

contribute to the discourse concerning the nuances of the clinical use of fomepizole. Rehman points out that the osmolal gap dissipates as ethylene glycol or methanol is metabolized. The reason is that only the parent compound contributes to this gap. Electroneutrality requires the acidic metabolites of ethylene glycol and methanol (primarily glycolate and formate), which are ionized to negatively charged carboxyl groups at physiologic pHs, to have a counter cation. In plasma, on a probabilistic basis, this would be a sodium ion. The equation for calculating the osmolal gap includes, for serum osmolality, a term that is two times the sodium concentration, accounting for both the sodium ion itself and, by virtue of the coefficient, the metabolite anion (Table 3 of the article). Thus, these metabolites, unlike the parent compounds, will not contribute to the calculated osmolality and hence decrease the osmolal gap. This event is important because the disappearance of the osmolal gap is evidence of metabolism of the parent compound, after which there is no benefit to inhibiting alcohol dehydrogenase. Rehman also correctly observes that the contribution of ethanol must be factored into the calculation of the predicted osmolality (also explained in Table 3 of the article).

González-Santiago and Garza-Ocañas speculate that the pharmacokinetic interaction between fomepizole and ethanol might exacerbate the potential adverse effects of the latter. When fomepizole is used, ethanol continues to be metabolized, but with a reduction in the clearance rate of approximately 40%.<sup>1</sup> There have been a number of instances in which fomepizole was administered to patients with blood alcohol concentrations in the intoxication range with no reported adverse effect, including one instance in which the fomepizole nullified an adverse effect of ethanol on a patient's level of consciousness.<sup>2</sup> I agree with González-Santiago and Garza-Ocañas that fomepizole should be used with caution if a patient is allergic to pyrazoles; however, no allergic reactions in patients with intolerance to this class of medications have been reported in the literature.

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## More on B-Cell–Depleting Induction Therapy and Acute Cellular Rejection

**TO THE EDITOR:** We read with interest the letter by Clatworthy et al. (June 18 issue)<sup>1</sup> describing their use of rituximab as compared with daclizumab for induction therapy in patients undergoing renal transplantation. We were surprised, however, not only by their finding that significantly more rejection episodes occurred in patients treated with rituximab but also by their interpretation of that finding, in which they state that rituximab may have had a rejection-provoking effect.

We have been using rituximab as part of a desensitization protocol,<sup>2</sup> and in 150 ABO-incompatible renal transplantations in Sweden we have not seen any increase in rejection episodes. On the

contrary, because the number of rejections in patients receiving rituximab has been exceptionally small, we recently published a report of a randomized, double-blind, placebo-controlled, multicenter study in which the effect of administering a single dose of rituximab was compared with the use of placebo as induction therapy in renal transplantation (ClinicalTrials.gov number, NCT00255593).<sup>3</sup> Working with a total of 140 enrollees, we found a tendency toward fewer (11.6% vs. 17.6%) and milder rejections during the first 6 months in the rituximab group and observed no increase in infectious complications or leukopenia in that group.

Our findings highlight the value of conducting randomized, blinded, placebo-controlled studies as opposed to drawing conclusions based on incidental observations.

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Drs. Tydén, Mjörnstedt, and Ekberg report receiving lecture fees and travel grants from Roche and Astellas to attend scientific meetings. No other potential conflict of interest relevant to this letter was reported.

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**TO THE EDITOR:** Clatworthy et al. report the premature termination of a clinical trial after the occurrence of acute rejection in five of six patients who received rituximab as B-cell–depleting induction therapy after renal transplantation. We are currently carrying out a study in which 280 patients undergoing renal transplantation will be randomly assigned to receive rituximab (375 mg per square meter of body-surface area) or placebo during surgery; patients in both study groups will also receive tacrolimus, mycophenolate mofetil, and corticosteroids (ClinicalTrials.gov number, NCT00565331). Prompted by the disquieting findings of Clatworthy et al., we analyzed the data we had on the first 65 patients in the trial who had reached a follow-up of 6 months. In the patients treated with rituximab, the relative risk of acute rejection confirmed by biopsy during the first 6 months after transplantation was 0.53 (95% confidence interval, 0.21 to 1.32). Tydén et al. recently reported an incidence of acute rejection with rituximab of 12% versus 18% with placebo (not sig-

nificant,  $P=0.32$ ) during the first 6 months after transplantation.<sup>1</sup> Like Clatworthy et al. and Tydén et al., we found no difference between the two groups in the frequency of infections or cancer (according to preliminary data). On the basis of these reassuring results, we are proceeding with the enrollment of patients.

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**THE AUTHORS REPLY:** In their letters, both Tydén et al. and van den Hoogen and Hilbrands report on recent studies in which the use of rituximab in patients undergoing transplantation did not result in an increase in acute rejection but rather resulted in nonsignificant trends toward a lower rejection rate. Although these results initially seem incompatible with our observation that rituximab may increase the rate of acute rejection, careful consideration of the different protocols used in the three studies indicates that this is probably not the case.

In our study, we proposed that the increase in rejection that we observed might be due to a mechanism through which B-cell depletion resulted in systemic release of cytokines, thereby enhancing T-cell activation and increasing the risk of acute rejection. This interpretation is consistent with the increased cytokine levels measured in our patients and with the transient increase in inflammation seen on the initiation of rituximab in the treatment of some autoimmune diseases. As we stated, if rituximab had been administered to patients before transplantation, as it is when used in desensitization or ABO-incompatibility protocols, such a cytokine storm would have resolved before transplantation and no increase in the risk of rejection would therefore be expected. We think it is also likely that ongoing corticosteroids

teroid therapy, used in the protocols of the studies conducted by Tydén et al. and van den Hoogen and Hilbrands, could suppress the effects of cytokine release, which would explain why the increase in acute rejection seen in our corticosteroid-free protocol was not observed in the other studies. Comparison of the three studies thus highlights the possibility that rituximab therapy can induce rejection, perhaps through systemic release of cytokines, if it is administered at the time of transplantation and without concurrent administration of corticosteroids.

Tydén et al. state that conclusions should not be drawn from our results. We disagree strongly with this statement. We did not claim that our study was conclusive but rather urged caution. Nonetheless, our results constitute a potentially clinically important, statistically significant ob-

servations made in a randomized, controlled study with the “hard” end point of rejection confirmed by biopsy. It is important to publish such results — indeed, their publication has made possible a comparison with the results reported by Tydén et al. and van den Hoogen and Hilbrands, providing insight into the circumstances in which, and the mechanism by which, rituximab might increase the risk of acute rejection, and suggesting strategies that might be used to avoid this outcome. The process has therefore been of potential benefit to patient care.

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## A Classic Twin Study of External Ear Malformations, Including Microtia

**TO THE EDITOR:** The pathogenesis of microtia, a rare congenital malformation of the external ear, remains elusive. Phenotypes range from minor deformities, such as preauricular tags, to anotia, the complete absence of the external ear (Fig. 1). Prevalence ranges from 0.66 per 10,000 in England to 17.4 per 10,000 in Quito, Ecuador.<sup>1</sup> The relative contributions of environmental and genetic factors to microtia were assessed in a classic twin study.

In this study, we identified 13 monozygotic and 22 dizygotic twin pairs at three microtia reconstruction centers, one in the United States, one in Ecuador, and one in Colombia. In other words, each twin pair was ascertained because at least one sibling had severe nonsyndromic microtia requiring surgical repair. There was no ear malformation in 40% of subjects, malformation of the right ear in 34%, malformation of the left ear in 13%, and bilateral anomalies in 12%. Ear-malformation laterality was indistinguishable among monozygotic and dizygotic twins. None of the twins reported first-degree relatives with ear mal-

formations, but 20% had at least one, more distant relative with an ear malformation.

The concordance rate for all auricular malformations was higher in monozygotic twins than in dizygotic twins (61.5% and 4.5%, respectively;  $P=0.003$ ) (Table 1). Concordance rates for microtia (excluding skin tags and minor pinna malformation) among monozygotic and dizygotic twins were also significantly different — 38.5% and 4.5%, respectively (Table 1).

A literature review identified 37 twin pairs with microtia in whom other auricular malformations were rarely reported.<sup>2-5</sup> When the results of this study are combined with those of our study of 35 twin pairs, the 72 sets of twins show significant differences in the concordance rate for monozygotic twins (10 in 38, or 26.3%), and the concordance rate for dizygotic twins (1 in 34, or 2.9%) (odds ratio, 11.5; 95% confidence interval, 1.4 to 93.7;  $P=0.023$ ).

In summary, twin studies indicate that there is a strong genetic contribution to malformations of the external ear. Shared genotype in monozy-