MICROSURGICAL RECONSTRUCTION OF LOWER EXTREMITY IN HOMOZYGOSITY OF *C677T* MTHFR GENE MUTATION: CASE REPORT AND REVIEW OF THE LITERATURE

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Summary

The presence of hypercoagulable states, either inherited or acquired, has been recognized as a risk factor for free flaps failure. Nevertheless, the actual risk of microvascular failure related to these conditions is yet to be determined. We present and discuss the case of a successful free tissue transfer in a 27-year-old man, with known homozygosity for the *C677T* polymorphism for the MTHFR gene mutation and anti-erythrocyte panagglutinin with a review of the literature.

Key words: free flap reconstruction, Latissimus Dorsi flap, thrombophilia, MTHFR

INTRODUCTION

Hypercoagulable states represent a risk factor for microvascular thrombosis and failure of microsurgical flaps [1,2]. While technical errors were once considered the most common cause of microvascular failure, to date the widespread use of microsurgical techniques has significantly reduced the rate of flap loss attributable to the surgical procedure [3]. Nevertheless, even under the best surgical circumstances, inherited or acquired individual factors, may compromise the outcome of a free flap reconstruction. While acquired conditions may be ascertained with a detailed patient history, inherited factors predisposing to blood clotting are commonly unknown to both patient and surgeon prior to a thrombotic complication. In literature, free flap failure has been related to factor V Leiden mutation, prothrombin A20210G gene mutation, MTHFR gene mutation, PAI-1 gene mutation, protein C and S activity deficiency, antithrombin III deficiency, lupus anticoagulant, plasma homocysteine level, anticardiolipin antibody, antiphospholipid antibody and ß2-glycoprotein antibody [4,5]. To date, there are no standardised guidelines for the preoperative screening of hypercoagulable

states. The multitude and the heterogenicity of genetic factors predisposing to blood clotting makes the identification of a comprehensive panel of preoperative tests difficult, and its cost-effectiveness may not be favourable [6,7]. Furthermore, even in the presence of a known diagnosis of thrombophilia, scant literature data does not enable to define the actual risk of microvascular complications. Here we present the details and perioperative management of a successful microsurgical reconstruction in a patient diagnosed with homozygosity for the *C677T* polymorphism of the MTHFR gene and anti-erythrocyte pan agglutinizing antibody. A review of the literature on peri-operative thrombotic complications in patients with MTHFR gene mutations, undergoing free flap surgery was performed.

CASE REPORT

We report the case of a 27-year-old healthy male who sustained severe polytrauma following a car accident. At admission at our institution, the patient presented with a subamputation of the right leg, open fracture of the left femoral condyle, open fractures of the right radial and ulnar bones and fracture of the right scaphoid bone. For the present report, the authors will provide only details pertaining the management of the right leg injury. In the emergency room, clinical evaluation revealed a Gustilo-Anderson IIIB open fracture of both tibia and fibula (Fig. 1A-B). CT angiography scan showed no major vascular lesions, with integrity of the anterior tibial, posterior tibial and peroneal vessels. Primary management, led by the orthopaedic team, included intravenous antibiotics, irrigation of the wound and thorough debridement of contaminated and devitalized tissue. The tibial fracture was reduced and stabilized with an external fixator. The instability of patient's conditions did not enable to perform an immediate reconstruction of the soft tissue defect, thereby treatment with VAC therapy was initiated. The patient underwent multiple wound debridement procedures, and regular change of VAC therapy over the first three months following the trauma. Wound swabs were taken and sent for microbiological examination at regular intervals. During hospitalisation, Klebsiella Aerogenes and Pseudomonas Aeruginosa infections of the wound were diagnosed and managed with adjustment of the intravenous antibiotic therapy based on susceptibility testing. At three months from the injury, despite significant growth of granulation tissue was observed at the wound, adequate coverage of the tibial shaft was not attained. After evaluation of the residual defect by the plastic surgeon (B.L.), a reconstruction with Latissimus Dorsi free flap was planned to provide adequate soft tissue coverage of the exposed bone and optimize

Figure 1. A) Photograph at patient's arrival at the hospital: sub-amputation of the right leg, with injury at its middle third, and fracture of the tibial and peroneal bones. The distal part of the leg is displaced and flexed and cannot be seen in this photograph; B) Photograph at patient's arrival at the hospital: repositioning of the distal segment of the lower limb reveals the partial amputation of the limb, with exposed bones and extensive soft tissue defect.

its vascular supply. Preoperative blood group typing revealed the presence of a pan agglutinizing anti-erythrocyte antibody, previously unknown. The activity of the anti-E alloantibody was found until a 1:4 dilution of the patient serum. Given the incidental finding, a coagulation screen was performed, unveiling patient's homozygosity for the *C677T* polymorphism of the MTHFR gene mutation, associated with high blood homocysteine. The patient referred no known personal nor familiar history of arterial or venous thrombosis. Routine coagulation analyses were not altered. Prior to surgery, the patient underwent normovolemic haemodilution, through combined crystalloid and colloid intravenous fluid infusion at a rate of 1.5 mL/kg per hour. During surgery, the Latissimus Dorsi muscle flap was harvested with the patient in left lateral decubitus. The muscle was dissected from its origin at the iliac crest to its tendinous insertion, preserving the thoracolumbar fascia. The thoracodorsal vessels were identified and isolated until their origin. During dissection, the vascular branch to the Serratus Anterior muscle and the thoracodorsal nerve were identified and divided. At the recipient site, a curettage of the wound was performed. The anterior tibial artery with its comitant veins were identified and isolated, with care to preserve the deep peroneal nerve. After preparation of the recipient site, the Latissimus Dorsi pedicle was divided, and the flap transferred from the donor to the recipient area. The thoracodorsal vessels were tunnelled

Figure 2. Intraoperative photograph: right leg soft tissue defect prior to reconstruction. The presence of granulation tissue formed at the wound site after 3-month of VAC therapy can be observed. The tibial shaft is exposed with no adequate soft tissue coverage.

at the posterior aspect of the tibialis anterior muscle to protect the flap pedicle and the microvascular anastomoses. End-to-end anastomoses between the thoracodorsal artery and the anterior tibial artery, and between the thoracodorsal vein and one of the venae comitantes of the anterior tibial pedicle were performed. No signs of intraoperative thromboses were detected. Wound edges were undermined to improve flap's contour (Fig. 2). Two closed suction drains were placed, one at the donor and one at the recipient site. A meshed split-thickness skin graft, harvested from the anterior aspect of the ipsilateral thigh was used to provide cutaneous coverage of the muscular flap (Fig. 3). At the end of surgery, total amount of blood loss was 180 mL with a value of haemoglobin of 8 g/dL (preoperative value was 9.5 g/dL) and no transfusions of red blood cells were needed. Postoperatively a fluid therapy protocol was initiated. Intravenous infusion of 2500 mL of crystalloids daily (1500 mL Ringer's lactate solution and 1000 mL electrolyte solution) was started

Figure 3. Intraoperative photograph: reconstruction of right soft tissue defect with free Latissimus Dorsi muscle flap. A meshed split-thickness skin graft harvested from the anterior aspect of the ipsilateral thigh was used to provide coverage for the muscle flap.

following the surgery to maintain blood haematocrit at 22-25% range, ensuring a urinary output of 1 ml/kg/24h, for the first five postoperative days and titrated over the following days. Thromboembolic prophylaxis with 4000 UI of low molecular weight heparin daily started 6 hours after surgery. The patient was monitored with hand-held doppler ultrasound for 10 days, with no evidence of postoperative vascular impairment. The patient recovered well with no major complications inherent to the microsurgical reconstruction, although definitive bone fixation was delayed due to chronic osteomyelitis of the tibial bone (Figs. 4-5). Blood tests for the search of anti-erythrocyte agglutinin were repeated, with confirmed positivity for anti-E alloantibodies.

DISCUSSION

Hypercoagulable states, either inherited or acquired, may have a role in free flap failure. Polymorphisms of the MTHFR gene (*C677T* and *A1298C*) are associated with a reduced activity of the Methylenetetrahydrofolate reductase enzyme, resulting in impaired folate metabolism and hyperhomocisteinemia [8]. High blood levels of homocysteine determine an increased risk of venous thromboembolism, and may have detrimental effect on the vessel endothelium, through oxidative damage, favouring platelet adhesion and thrombosis [9,10]. There are only few reports in literature addressing the occurrence of microvascular complications in patients with thrombophilia and MTHFR gene mutations (Tab. I) [11-13]. Davison et al. reported four cases of free flap failure in patients undergoing head and neck reconstruction, caused by undiagnosed thrombophilia. Three among these patients were diagnosed with MTHFR gene mutations, two being compound heterozygous for the *C677T* and *A1298C* polymorphisms, and one homozygous for the *C677T* polymorphism. Except one patient with compound heterozygosity, the other two patients presented additional pro-coagulant alterations, including elevated factor VIII activity, elevated PAI-1 activity, factor V Leiden mutation (*R506Q* polymorphism), and elevated anti-phosphatidyl IgM and IgG antibodies. In all three patients flap surgery was complicated by intraoperative arterial thrombosis. Furthermore, one patient experienced deep venous thrombosis, and one patient had ischemic complications unrelated to the flap [12]. De Fazio et al. presented a case series of patients with thrombophilia undergoing lower limb free tissue transfer. Among the cohort of ten patients who experienced perioperative thrombotic complications, seven were diagnosed with MTHFR gene mutations; of these, two patients had postoperative venous thrombosis, and five patients experienced intraoperative arterial

Figure 4. A) Postoperative photograph: outcome at 6 months from surgery, medial view; B) Postoperative photograph: outcome at 6 months from surgery, frontal view; C) Postoperative photograph: outcome at 6 months from surgery, lateral view.
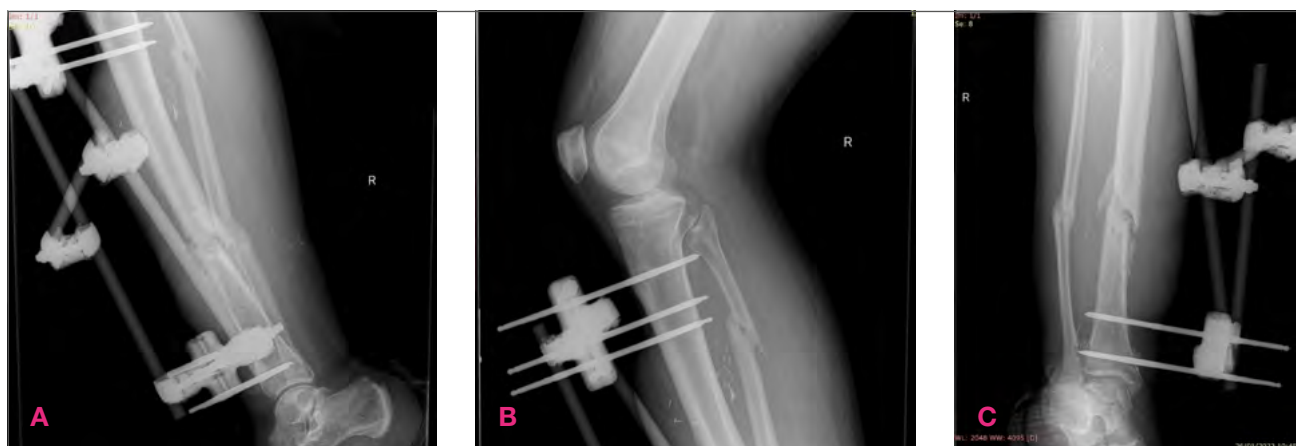

Figure 5. Postoperative x-rays: bone fixation at 6 months from surgery. A-B) Medial views; C) Posterior view.

thrombosis. MTHFR gene mutation and high homocysteine levels were detected in five of the six patients with failed free flaps [13]. Vekris et al. presented the case of a young patient who underwent two consecutive unsuccessful reconstructions for a forearm defect, with the right and left gracilis muscle free flaps, both complicated by postoperative thrombosis and flap failure. Following the two failures, genetic analysis revealed that the patient was homozygous for the MTHFR *C677T* mutation and heterozygous for the mutations of factor V, G1691A-Leiden. Besides flap failures, the patient had deep venous thrombosis following the first

reconstructive attempt, and pulmonary embolism after the second surgery [14].

Among the cohort of cases retrieved from literature, most patients were diagnosed with multiple genetically inherited alterations, that comprehensively account for the high number of flap vessel thrombosis and systemic thrombotic complications. However, the presence of multiple factors predisposing to clot formation, makes any corelation of single blood alterations with microvascular complications of difficult interpretation.

Beyond the presented reports focusing on MTHFR gene mutations, there is more literature data on microvascular

Table I. Free flap failure in patients with MTHFR gene mutation.

Author	Patient age, sex	Coagulation alteration	Microsurgical reconstruction	Flap complications	Other complications
Vekris et al., 2007 [9]	18, M	Heterozygosity for Factor V Leiden, *A4070G* Homozygosity (*C677T*) MTHFR gene	I: Gracilis flap (right) II: Gracilis flap (left)	I: flap venous thrombosis II: thrombosis graft vessels	I: Deep venous thrombosis II: Pulmonary embolism
Davison et al., 2009 [10]	43, F	Elevated PAI-1 activity Elevated Factor VIII activity Elevated anti-phosphatidyl IgM and IgG antibodies Compound heterozygosity (*C677T, A1298C*) MTHFR gene. Heterozygosity for factor V Leiden gene (*R506Q* polymorphism)	I: SIEA flap after oncologic resection II: DIEP salvage flap	I: Intraoperative arterial thrombosis II: Postoperatory thrombosis (12 h) at arterial anastomosis of DIEP salvage flap	N/A
	29, F	Elevated Factor VIII activity Homozygosity (*C677T*) MTHFR gene	I: RFFF flap after oncologic resection II: RFFF after oncologic resection III: DIEP salvage flap	I: Intermittent flow reductions II: Intra-operative thrombosis	Donor site ischemia following flap harvest Deep vein thrombosis at CVC
	42, F	Compound heterozygosity (*C6777T/A1298C*) MTHFR gene	I: RFFF after oncologic resection II: ALT flap	I: Intraoperative arterial thrombosis of flap artery II: Postoperative recurrent thrombosis of flap artery; partial necrosis of the flap	Toe gangrene distal to cannulated artery
De Fazio et al. [11]	N/A	Heterozygosity (*G20210A*) Prothrombin gene Compound heterozygosity (*C6777T/A1298C*) MTHFR gene Hyperhomocysteinemia Lupus Anticoagulant	I: ALT flap	I: Postoperatory venous thrombosis (POD2) (Failed)	N/A
	N/A	Compound heterozygosity (*C6777T/A1298C*) MTHFR gene Hyperhomocysteinemia PAI-1 45/5G Lupus Anticoagulant Elevated Factor VIII	I: Gracilis flap	I: Intraoperative arterial thrombosis, mixed postoperatory thrombosis (POD7) (Failed)	N/A
	N/A	Compound heterozygosity (*C6777T/A1298C*) MTHFR gene Hyperhomocysteinemia	I: ALT flap	I: Postoperatory venous thrombosis (POD2, POD11) (Failed)	N/A
	N/A	Compound heterozygosity (*C6777T/A1298C*) MTHFR gene Hyperhomocysteinemia PAI-1 45/5G Heterozygosity (*G1691A*) Factor V Leiden	I: Rectus Femoris flap	I: Intraoperative arterial thrombosis, postoperative mixed thrombosis (Failed)	N/A
	N/A	Compound heterozygosity (*C6777T/A1298C*) MTHFR gene Hyperhomocysteinemia	I: ALT flap (distal third of the leg)	I: Intraoperative arterial thrombosis and postoperative arterial thrombosis (POD 4) (Failed)	N/A

▶

Table I. *continues.*

Author	Patient age, sex	Coagulation alteration	Microsurgical reconstruction	Flap complications	Other complications
De Fazio et al. [11]	N/A	Heterozygosity (*A1298C*) MTHFR gene PAI-1 45/5G	I: ALT flap (distal third of the leg)	Intraoperative arterial thrombosis (Salvaged)	N/A
	N/A	Heterozygosity (*A1298C*) MTHFR gene PAI-1 45/5G Hyperhomocysteinemia	I: Medial femoral condyle flap (forefoot)	Intraoperative arterial thrombosis (Salvaged)	N/A

Abbreviations: N/A: not applicable; SIEA: superficial inferior epigastric artery; DIEP: deep inferior epigastric artery perforator; RFFF: radial forearm free flap; ALT: anterolateral thigh; PAI-1: plasminogen activator-inhibitor-1; MTHFR: methylenetetrahydrofolate reductase.

complications occurring in patients with common inherited thrombophilia, including Factor V Leiden, prothrombin gene mutation, protein C and protein S deficiencies, elevated factor VIII, and antithrombin III deficiency [15,16]. Nevertheless, for all these hypercoagulable states, the absence of a control group of patients who have not experienced thrombotic complications after free flap surgery, prevents an estimation of the actual risk.

Our patient was found to be homozygous for the *C677T* polymorphism of the MTHFR mutation gene. Furthermore, preoperative blood tests revealed the presence of a pan agglutinizing anti-E Rh antibody, also confirmed at 6 months postoperatively. Anti-erythrocytes alloantibodies are often associated with pregnancy-induced alloimmunization and history of chronic blood transfusions in patients with haemolytic disorders as occurred in our patient. However natural anti-E autoantibodies may appear in patients with no known history of transfusion, nor pregnancy [17]. Given the severity and the extent of the defect, a free flap reconstruction was indicated [18-21]. The patient was informed on the risks inherent to the procedure and his hypercoagulable state and accepted to undergo the surgery. A free Latissimus Dorsi muscle flap reconstruction was planned to provide a large soft tissue coverage of the exposed bone and prevent complications. Given the integrity of the anterior tibial, posterior tibial, and peroneal vessels, end-to-end anastomoses between the pedicle vessels and the anterior tibial vessels were performed to maximize the blood flow and decrease the risk of vascular thrombosis. Prior to surgery, the patient underwent normovolemic haemodilution to optimize blood perfusion to the microsurgical flap and reduce the risk of perioperative thrombosis. The efficacy of normovolemic and hypervolemic haemodilution in microsurgical reconstruction is still debated, and in this regard, literature lacks randomized, prospective studies [22]. Nonetheless, experimental studies have proven the benefits of haemodilution in reducing the rate of thrombotic complications in free flap surgery, suggesting its potential benefits on free flap survival [23-26]. As regards fluid therapy protocols, crystalloids or a combination of colloidal and crystalloid solutions are commonly utilized for hemodilution. Some literature evidence shows that the integration of colloids in the fluid therapy guarantees the maintainance of adequate flap perfusion, reducing tissue oedema and pressure on the microcirculation [27,28]. A target haematocrit between 22-25% was set preoperatively and maintained for the first five postoperative days to reduce blood viscosity and shear stress on the endothelium.

CONCLUSIONS

Although hypercoagulable states pose a risk for microvascular thrombosis and perioperative systemic thrombotic complications, in our opinion these should not preclude the attempt of a microsurgical reconstruction. The presented case is an example of successful free flap reconstruction in a patient diagnosed with homozygosity for the *C677T* polymorphism of the MTHFR mutation gene and pan-agglutinizing anti-erythrocyte alloantibody. The perioperative management of high-risk patients undergoing free flap surgery might require haematological and anaesthesiologic referral, with the aim of preparing the patient to the surgery and tailoring preoperative and postoperative prophylactic measures to minimize the risk of complications. Perioperative fluid management is key in the control of flap perfusion, tissue oedema and pressure on the vascular anastomoses. In our patient the adoption of a normovolemic haemodilution protocol and the maintenance of low haematocrit values were successful in securing adequate flap flow, with no need for transfusions and no reported complications. Literature evidence on microsurgical reconstruction outcomes in patients with thrombophilia is scant, and further investigation is required to better define the relative risk of complications in this cohort of patients.

References

[1] Srikanthan K, Viswanathan N, Yuen JC. Free-flap failure in thrombophilia: case report and systematic review of the literature. Ann Plast Surg 2013;71:675-681.

[2] Wang TY, Serletti JM, Cuker A, et al. Free tissue transfer in the hypercoagulable patient: a review of 58 flaps. Plast Reconstr Surg 2012;129:443-453. https://doi.org/10.1097/prs.0b013e31823aec4d

[3] Khouri RK, Cooley BC, Kunselman AR, et al. A prospective study of microvascular free-flap surgery and outcome. Plast Reconstr Surg 1998;102:711-721.

[4] Pannucci C, Kovach S, Cuker A. Microsurgery and the hypercoagulable state: a hematologist's perspective. Plast Reconstr Surg 2015;136:545e-552e. https://doi.org/10.1097/PRS.0000000000001591

[5] Herrera F, Lee C, Kryger G, et al. Microsurgery in the hypercoagulable patient: review of the literature. J Reconstr Microsurg 2012;28:305-312. https://doi.org/10.1055/s-0032-1311687

[6] La Padula S, Hersant B, Meningaud JP. Intraoperative use of indocyanine green angiography for selecting the more reliable perforator of the anterolateral thigh flap: a comparison study. Microsurgery 2018;38:738-744. https://doi.org/10.1002/micr.30326

[7] Ruiz R, Hersant B, la Padula S, Meningaud JP. Facelifts: improving the long-term outcomes of lower face and neck rejuvenation surgery: the lower face and neck rejuvenation combined method. J Craniomaxillofac Surg 2018;46:697-704. https://doi.org/10.1016/j.jcms.2018.01.013

[8] Liew SC, Gupta E das. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. Eur J Med Genet 2015;58:1-10. https://doi.org/10.1016/j.ejmg.2014.10.004

[9] Zeng J, Zeng Q. Correlations between methylenetetrahydrofolate reductase gene polymorphisms and venous thromboembolism: a meta-analysis of 99 genetic association studies. Eur J Prev Cardiol 2019;26:120-134. https://doi.org/10.1177/2047487318799467

[10] Esse R, Barroso M, Almeida I, et al. The contribution of homocysteine metabolism disruption to endothelial dysfunction: state-of-the-art. Int J Mol Sci 2019;20:867. https://doi.org/10.3390/ijms20040867

[11] Vekris MD, Ovrenovits M, Dova L, et al. Free functional muscle transfer failure and thrombophilic gene mutations as a potential risk factor: a case report. Microsurgery 2007;27:88-90. https://doi.org/10.1002/micr.20312

[12] Davison S, Kessler C, Al-Attar A. Microvascular free flap failure caused by unrecognized hypercoagulability. Plast Reconstr Surg 2009;124:490-495. https://doi.org/10.1097/PRS.0b013e3181adcf35

[13] DeFazio M v, Economides JM, Anghel EL, et al. Lower extremity free tissue transfer in the setting of thrombophilia: analysis of peri-operative anticoagulation protocols and predictors of flap failure. J Reconstr Microsurg 2019;35:270-286. https://doi.org/10.1055/s-0038-1675145

[14] Vekris MD, Ovrenovits M, Dova L, et al. Free functional muscle transfer failure and thrombophilic gene mutations as a potential risk factor: a case report. Microsurgery 2007;27:88-90. https://doi.org/10.1002/micr.20312

[15] Khansa I, Colakoglu S, Tomich DC, et al. Factor V Leiden associated with flap loss in microsurgical breast reconstruction. Microsurgery 2011;31:409-412. https://doi.org/10.1002/micr.20879

[16] Sezgin B, Ayhan MS, Tuncer S, et al. Hypercoagulability in microvascular breast reconstruction: an algorithmic approach for an underestimated situation. J Reconstr Microsurg 2012;28:515-520. https://doi.org/10.1055/s-0032-1315771

[17] Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood 2019;133:1821-1830. https://doi.org/10.1182/blood-2018-08-833962

[18] Cervelli V, Longo B. Plastic and reconstructive surgery: roots and future direction of a constantly evolving discipline. PRRS 2022;1:49-50. https://doi.org/10.57604/PRRS-090

[19] Longo B, D'Orsi G, Orlando G, et al. Recurrent dermatofibrosarcoma protuberans of the clavicular region: radical excision and reconstruction with Latissimus Dorsi myocutaneous flap. PRRS 2022;1:14-19. https://doi.org/10.57604/PRRS-002

[20] Schonauer F, Cavaliere A, Pezone G, et al. Letter to the Editor in Chief. Thoraco-acromial artery perforator (TAAP)

flap for reconstruction of a recurrent dermatofibrosarcoma protuberans of the clavicular region. PRRS 2022;1:79-81. https://doi.org/10.57604/PRRS-083

21 Longo B, D'Orsi G, Pistoia A, et al. T-inverted shaped rectus abdominis myocutaneous (Ti-RAM) flap for chest wall reconstruction. PRRS 2022;1:64-68. https://doi.org/10.57604/PRRS-028

22 Amoroso M, Apelgren P, Elander A, et al. The effect of hemodilution on free flap survival: a systematic review of clinical and experimental studies. Clin Hemorheol Microcirc 2020;75:457-466. https://doi.org/10.3233/CH-200832

23 Atchabahian A, Masquelet AC. Experimental prevention of free flap thrombosis. II: normovolemic hemodilution for thrombosis prevention. Microsurgery 1996;17:714-716. https://doi.org/10.1002/(SICI)1098-2752(1996)17:12<714::AID-MICR10>3.0.CO;2-X

24 Farina Jr J, Piccinato C, Rossi M, et al. Effect of isovolemic hemodilution with 3% albumin on thrombus formation at venous microanastomosis in rats. Microsurgery 2002;22:152-157. https://doi.org/10.1002/micr.21743

25 Farina Jr. JA, Piccinato CE, Campos AD, et al. Comparative study of isovolemic hemodilution with 3% albumin, dextran-40, and prophylactic enoxaparin (LMWH) on thrombus formation at venous microanastomosis in rats. Microsurgery 2006;26:456-464. https://doi.org/10.1002/micr.20270

26 Amoroso M, Özkan Ö, Özkan Ö, et al. The effect of normovolemic and hypervolemic hemodilution on a microsurgical model: experimental study in rats. Plast Reconstr Surg 2015;136:512-519. https://doi.org/10.1097/PRS.0000000000001525

27 Hagau N, Longrois D. Anesthesia for free vascularized tissue transfer. Microsurgery 2009;29:161-167. https://doi.org/10.1002/micr.20584

28 Zhong T, Neinstein R, Massey C, et al. Intravenous fluid infusion rate in microsurgical breast reconstruction: important lessons learned from 354 free flaps. Plast Reconstr Surg 2011;128:1153-1160. https://doi.org/10.1097/PRS.0b013e318221da56