Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *2023 24(6):524-529*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Correspondence

https://doi.org/10.1631/jzus.B2200451

Check for updates

Outcomes of allograft from donor kidney microthrombi and secondary recipient thrombotic microangiopathy: should we consider loosening the belt?

Yamei CHENG[1,2,3,4,5*], Luying GUO[1,2,3,4,5*], Xue REN[1,2,3,4,5], Zhenzhen YANG[1,2,3,4,5], Junhao LV[1,2,3,4,5], Huiping WANG[1,2,3,4,5], Wenhan PENG[1,2,3,4,5], Hongfeng HUANG[1,2,3,4,5], Jianyong WU[1,2,3,4,5], Jianghua CHEN[1,2,3,4,5], Rending WANG[1,2,3,4,5]✉

[1]*Kidney Disease Center, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China*
[2]*Key Laboratory of Kidney Disease Prevention and Control Technology, Zhejiang Province, Hangzhou 310003, China*
[3]*National Key Clinical Department of Kidney Diseases, Hangzhou 310003, China*
[4]*Institute of Nephrology, Zhejiang University, Hangzhou 310003, China*
[5]*Zhejiang Clinical Research Center of Kidney and Urinary System Disease, Hangzhou 310003, China*

There is currently a huge worldwide demand for donor kidneys for organ transplantation. Consequently, numerous marginal donor kidneys, such as kidneys with microthrombi, are used to save patients' lives. While some studies have shown an association between the presence of microthrombi in donor kidneys and an increased risk for delayed graft function (DGF) (McCall et al., 2003; Gao et al., 2019), other studies have demonstrated that microthrombi negatively impact the rate of DGF (Batra et al., 2016; Hansen et al., 2018), but not graft survival rate (McCall et al., 2003; Batra et al., 2016; Gao et al., 2019). In contrast, Hansen et al. (2018) concluded that fibrin thrombi were not only associated with reduced graft function six months post-transplantation but also with increased graft loss within the first year of transplantation. On the other hand, Batra et al. (2016) found no significant differences in the DGF rate or one-year graft function between recipients in diffuse and focal microthrombi groups. To date, however, the overall influence of donor kidney microthrombi and the degree of influence on prognosis remain controversial, necessitating further research.

We retrospectively reviewed a total of 1735 deceased donor kidney transplants performed at our hospital between January 2013 and October 2020. A total of 33 (out of 1528) recipients with post-reperfusion kidney biopsies exhibited microthrombi in glomerular capillaries. The incidence rate of microthrombi in donor kidneys was 2.2%. Microthrombi were classified as diffuse or focal if at least 50% or <50% of glomeruli had microthrombi, respectively. Based on this, we stratified the recipients into diffuse (n=18) and focal (n=15) groups. We found no statistically significant differences between the focal and diffuse groups with regard to donor or recipient clinical characteristics (Table S1). Recipients in the diffuse group had significantly higher DGF rates than those in the focal group (55.6% (10/18) versus 13.3% (2/15), P=0.027). Notably, allograft function was recovered in 11 of the 12 recipients who developed DGF; the remaining allograft with 100% glomerular microthrombi was lost due to perirenal hematoma in combination with renal vein thrombosis. After excluding the patient with graft loss, the average time of disengaged dialysis in the diffuse and focal groups was 16 and 11 d, respectively, although the difference was not statistically significant (P=0.391). Recipients in the diffuse group had significantly higher serum creatinine (S-Cr) (Fig. 1a) than those in the focal group at 1 month (P=0.024) and 6 months (P=0.028), but no significant differences were observed at 3 months

✉ Rending WANG, rd_wangjia@zju.edu.cn
* The two authors contributed equally to this work
Rending WANG, https://orcid.org/0000-0002-2063-8533

Received Sept. 12, 2022; Revision accepted Jan. 30, 2023;
Crosschecked Mar. 22, 2023; Published online Apr. 15, 2023

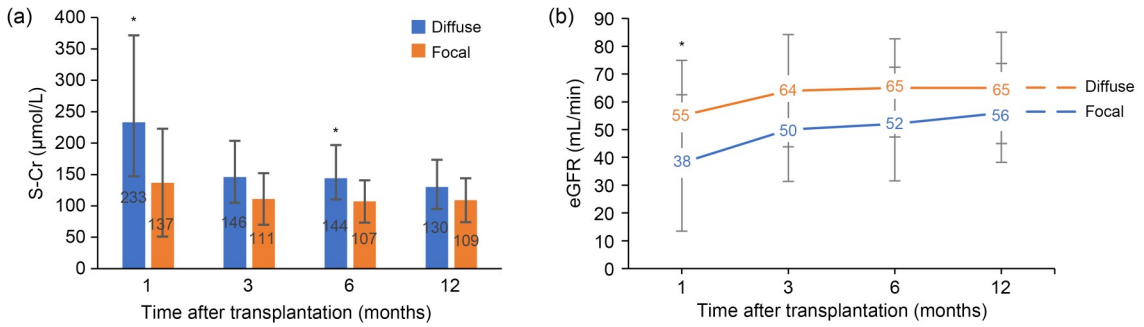

Fig. 1 Degree of microthrombi and graft function. (a) Serum creatinine (S-Cr); (b) Estimated glomerular filter rate (eGFR). Data are expressed as mean±standard deviation (SD), n=33. * P<0.05 between the groups.

or 12 months after transplantation. Recipients in the diffuse group exhibited significantly lower estimated glomerular filter rate (eGFR) levels than their focal counterparts only at 1 month post-transplantation (P=0.042); no statistically significant differences were observed at 3, 6, or 12 months after transplantation (Fig. 1b). Notably, we found no statistically significant differences between the diffuse and focal groups with regard to patient survival rates (100.0% versus 100.0%) or graft survival rates (94.4% versus 100.0%, P>0.999) at one year post-transplantation. Clinical doctors are usually reluctant to use donor kidneys with thrombi, especially donor kidney with diffuse microthrombi, owing to poor outcomes (Nghiem et al., 2009). Our results showed that donor kidneys with either focal or diffuse microthrombi were associated with favorable prognosis, despite a link between diffuse microthrombi and high DGF incidence. Notably, 32 out of our 33 patients recovered, indicating that donor kidneys with microthrombi are a safe option for transplantation.

Is anticoagulation needed for donor kidney microthrombi, especially for diffuse microthrombi? Several studies have demonstrated that donor glomerular microthrombi can be cleared by the fibrinolytic system of recipients after transplantation (McCall et al., 2003; Batra et al., 2016; Gao et al., 2019). In the present study, seven recipients received a second biopsy on the 5th, 8th, 11th, 13th, 20th, 20th, or 24th days post-transplantation, and microthrombus residues were found in three cases: the biopsies performed on the 5th, 11th, and 20th days post-transplantation. Our unpublished systemic review showed that 110 of 120 recipients of a microthrombus-positive graft who received second biopsies between 4 and 180 d after transplantation (but mostly within 30 d) had no residual thrombi. Immediate anticoagulation may exacerbate the risk of bleeding. Two previous studies evaluated ex vivo thrombolysis, using pulsatile perfusion with a tissue-plasminogen activator; in the first (Nghiem et al., 2009), glomerular thrombi decreased from 50% to 23%, and in the second (Sibulesky et al., 2013) from 75%–80% to 50%. Notably, only two of our 33 recipients were given anticoagulation medication post-transplantation, and both of them developed perirenal hematomas. The other 31 recipients, who did not receive anticoagulation drugs post-transplantation, have better outcomes. Therefore, further investigation of the effects of ex vivo anticoagulation and anticoagulation post-transplantation is called for.

Interestingly, most donors in our cohort suffered severe head injuries leading to their death. Severe head injury, especially caused by trauma, is commonly associated with a high incidence of coagulopathy, resulting in disseminated intravascular coagulation (DIC), even excessive microthrombus formation (Gao et al., 2019). Microthrombi in donor kidney can be accompanied by hemolytic anemia and thrombocytopenia in recipients immediately after transplantation (Garrouste et al., 2019). A multicenter study reported that 30 out of 137 recipients of a DIC-positive (DIC^+) graft developed decreased platelet (PLT) count (Garrouste et al., 2019). A previous study showed that 16 out of 44 recipients who received kidneys from DIC^+ donors developed thrombocytopenia which was independently associated with DGF or slow graft function, but that had no impact on renal function at one year (Wang et al., 2011). However, whether recipients developed anemia or not was not mentioned in these two studies. In the prevailing literature, only a few cases mention thrombocytopenia and/or anemia in individuals receiving kidneys from donors with microthrombi (Pastural et al., 2001; Revollo et al., 2015; Soares et al., 2017). At

present, the effects of donor glomerular microthrombi on recipient PLT count, hemoglobin (Hb), or kidney allograft function are not well understood.

Thrombotic microangiopathy (TMA), characterized by endothelial dysfunction and the presence of thrombi in small blood vessels, clinically presents with micro-angiopathic hemolytic anemia, absolute or relative thrombocytopenia, schistocytes on peripheral smear, elevated lactate dehydrogenase (LDH), and acute kidney injury (Ávila et al., 2021). The phenomenon of decreased PLT and Hb in recipients resulting from donor microthrombi can be clinically termed "donor-induced recipient TMA" (dir-TMA). However, only a handful of our recipients had schistocyte testing on peripheral smear after transplantation. Therefore, we mainly identified dir-TMA by the recipients' anemia status, thrombocytopenia, and elevated LDH, based on donor kidneys with microthrombi. Typical morphology images of diffuse microthrombi and subsequent TMA are shown in Fig. S1. We divided the 33 recipients who received donor kidneys with microthrombi into dir-TMA (n=20) and non-dir-TMA (n=13) subgroups, based on the presence or absence of dir-TMA. A summary of donor and recipient clinical characteristics of the dir-TMA and non-dir-TMA subgroups is presented in Table 1.

The trajectory of PLT and Hb in recipients in the dir-TMA and non-dir-TMA subgroups from one day before transplantation to one year post-transplant is presented in Figs. 2a and 2b. PLT counts in both groups

Table 1 Donor and recipient clinical characteristics by dir-TMA status

Characteristics	Dir-TMA (n=20)	Non-dir-TMA (n=13)	P
Donors			
Gender, male	17 (85.0%)	6 (46.2%)	0.026
Age (years)	46.5±16.3	46.8±14.3	0.954
BMI (kg/m^2)	23.7±3.5	23.4±3.9	0.819
Death from brain trauma	16 (80.0%)	10 (76.9%)	1.000
CIT (h)	7.4±2.7	10.4±3.1	0.008
Warm ischemia (min)	14.4±4.4	11.8±8.5	0.328
Recipients			
Age (years)	45.9±11.6	44.6±11.4	0.767
Gender, male	13 (65.0%)	6 (46.2%)	0.472
BMI (kg/m^2)	20.6±2.3	21.8±2.2	0.158
Preoperative PLT ($\times10^9$ L^{-1})	220±86	243±71	0.426
Preoperative Hb (g/L)	113±12	119±17	0.256
Intraoperative blood loss (mL)	103±26	104±14	0.880
Intraoperative blood transfusion	0	0	
PLT 3 h after operation ($\times10^9$ L^{-1})	154±60	211±78	0.025
Hb 3 h after operation (g/L)	100±13	107±15	0.182
RBC transfusion	5 (25.0%)	2 (15.4%)	0.676
LDH (U/L)	689±424	252±51	0.007
Renal hematoma posttransplant	4 (20.0%)	2 (15.4%)	1.000
Plasma exchange	3 (15.0%)	0	0.244
Plasma transfusion	6 (30.0%)	0	0.027
HLA mismatch	3.6±1.2	3.1±1.6	0.333
Induction therapy			0.142
ATG (%)	15 (75.0%)	6 (46.2%)	
Basiliximab (%)	5 (25.0%)	7 (53.8%)	
Maintain immunosuppression, FK506+MMF+Pred	20 (100.0%)	12 (92.3%)	0.394

Data are expressed as mean±standard deviation (SD) or number (percentage). Dir-TMA: donor-induced recipient thrombotic microangiopathy; BMI: body mass index; CIT: cold ischemia time; PLT: platelet; Hb: hemoglobin; RBC: red blood cell; LDH: lactate dehydrogenase; HLA: human leukocyte antigen; ATG: anti-thymocyte globulin; FK506: tacrolimus; MMF: mycophenolate mofetil; Pred: prednisone.

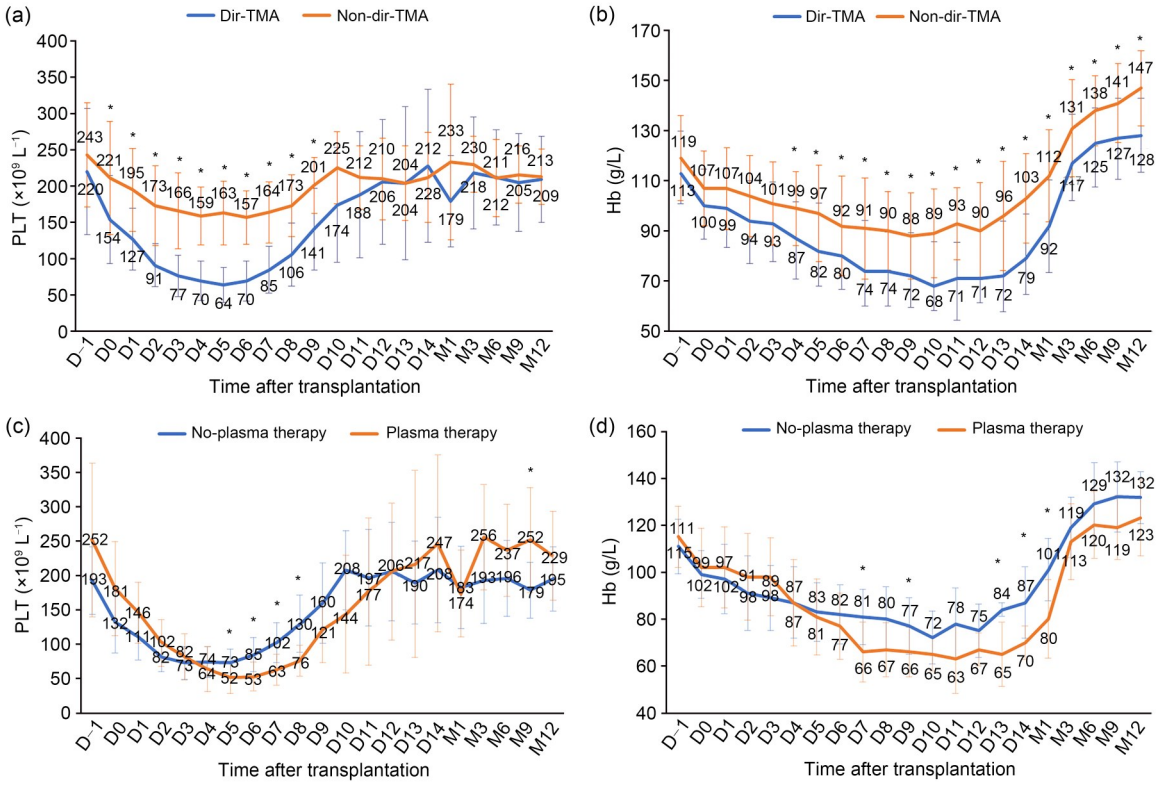

Fig. 2 Change trend of platelet (PLT) count and hemoglobin (Hb) level from 1 d pretransplant to 14 d posttransplant and 1 to 12 months after transplantation. (a, b) Change trend of PLT (a) and Hb (b) between dir-TMA and non-dir-TMA subgroups; (c, d) Change trend of PLT (c) and Hb (d) between plasma therapy and no-plasma therapy groups. Dir-TMA: donor-induced recipient thrombotic microangiopathy. D: day; M: month. D0 is three hours after operation. Data are expressed as mean±standard deviation (SD), n=33. * P<0.05 between the groups.

decreased within 4 d after operation, and began to steadily rise on the 5th day. PLT counts in the dir-TMA subgroup were significantly lower than those in the non-dir-TMA subgroup from 3 h to the 9th day after operation. PLT counts in the non-dir-TMA subgroup remained normal across all time points. Hb levels began to increase on the 11th day after operation, with those in the dir-TMA subgroup found to be significantly lower than those recorded in the non-dir-TMA subgroup between the 4th and 14th days after operation. Follow-up at 1, 3, 6, and 12 months post-transplantation revealed comparable PLT counts in the two subgroups. However, although Hb counts increased to 128 g/L in the dir-TMA subgroup, they remained significantly lower than those in the non-dir-TMA subgroup (all P<0.05).

Plasma exchange, an important therapy for TMA, may result in graft salvage in about 80% of cases (Karthikeyan et al., 2003). Of our 20 recipients, six and three with dir-TMA were treated with plasma transfusion and plasma exchange, respectively. We stratified recipients with dir-TMA into plasma therapy (n=9) and no-plasma therapy (n=11) subgroups. A summary of donor and recipient characteristics in these groups is given in Table S2. Changes in mean PLT counts and Hb levels among recipients in both subgroups are shown in Figs. 2c and 2d. In brief, patients in the plasma therapy group had significantly lower PLT counts than their no-plasma therapy counterparts between 5 and 8 d post-transplantation (all P<0.05). Subjects in the plasma therapy subgroup had significantly lower Hb levels than those in the other subgroup on Days 7, 9, 13 and 14, and 1 month post-transplantation (all P<0.05).

Moreover, recipients with dir-TMA had higher (but statistically insignificant) DGF rates than the non-dir-TMA subgroup (50.0% versus 15.4%, P=0.067). The dir-TMA subgroup recorded significantly higher S-Cr levels (Fig. 3a) and lower eGFR levels (Fig. 3b) than the non-dir-TMA subgroup at 1, 3, 6, and 12 months post-transplantation. Early drops in recipient PLT and Hb may be a sign of ongoing thrombosis in recipients, which contributes to poor graft function. Additionally, among individuals who developed dir-TMA,

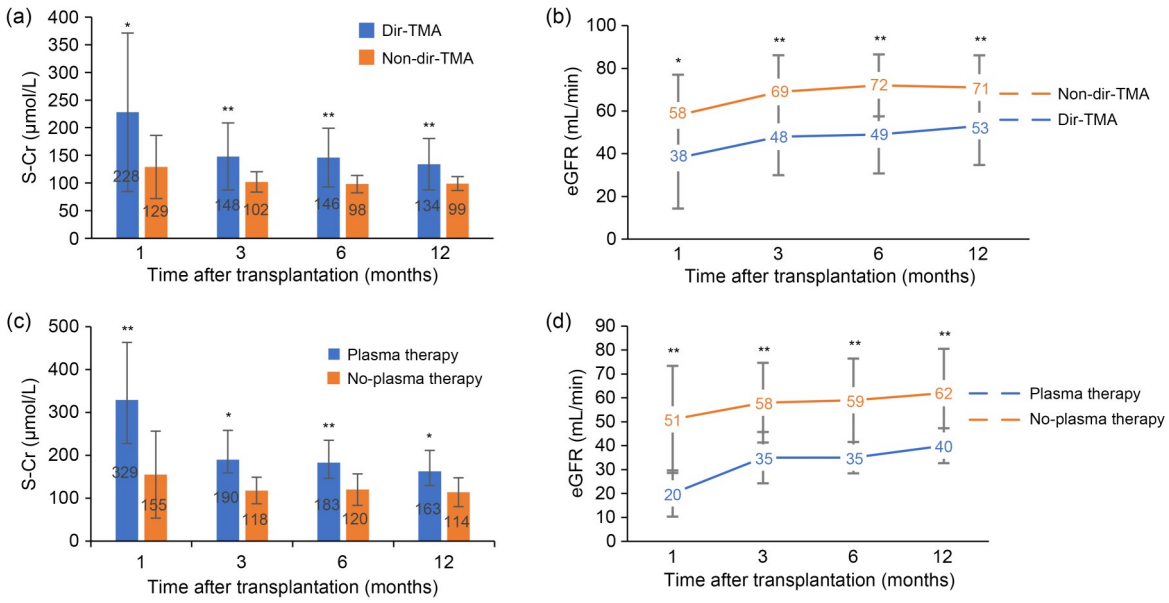

Fig. 3 Graft function between different groups. (a, b) The levels of serum creatinine (S-Cr) (a) and estimated glomerular filter rate (eGFR) (b) between dir-TMA and non-dir-TMA subgroups; (c, d) The levels of S-Cr (c) and eGFR (d) between plasma therapy and no-plasma therapy groups. Data are expressed as mean±standard deviation (SD), n=33. * P<0.05, ** P<0.01 between the groups. Dir-TMA: donor-induced recipient thrombotic microangiopathy.

recipients with severe anemia (Hb≤60 g/L) had worse graft function compared with those without, although the difference was not statistically significant (Fig. S2). Recipients in the plasma therapy subgroup had significantly higher DGF incidence rates than those who did not receive plasma therapy (88.9% (8/9) versus 18.2% (2/11), P=0.005). Notably, S-Cr (Fig. 3c) and eGFR (Fig. 3d) levels at 1, 3, 6, and 12 months post-transplantation were significantly worse in the plasma therapy subgroup than in the no-plasma therapy subgroup (all P<0.05). There were no statistically significant differences between the two groups with regard to patient (100.0% versus 100.0%) or graft (95.0% versus 100.0%, P>0.999) survival rates at one year.

Based on the above results, recipients with dir-TMA do not appear to benefit from plasma therapy. The main mechanism underlying thrombosis in a deceased donor kidney is disordered activation of coagulation and the fibrinolysis system caused by endothelial injury, which also activates the immune system and complementary pathways. Endothelial damage in donors, due to insufficient effective circulating capacity or other reasons (particularly traumatic brain injury), caused disorders of coagulation and fibrinolysis. Persistence of this condition may eventually lead to necrosis of the renal parenchyma. Disappearance of microthrombi in allograft immediately after kidney transplantation from the thrombotic environment of the donor to the non-thrombotic recipient environment may suggest that the active fibrinolytic system of the recipient dissolves the microthrombi due to removal of inducement. Although activation of the complementary pathway and immune system, along with other causes, may lead to occurrence of dir-TMA at the beginning, the recipient's own fibrinolytic system has a strong ability to effectively clear the microthrombi. Although plasma therapy is strongly recommended and is effective in the treatment of TMA in the primary kidney, its efficacy in dir-TMA may be limited. Further research is needed to clarify the effect of plasma therapy on dir-TMA. For patients who are ready to donate organs, their PLT counts, Hb and LDH levels, and any schistocytes on peripheral smear, combined with urine volume, urine color, and urinary RBC count, among other aspects, should be examined during the early stages to determine whether thrombosis has occurred. This is extremely important for expanding the supply of, and maximizing utilization of, such donors.

In summary, our findings show that a severe extent of donor kidney thrombi is associated with increased risk of DGF, although it does not affect allograft function. Donor-derived microthrombi initiate an early decrease in recipient Hb and PLT, which may be a sign of poor allograft function; thus, it should

not be ignored. We would not strongly recommend plasma therapy for dir-TMA, but further studies are needed to clarify this critical issue.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

This study was supported by the Science and Technology Department of Zhejiang Province (No. 2019C03029), the National Natural Science Foundation of China (Nos. 82070766, 81870510, and U21A20350), and the Bethune Charitable Foundation (No. GX2019010112), China.

Author contributions

Yamei CHENG, Luying GUO, and Rending WANG were responsible for study concept and design. Yamei CHENG, Luying GUO, Xue REN, Zhenzhen YANG, and Junhao LV were responsible for the collection of clinical data. Yamei CHENG and Luying GUO were responsible for drafting of the manuscript and statistical analysis. Huiping WANG, Wenhan PENG, Hongfeng HUANG, Jianyong WU, Jianghua CHEN, and Rending WANG were responsible for critical revision of the manuscript for important intellectual content. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yamei CHENG, Luying GUO, Xue REN, Zhenzhen YANG, Junhao LV, Huiping WANG, Wenhan PENG, Hongfeng HUANG, Jianyong WU, Jianghua CHEN, and Rending WANG declare that they have no conflicts of interest.

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University (No. 2019-335) and with the Helsinki Declaration of 1975, as revised in 2008 (5). All procedures were carried out in accordance with the relevant guidelines and regulations. Informed consent was obtained from all patients. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

References

Ávila A, Gavela E, Sancho A, 2021. Thrombotic microangiopathy after kidney transplantation: an underdiagnosed and potentially reversible entity. *Front Med*, 8:642864. https://doi.org/10.3389/fmed.2021.642864

Batra RK, Heilman RL, Smith ML, et al., 2016. Rapid resolution of donor-derived glomerular fibrin thrombi after deceased donor kidney transplantation. *Am J Transplant*, 16(3):1015-1020. https://doi.org/10.1111/ajt.13561

Gao GF, Chen LX, Brown IE, et al., 2019. Donor characteristics, recipient outcomes, and histologic findings of kidney allografts with diffuse donor-derived glomerular fibrin thrombi. *Transplantation*, 103(9):1921-1927. https://doi.org/10.1097/TP.0000000000002578

Garrouste C, Baudenon J, Gatault P, et al., 2019. No impact of disseminated intravascular coagulation in kidney donors on long-term kidney transplantation outcome: a multicenter propensity-matched study. *Am J Transplant*, 19(2): 448-456. https://doi.org/10.1111/ajt.15008

Hansen D, Rørvig S, Andersen CB, et al., 2018. Fibrin thrombi in deceased donor kidneys: prevalence and influence on graft function and graft survival in transplanted patients. *APMIS*, 126(1):3-8. https://doi.org/10.1111/apm.12781

Karthikeyan V, Parasuraman R, Shah V, et al., 2003. Outcome of plasma exchange therapy in thrombotic microangiopathy after renal transplantation. *Am J Transplant*, 3(10): 1289-1294. https://doi.org/10.1046/j.1600-6143.2003.00222.x

McCall SJ, Tuttle-Newhall JE, Howell DN, et al., 2003. Prognostic significance of microvascular thrombosis in donor kidney allograft biopsies. *Transplantation*, 75(11):1847-1852. https://doi.org/10.1097/01.Tp.0000063126.88887.68

Nghiem DD, Olson PR, Sureshkumar KK, 2009. Role of pulsatile perfusion with tissue plasminogen activator in deceased donor kidneys with extensive glomerular thrombosis. *Transplant Proc*, 41(1):29-31. https://doi.org/10.1016/j.transproceed.2008.08.150

Pastural M, Barrou B, Delcourt A, et al., 2001. Successful kidney transplantation using organs from a donor with disseminated intravascular coagulation and impaired renal function: case report and review of the literature. *Nephrol Dial Transplant*, 16(2):412-415. https://doi.org/10.1093/ndt/16.2.412

Revollo JY, Cuffy MC, Witte DP, et al., 2015. Case report: hemolytic anemia following deceased donor renal transplantation associated with tranexamic acid administration for disseminated intravascular coagulation. *Transplant Proc*, 47(7): 2239-2242. https://doi.org/10.1016/j.transproceed.2015.05.027

Sibulesky L, Gohh R, Charpentier K, et al., 2013. Kidney transplantation from donors with severe disseminated intravascular coagulation. *ISRN Transplant*, 2013:646310. https://doi.org/10.5402/2013/646310

Soares KC, Arend LJ, Lonze BE, et al., 2017. Successful renal transplantation of deceased donor kidneys with 100% glomerular fibrin thrombi and acute renal failure due to disseminated intravascular coagulation. *Transplantation*, 101(6):1134-1138. https://doi.org/10.1097/tp.0000000000001386

Wang CJ, Shafique S, Mccullagh J, et al., 2011. Implications of donor disseminated intravascular coagulation on kidney allograft recipients. *Clin J Am Soc Nephrol*, 6(5):1160-1167. https://doi.org/10.2215/cjn.07280810

Supplementary information

Tables S1 and S2; Figs. S1 and S2; Materials and methods