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A Flow Dynamic Rationale for Accelerated Vascularized Composite Allotransplant Rejection

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Background: From 1996 to 2000, Diefenbeck et al. carried out six knee vascularized composite allotransplants. The allotransplants were composed of bone, soft tissue, and femoral vascular pedicle (25 to 40 cm). All rejected between 14 and 56 months. Failures were attributed to chronic rejection. In 2008, the Louisville team lost their fourth patient's hand transplant at 8 months. During the rejection workup, intraoperative findings noted a thickened arterial pedicle attributed to intimal hyperplasia with significant fibrotic perivascular tissue and a near "no-flow phenomenon." No cutaneous rejection was appreciated and failure was attributed to chronic rejection.
Methods: Data were collected from two teams, one in Germany and the other in Louisville, Kentucky. The population under study consisted of the six knee and one hand transplants. The factor of interest was the long donor arterial pedicle. The outcome measurements were transplant survival time and histopathologic results.
Results: There are only seven published vascularized composite allotransplant cases where a donor artery longer than 25 cm was used. This cohort represents a 100 percent accelerated failure rate. The cause of these losses remains unexplained. The donor arteries suffered from T-cell–mediated rejection and ischemia-induced media/adventitial necrosis.
Conclusions: We hypothesize that the donor artery rejected at an accelerated rate because of ischemia caused by disruption of the external vasa vasorum in conjunction with intimal hyperplasia induced by T-cell–mediated rejection that led to disruption of the Windkessel effect. Loss of this effect presented as intimal hyperplasia accelerated by ischemia causing an expedited transplant failure. (*Plast. Reconstr. Surg.* 143: 637e, 2019.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

The first modern vascularized composite allotransplants were a pioneering series of knee joint transplants carried out between 1996 to 2004 by Drs. Hofmann, Diefenbeck, and their team.[1–7] Hofmann et al. pioneered the concept that it was possible to transplant the joint, the surrounding synovial lining, and the feeding artery and vein. This approach spared the recipient surrounding tissues and vital structures.

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These early pioneers are rarely identified as the first to produce a modern vascularized composite allotransplant, perhaps because all of the knee joint transplants were lost within 56 months. The definitive cause for the losses remains a mystery to this day. Without a clear explanation as to why these knee joint transplants failed, knee joint transplants have ceased to be continued in the clinical setting. Instead, focus has shifted primarily to hand and face transplantation, with much improved long-term survival.

Why have so many other types of vascularized composite allotransplants established long-term survival whereas knee joint transplants have not? The answer may lie in a familiar case performed in hand transplantation by the senior author of this article, Warren C. Breidenbach, M.D., M.S. When comparing the hand transplantations performed by the Louisville team and the knee joint transplants performed in Germany, one theme begins to emerge as a possible cause for the accelerated vascularized composite allotransplant failure.

The hypothesis of this article is that a vascularized composite allotransplant with a lengthened donor artery will reject at an accelerated rate because of ischemia of the donor artery caused by disruption of the external vasa vasorum in conjunction with intimal hyperplasia induced by T-cell–mediated rejection, leading to the destruction of normal physiologic blood flow. We propose that any vascularized composite allotransplant with an extremely long donor artery (>25 cm) has a high probability of an expedited loss secondary to T-cell–mediated rejection leading to intimal hyperplasia. We would like to emphasize that we believe the length of the donor artery, and not the type of vascularized composite allotransplant, is the primary concern.

This is a case series study where data were collected from publications by two teams, one in Germany and the other in Louisville, Kentucky. A total of seven long arterial pedicles were evaluated (six knee joint transplants and one hand transplant) for evidence of T-cell–mediated rejection, antibody-mediated rejection, and intimal hyperplasia. The factor of interest is the long donor pedicle artery.[2–4,6–8] The outcome measurement will be the extent of T-cell–mediated rejection, antibody-mediated rejection, ischemic injury, capillary injury to the long donor arteries, and vascularized composite allotransplant survival. In all of these cases, the vascularized composite allotransplant was lost early. The details of these cases are as follows.

PATIENTS AND METHODS

Knee Transplants

Hofmann and Diefenbeck's team carried out six knee joint transplants. The arteries to these vascularized composite allotransplants were stripped of their surrounding soft tissue, leaving the bone, cartilage, and synovial lining of the joint with a 25- to 45-cm donor pedicle.[2–4,6,7] [**See Figure, Supplemental Digital Content 1**, which shows the diagram of the methodology used by the German team to carry out a knee transplant. The femur, proximal tibia synovial tissue, along with the superficial femoral artery and the descending geniculate arteries are harvested and separated from the surrounding tissue. Therefore, the bone, synovia of the knee joint, and artery are skeletonized. (From Diefenbeck M, Wagner F, Kirschner MH, Nerlich A, Mückley T, Hofmann GO. Outcome of allogeneic vascularized knee transplants. *Transpl Int.* 2007;20:410–418. Used with permission from John Wiley & Sons, Inc.), ***http://links.lww.com/PRS/D328***.] The outcomes of the six knee joint transplants are detailed in Table 1. Patients 3 and 4 were lost to infection and noncompliance, respectively. The remaining knee joint transplants were lost between 14 and 54 months. Clinically, the knees were described as becoming unstable, with decreased range of motion. Evaluation of the knees after explantation revealed necrosis of the bone. The team stated that the long donor artery was not evaluated because of there not being enough viable tissue for appropriate evaluation.[4]

This leaves the last knee joint transplant patient with the sentinel vascularized composite allograft for a thorough evaluation. At 18 months, the tibial nail was removed, and biopsy specimens of the cartilage, bone, and synovium were obtained. At this 18-month mark, synovial vessels indicated mild proliferation of the intima with infiltration of mononuclear cells—determined to be "mild rejection with no need for intervention."[4] No cutaneous changes were noted in the sentinel skin graft at the time. At 28 months, the "sole" episode of acute rejection of the sentinel flap occurred. Skin and knee joint synovial biopsy specimens were obtained indicating acute T-cell–mediated rejection. The patient was treated with high-dose intravenous methylprednisolone for 3

Table 1. Summary of the Six Knee Transplants Performed by Diefenbeck et al. with Date of Surgery, Outcome, and Total Survival Time*

Patient	Date of Surgery	Outcome of KT	Total Survival Time of KT
1	April of 1996	Amputation secondary to rejection	16 mo
2	November of 1996	Amputation secondary to rejection	3 yr
3	December of 1996	Removal of KT secondary to infection	5 wk
4	July of 1997	Amputation secondary to noncompliance	24 mo
5	February of 1998	Amputation secondary to rejection	14 mo
6	April of 2002	Amputation secondary to rejection	56 mo

KT, knee joint transplant.
*Two of the patients are excluded (patients 3 and 4) because of infection and noncompliance, respectively, which added a confounding variable to the cause of graft failure.

days followed by a 2-week taper, and the sentinel flap indicated that the rejection episode resolved. The sentinel flap indicated no other episodes of sentinel vascularized composite allotransplant rejection until 36 months postoperatively, when a portion of the sentinel vascularized composite allotransplant necrosed. [**See Figure, Supplemental Digital Content 2**, which shows a biopsy specimen of sentinel skin graft at 36 months postoperatively: extensive intimal hyperplasia was noted in small arterioles. (From Diefenbeck M, Nerlich A, Schneeberger S, Wagner F, Hofmann GO. Allograft vasculopathy after allogeneic vascularized knee transplantation. *Transpl Int.* 2011;24:e1–e5. Used with permission from John Wiley & Sons, Inc.), ***http://links.lww.com/PRS/D329***.] No clinical evidence of rejection in the sentinel vascularized composite allotransplant was appreciated before this result, with biopsy specimens revealing Banff grade I to II rejection in the dermis. The knee joint transplant sentinel vascularized composite allotransplant steadily worsened. By 50 weeks, knee function started to decrease. Deep biopsy specimens indicated bone and cartilage necrosis in the knee, with increasing intimal hyperplasia in the arterioles. [**See Figure, Supplemental Digital Content 3**, which shows a biopsy specimen of the synovium at 50 months postoperatively: extensive concentric intimal hyperplasia with nearly complete occlusion of the lumen was noted. (From Diefenbeck M, Nerlich A, Schneeberger S, Wagner F, Hofmann GO. Allograft vasculopathy after allogeneic vascularized knee transplantation. *Transpl Int.* 2011;24:e1–e5. Used with permission from John Wiley & Sons, Inc.), ***http://links.lww.com/PRS/D330***.] This was followed by a deep surgical infection that started with the necrotic sentinel vascularized composite allotransplant. At 56 months, the leg was amputated.

It is noteworthy to reiterate that the sentinel flap never indicated a rejection episode (grade III to IV) before the skin island became frankly necrotic. Repeatedly, the literature claims skin to be the most important marker of rejection in the deep tissues.[9–22] We believe this has significant implications for this concept as reviewed in another of our articles ("Is Skin the Most Allogenic Tissue in Vascularized Composite Allotransplantation and a Valid Monitor of the Deeper Tissues?").[23]

Hand Transplant

The fourth hand transplantation carried out in Louisville by the senior author (W.C.B.) was lost not to chronic rejection but to accelerated intimal hyperplasia of the long donor artery leading to ischemia of the hand.[8,24] At 8 months after transplantation, the patient presented with increasing blue discoloration of the hand but with no classically described evidence of clinical rejection. [**See Figure, Supplemental Digital Content 4**, which shows a hand transplant several days before amputation. There is no clinical evidence of rejection. There is evidence of ischemia appreciated by cyanotic discoloration. One of three biopsy specimens demonstrated Banff grade I rejection. The remaining two were normal. (From Kaufman CL, Ouseph R, Blair B, et al. Graft vasculopathy in clinical hand transplantation. *Am J Transplant.* 2012;12:1004–1016. Used with permission from John Wiley & Sons, Inc.), ***http://links.lww.com/PRS/D331***.] Biopsy specimens of the skin several days before amputation demonstrated only a Banff grade I rejection. An arteriogram was carried out, which indicated that the donor artery to the vascularized composite allotransplant was patent. Clinically, the hand transplant skin became increasingly cyanotic.

The senior author of this article surgically explored the donor pedicles (radial and ulnar) and found the arteries to be patent, with minimal flow. The artery adventitia and possibly the media were extremely sclerotic. A recipient vein graft was used to attempt reconstruction of the

donor artery. Blood flow to the hand was reestablished but the flow repeatedly ceased after a 10- to 15-minute period. There was no evidence of thrombosis noted. Several attempts at vein grafting reconstruction were carried out, always with the same outcome of loss of flow. Intraoperatively, a diagnosis was made of "no flow" to the vascularized composite allotransplant and the incision was closed. The hand cyanosis progressed severely over the following 48 hours, leading to amputation.

No clinical evidence of cutaneous rejection in the hand transplant was appreciated before amputation. After explanation, histologic evaluation demonstrated that there was extensive intimal hyperplasia in the donor artery to the extent that the lumen appeared obliterated. [**See Figure, Supplemental Digital Content 5**, which shows hematoxylin and eosin stain of radial artery at the time of amputation. No evidence of mononuclear cell infiltration is seen. (From Kaufman CL, Ouseph R, Blair B, et al. Graft vasculopathy in clinical hand transplantation. *Am J Transplant.* 2012;12:1004–1016. Used with permission from John Wiley & Sons, Inc.), ***http://links.lww.com/PRS/D332***.] Even to the level of the digital arteries, nearly complete occlusion was demonstrated on histopathologic evaluation. Histologic evaluation did not demonstrate evidence of T-cell–mediated rejection in the skin or arteries. Thus, it is unlikely that the extensive and rapid development of intimal hyperplasia in the donor artery to the vascularized composite allotransplant was attributable solely to T-cell–mediated rejection. So why did this one case, unlike all the other cases performed in Louisville, develop severe intimal hyperplasia with no evidence of early or late T-cell–mediated rejection?

DISCUSSION

No other team in the world with five or more vascularized composite allotransplants have reported a 100 percent failure rate. Therefore, empirically, there is a significant difference between our vascularized composite allotransplant cohort compared to the worldwide vascularized composite allotransplant experience. What is the cause of this difference? We believe it is reasonable to hypothesize that the factor that distinguishes this cohort is the use of a long donor pedicle skeletonized from its feeding external vasa vasorum.

The first knee joint transplant pioneered the way forward for vascularized composite allotransplant years before the first hand and face transplant. However, all knee joint transplants were lost within 14 to 56 months. Because of this outcome, no knee joint transplant has been clinically attempted since 2002. Now, 15 years since the last attempted knee joint transplant, it appears that most teams have given up on the possibility of clinical joint transplantation. These previous failures should be appropriately understood to continue a greater pursuit for success in the future.

The two commonalities in our cohort under study that are not reported as present in any other vascularized composite allotransplant cohort are as follows: (1) use of a long donor artery of at least 25 cm or more and (2) accelerated loss of all vascularized composite allotransplants in a program. These stark differences in outcomes should focus our attention on the most likely variable, the long donor artery pedicle, as the cause of failure.

Multiple Studies Have Demonstrated That Damage or Separation of the External Vasa Vasorum from an Artery Produces Intimal Hyperplasia

We believe we know why all knee joint transplants failed and what can be done to achieve long-term joint vascularized composite allotransplant survival. Our hypothesis is that a long donor artery is prone to develop intimal hyperplasia caused by both ischemia and T-cell–mediated rejection. The former appears to exponentially increase the rate of failure. Simply put, the greater initial danger to a vascularized composite allotransplant with a long donor artery is ischemia to the vasculature of the graft, followed by T-cell–mediated rejection.

Using a long donor pedicle mandates separation of the external vasa vasorum from the donor. [**See Figure, Supplemental Digital Content 6**, which shows a corrosion cast preparation of the anterior descending artery in a porcine heart. The white arrows point to the external vasa vasorum. If the artery is elevated out of its bed, it becomes mandatory that these external vasa vasorum be transected. (From Harnoss JM, Krackhardt F, Ritter Z, et al. Porcine arteriogenesis based on vasa vasorum in a novel semi-acute occlusion model using high-resolution imaging. *Heart Vessels* 2017;32:1400–1409. Reprinted by permission from Springer), ***http://links.lww.com/PRS/D333***.] Numerous publications have demonstrated that separation of the external vasa vasorum from the artery produces ischemia, which leads to development of intimal hyperplasia, media necrosis, and other ischemic pathologic

changes.[25–33] This can be experimentally accomplished by simple elevation of the artery out of its bed, creating separation of the artery from the external vasa vasorum with a silastic cuff or some other blocking mechanism.

We are confident that in the knee joint transplants and one hand transplant, the external vasa vasorum were separated over a significant portion of the donor artery. This could logically be the inciting source for the accelerated intimal hyperplasia development. However, ischemia causing intimal hyperplasia in the donor artery would most likely not result in complete obstruction of the artery as reported by Hautz et al.[8] The occlusion of the radial and ulnar artery, demonstrated by Kaufman et al., is likely an artifact caused by formalin preparation of the biopsy specimens, creating a false-positive diagnosis of occlusion. In hindsight, this finding should have been noted initially because of the arteriogram, obtained 3 days before amputation, demonstrating that the radial and ulnar arteries were functionally patent.

If the arteries were patent, why was there ischemia of the hand? How do we reconcile a no-flow phenomenon with a patent artery? The answer lies in the understanding of fluid dynamics through the formulaic components of the Windkessel effect.

The Importance of the Windkessel Effect in Vascularized Composite Allotransplantation

The stark differences in survival of a vascularized composite allotransplant based on the above-described donor pedicles can be supported by a mathematical fluid dynamics discussion. The formulas listed below define flow (Q) and resistance (R) found in the cardiac and peripheral vascular systems. Initially, these formulas were derived to explain flow dynamics in a nonideal fluid state by Hagen-Poiseuille in the mid 1800s:

$$Q = \Delta P \div R \text{ and } R = 8L\eta \div \pi r^4.$$

When the equations are evaluated, it can be appreciated that flow through a particular vessel is inversely proportional to resistance. Evaluating the equation for resistance can then exemplify how resistance in a vessel is directly related to length (L) of that vessel. The vessel length and radius (r) are the only undefined variables preoperatively, as the remaining factors largely remain constant when applied within the same patient. Postoperatively, the radius is primarily affected by development of intimal hyperplasia caused by the vessel's response from insult of the immune system and the damage to the external vasa vasorum during procurement. When solving the equation for flow, the radius is seen to be directly proportional to flow ($\uparrow r \uparrow Q$), whereas length of the affected vessel is seen as an inverse relationship with blood flow to the vascularized composite allotransplant ($\uparrow L \downarrow Q$).

It has been well established in the medical and physiology literature that as vessels develop, intimal hyperplasia they will inherently lose vessel elasticity and therefore compliance will decrease. With this decrease in compliance, the vessels lose the ability to maintain appropriate diastolic pressure, and propagation of the diastolic vascular phase in the graft will be severely hindered. This significant aspect appears to cause the ischemic changes and damage to the vascularized composite allotransplant over time:

$$C = \Delta V \div \Delta P.$$

This then presents us two concepts that can be incorporated into a single physiologic system defined by the Windkessel effect derived by Otto Frank in the late 1800s. For the purposes of our hypothesis, it does not require an extensive understanding of the intricacies of the formula, but we highlight the two primary variables that affect the flow through the vessel, and therefore a deviation from its normal physiologic function will be seen: resistance and compliance as affected by the previously discussed variables. Ultimately, the two variables have been altered by the surgeon (resistance and compliance) and the recipient's immune response to the vascularized composite allotransplant (compliance):

$$P(t) = P(td)e(-t / RC).$$

The Hagen-Poiseuille equation and the Windkessel effect clearly define the consequences that can occur with alterations in both resistance and compliance. The two variables that serve as the basis for our hypothesis are affected by length of the vessel harvested and the development of intimal hyperplasia as the vascularized composite allotransplant undergoes repeated insults from the immune system and ischemia caused by surgical dissection. Therefore, we hypothesize that the variation in the vascular pedicle in the above vascularized composite allotransplant cases leads to an accelerated low-flow or functional no-flow state in the end organ, ultimately leading to vascularized composite allotransplant failure.

We believe this phenomenon was overlooked in the hand transplant described in this article, where Dr. Breidenbach improperly decided to use a long donor artery pedicle (>25 cm). This led to

a sclerotic donor vessel secondary to ischemia and T-cell–mediated rejection. This loss of elasticity in the donor artery and subsequent reduction in the Windkessel effect was amplified by the length of the artery, leading to a marked decrease of blood flow to the hand. The hand became ischemic once diastolic blood flow reached a critically low level. Ultimately, this led to a functional no-flow phenomenon in which the tissues in the hand suffered extreme ischemia followed by vascularized composite allotransplant failure.

To further solidify this concept, one can compare two sentinel cases not listed above: the first face transplant, performed by Devauchelle et al.[34]; and the first Louisville hand transplant, performed by Breidenbach et al.[35] The length of the donor pedicle is unknown to us in the face transplant, but what can be assumed is that the diameter of the arterial pedicle was inherently smaller in the facial graft (as the facial artery was used) compared with the radial artery in the hand. Hypothetically, if the facial artery was 2 mm versus the radial artery's 4-mm diameter, these differences are exponentiated by a factor of 4 (i.e., $r^4 \rightarrow 16$ versus 256). As intimal hyperplasia develops in these vessels, the flow will significantly decrease over a shorter period in the smaller vessel, which might explain why the facial graft lasted 10 years before failure versus the hand transplant, which continues to survive at 19 years.

Lastly, we understand that transplantation does not occur in a vacuum. There are many factors that could not be controlled, such as patient noncompliance, microtrauma to grafts, denervation of the knee joints, and others. We also note that extensive research has been performed on loss of sympathetic innervation to the arterial structures that leads to loss of normal function affecting the fluid dynamics. These are all factors that likely contributed to graft failure, but we hypothesize that the explanation above had the greatest compounding detrimental effect leading to accelerated graft loss.

CONCLUSIONS

We are proposing in this publication that a knee joint transplant should be possible if one properly manages the length of the donor and recipient pedicles. The principle should be to keep the donor pedicle as short as possible (<10 cm) and minimize the skeletonization of the pedicles during dissection. Both donor and recipient pedicles will suffer accelerated ischemia as their length increases. The donor pedicle will be subjected to both ischemia and rejection, whereas the recipient pedicle will be subjected only to ischemia. The longer the donor pedicle, the more external vasa vasorum will be separated, until a critical distance is reached where adventitial and media blood flow will start to decrease.

Over time, intimal hyperplasia will form in both the donor and recipient arteries. This will result in stiffening of the donor and recipient arteries that will result in loss of the Windkessel effect. The loss of the Windkessel effect will be most prominent in the donor artery (thus the importance of keeping the donor pedicle short).

The concepts we have outlined above should and will be tested in our laboratories. A vascularized composite allotransplant is being carried out using large animals with routine deep wide biopsies, skin biopsies, and various vascular parameters to measure vasculature intimal hyperplasia development, various fluid dynamic values, and functional changes. This research, we believe, will provide extremely valuable data that we hope to publish in the future validating the hypothesis and provide rapid, translatable techniques for earlier diagnosis of graft rejection.

As vascularized composite allotransplant surgeons, we need to be cognizant of the complex vascular and immunologic responses that are necessary for a successful vascularized composite allotransplant. We need to work closely with the vascular surgeons to enhance our understanding of vascular fluid dynamics, the Windkessel effect, and their impact on the outcomes of our patients. This model of multidisciplinary medicine will produce a shared anatomical and physiologic understanding that will provide our vascularized composite allotransplant patients a greater chance at long-term success.

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