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Alterations in the Coagulation Profile in Renal Pig-to-Monkey Xenotransplantation

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Five monkey recipients of a porcine renal xenograft were studied to determine the relationship between fibrin formation in acute humoral xenograft rejection (AHXR) and procoagulant and anticoagulant factor levels to establish whether changes in coagulation parameters could be used to predict AHXR and determine whether AHXR is associated with overt disseminated intravascular coagulopathy (DIC) in this model.

Variable degrees of compensated consumptive coagulopathy were observed in each primate. Elevated thrombin-antithrombin (TAT), F_{1+2} and D-dimer levels consistent with thrombin generation and fibrin formation were recorded. There was no consumption of the main clotting inhibitors (including antithrombin) or a progressive, severe drop in fibrinogen levels and platelet counts, although grafts were left *in situ*. After transplantation, D-dimer levels remained persistently high, so they were of limited value in defining this coagulopathy. At post mortem, no cases of multiorgan involvement typical of overt DIC were observed. The lack of a rapid postoperative recovery of clotting inhibitor levels after transplantation was invariably associated with early poor outcome.

This study shows that AHXR is associated with various degrees of compensated consumptive coagulopathy in our pig-to-primate model. No clear relationship was found between coagulation parameter levels and graft outcome.

Key words: Coagulopathy, pig, primate, xenotransplantation

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Introduction

Activation of the clotting cascade, fibrin deposition and thrombosis are key features of the rejection process that takes place when pig organs are transplanted into primates (1–6) and some consider this coagulopathy as a real barrier to the long-term survival of pig organs transplanted into primates (1–3).

Lin and colleagues recently demonstrated that continuous antibody removal by plasmapheresis after cardiac pig-to-primate xenotransplantation was able to prevent the onset of acute humoral xenograft rejection (AHXR) in the explanted xenografts (7). No thrombosis was observed in these grafts, which were explanted while still beating, suggesting that AHXR and activation of the clotting cascade are closely related events.

It is well known that several anticoagulant systems exist with a key role in maintaining the thrombotic hemostatic balance (8). To date, however, there is no clear evidence on the role of clotting inhibitor levels and of any imbalance of these molecules in the onset of AHXR. The imbalance in clotting homeostasis tending towards a hypercoagulability and the excessive generation of thrombin observed in AHXR could be due to a rise in the levels of procoagulant factors (fibrinogen, thrombin, prothrombin, factor X), or a reduction in the physiological inhibitors, such as protein C, protein S and antithrombin (AT), or both.

We tried to address this issue in a longitudinal study on a group of nephrectomized primates that received a life-supporting kidney from a pig transgenic for human decay-accelerating factor (hDAF). We also investigated whether any change in the clotting parameter levels evaluated could be used as a marker of impending or ongoing rejection and thereby predict the outcome of porcine organs transplanted into primates.

Materials and Methods

Animals

All experiments and procedures were conducted in accordance with the Italian Animals Act (law no. 116 of 27/1/1992) and were authorized by a

special decree of the Italian Ministry of Health. Five ABO-matched Large White Landrace hDAF transgenic pigs (Imutran-Novartis) (9), 3–5 weeks of age and weighing between 7.2 and 13.7 kg, were used as kidney donors. Five 3–4 year-old purpose-bred male cynomolgus monkeys (*Macaca fascicularis*) from Mauritius, weighing between 4.1 and 6.2 kg, were used as recipients in the transplant studies.

Renal xenotransplantation

The surgical procedure has been described in detail elsewhere (10). All xenotransplant recipients were pretreated with GAS914 (Novartis Pharma AG, Basel, Switzerland), an injectable polymer expressing the carbohydrate moieties Galα1–3Galß1–4GlcNAc-R, at a dose of 1 mg/kg sc on days –3, –2, –1 and on the day of transplantation (day 0; 11). To take advantage of the 'protective' function of carbon monoxide against ischemia-reperfusion injury (12), all donors under general anesthesia were exposed to carbon monoxide for 4 h during the pretransplant phase, at the dosage needed to keep the concentration of CO-hemoglobin between 12 and 18%. In addition, each primate was given immunosuppression consisting of up to four doses of cyclophosphamide (Endoxan®, Asta Medica Oncology, Milan, Italy) for a total of 85–97 mg/kg i.v. perioperatively, cyclosporine A (Neoral®, Novartis Pharma AG), steroids and mycophenolate sodium (MPS) (Myfortic®, Novartis Pharma AG), as described elsewhere (13).

Episodes of graft function deterioration were treated as reported elsewhere (13). Post-transplant clinical monitoring included analyzing daily blood smears prepared using standard methods and assaying hemolytic antipig antibody levels (APA).

Coagulation studies

Blood samples: Blood samples were drawn in 1 : 10 (v/v) sodium citrate solution from naive animals at least 3 weeks before transplantation, daily from day –3 to day 7 and every other day thereafter, the last one being at the time of euthanasia. Plasma obtained after centrifugation was stored in aliquots at –80 °C until use.

Activated partial thromboplastin time (aPTT): Determined by the Cephotest (Axis, Shield, Norway), on the automated coagulometer analyzer (ACL 3000, IL, Milan, Italy). Normal ranges (from 24.7 to 32.2 s) were obtained using the plasma from nine healthy cynomolgus monkeys from Mauritius.

Fibrinogen: Determined using a fibrinogen kit by Roche Diagnostics GmbH (Mannheim, Germany), according to the manufacturer's instructions.

Prothrombin antigen (Ag): Evaluated by ELISA, as described elsewhere (14). A reference curve was obtained using pooled plasma from five healthy cynomolgus monkeys (from Mauritius and the Philippines). Normal ranges obtained in nine healthy cynomolgus monkeys from Mauritius were from 27 to 131%.

Factor X Ag: Evaluated by ELISA as described for prothrombin Ag using anti-Factor X polyclonal antibodies (15). Normal ranges were from 45 to 75%.

Antithrombin (AT): Activity was assessed using the antithrombin III kit from Roche Diagnostics GmbH (Mannheim, Germany) on an ACL 9000 (IL). An internal standard of known AT concentration was used.

ELISA for protein C (PC) antigen: This assay was performed as reported elsewhere (14). A reference curve was obtained from 1 : 50 to 1 : 3200 dilutions of plasma from four healthy subjects.

ELISA for total and free protein S (PS) antigen: The ELISA for total PS antigen was performed using the same method as for PC antigen, using anti-PS polyclonal antibody from DAKO (Milan, Italy) and anti-PS polyclonal antibody conjugated with HRP (DAKO) as the catching and second antibodies, respectively. The same assay was used to detect free PS antigen, after PEG8000-precipitation of plasma PS-C4b-bp complex (16). Pooled normal plasma, obtained as described earlier, was treated in the same way to obtain a reference curve for free PS antigen.

Platelet counts: Determined by the standard method on an automated multiple parameter analyzer (Celldyn 3500, Abbott, Rome, Italy).

D-dimer: Determined by ELISA (Asserachrom, Diagnostica Stago, Asnières, France). The assay was performed according to the manufacturer's instructions. Incidentally, similar measurements using a different ELISA kit (D-Dimer Biopool International, Dasit, Milan, Italy) did not work in this species.

Thrombin-antithrombin (TAT) complexes: Determined using a specific enzymatic immunoassay (Affinity Biological, Ancaster, Ontario, Canada), according to the manufacturer's instructions.

Prothrombin F_{1+2}: Determined using a highly specific immunoassay as described by the manufacturer (Enzygnost F_{1+2} micro, Dade Berhing, Marburg, Germany).

Diagnosis of overt disseminated intravascular coagulation (DIC): Diagnosed on the grounds of clinical and laboratory findings. As far as the latter are concerned, the following parameters were taken into account: prolongation of clotting time(s) and/or progressive drop in platelet counts to values less than 100 000 platelets/mm^3, and/or evidence of fibrinogen consumption (to values less than 100 mg/dL), and/or severe reduction in antithrombin levels (less than 70%) and/or an increase in D-dimer levels; severe consumption of clotting factors and inhibitors could also be present. As DIC has to be considered an ongoing phenomenon, these parameters may be altered to a different extent at various stages of DIC. Clinical findings required for the diagnosis of DIC included evidence of hemorrhage or thrombosis, or both, occurring in an appropriate clinical context (17).

Histopathology and immunohistochemistry

Tissue sections from all explanted kidneys were stained with hematoxylin and eosin (H&E) and tested for the presence of edema, hemorrhage, necrosis, microvascular thrombosis and the nature of the cellular infiltrate. Xenografts were also examined by immunohistochemistry for the presence of C5b-9, DAF, fibrin, IgG and IgM, as reported elsewhere (18). The histology of the transplanted organs was evaluated independently by two board-certified pathologists with expertise in organ transplantation who were unaware of the animal's clinical conditions and laboratory data at the time of euthanasia. The findings of both pathologists were recorded. In the event of any discrepancy in the evaluation, the relevant slides were jointly reviewed by the two pathologists and a consensus was reached. Acute humoral xenograft rejection was graded according to the extent of tissue damage, as described elsewhere: Grade I = damage affects < 20% graft; Grade II = damage affects 20–50% graft; Grade III = damage affects > 50% graft (13).

In addition, a complete macroscopic and histopathological evaluation was undertaken of the kidney, lung, liver, stomach, small intestine, large bowel, pancreas, heart and bladder explanted from each primate involved in the study.

Table 1: Survival, cause of death and histopathological findings

Animal	Survival (days)	Cause of euthanasia	Graft histology at autopsy*
Y034	2	Renal failure/renal artery thrombosis	HAR?
W922	5	Cardiac arrest, renal failure secondary to hydronephrosis	Minor deposits of IgM in some glomeruli
Y186	12	Severe diarrhea/animal welfare reasons	AHXRII
W946	27	Renal failure	AHXRIII
W918	37	Animal welfare reasons/sepsis	AHXRII-III

*AHXR = acute humoral xenograft rejection (Grade I = damage affects < 20% graft; Grade II = damage affects 20–50% graft; Grade III = damage affects > 50% graft); HAR = hyperacute rejection.

Results

Transplantation studies

The animals in this study survived from 2 to 37 days, with a mean survival of 16.6 ± 14.9 days (median: 12 days). Recipient survival, causes of euthanasia and graft histology at autopsy are given in Table 1.

Three animals (W922, W946 and Y034) were euthanized due to kidney failure between 2 and 27 days after transplantation. Animal W922 had a cardiac arrest during parenteral infusion with calcium gluconate for hypocalcemia secondary to pancreatitis. This animal also had hydronephrosis. Kidney failure in animal W946 was associated with anemia secondary to a severe hemorrhagic cystitis related, at least initially, to drug toxicity. In animal Y034, an exploratory laparotomy on day 2 revealed hemoperitoneum, a necrotic kidney and thrombosis of the renal artery (Figure 1). The presence of hyperacute rejection (HAR) in this animal could not be ruled out. The two remaining animals (Y186 and W918) were sacrificed for animal welfare reasons and severe diarrhea. One of them (W918) also had a hemorrhagic colitis related to cytomegalovirus infection and acute pneumonitis. The terminal blood culture in this animal was found positive for pneumococcus. In three cases, fibrin deposition and thrombosis were present in the explanted xenografts, in agreement with the previously proposed criteria for defining AHXR (Figure 2; 19). Furthermore, no histological signs compatible with DIC could be seen in any of the organs examined at autopsy (Figure 3). We observed six rejection episodes in the three longest-surviving animals treated with 3–5 doses of 15 mg/kg i.v. methylprednisolone.

Coagulation studies

aPTT, fibrinogen, prothrombin and Factor X

After an initial prolongation in the perioperative period, aPTT levels tended to stabilize within normal ranges in the longest-surviving animals (Figure 4A). In all cases, a progressively longer above-normal aPTT was invariably associated with poor outcome (even in the longest survivors), but aPTT prolongation could never be correlated with a significant reduction in fibrinogen (Figure 4D). Indeed, fibrinogen levels consistently remained greater than the lower limit of the normal range in all animals during follow up. In fact, after an initial perioperative drop, fibrinogen rose immediately after transplantation, reaching normal values (> 200 mg/dL in all tests) by the end of the first or second week and remaining high even in the late survival period. In addition, prothrombin antigen levels tended to remain greater than 70% in the three longest-surviving animals, rapidly increasing at the time of euthanasia in all but one case (Figure 4B). Factor X levels roughly mimicked the trend of prothrombin Ag, but the values were more stable than prothrombin Ag and remained within pretransplant ranges (Figure 4C) in the three longest-surviving animals.

Physiological clotting inhibitors

Pre-transplant AT activity was between 107 and 150% of the standard control samples. Figure 5(A) shows AT activity levels in the five animals. Persistently reduced AT levels on postoperative days 1–3 was associated with early poor outcome (animals Y034 and W922). Antithrombin levels returning to normal values by day 5 appeared to be a good indicator of prolonged xenograft survival. The three animals surviving the longest had physiological or supra-physiological levels of AT from day 4 onwards. Thus, no evidence of severe AT consumption coincided with AHXR.

Protein C Ag levels are shown in Figure 5(B). After an initial drop, these levels only returned to normal by day 5 in the two animals surviving the longest, both of which revealed a trend towards a normalization of PC, which again suggests that there is no severe consumption of this inhibitor in AHXR.

Of the two plasma PS components analyzed (total and free; Figures 5C,D, respectively), total PS Ag levels showed an initial drop followed by a substantial rise, observed in all animals between days 1 and 3. An early, rapid recovery of total PS Ag to normal plasma levels was associated with a prolonged survival. Free PS Ag levels presented with a similar initial behavior to total PS Ag, but substantially higher levels were seen in all animals soon after transplantation regardless of outcome. All animals also had normal or higher levels of free PS Ag by day 3, irrespective of outcome. Neither total PS nor free PS Ag appeared to drop in association with AHXR.

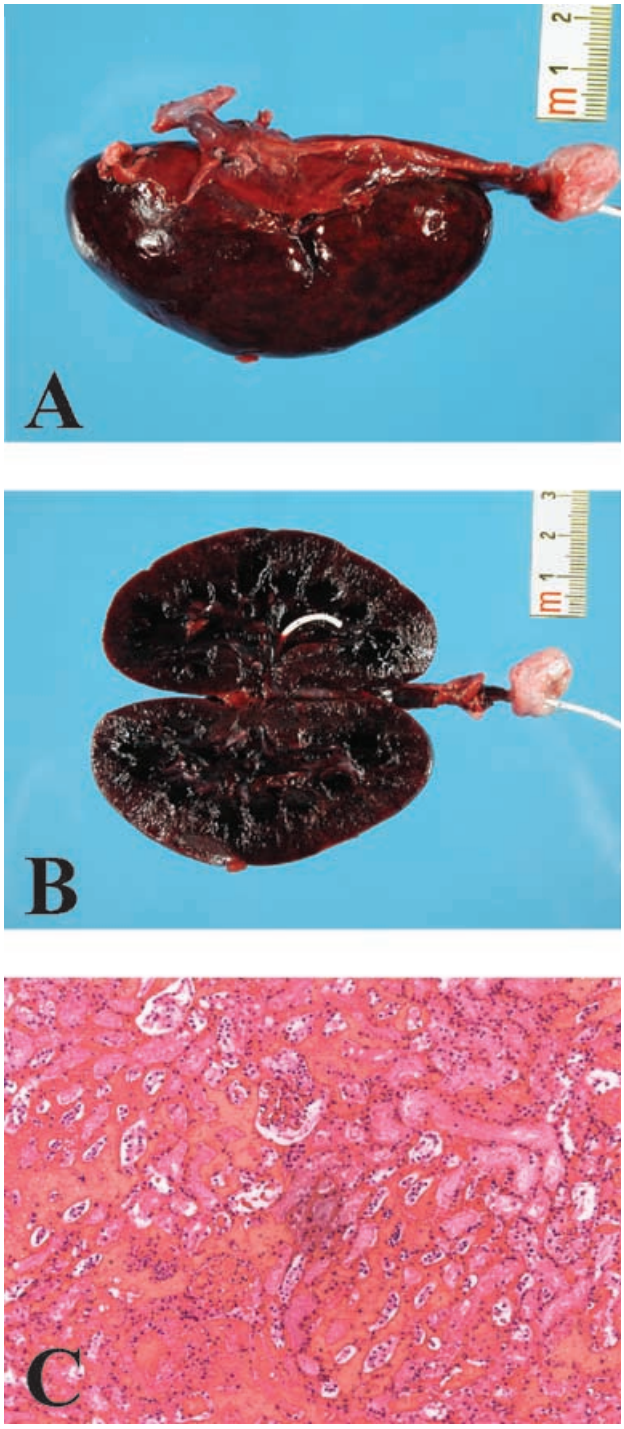

Figure 1: Gross findings and histological changes at post mortem in the xenograft of animal Y034. Note that the kidney was frankly necrotic at the time of autopsy (A,B). H&E staining shows extensive hemorrhage, tubular necrosis and mild infiltration of neutrophils (C; original magnification, ×25). In this case hyperacute rejection (HAR) could not be ruled out.

Platelets

Figure 4(E) shows the platelet counts for the five animals. Platelet counts varied considerably in the three longest-surviving animals, remaining between normal and high even when AHXR occurred. In contrast, the platelet counts dropped rapidly in the two animals that survived for less than 1 week. The platelet counts never reached values lower than 130 000 cells/mL, however.

Neither the fibrinogen levels (see above) nor the platelet counts were markedly reduced in the animals that had AHXR at the time of euthanasia.

D-dimer

D-dimer levels are shown in Figure 6(A). Pretreatment levels were between 508 and 942 ng/mL. The levels increased considerably during the induction phase but were only moderately elevated in the three animals analyzed on the day of transplantation. Immediately after transplantation, D-dimer levels increased rapidly to greater than 3000 ng/mL and remained consistently greater than these levels for the whole follow-up period in all animals, irrespective of outcome.

TAT and F_{1+2}

Thrombin-antithrombin and F_{1+2} levels, as markers of thrombin generation, are shown in Figures 6(B,C). During the induction phase, an increase was seen in both markers in some cases. After transplantation, F_{1+2} increased (as expected) to higher than the basal levels. After an initial rise postoperatively, F_{1+2} levels dropped and reached a nadir around days 2–3 in all animals except Y034. Subsequently, there was a substantial increase in three of the four surviving animals from days 3–4 onwards and these levels remained consistently high throughout the follow up. In contrast, the longest-surviving animal exhibited very modest and sporadic increases in F_{1+2} during the post-transplant period.

After the transplant, rises in TAT levels were seen in all animals and often correlated with poor outcome. The three longest-surviving animals had normal TAT levels between days 3 and 5, then a peak in TAT levels was observed between days 5 and 9, followed by a return to normal by day 9 in the two animals that survived the longest. As with F_{1+2}, TAT levels remained in the normal range for the whole post-transplant period in the longest-surviving animal.

Increases in APA titers were found to immediately precede rising thrombin activation markers (Figure 6D) and graft function impairment (Figure 6E). No findings compatible with the presence of fragmented red blood cells (schistocytes), characteristic of hemolytic microangiopathy, were observed in the blood smears from these animals. Postoperative hemoglobin levels are given in Figure 6(F).

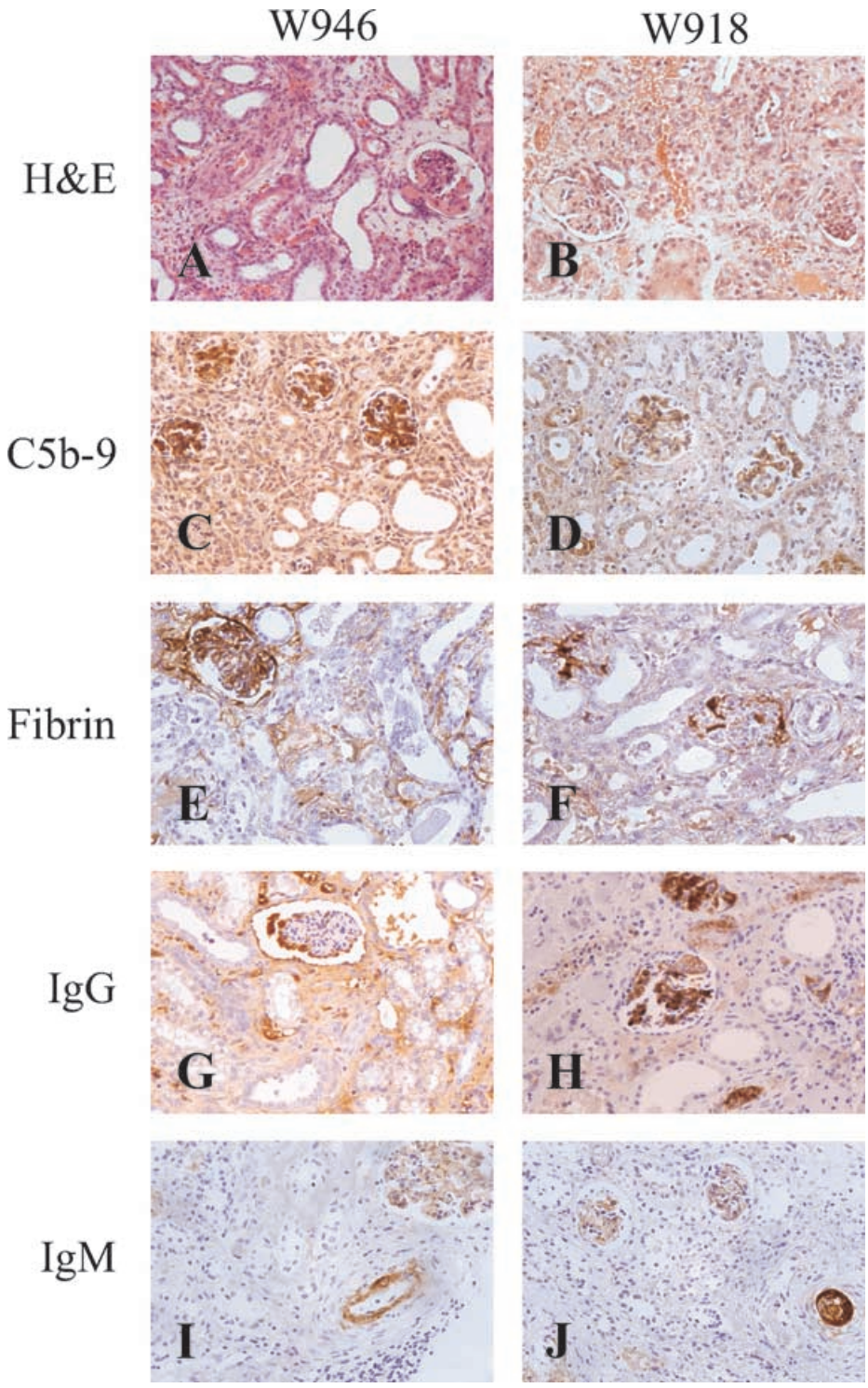

Figure 2: H&E stains (A,B) and immunopathology (C–J) of hDAF kidneys removed at autopsy from the two longest-surviving animals. Significant deposition of complement component C5b-9 (C,D), fibrin (E,F), IgG (G, H) and IgM (I,J) could be seen in both xenografts when explanted in the presence of AHXR (original magnification, × 50).

Discussion

In the last few years, considerable attention has been paid to characterizing the clotting profile in primate recipients of porcine xenografts (1–5,20). To date, most of the available information has been generated primarily in the baboon as the recipient species and the alterations observed, ranging from different degrees of coagulopathy to overt DIC, have generally been related to xenograft rejection (1,2,5). It is not yet clear, however, whether these

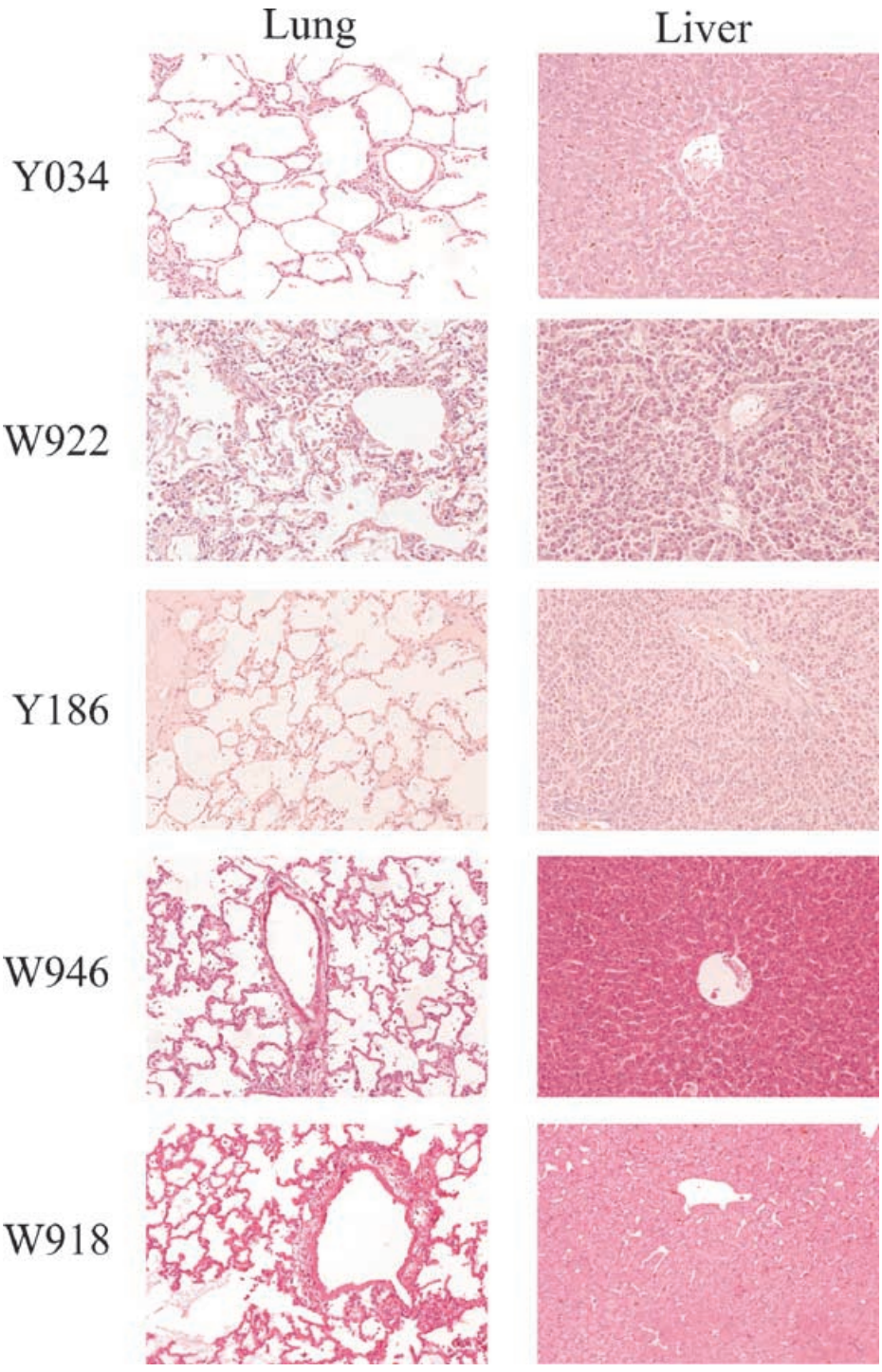

Figure 3: H&E stains in the recipients' lungs and livers at autopsy. Only mild histological changes compatible with interstitial liver edema and lung congestion were usually observed. Animal Y034 also presented a mild pigmentation of Kuppfer cells in the liver. In addition, a mild discoloration of the cytoplasm was observed in the liver of the two longest-surviving animals (W946 and W918) (original magnification, × 25).

coagulopathies (e.g. thrombocytopenia, clotting factor consumption or DIC) are complications relating to the rejection process, or whether they may even be responsible for inducing, or at least exacerbating rejection. Some investigators have recently attempted to analyze the correlation between changes in the clotting cascade and clinical outcome of the xenograft. They report that DIC can occur in the absence of AHXR (21) and suggest that porcine cytomegalovirus infections may predispose to coagulopathy, although DIC may occur independently of such infections. There is also evidence that many animals with graft rejection do not die of DIC (10,12,18,22–25) and even animals without DIC may have alterations in the coagulation system (4,10).

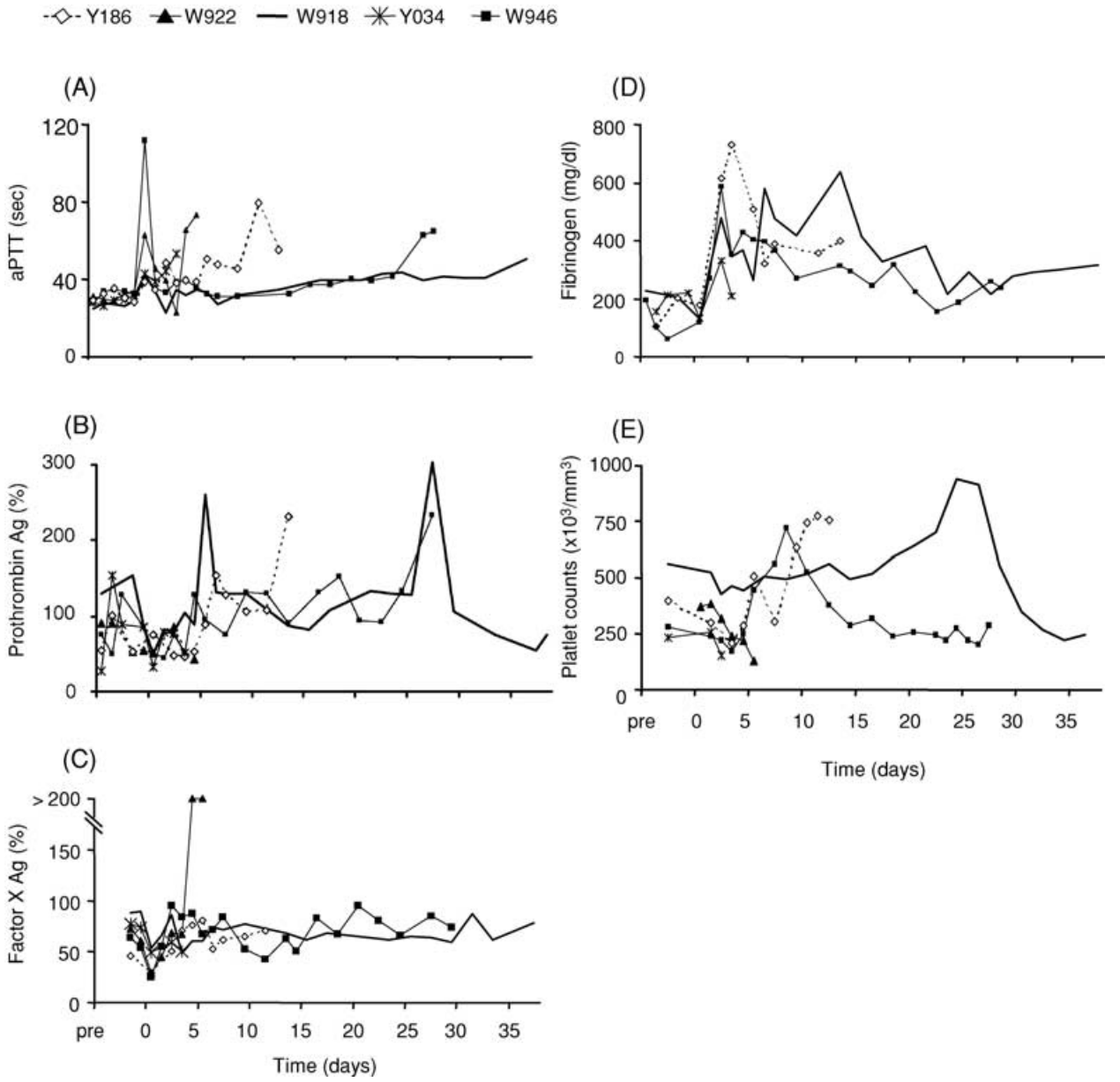

Figure 4: Daily activated partial thromboplastin time (aPTT) (A), prothrombin (B), factor X (C), and fibrinogen (D) levels and platelet counts (E) in the five xenograft recipients.

In our previous studies using an hDAF pig-to-cynomolgus monkey renal xenotransplantation model, DIC definable as a laboratory or clinical entity (based on histological or macroscopic findings) was only rarely observed, though changes in coagulation parameters (platelet counts or FDP) were common (10). As a full interpretation of these findings was hampered by an incomplete evaluation of the clotting profile, we extensively studied the coagulation variables in our pig-to-cynomolgus monkey model. In addition, to gain insight on whether the coagulation parameters investigated could predict graft outcome, we tried to correlate their postoperative levels with graft function and survival.

To this end, a standard immunosuppressive protocol was administered to our recipients in this series and coagulation parameters were evaluated pre- and postoperatively throughout the postoperative period.

The data generated in the present study confirm that AHXR is not associated with clear evidence of overt DIC in our

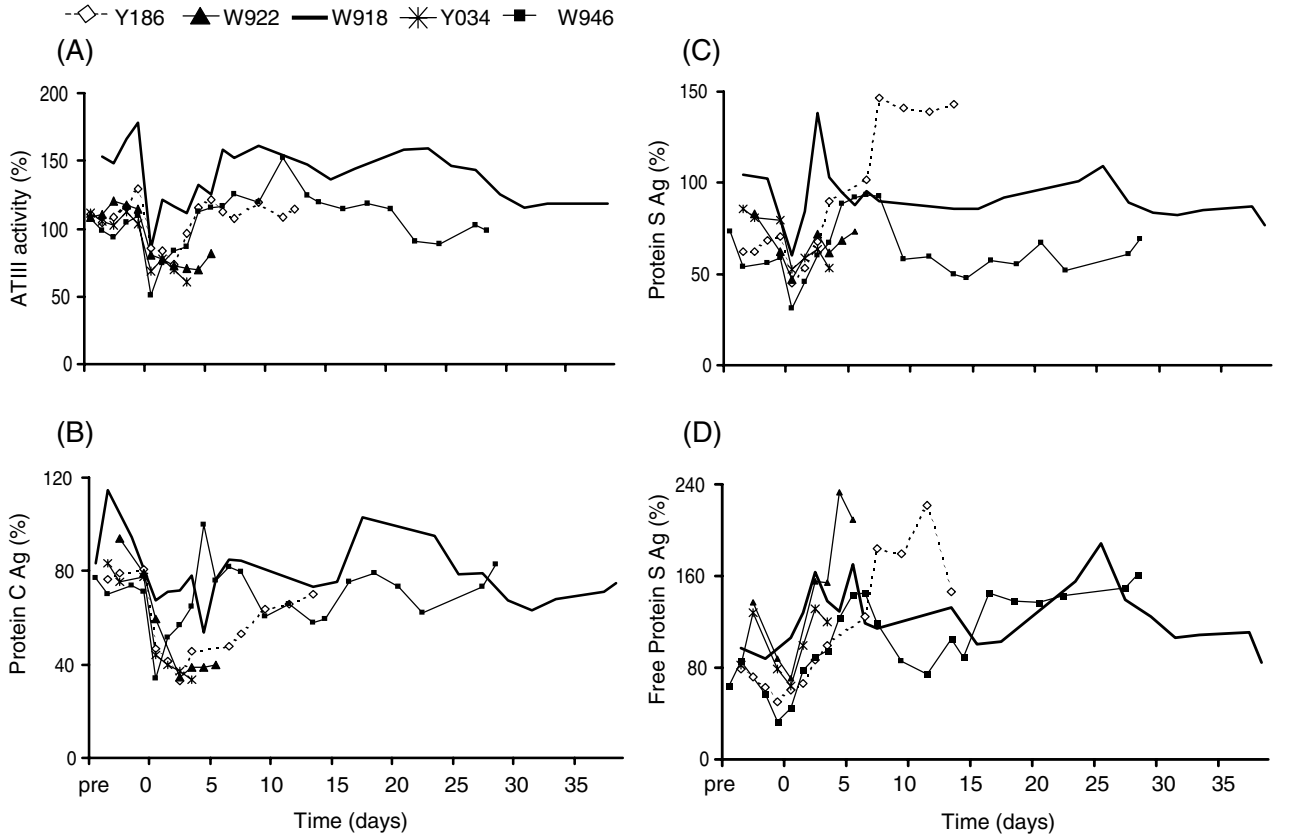

Figure 5: Daily levels of physiological clotting inhibitors in the five xenograft recipients: AT activity (A), protein C antigen (B), protein S antigen (C) and free protein S antigen (D).

pig-to-primate model (10), although it is undeniable that variable degrees of alteration in the coagulation profile were observed in all the animals studied. Several of our findings deserve careful thought, however. First, no consumption of major physiological clotting inhibitors (AT, protein C and protein S) could be demonstrated in these xenograft recipients. While this is rather surprising – as the consumption of physiological clotting inhibitors, and particularly AT, is one of the key criteria for the laboratory diagnosis of DIC in humans (17,26,27) – it does not rule out the possible existence of a low-grade consumptive coagulopathy and more effective compensatory mechanisms in the primate species considered. Second, there was no convincing evidence that the variations in platelet counts and fibrinogen levels seen in our study were related to the progressive coagulopathy usually observed in DIC. On the contrary, persistent or raised fibrinogen levels and platelet counts were seen in the longest-surviving animals. It should be noted that a progressive and severe drop in fibrinogen levels and platelet counts consistent with DIC was often observed in pig-to-baboon xenotransplantation and was reversed only after graft excision (1,4,28). This did not occur in our model. Although an acute-phase reaction after surgery may partially justify the presence of sustained levels of fibrinogen [see (1)] and platelet counts even in the presence of DIC, it seems unlikely that an overt DIC process could have lasted for lengthy periods of time (up to 37 days in the longest-surviving animal) without determining a marked consumption of these factors. Once again, however, these findings do not rule out the possibility of a concomitant, but well-compensated consumptive coagulopathy, or the risk of this precarious situation precipitating, at some stage, into overt DIC. Third, markers of thrombin generation, such as F_{1+2} and TAT, are likely to rise in the presence of massive clotting cascade activation during DIC (17,26,27). These findings must be interpreted with care, however, as F_{1+2} and TAT elevations are often seen in many conditions other than DIC, in which the hemostatic/thrombotic equilibrium is disrupted: hypercoagulability states represented by inherited deficiencies (as seen in humans) or acquired conditions (such as surgery, as in our model) may account for different degrees of thrombin activation (29,30). Thrombin generation can be counterbalanced by clotting inhibitors and AT is usually consumed and found to be severely reduced in overt DIC (17,27). That is why AT concentrates were originally given to humans with DIC in an attempt to restore normal levels in the plasma (27,30). In our pig-to-primate model AT was not reduced even in the presence of significant thrombin generation. Combined with the lack of a progressive and irreversible

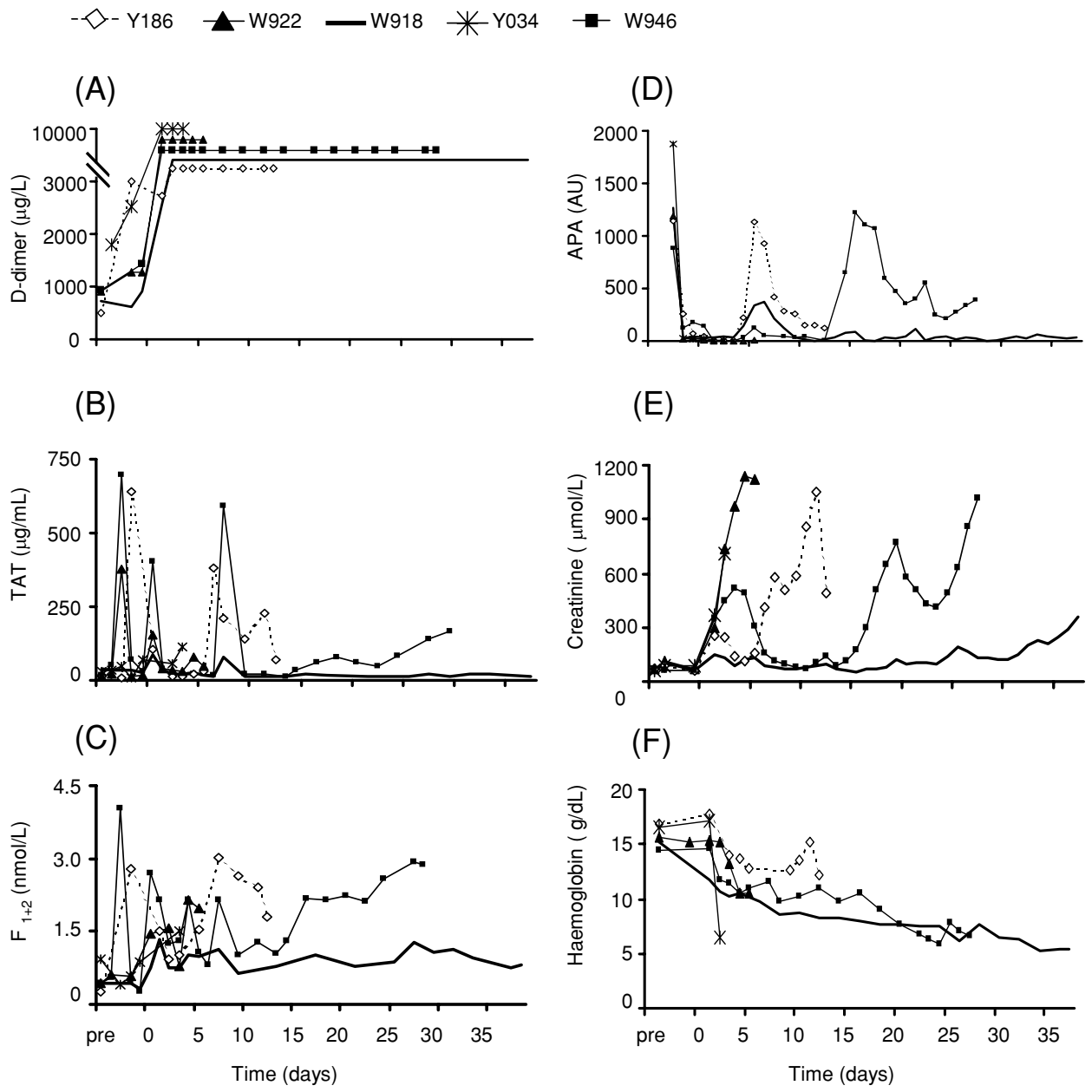

Figure 6: Daily levels of D-dimer (A) thrombin-antithrombin (TAT) complexes, (B), F_{1+2} (C), hemolytic antipig antibody (APA) levels (D), creatinine (E) and hemoglobin (F) in the five xenograft recipients.

consumption of fibrinogen and platelets, this finding is not in favor of a diagnosis of overt DIC in our model.

Fourth, high D-dimer levels have previously been interpreted as a potential marker of DIC (17,26,27). Like high F_{1+2} and TAT levels, increased D-dimer levels are also observed in uncomplicated surgery (31). The utility of elevated D-dimer levels in the diagnosis of DIC may consequently be limited, particularly if there is no return to basal (normal) levels after the surgical procedure. This seems to be the case in our and other pig-to-primate models, where persistently and markedly elevated D-dimer levels were observed after surgery and throughout the postoperative period (1,3,5). Thus, we favor the hypothesis of a compensated consumptive coagulopathy in our (longest-surviving) animals that may account for the formation and deposition of a moderate amount of fibrin in the transplanted organ. Even in the longest-surviving animals, moreover, neither the macroscopic nor the histological findings at post mortem showed the multiorgan involvement usually observed in overt DIC. Disseminated intravascular coagulopathy could not be ruled out in animal Y034, which died

on day 2 (possibly having developed hyperacute rejection). The liver and lung specimens from this animal were normal at histology, however.

The absence of overt DIC in our model is unlikely to be related to the use of high-dose cyclophosphamide. Indeed, Buhler and colleagues used even higher doses of cyclophosphamide perioperatively and recorded DIC in three out of five cases (4).

Clinical and laboratory findings similar to those reported here have recently been recorded at our laboratories in xenografted monkeys administered the same immunosuppressive regime but without exposure to carbon monoxide (Cozzi, unpublished observations), suggesting that the absence of overt DIC in our model is not related to any modulating effect of CO on vascular inflammatory responses. On the other hand, in the absence of data on the effect of a brief induction treatment with GAS 914 like ours in baboons, we cannot say whether the use of this polymer has any long-term protective effect on these monkeys.

On the whole, these data indicate that, in our model at least, the coagulopathy associated with AHXR is not consistent with overt DIC. Assuming that this non-DIC-related coagulopathy can exacerbate AHXR, the strategies normally used to prevent DIC may consequently be inappropriate for preventing or mitigating this form of rejection. On the other hand, as fibrin deposition was detected in the explanted xenografts of the three longest-surviving animals, approaches capable of preventing its deposition may indeed help to prevent the onset of AHXR.

The thorough evaluation of the coagulation parameters undertaken in this work differs in many aspects from previous studies. We focused our attention on the cynomolgus monkey as the recipient species. The immunosuppression we used was different from that used by other groups and we also evaluated different clotting parameters, particularly clotting inhibitors. Indeed, the lack of such measurements in the other studies published to date makes it impossible for us to compare our findings with those of other investigators, or identify any species-related differences. Finally, the assays used for the laboratory investigations differed, often substantially, between centers. In our hands, for instance, only one of the two commercially available kits we tested for the immunoenzymatic determination of D-dimer proved suitable for the species considered in this study.

No definite cases of hyperacute rejection were observed in our series, so we were unable to draw any conclusions regarding the coagulopathy potentially accompanying this form of rejection.

Our findings indicate that the xenograft recipient's failure to recover protein C, total protein S and AT levels rapidly is invariably associated with a poor outcome, and the same goes (even in the long term survivors) for a progressive prolongation of the aPTT beyond normal ranges. In our hands, the levels of procoagulant and physiological anticoagulant factors, D-dimer and platelet counts did not correlate clearly with AHXR and enable us to predict impending rejection. However, throughout the study increases in APA titers often coincided with, or immediately preceded, a rise in thrombin activation markers and an impaired graft function, suggesting that immunological events may underlie the activation of the clotting cascade. At this stage, we cannot rule out the hypothesis advanced by others (1) that complement activation (demonstrated by C3b and C5b-9 deposition in grafts explanted with AHXR) may play a part in the activation of the clotting cascade, with the production of porcine tissue factor.

Moreover, the activity of the clotting cascade inhibitory system might be impaired even though the levels of physiological coagulation inhibitors were normal possibly as a consequence of molecular incompatibility between pigs and cynomolgus monkeys.

Unfortunately we were unable to assess the prothrombin time in this study, but it is worth noting that the levels of two vitamin K-dependent clotting factors normally correlating with PT values (factor II and factor X) remained substantially normal during the follow up in the longest-surviving animals.

In conclusion, this study demonstrates that AHXR in our pig-to-primate model is associated with various degrees of compensated consumptive coagulopathy. Evaluating early postoperative levels of clotting inhibitors may help to predict the clinical outcome of xenograft recipients, though no clear relationship could be found between coagulation parameters and graft outcome. To date, none of the immunosuppressive regimens adopted have been able to prevent the production of elicited antixenograft antibodies observed in AHXR. It consequently remains to be seen whether the prevention of AHXR will involve interfering with the production of antipig antibodies, inhibiting the coagulation cascade, or both.

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