Hemostatic changes and antithrombotic management in kidney transplantation

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Summary, General Discussion and Future perspectives

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SUMMARY

This thesis discusses 1) thrombotic complications and the current evidence and controversies considering antithrombotic management in kidney transplantation, 2) hemostatic changes in kidney transplant recipients and 3) recent international developments in transplant research.

Chapter 1 provides a general introduction of hemostasis in physiological conditions and the pathological changes in hemostasis in end-stage renal disease (and eventually in kidney transplant recipients) that cause the paradoxical situation of a simultaneously increased thrombosis and bleeding risk. It also outlines the various antithrombotic agents and their clinical use in kidney transplantation and describes surgical options for the treatment of renal graft thrombosis and postoperative bleeding.

In **Part I, Chapter 2**, we performed a retrospective analysis of 2000 kidney transplant recipients transplanted between 2011 and 2016 in the two largest transplant centers of the Netherlands. This analysis showed a 1.1% incidence of thromboembolic complications and a 4.4% incidence of significant postoperative bleeding in the first week after transplantation, with a peak in the first two days. Obesity and multiple renal arteries were transplant characteristics that increased the risk for thrombosis, whereas cardiovascular disease and preemptive transplantation were independent risk factors for postoperative bleeding. Regarding antithrombotic management, we observed that intraoperative administration of unfractionated heparin is safe, while postoperative heparin infusion or the continuation of vitamin K antagonists clearly increased the risk for significant postoperative bleeding. It was intriguing that antithrombotic management already differed between the two centers that participated in this retrospective analysis. Therefore, in **Chapter 3** we sought to obtain a European view on the different thromboprophylactic strategies used in kidney transplantation. In an online survey, addressed to kidney transplant professionals in Europe, we observed that some form of thromboprophylaxis is preferred by 78% of respondents, and 32% administer unfractionated heparin intraoperatively. However, timing, agent and dosage varied considerably, which is most likely due to the paucity of high-quality studies.

Part II focused on hemostatic changes in kidney transplant recipients and during organ preservation. As mentioned before, centers apply different antithrombotic strategies based on known risk factors. In the University Medical Center Groningen, the protocol involves the administration of 5000 international units of unfractionated heparin prior to clamping of the vessels during kidney transplantation. However, this only concerns preemptive kidney transplant recipients due to a presumed increased bleeding risk for dialysis-dependent recipients. Therefore, in **Chapter 4**, we questioned whether this distinction was justified, as recent insights have shown that dialysis-dependent recipients are not only at increased risk

for bleeding but also for thrombosis. We performed extensive hemostatic profiling of plasma samples from the VAPOR-1 trial, which included kidney transplant recipients and their healthy living donor. Not only had preemptive and non-preemptive (dialysis-dependent) recipients a comparable hemostatic state prior to transplantation, they also showed a hypercoagulable state compared to healthy donors, as evidenced by increased plasma levels of platelet factor 4, prothrombin fragment 1+2 and D-dimer, and impaired fibrinolysis, as evidenced by longer clot lysis times.

Chapter 5 describes fibrin depositions and microthrombi in human kidneys after static cold storage (SCS) preservation. The presence of microthrombi in deceased donor kidneys was not unexpected, especially since brain death and donation after cardiac arrest have been shown to activate coagulation and dysregulate fibrinolysis. Remarkably, kidneys from living donors also exhibited a steady increase in microthrombi over the course of the transplantation procedure. The increase in microthrombi in kidneys from living donors may be explained in part by manipulation of the kidney during hand-assisted donor nephrectomy. However, it is also expected that microthrombi results from activation of hemostasis as a consequence of ischemia-reperfusion injury. As microthrombi and fibrin depositions cause local perfusion disorders, and may function as an accumulation site for larger thrombi, graft function may be threatened.

For kidney transplantation in the Netherlands, hypothermic machine perfusion (HMP) has been the gold standard for preservation of deceased donor kidneys since 2018. Although HMP has been proven superior to SCS, the exact mechanisms underlying this superiority have only been studied to a limited extent. In **Chapter 6** we hypothesized that HMP serves as a prolonged and better flush, facilitating removal of microthrombi that have formed due to brain death, withdrawal of life support or cardiac arrest. Viable porcine kidneys from a local abattoir were exposed to 35 minutes of warm ischemia and flushed with heparinized University of Wisconsin-solution to mimic a donation after circulatory death (DCD)-model. Afterwards, they were assigned to either SCS, HMP or HMP with the addition of alteplase. Analyses of the perfusate, taken at several timepoints, showed that while the endothelium was not overtly activated by HMP, displayed by low levels of Von Willebrand factor and absent staining of VWF in biopsies, D-dimer levels were significantly higher in machine perfused kidneys, indicating increased clot breakdown. Histological evaluation also showed that machine perfused kidneys had numerically less microthrombi compared to SCS. The addition of a thrombolytic agent to HMP did not appear to be of added value.

Part III: In the last century, major breakthroughs in solid organ transplantation have followed each other in rapid succession. Meanwhile, the scientific community is changing as well, and, in recent years, several positive and negative changes have occurred, which affect transplant research as well.

In 2016, the United Kingdom (UK) held a referendum on whether to leave the European Union and with 52% of the votes, the leave-party won. "Brexit" was imminent and concerns were raised regarding the potential impact it would have on the National Health Service and the UK's scientific community. In **Chapter 7,** we performed a bibliometric analysis to assess the UK's involvement in solid organ transplantation research, especially in relation to the European Union and their funding. Using data from the Publications Office of the European Union (CORDIS) database, it was clear that the UK transplant community is highly dependent of EU-funding and international collaborations, as 20% of their publications involved international collaborations and almost 50% were funded by the EU.

In addition, over the years, open access publishing has been a growing method of publishing. In response to this development, some traditional journals have set up open access sibling journals. Apart from direct submission, these sibling journals provide the opportunity to offer transfer of manuscripts which they cannot publish for priority reasons. In 2015, the Transplantation Society and Wolters Kluwer established *Transplantation Direct* as the sibling journal of *Transplantation*. In **Chapter 8,** the destination of manuscripts offered transfer to Transplantation Direct between 2015 and 2019 was studied. Although many authors did not take up on this offer and sought publication in other traditional journals, it was shown that accepting transfer to a paired sibling journal offers a reliable and time efficient opportunity for authors to publish their research.

GENERAL DISCUSSION

For a long time, it was thought that kidney transplant recipients, and especially those who were dialysis-dependent at the time of transplant, have an increased risk of perioperative bleeding. However, it has also become widely acknowledged that these patients are also at risk of perioperative thrombosis due to various hemostatic changes caused by end-stage kidney disease (ESKD), dialysis and the surgical procedure.[1,2] Nowadays, renal graft thrombosis (RGT) is the main cause of early graft loss after kidney transplantation. Yet, research does not tend to focus on prevention of RGT. Owing to the paucity of studies on antithrombotic therapy in kidney transplant recipients, or ESKD patients for that matter, major thrombosis guidelines have to rely on low-grade evidence, resulting in weaker recommendations for prevention. Subsequently, this complicates clinical decision making, and results in varying approaches to antithrombotic management, even within individual centers, as shown by research presented in this thesis.[3]

In case of the University Medical Center Groningen, the absence of international guidelines has led to a protocol in which preemptive recipients do and dialysis-dependent recipients do not receive heparin intraoperatively, based on the historical belief that dialysis causes a bleeding diathesis. At the start of this PhD trajectory, we aimed to evaluate whether this distinction was indeed justified. Notwithstanding that we were unable to assess platelet function in stored plasma samples, a comprehensive analysis of functional hemostatic tests and markers of in vivo activation of coagulation, showed that the assumptions on which this protocol were based, were invalid.

Identifying the patient at risk of renal graft thrombosis

Only some recipients experience renal graft thrombosis, and over the years, several groups have tried to identify the patient at risk by studying known and unknown risk factors of thrombosis and bleeding in kidney transplant patients, compared to general surgery patients.[4–9] Unfortunately, most studies are underpowered to draw firm conclusions and high heterogeneity limits the possibility to perform thorough multivariate and meta-analyses, but several independent risk factors have been identified. Well-known, non-modifiable risk factors are: the extremes of age and renal atherosclerosis in both donor and recipient, diabetic nephropathy, past VTE events, and hemodynamic instability. Technical difficulty and longer cold ischemia (>24h) are independent risk factors as well, but surgical difficulty is highly operator-dependent and long cold ischemia can be actively avoided.[4,5] Importantly, although chronic kidney disease cannot be investigated as a potential risk factor in the kidney transplant setting (because all patients suffer from it), it was identified as an independent contributor to venous thrombosis in a surgical setting.[10]

While our analysis in Chapter 2 did not identify unknown *independent* risk factors of RGT, it does describe an apparent increased risk for patients with obesity (BMI>30 kg/m^2). A previous finding that high BMI protects against bleeding is supportive of this outcome.[9] Although BMI probably interacts with other patient characteristics, as was recently reported,[11] it is an important factor to include when identifying the patient at risk and for patient education, especially since there is a global rise in obesity. In 2022, more than 1 in 5 adult Dutch citizens were obese, and far more are overweight,[12] and in the last years, patients with high BMI are increasingly transplanted. Many centers use a BMI cut-off of 35 kg/m^2, and in the United States sometimes even 40 kg/m^2. When acquiring informed consent for the transplantation, preoperative consultations now often specifically mention postoperative bleeding as one of the main complications, apart from rejection. The results presented here suggest that in obese patients more emphasis should be put on the occurrence of renal graft thrombosis, rather than postoperative bleeding. Furthermore, work in this thesis showed that all kidney transplant recipients, regardless of dialysis modality, have a comparable and procoagulant hemostatic state prior to transplantation. In addition, preemptive recipients show a higher risk of postoperative bleeding, independent of the existing heparin-protocol in place, most probably related to circulating uremic toxins. Deciding on the right antithrombotic treatment is thus far more complex than just a preemptive or dialysis-dependent state, and all risk factors should be reckoned with before implementing new strategies, such as protocolizing intraoperative administration of 5000 IU of heparin to all recipients.

Antithrombotic therapy and its role in prevention of renal graft thrombosis

Antiplatelet and anticoagulant therapies are, in accordance with their function and purpose, known risk factors for bleeding complications. However, as mentioned before, reports investigating the actual risks or benefits of continuing prophylactic or therapeutic antithrombotic therapy in kidney transplant recipients are scarce and there are no randomized-controlled trials that assess the efficacy on preventing RGT. The literature that is available, including our own analysis in Chapter 2, is more focused on the (un)safety of antithrombotic drugs, due to the higher incidence of bleeding complications, than the beneficial effects of preventing thrombosis. Additionally, different definitions, e.g. what is considered significant bleeding, are used in retrospective studies, which makes it difficult to make comparisons. However, management options for bleeding are readily available, whereas RGT almost always results in graft loss. Therefore, by combining general practice of subject experts, as obtained in this thesis, and the existing literature we are confident to put up a framework to work with:

Antiplatelet therapy

In CKD patients, thromboembolic prevention is mainly managed with acetylsalicylic acid, its derivative carbasalate calcium (best known as Ascal®), and $P2Y_{12}$-inhibitors (including clopidogrel and ticagrelor). However, ESKD (CKD stage 4-5) patients are often underrepresented

in studies investigating antiplatelet therapies.[13] Some patients receive dual antiplatelet therapy, in which case most transplant centers discontinue one of these prior to kidney transplantation, but single antiplatelet therapy is generally accepted (Chapter 3).[3] Hernandez *et al.* reported 6% more bleeding complications in patients on antiplatelet therapy, but emphasize the importance of additional risk factors, such as complicated bench (ex-vivo) surgery.[14] In Chapter 2, our group analyzed and published a series of 2000 kidney transplant recipients and found no increased bleeding risk when antiplatelet therapy was continued.[15] Eng *et al.* also found no effect of preoperative clopidogrel on bleeding risk, although this might be biased by low number of events and users (2 events out of 10 users, in a total cohort of 327 patients).[16] As mentioned above, continuation of antiplatelet therapy must be weighed against other risk factors such as preexisting cardiovascular disease or expected high blood loss.[15]

Heparin

Ng and co-workers investigated different heparin protocols in the post-kidney transplant period.[17] Although therapeutic use of unfractionated heparin showed an increased incidence of significant postoperative bleeding, administration of a prophylactic dose (5000 IU s.c.) was not associated with increased bleeding. This finding is consistent with our analysis in Chapter 2 in which intravenous administration of 5000 IU was safe. In Chapter 5 we also showed that kidneys from patients treated with 5000 IU unfractionated heparin intravenously, prior to reperfusion, had less microthrombi compared to the no-heparin group.[18] Although its effectiveness remains unclear, it appears that prophylactic doses of unfractionated heparin (e.g., 5000 IU intraoperatively) can be safely used without increasing the incidence of significant postoperative bleeding.[15,17,19]

Vitamin K antagonists

Several studies have concluded that vitamin K antagonists are an independent risk factor for severe postoperative bleeding, with a nearly 7-fold increased risk,[15,16,20] although one report found no effect of warfarin on bleeding.[16] For ESKD patients the international normalized ratio (INR-) target should be lowered, as reduced kidney function is associated with significantly increased bleeding risk.[21] Furthermore, VKA are associated with progression of vascular calcification through inhibition of vitamin K-dependent matrix gla protein (MGP), which inhibits vascular calcification.[22] Many kidney transplant recipients are on a VKA-regimen due to cardiovascular comorbidities and most transplant professionals discontinue its use or bridge with heparin prior to transplantation, which in deceased donor KTx, is not always possible from a time perspective.[15] However, patients on VKA usually have a vital indication, e.g., high CHA_2DS_2-VASc prediction score, which makes it difficult to adjust the treatment or dosage.

Direct oral anticoagulants (DOACs)

For CKD stage 1 to 3 DOACs are considered safe, but for CKD 4 or 5 results are poor and conflicting.[23] The half-time of dabigatran, a direct thrombin inhibitor, is severely influenced by renal function as this compound is 85% cleared by the kidney. Therefore, dabigatran is contraindicated in CKD patients. Direct factor Xa inhibitors (Rivaroxaban, Edoxaban, Apixaban) have a half-life between 5-12 hours and are only partially cleared through renal excretion, but it is expected that this half-life is prolonged in severe CKD stages as well.[24] For now it appears that DOACS do not have a place in the antithrombotic management strategy for kidney transplant recipients and especially not in the prevention of RGT.

Microthrombi – The smallest things do matter.

As mentioned above, Chapter 5 showed that microthrombi are present in human donor kidneys after static cold storage preservation and increase in number after reperfusion. In Chapter 6 we showed that HMP facilitates clearance of microthrombi in a DCD porcine model. Historically, it is often debated whether the existence of microthrombi has clinical implications, but recent studies showed that microthrombi cause local perfusion disorders and that thrombolysis or inhibition of coagulation improves local perfusion and graft function.[25–29] It thus appears that microthrombi are formed in deceased donor organs prior to or during the initial cold flush, then again after reperfusion, and are not of trivial importance, based on the improvement in local perfusion as described above. One of the major improvements after the implementation of HMP was observed in delayed graft function (DGF) rate and early graft function, while, owing to the increased use of marginal kidneys for transplantation, DGF incidence has gone up.[30] Both these outcomes have been mentioned repeatedly as a potential consequence of microthrombi formation.[30,31] Thus, hypothetically, the improved outcomes after HMP could simply be the result of a better flush out of microthrombi. However, we have also shown that with the current addition of heparin to the initial cold flush, microthrombi still form during the cold flush and that they are not removed completely. Probably the only way to effectively prevent microthrombi formation during preservation is to anticoagulate the donor.

Academic developments in the recent years

As the world evolves, a lot can change during the course of a PhD and sometimes it is good to look beyond the general scope of a thesis. Apart from research aimed at prevention of thromboembolic complications of the renal graft, this thesis also contains two bibliometric analyses. These were performed to answer questions regarding the academic environment in which transplantation research takes place, in light of recent academic developments that might affect transplantation research.

Bibliometric analyses may provide insight into the terms on which research has been conducted and expose selection bias, which potentially influence research results, refraining us from certain advancements. For example, a recent publication in the "Lancet" from Santema *et*

al. showed that women require lower doses of medication for heart failure with reduced ejection fraction.[32] The reason why this was breaking news, was because studies investigating the effects and clinical application of angiotensin-converting-enzyme inhibitors, angiotensin-receptor blockers, and β blockers were mainly conducted on men, and thus women had been "overdosed" for years. Bibliometric analyses can play an important part in identifying and uncovering such cases where there is a high risk of selection bias. One example of such a bibliometric analysis was performed by the United States Food and Drug Administration, which showed that of all participants in clinical trials between 2015 and 2019, only 11% were Asian and 7% were Black,[33] whereas Asia alone accounts for nearly 60% of the world population. Caution is thus needed when using the results of these clinical trials for Asian or Black patients. Another example was performed by our own group and showed that there is a persisting gap in citations and funding for female authors of transplant-related research. Not only are female transplant scientists less cited and less funded, they also remain underrepresented when set against the gender ratio in general medicine and transplant surgery.[34] The heart failure medication example above might have been prevented by a more diverse research workforce, as a diverse healthcare team has been shown to improve patient care and outcomes.[35]

However, bibliometric analyses can also tell us more about how a scientific community is functioning. How open and transparent it is and where it can still improve. It can also guide in decision-making through estimating the effect of a change by looking at its historical proceeds.

Brexit

As of January 31st, 2020, the United Kingdom left the European Union. In 2019, we described 3 possible scenarios: (1) a withdrawal agreement (deal), (2) no deal; or (3) a revoke of article 50 (no Brexit).[36] A withdrawal agreement was established and EU-funding and collaborations remained accessible until December 31st 2020. However, arrangements regarding Horizon Europe, the EU grant funding scheme, had yet to be made. On December 24, 2020, it was released that via a Trade and Cooperation Agreement (TCA), the UK and EU made sure that UK entities are able to respond to all calls in Horizon Europe with exception of the European Innovation Council fund.[37–39] Due to delays in the formalization of the agreement, the UK government established guaranteed funding of successful applicants to Horizon Europe for the first 2 waves.[40] Although it cannot match the level of symbiosis the UK had when it was a member-state of the European Union, the TCA allows, amongst other things, to participate in EU programs, such as Horizon Europe, in a broader aspect than traditional free trade arrangements.[38] For transplantation research in particular, this is of great importance, considering the tremendous contribution to transplant science UK researchers make every year, as is also reflected in Chapter 7.

The birth of open access sibling journals

In the recent years, there has also been a trend of traditional journals establishing sibling open access journals, in response to the call of the European Union, the World Health Organization

and other large governmental bodies. In Chapter 8, we showed that many authors still seek the comfort of a traditional journal, when their manuscript submitted to *Transplantation* was rejected but offered transfer to its sibling journal *Transplantation Direct*. We discussed several reasons that could influence this decision. Although there is a growing recognition that open access is the future, there are still many barriers to overcome, one of which is the paywall. One way or the other, someone has to pay to make the research accessible to the public. In the case of open access, it is the author. This makes it less attractive for authors in developing countries where universities do not have agreements, but it also becomes increasingly expensive for libraries in wealthier countries to establish deals with all publishers. Furthermore, in this open access system in which the author pays, the risk of financial bias is lurking, potentially threatening the quality of the peer-review process, especially by a growing group of predatory journals. The most radical decision would be to make all research free i.e. through funding via governmental funding schemes such as Horizon Europe.

Although the European Commission mandated all science projects funded through Horizon Europe to be made open access, they do not yet offer to cover open access fees to field specific journals. They did however, establish a collaboration with F1000 Research to cover peer-reviewed open access publishing of Horizon Europe grantees in Open Research Europe.[41]

Conclusion

Although this thesis does not provide definitive answers on how to prevent renal graft thrombosis and other thromboembolic complications in kidney transplant recipients, we were able to get a better understanding of the altered hemostatic state in kidney transplant recipients and provide the groundwork for future research. Most importantly, it has become clear that a one-size-fits-all principle cannot be applied to antithrombotic treatment in kidney transplant recipients. All available anticoagulant drugs are more or less associated with bleeding, or, if not, their effect on thrombosis reduction has not yet been sufficiently demonstrated, even if they appear safe. However, postoperative bleeding is readily detectable and several treatment options are available, whereas renal graft thrombosis usually results in graft loss. Overall, there seems to be some evidence to indicate that intraoperative administration of unfractionated heparin prior to reperfusion is safe and can aid in decreasing the thrombotic risk.[15,18,19,42] Antiplatelet therapy can be continued safely with minimal risk of postoperative bleeding.[15]

Furthermore, in order to tackle problems such as renal graft thrombosis, it is important to have an optimal research environment. Academic developments directly and indirectly impact the results generated for clinical research objectives. There is a growing recognition that it is important to facilitate access to transplantation research in the broadest sense. Transplantation research will benefit from the best possible circumstances: free open access, unrestricted international collaborations and research targeted to all members of society by a diverse workforce, that is assessed and rewarded based on the quality of their research.

FUTURE PERSPECTIVES

This thesis highlights the complex changes in the hemostatic system of kidney transplant recipients and the deleterious effect of renal graft thrombosis warranting appropriate antithrombotic therapy. For the near future, a Delphi study, in which kidney transplant experts draft a guideline based on a general consensus, could offer a solution for the center-wide differences in antithrombotic therapy. That is, at least until adequate randomized controlled trials or well-powered retrospective studies are conducted on which robust recommendations can be based. In the end, we do not only want to prevent RGT, but also the unwanted activation of the coagulation cascade and connected systems of inflammation and injury, without compromising on safety. ESKD patients and kidney transplant recipients perhaps require a different approach, but we should be cautious of persisting dogmas about the bleeding tendency of patients with impaired renal function, as they might benefit from anticoagulant therapy as well.

A first step would be to investigate the merit of heparin in the prevention of RGT. Based on the evidence presented in this thesis and supporting studies, prophylactic dosing of unfractionated heparin appears safe. A prospective trial investigating the effect of intraoperative intravenous unfractionated heparin on the incidence of RGT is needed, but considering a reported incidence of roughly 1%, this would entail a long-term multicenter project. Notwithstanding the many confounders, it would also be quite a logistical challenge. In recent years, however, efforts have been made to unite surgical kidney transplant research in the Netherlands. As a result, the *Dutch Kidney Transplant Study Group*, which was founded in 2017, involves members from all Dutch transplant centers. With approximately 1000 kidney transplant per year, we would not have to cross borders and we could perform a trial in which patients are given heparin at random, independent of donor type and dialysis modality. A few exclusion criteria would be required: patients with a specifically increased preoperative risk of bleeding, e.g., due to vitamin K antagonists or known bleeding disorders do not have a suitable benefit-risk ratio. Required funding would be limited to the costs for a full-time PhD candidate with minimal research expenses, as heparin for its use in kidney transplantation is covered through the Dutch health insurance. The results could further aid in personalized prevention, by identifying the patients that would benefit the most from the heparin treatment.

Additionally, the proposed trial could provide answers on other research objectives, including the potential effect of heparin on the incidence of DGF and antibody-mediated rejection. Heparin and other heparinoids have been shown to inhibit parts of the complement system, which is directly linked to the coagulation system and an important factor for ischemia-reperfusion injury and antibody-mediated rejection.[43,44] A recent non-human primate study showed that targeted inhibition of complement together with heparin significantly reduced DGF after DBD donation. However, apart from observational studies, future research should

also include additional in vitro studies to investigate these pleiotropic effects of heparin on inflammation in the human population.

Furthermore, in Chapter 4 we have shown that dialysis-dependent and preemptive recipients have a comparable hypercoagulable state prior to transplantation. Yet, preemptive transplantation was shown to be an independent risk factor of postoperative bleeding in Chapter 2. These results are conflicting and indicate that there is still a lot to learn about the hemostatic changes occurring in kidney transplant recipients, such as the formation of neutrophil extracellular traps (NETs). These web-like structures containing DNA, histones, and antimicrobial proteins, are expelled from neutrophils undergoing NETosis and are usually recruited in case of micro-organism invasion. However, excessive formation of NETs has been described in chronic kidney disease patients, hemodialysis, ischemia-reperfusion injury and thrombosis.[45–47] Following the results presented in this thesis, we hypothesized that the combined effects of CKD, hemodialysis and ischemia-reperfusion injury led to a more pronounced prothrombotic state in dialysis-dependent recipients compared to preemptive recipients. Preliminary results from our group show a significantly increased release of the specific NETs-marker myeloperoxidase-DNA in dialysis-dependent recipients after reperfusion. However, correlation analyses with the previously found hypercoagulable state need to be finalized to draw definitive conclusions.

The results in Chapter 6 also show that, during the initial cold flush, microthrombi form as a possible consequence of left-over plasma and vasoconstriction in response to the cold. The results suggest that by optimization of the initial flush, we could benefit from the positive effects of HMP even in SCS preservation. In response to this, our research group has moved forward to investigating the merit and applicability of performing a short-term normothermic flush before cooling down the graft in order to avoid vasoconstriction in the early stage of the flush. Of course, warm ischemia should be kept to a minimum and further research is needed to establish the viability of performing such a flush, especially outside the experimental setting. In addition to the formation of microthrombi without heparin, we observed that even with addition of heparin to the perfusate, microthrombi still form during the initial cold flush in DCD kidneys. Antemortem heparinization seems to be the only way to prevent this formation. However, in the Netherlands, the discussion on antemortem heparinization in the DCD donor is fed by mixed feelings and views, as it may expedite the anticipated death in case of bleeding, despite it already being performed in Belgium, France, Spain, Italy and Norway.[48] As of this writing, the Dutch Organ Procurement Committee has approved antemortem heparinization and it will likely be implemented in the Netherlands by the end of 2022. Apart from obvious outcome measures such as DGF and graft function, it would be highly interesting to evaluate the effect of this implementation on the incidence of RGT.

The promise of new antithrombotic drugs

Another solution might be provided by the development of more suitable drugs. Current anticoagulants mainly block proteases of the common pathway and thus affect all three stages of the coagulation process: inhibition, propagation and amplification. As a result, bleeding complications remain a serious side-effect. However, coagulation factors in the intrinsic pathway, such as FXI, appear to be key players in thrombosis but have a minimal role in bleeding and thus individual intrinsic coagulation factors are suggested to be suitable targets for effective and relatively safer anticoagulant drugs.[49]

Many research groups work on FXI(a), as evidenced by a large number of patents in this field,[50,51]and several compounds have already moved on to different stages of clinical trials. For example, the AXIOMATIC-TKA and ANT-005 TKA trials showed superior DVT prevention by FXIa-inhibitor milvexian and anti-FXI antibody abelacimab in patients undergoing total-knee arthroplasty compared to enoxaparin.[52,53] Several studies investigating the efficacy of FXI(a)-inhibitors Ionis FXI-LRx (NCT04534114), MK-2060 (NCT05027074) and osocimab (NCT03787368 and NCT04523220) in ESKD patients are currently planned or running. In the PACIFIC-AF study, asundexian (BAY2433334) was compared with apixaban in atrial fibrillation patients and showed a reduced bleeding risk in favor of asundexian. Although the differences between groups were small and the study was not powered to report on thrombosis incidence, these results are very promising, especially since 29% of the patients enrolled had CKD[54].

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