particular C-reactive protein (CRP), in patients with chronic kidney disease (CKD) adds to their prognostic evaluation in terms of mortality. They conclude that the determination of inflammatory markers is not helpful if age and comorbidity are taken into account. It would, however, be appropriate if some further analyses were performed before such a conclusion is advanced. The conclusion of Caravaca et al. [1] is based on a multivariate Cox-regression analysis in which the significance of CRP as a predictor of mortality is lost after adjustment for other variables. For this analysis, CRP and other inflammatory markers are dichotomized, while other variables, including age, are left unchanged with retention of full variance. Entrance of dichotomized and continuous variables together in one Cox-regression model is like amputating a swimmer’s legs before competition. If a predictor, like CRP, has a continuous distribution, dichotomization along the median results in a loss of predictive power by 35% [2,3]. Another issue is that of multicollinearity. When different domains of the same phenomenon, such as inflammation, are studied simultaneously, the variables tend to correlate with each other, thus violating the assumptions of multivariate regression models [4]. Caravaca et al. [1] do not report whether or not there were significant correlations between CRP, white blood cell counts and counts of polymorphonuclear leukocytes. However, significant correlations of all these parameters with the negative acute phase response marker, serum albumin, existed. The authors should thus at least repeat their multivariate analysis with inflammatory markers as continuous variables, in the absence of serum albumin as a variable in the model. If the other inflammatory markers are found to be correlated, this analysis should ideally be repeated for each of the markers separately. Then, if the authors still find the same loss of significance of each of the inflammatory markers as a predictor of mortality in the multivariate model(s), their conclusion is valid.

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Reply

Sir,

We would like to apologize for not showing a more complete presentation of the analyses originally done in our study. Of course, C-reactive protein (CRP) was analysed as a continuous variable, log-transformed for normalization of its distribution. Different Cox-regression models were performed with varying degrees of covariate adjustment. The first multivariate model included the age and comorbidity index. In order to establish the predictive information added by CRP levels, this variable was entered into this first model in several forms: as a log-transformed continuous variable, and as nominal scale covariates (above or below median, tertiles, above or below 3 mg/l, or as above or below the best predictive value over mortality). None of these covariates added predictive information to that provided by age and comorbidity index. For instance, the hazard ratio of log-CRP in this model—in which serum albumin or other inflammatory markers were not included—was 1.28 (95% CI: 0.90–1.82).

We would like to emphasize that the dichotomization of CRP values in this study was performed after assessing the cut-off values best related with the mortality of the study group. By doing that, and using the same metaphor which Dr Bakker used, we did not intend to amputate the leg of the swimmer, but provide him a couple of flippers.

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Xenotransplantation—will tolerance be essential?

Sir,

I enjoyed reading the short review by Sprangers et al. [1] in your April 2006 issue. However, I wish to question one of their conclusions.

They state that successful application of xenotransplantation will still require induction of robust T- and B-cell xenotolerance.

I doubt if they have clear evidence for making this statement. In the articles they review, related to the transplantation of hearts and kidneys from α1,3-galactosyltransferase gene-knockout (GT-KO) pigs into baboons [2,3], the heart transplants were protected from rejection by an immunosuppressive regimen. These grafts functioned for ~2–6 months, with no recipient baboon suffering any infectious or other immunosuppressive drug-related complication. This would suggest that the immunosuppressive therapy was not excessive, and would also suggest that with further genetic manipulation to the organ-source pig, prolonged graft survival of clinical relevance might be obtained with pharmacological immunosuppression alone.
In an effort to achieve graft tolerance, the kidney transplants were accompanied by donor thymus transplants [3]. Survival did not extend beyond 83 days, and all recipients died of complications of the ‘tolerance-inducing’ regimen. Although survival of kidney transplants in baboons that received only pharmacological immunosuppression (and did not also receive thymic grafts) was poor in this study [3], others have reported better survival. Kidney grafts have survived in monkeys for up to 90 days [4] and in baboons for up to 75 days [5] on pharmacological immunosuppression alone. Although the ultimate goal of xenotransplantation, as with allotransplantation, is the induction of tolerance, there is little evidence that the addition of the thymus graft contributed to prolonged survival, or that tolerance can be achieved.

In the heart transplant group [2] there was little T-cell infiltration into the grafts and no documented elicited T-cell-dependent antibody response, and the mixed lymphocyte reaction was completely suppressed throughout the course of the experiment until immunosuppressive therapy was discontinued. Although T-cells can certainly be found in the coagulation systems between primates and pigs may be far more important.

We worked from several groups in rodent models would indicate that genetic manipulation of the donor, in the form of the introduction of ‘anticoagulant’ genes [6] or the deletion of ‘coagulant’ genes [7] that correct the coagulation dysregulation may be as important, if not more important, as what we normally consider as an immunological response. For example, Chen et al. [6] reported that, whereas control mouse hearts in immunosuppressed rats failed from acute humoral xenograft rejection after 6 days, hearts from transgenic mice expressing membrane-tethered fusion proteins of human tissue factor pathway inhibitor survived indefinitely (>100 days). No features of rejection were identified histologically despite antibody and complement deposition on the xenograft endothelium. This surprising result suggests that coagulation dysregulation may be playing a primary role in graft failure, and not the secondary role hitherto inferred based on kinetics and histology.

We are awaiting the birth of GT-KO pigs that are additionally transgenic for human tissue factor pathway inhibitor or other ‘anticoagulant’ gene. The evidence is that the availability of such pigs may play a much greater role in extending pig xenograft survival than further manipulation of the adaptive immune response.

Clearly, efforts to induce tolerance should continue in both allotransplantation and xenotransplantation, but this has proved an elusive goal. Furthermore, it does not preclude the fact that, as with allotransplantation during the past 50 years, clinically valuable and acceptable results may be achieved with chronic immunosuppressive therapy while we await breakthroughs that might induce tolerance.

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Reply

Sir,

We appreciate Cooper’s opinion, which is based on a large experience in the field. Undoubtedly, major progress has been made in the pre-clinical development of xenotransplantation, as evidenced from the studies we review.

We agree that, in Kuwaki et al.’s study [1] serious treatment-related adverse events did not occur, suggesting that—although intense—pharmacological therapy was not excessive in this baboon model. However, despite prolonged survival, ultimately, five of eight heart xenografts were rejected with evidence of acute humoral xenograft rejection. Even if little T-cell infiltration was noted in the grafts, deposition of not only immunoglobulin (Ig)M but also IgG does suggest T-cell xenoreactivity. In addition, the rise in mixed lymphocyte reaction-responsiveness following cessation of treatment indicates that tolerance is not induced and that long-term xenograft survival will necessarily require ongoing immunosuppressive therapy. Also, the difference in kidney xenograft survival between the Yamada et al. study [2] and the Chen et al. study [3] in which a minimally immunosuppressive ‘clinical’ regimen was used, indicate that immunosuppressive therapy will need to be profound, and the effect of including xenothymus transplantation and T-cell tolerance-inducing anti-CD154 monoclonal antibody underscores the importance of suppressing xenoreactive T-cells.

It may be difficult to compare the effect of pharmacological immunosuppression only on the survival of different types of genetically engineered xenografts. In our opinion, the observation that—in the particular model of Gal-/-/xenokidney grafting that is studied by Yamada et al.—the combination of xenothymus grafting with pharmacological suppression strictly prevented acute