OR were estimated with good precision.
- 95% CI OR was smaller using GEE, this advocates the use of this method at least when subject-specific parameter estimates are not of special interest.
- GEE and GLMM models described only the transition 1 (Figure 1) while the Markov model allows additionally the study of the reverse transition from anemia to a normal Hb level >10 g/dl (transition 2). MPA AUC values were not a significant covariate in this reverse transition. The erythropoietin (EPO) is a potential covariate for this transition, but due to numerous missing data (>50%) this covariate could not be tested in this analysis.

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