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**ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ**

# Vascularized Composite Allotransplantation: Current challenges and future perspectives

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**Abstract**

Vascularised composite allotransplants (VCAs) have unique properties because of diverse tissue components transplanted en mass as a single unit. Modern microsurgical techniques have made possible a broad spectrum of novel means for the reconstruction of complex bone and soft tissue defects. (1) These techniques, in combination with developments in transplant immunology, have led to successful allotransplantation and achievement of the highest rung in the reconstructive ladder - truly replacing like with like. The utilization of contemporary microsurgical technique in the context of VCA permits successful technical execution and feasibility of VCA, facilitates the study of immunologic tolerance in VCA preclinical models, and optimizes functional VCA outcomes. (2)

In addition to surgery, this type of transplant also faces enormous immunological challenges that demand a detailed analysis of all aspects of alloimmune responses, organ preservation, and injury, as well as the immunogenicity of various tissues within the VCA grafts to further improve graft and patient outcomes. Moreover, the side effects of long-term immunosuppression for VCA patients need to be carefully balanced with the potential benefit of a non-life-saving procedure. (3)

Vascularized Composite Allografts offer the opportunity for life-improving function to patients with tissue loss unable to be reconstructed through traditional techniques. Currently, most immunosuppressive regimens reported to date in clinical VCA have used CNIs, with most of the VCA recipients experiencing side effects. (4, 5) Since VCA is typically employed for non-life-threatening conditions, the need of a CNI-free regimen is needed.

In this review article, we provide a comprehensive update with a specific emphasis on the alloimmune responses to VCA, established and novel immunosuppressive treatments, and patient outcomes.

## *1. Introduction*

Vascularized composite allograft (VCA) has emerged as a reconstructive option for patients who have suffered severe tissue loss due to trauma, burns, tumor resection, or congenital malformation. Although VCA is considered a relatively young field, the notion of replacing “like with like” to repair severe tissue defects is hardly new. St. Cosmas and St. Damian, the patron saints of medicine, appeared in a dream to physicians who have removed a cancerous leg from a patient and replaced it with the leg of a cadaver. It would not be until several centuries later that that dream would become a reality .

***(Figure 1)*** **Alonso de sedano, ca.1495**

Vascularized composite allograft has become a valid therapeutic and restorative option for patients with severe tissue defects not amendable to conventional reconstruction. It involves the transfer of multiple tissue types—including skin, muscle, nerve, tendon, bone, and blood vessels—as a single unit using advanced microsurgical techniques (6). Technical success has been achieved in several forms of VCA and since then the field of VCA has expanded to include face (7), uterus (8), abdominal wall (9), larynx (10), penile (11), and other transplants (12). Although the technical aspects are distinctive, minimizing immunosuppression in VCA, however, continues to be of utmost importance as VCA is a life-enhancing transplant (13) **.**

***(Figure 2)***

The field of transplantation has been rapidly progressing since the safety of solid organ transplantation was demonstrated and established in the 1970s with the implementation of immunosuppression. Clinical vascularized composite allotransplantation had been attempted as early as 1964 (14). Although technically successful and despite the use of chemical immunosuppressants, the first allograft failed (14) due to irreversible acute rejection (15). However, early clinical results in addition to aggregated experimental experience led investigators to the belief that the skin's potent immunogenicity would prevent the success of VCAs (16), resulting in a hiatus of three decades without major advances (17). It was not until 1998 that the first successful VCA was performed (18). Since that time, there have been over 200 upper extremity (19) and 30 face transplants (20) have been performed around the world, due to improvement in surgical and immunological outcomes (21). In the 1990s, the advent of more potent immunosuppressants rekindled the interest and successful experimental trials in rodents and pre-clinical large animal VCA models were performed. Significant advances in both basic science and translational research over the last decade paired with highly encouraging functional and immunological clinical outcomes in the majority of patients have lead VCA to transition from an experimental and sometimes controversial procedure to an accepted and rapidly expanding field with great promise (21).

**Table 1** highlights the milestones of VCA since its introduction to the transplant

| Year | Milestone |
|---|---|
| 1964 | Clinical vascularized composite allotransplantation (VCA) first attempt |
| 1998 | First succesful VCA |
| 2000 | First Uterine Transplantation |
| 2007 | Banff Classification of clinical rejection in VCA |
| 2015 | Belatacept in VCA |

## *2. VCA and Mechanisms of Rejection*

Rejection remains a considerable problem in VCA, though specific incidence is not yet known. Approximately 85% of all patients have been reported to experience at least one episode of acute rejection early in the post-transplant period, with over 50% experiencing multiple episodes (22). These episodes of rejection seem to happen with higher frequency than seen in solid organs, possibly secondary to the skin component in most VCAs, unique as both an immunogenic target and as a means for monitoring (20).

In 1980, Dvorak et al demonstrated that microvascular endothelium is the critical target of the immune response, and that VCA rejection manifests largely by vascular damage followed by ischemic infarction (23). They provided further evidence that, along these vascular changes, both major T-cell subsets, $CD4^+$ (helper/inducer), and $CD8^+$ (cytotoxic/suppressor), infiltrate the skin forming perivascular cuffs (24). This model is partly similar to a VCA with rejection histological changes appearing initially in form of perivascular infiltrates in the dermis; however, major immunologic differences exist. First, a variety of immunosuppressive and immunomodulatory protocols are used in VCA which

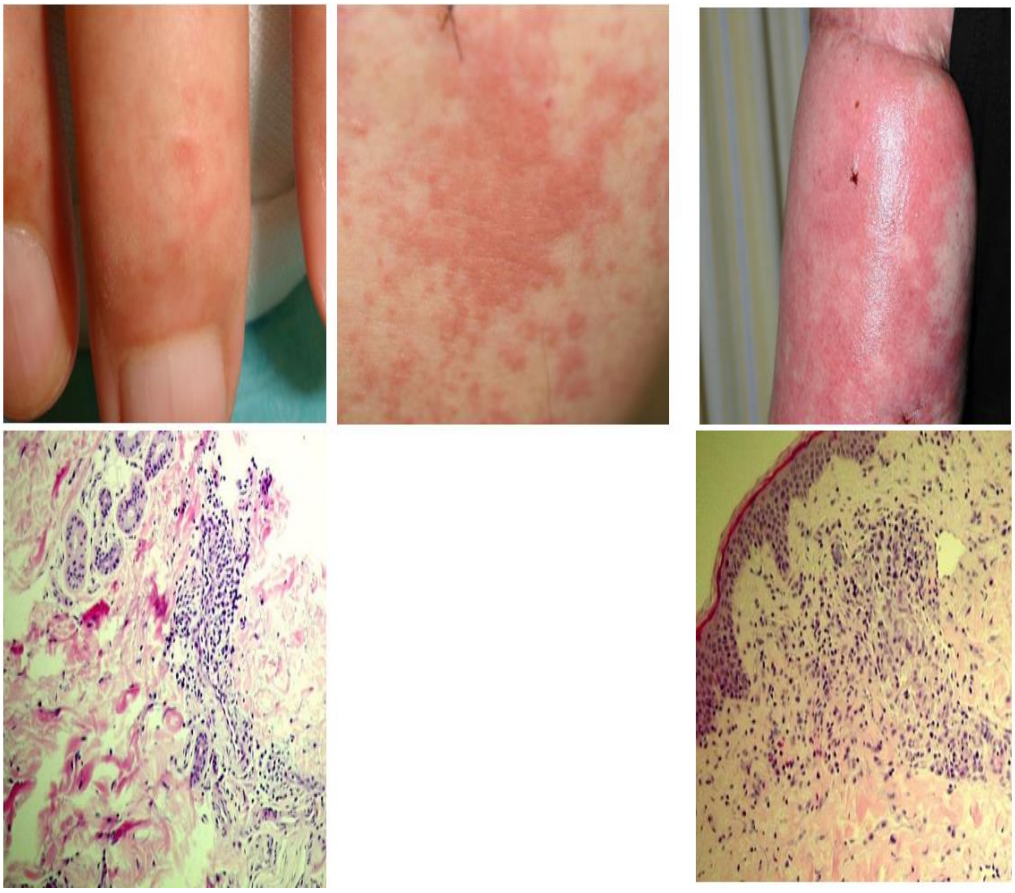
impacts the dynamics rejection; second, the skin, being transplanted with other components in a VCA, is rendered less antigenic which might alter the timing and intensity of rejection episodes (25).

### 2.1 **Cell-Mediated Rejection**

The most common and best understood mechanism of rejection is that of cell-mediated rejection as a function of innate and adaptive immune responses. This form of rejection, most often presenting as an acute episode, relies on cell-based rejection through the direct and indirect pathways of antigen recognition with consecutive T-cell priming (alloantigen-specific T cell activation). Acute response begins at the time of transplant, where tissue damage from preservation, ischemia or trauma from the operation leads to generation of damage-associated molecular patterns that are recognized by pathogen-associated pattern recognition receptors on recipient innate immune cells (26). This leads to upregulation of genes involved in the inflammatory response with concomitant increase in chemokines and preformed P-selectin that promote the recruitment of additional innate cells as well as activated lymphocytes (26). Recruited macrophages secrete additional inflammatory cytokines [Interleukin (IL)-1 and IL6] which further increase response to the graft as well as recruitment and activation of antigen-presenting cells (APCs) (27).

One of the most potent forms of cell-mediated rejection is the response to mismatched major histocompatibility complex (MHC) molecules, heavily reliant on $CD4^+$ and $CD8^+$ T cells. Through APCs such as dendritic cells or macrophages, presentation of nonself peptide antigens via MHC class II ($CD4^+$) or MHC class I ($CD8^+$) drives activation of alloreactive T cells that rapidly divide and differentiate into effector cells. Upon reaching the transplanted tissues, effector T cells orchestrate

a vast graft destructive program based on secreted cytokines leading to an increase in inflammatory cell response as well as apoptosis of graft cells through $CD8^+$ cell release of cytotoxins (26). In VCA, acute rejection typically presents clinically with mainly skin changes including a maculopapular variably erythematous rash with edema (28) and with histologic findings of perivascular or interstitial mononuclear cell infiltration with epidermal and/or adnexal involvement dependent on the grade of rejection (29) .



***(Figure 3)***

The skin component of the allografts shows clinical signs of rejection before other graft tissues and is thought to be a powerful producer of immune response and the major target of acute rejection (30). T cells have been implicated in acute rejection of skin allografts since the 1980s with many subsequent studies confirming the presence of T cells within target damaged tissue (31).

Preclinical studies have identified gene expression markers that differentiate T cell infiltrates in VCA that differ from those in T cell-mediated skin inflammation from other causes (32), and inhibition of T cell activation through regulatory T cells and other pathways has successfully prevented rejection (33). However, in human VCAs, investigation into T cell-mediated rejection has been somewhat limited. Hautz et al (34) described the predominance of $CD3^+$ cells in infiltrate and showed increases in CD68, Foxp3, indoleamine-2–3-dioxygenase and CD4/CD8 with worsening rejection along with upregulation of lymphocyte adhesion markers including ICAM1 and E-selectin. Another recent study looked at serial biopsies from multiple face transplant recipients at standardized time points including periods of clinical rejection and normal intervals (35). Pathologic findings during episodes of rejection uniformly consisted of perivascular lymphoid infiltrates, cell sloughing into vessel lumen, termed lymphoid vasculitis, lymphocyte migration into the epidermis and pilosebacious structures and epithelial apoptosis spatially associated with infiltrating lymphocytes (35). T cells predominated in the infiltrate, increasing in number during rejection episodes, supporting that the episodes are T cell driven. Interestingly, the majority of the T cells were found to be of donor origin when checked by biomarkers, hypothesized to be due to donor resident memory T cell response to MHC targets from the homing of recipient lymphocyte/polymorphonuclear leukocyte to microenvironments.

## 2.2 **Presensitization and antibody-mediated rejection**

With initial exposure to antigen in lymphoid tissue, B cells are activated by T helper cells with subsequent cytokine secretion by T cell that furthers B cell activation, differentiation and antibody production (26). One of the major hurdles in VCA is the amount of sensitization present in the recipient candidates. After injuries so devastating such that they would warrant a limb or face transplant, patients often receive numerous blood transfusions and/or skin grafts, leading to sensitization and the presence of donor-specific antibodies (DSAs) able to cross-react with human leukocyte antigen (HLA) antigens of transplant origin (22). In the field of solid organ transplantation, presensitization is known as the greatest risk factor for allograft rejection and long-term graft loss despite immunosuppression, and it frequently causes patients with DSA to be excluded as candidates for transplantation (36).

In a study of patients with extensive burn-related injuries who had received blood transfusions and allograft coverage, a current standard of initial care, it was shown that this number is even higher for VCA candidates. In this report, over 50% of patients with extensive third-degree burns evaluated for VCA presented with a panel-reactive antibody (PRA) level of more than 85% and were thus considered contra indicated for transplantation due to HLA-hypersensitization (37). Even if the pretransplant crossmatch result is negative and a sensitized patient eventually is transplanted, there is an increased risk for antibody-mediated allograft injury and graft loss (38). The most common antigenic target of preexisting alloantibodies are MHC mismatches, though recognition of ABO incompatible antigens, minor histocompatibility complexes and endothelial cells has also been shown to contribute to rejection (39). Mechanistically, alloantibody-mediated tissue damage occurs

primarily through complement fixation as well as through antibody-dependent cellular toxicity, which follows natural killer cells and macrophage receptor binding of the Fc region of the antibody stimulating these cells to induce donor cell death (39). If preformed antibodies (DSA) exist, activation of complement and coagulation cascades can result in vascular thrombosis and infarction and thus hyperacute rejection and graft loss. The clinical impact of such a scenario has been strikingly evidenced by the most recent publication of the first demonstration of a case of AMR in a highly sensitized face transplant recipient (40).

Molecular diagnostics showed an increase in multiple genes during the episodes of rejection when compared to nonrejection times, including mainly activation of genes responsible for signaling pathways such as the CXCR3/CCR5 pathway, interferon gamma signaling and genes like GZMB, which are involved in the recruitment of cytotoxic cells and chemokine production as well as immune effector function (41). When analyzed for differences between the AMR episode and three TCMR episodes, statistically significant difference in upregulation of genes involved in leukocyte–endothelial cell interactions (i.e. ICAM1) was noted during the AMR episode, whereas increase in those associated with cytotoxicity was seen during TCMR (i.e. GZMB) (42). Morelon *et al* (43) reported another case of AMR with partial graft loss in a face transplant patient. This patient had two prior episodes of acute rejection in the first year posttransplant characterized by skin changes that were successfully treated with pulsed steroids. In the eighth posttransplant year, the patient was found to have DSA without clinical or pathologic signs of rejection; 4 months after the initial detection of antibodies, the patient had skin changes and edema with increase in DSA level but without evidence of C4d staining, capillary thrombosis or graft vasculopathy on biopsy. Although clinical improvement was seen, the patient

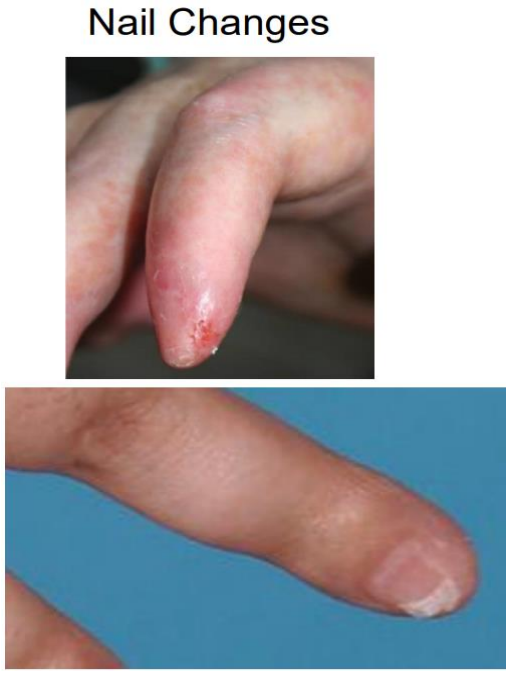
had another episode 9 months later at which point further increase in antibody was found as well as biopsy presence of C4d, dermal vascular changes and thickening of the pedicle vessel in the sentinel graft. Treatment was initiated against AMR (steroids, IVIG, plasmapheresis, bortezomib and eculizumab) without successful rescue, resulting in necrosis and partial graft loss (44). This case suggests both that the two mechanisms of rejection are different in their impact on the graft – namely, cellular rejection affects skin and vasculopathy is seen in AMR – and that low titers of anti-HLA antibodies below detection thresholds and antibodies that develop *de novo* after transplantation also injure allograft tissue.

Both preexisting DSA but especially de-novo DSA are currently discussed as main contributors to late allograft injury and graft failure (45). The Innsbruck group recently reported the first case of a primarily B-cell-driven rejection episode with the development of de-novo DSA indicative of AMR in a patient after forearm transplantation at 9 years post-transplant (46). One of the major findings was the evidence of lymphoid neogenesis in the dermis of both grafts reminiscent of tertiary lymphoid organs. However, diagnosis of AMR remains incompletely described, as staining for C4d and DSA titers has been shown to be unreliable in VCA (47, 36).

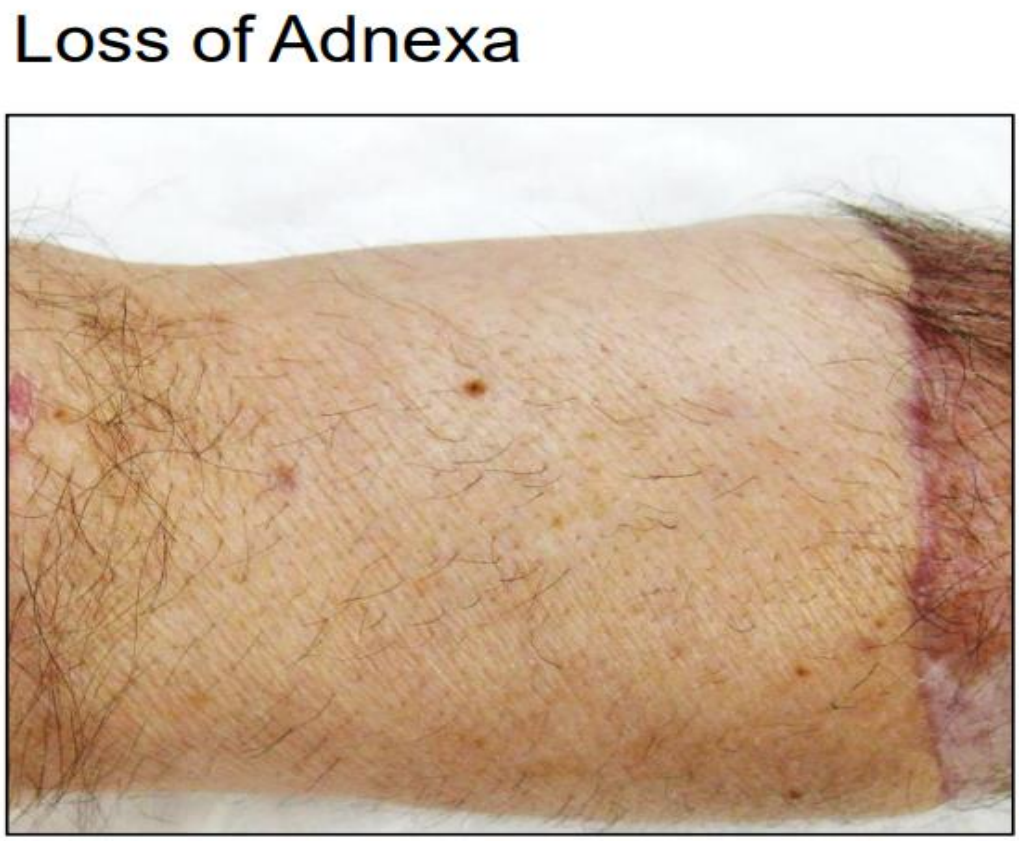
### 2.3 **Chronic rejection**

In VCA, chronic rejection was initially felt to be less common than in renal transplant, though the paucity of grafts and lack of significant follow-up time limited analysis. There have now been multiple cases reported of chronic rejection in VCA, particularly in hand and face transplantation (48,49). Because VCA is a relatively new field, limited data are available with regard to the long-term outcomes, specifically as

they relate to chronic rejection. Clinicopathologic signs felt to be indicative of chronic rejection included vascular narrowing, loss of adnexa, skin and muscle atrophy, fibrosis of deep tissue, myointimal proliferation and nail changes (50).




***(Figure 4)***

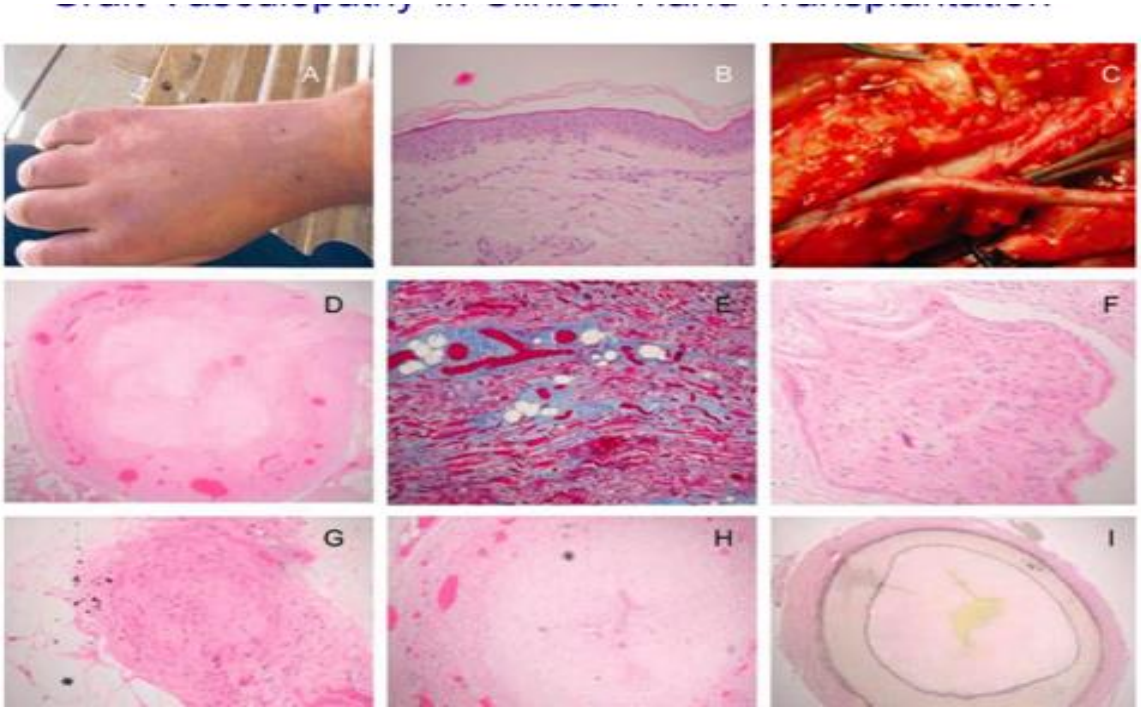



***(Figure 5)***

With longer graft survival in an increasing number of transplants, chronic changes such as dermal capillary microthromboses have been seen (51). This vasculopathy is thought to be caused by low-grade inflammation-induced endothelial damage and chronic remodeling of smooth muscle cells in an attempt to heal this damage. This induces neointimal thickening from both chronic adventitial inflammation and excess deposit of perivascular collagen (52). Immunologic contributors likely include HLA-mismatches, number and severity of acute rejection episodes, AMR, the presence of skin/vascularized bone marrow within VCA, cytomegalovirus status and infection (50).

Despite low number of human cases, there have been multiple preclinical studies that have demonstrated findings of chronic rejection in VCA. When compared

to isografted and consistently immunosuppressed rat hind–limb transplants, those that had pulse steroid/cyclosporine rescue from episodes of acute rejection were found to have graft vasculopathy and degeneration, demonstrating both the relationship of chronic rejection with acute rejection episodes and the effect of this chronic rejection on the histopathologic findings within vessels(53) .




***( Figure 6)***

A nonhuman primate study utilizing a face transplantation model in which immunosuppression was weaned in five long-surviving animals showed neointimal proliferation, vasculopathy, vessel wall fibrosis, progressive luminal occlusion and tertiary lymphoid follicles in serial biopsies of the transplanted grafts (54).

In human VCA recipients, reports of chronic rejection have been associated with cessation of immunosupression (55) or decrease in treatment because of medication side-effects (56) and have thus far correlated histopathologically with the preclinical models. Kaufman *et al* (57) reported on two patients who were found to have changes consistent with chronic rejection as described above. One patient had vascular symptoms without clinicopathologic skin changes, and vasculopathy progressed to the point of necessitating explant. Although not present prior, DSA did appear 2 days after explant. After this experience, the group began using deep tissue biopsies and ultrasound biomicroscopy (UBM) to monitor for vasculopathy in the hand transplant recipients. The second patient was found to have significant intimal thickening on screening UBM confirmed with deep biopsy, again DSA negative though C4d stains were positive. As the first had aggressive therapy and early and frequent use of the graft and the second patient had a complicated postoperative course (subsequent operations, wound dehiscence and infections) with DSA negative intimal hyperplasia, they postulate that nonimmune mechanisms may play a role in chronic rejection (58). The group out of Lyon, France reports on three cases of chronic rejection in VCA as well. One patient who voluntarily discontinued his treatment and required explant 13 years after transplant (43,59), one patient with just two acute rejection episodes in initial course who developed symptoms and vasculopathy 10 years after transplant requiring explant (60) and, most recently, partial loss of a facial transplant in which severe vasculopathy led to graft necrosis (43). Although all three patients had focal intimal thickening and luminal narrowing with surrounding infiltrate, the presence of DSA/AMR and C4d staining was variable.

**Table 2** summarizes the rejection mechanisms in VCA.

| **Type of Rejection** | **Findings** |
|---|---|
| Acute Rejection | Endothelial injury, CD4/CD8 infiltratates |
| Cell-Mediated | CD4/CD8, adhesion molecules, proinflammatory cytokines |
| Antibody-Mediated | B cells, C4d deposits |
| Chronic Rejection | microthromboses, |

| **Table 3** | **Banff Classification** | |
|---|---|---|
| **Grade** | **Pathology** | |
| 0 | No or rare inflammatory infiltrates | |
| I | Mild. Mild perivascular infiltration. No involvement of the overlying epidermis | |
| II | Moderate. Moderate-to-severe perivascular inflammation with or without mild epidermal and/or adnexal involvement (limited to spongiosis and exocytosis). No epidermal dyskeratosis or apoptosis | |
| III | Severe. Dense inflammation and epidermal involvement with epithelial apoptosis, dyskeratosis and/or keratinolysis | |
| IV | Necrotizing acute rejection. Frank necrosis of epidermis or other skin structures | |

## 3. Clinical Reflections

### *3.1 Hand transplantation*

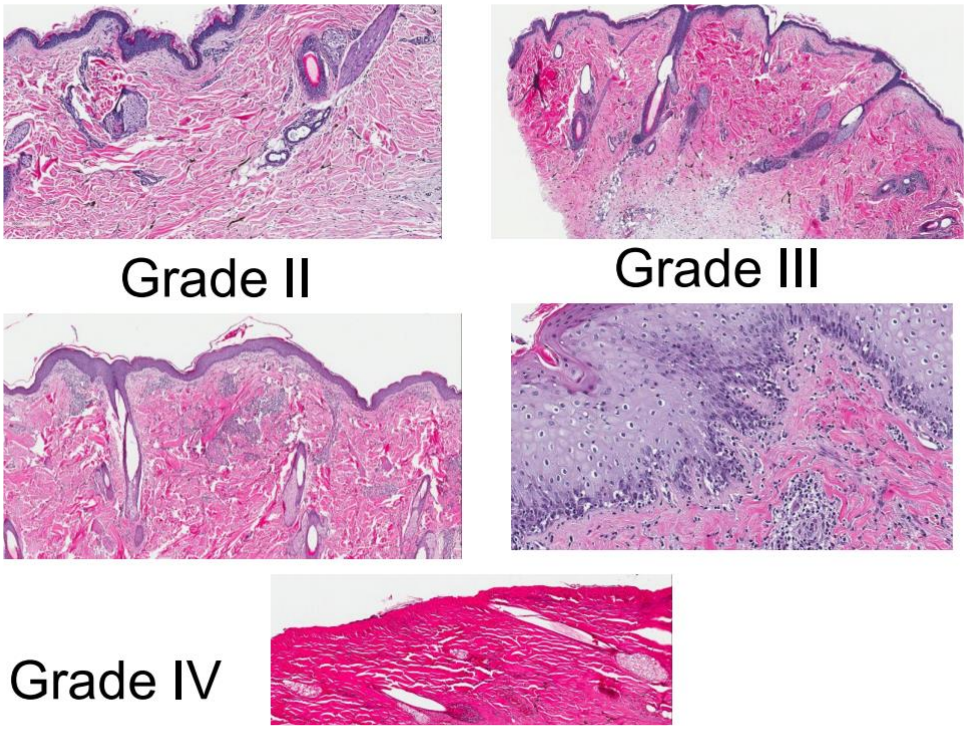
In hand transplantation, acute rejection manifests by changes either in the skin or less often in the palm and nail beds.

#### *3.1.1 Macroscopic features*

Macroscopic features of skin rejection include a maculopapular erythematous rash of diverse color intensities. It may be diffuse, patchy or focal, and with or without burning pain (61,62). It is distributed over the dorsal and volar aspects of the forearm and wrist, and the dorsum of the hand. This represents the “classical” pattern of rejection, sparing palmar skin and nails.

#### *3.1.2 Microscopic features*

As for the microscopic features, these are summarized by the Banff classification of hematoxylin-/eosin-stained sections (63): Grade I includes mainly lymphocytic perivascular aggregates in the dermis. In mild rejection stages, the inflammatory infiltrate is found in the interstitium and interphase between dermis and epidermis and/or adnexal structures. Moderate rejection is characterized by cellular infiltrate in the epidermis. Advanced stages are characterized by necrosis of keratinocytes resulting in focal dermal-epidermal separation, and finally necrosis with loss of the epidermis .




***( Figure 7)***

**3.2 Face transplantation**

In face transplantation, the high antigenicity of the oral/nasal mucosa compounds the immunologic challenge imparted by the skin. In a minority of cases, a sentinel skin graft (SSG) from the donor was transplanted for surveillance biopsies and monitoring of clinicopathologic signs of graft rejection (32,64).

*3.2.1 Macroscopic features*

These include skin redness, swelling, and appearance of nodules and papules (65). The oral mucosa is erythematous, and a SSG, when present, will display diffuse edema and erythema. In this situation, since the appearance of the facial graft (red macules) is different than that of the SSG (diffuse redness), it is important to differentiate rejection from various facial dermatoses manifesting with erythema. A

Periodic acid-Schiff stain of the oral mucosa is recommended not to miss a fungal infection (66).

*3.2.2 Microscopic features*

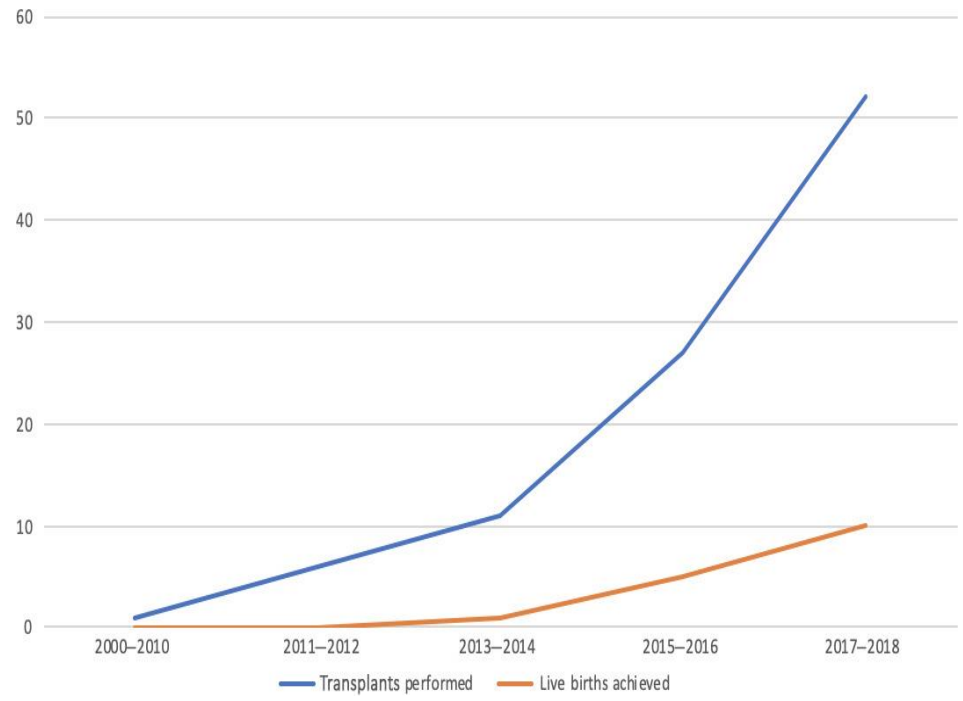
Microscopically, pathologic changes seen in skin and mucosal biopsies during rejection are qualitatively similar to those observed in hand rejection. The dermis shows edema and a predominantly lymphocytic inflammatory infiltrate of variable density; in the surface epithelium (epidermis or mucous membrane), intercellular edema, lymphocyte exocytosis, basal cell vacuolization, and keratinocyte apoptosis are noted (62). The severity of these changes can be assessed according to the same scoring system proposed for hand transplantation (67). Interestingly, biopsies of the oral mucosa show more severe changes than those seen on the SSG and the facial graft (68,69). The explanation for this observation is unclear; it could be due to a higher density of antigen-presenting cells (dendritic and Langerhans cells) in the mucosa as opposed to skin.

## 4. Uterus Transplantation

Absolute uterine factor infertility (AUFI) refers to women who are unable to conceive or maintain pregnancy because of the absence of a uterus, or the presence of one that is anatomically or physiologically dysfunctional. Women with AUFI experience involuntary childlessness or can acquire parenthood through adoption or surrogacy (70). However, neither option addresses the underlying structural issue, nor do they allow women to experience gestation. Moreover, adoption does not permit biological relation, and surrogacy is associated with religious and legal implications that make it unavailable to many. The advances in VCA techniques led to the

development of uterine transplantation (UTx) that was primarily motivated by the potential to ameliorate the suffering caused by the discrepancy between procreative ability and reproductive aspirations in women with AUFI. Although associated with greater risk, including multiple major surgeries and transient exposure to immunosuppression, UTx is the only option that provides women with AUFI the opportunity to conceive, gestate, and give birth to genetically related offspring themselves (70).

In 2014, almost 50 years after the initial concept was first considered, the first live birth following UTx was reported (71), signaling that UTx may be a feasible fertility-restoring intervention for women with AUFI. More than 60 UTx operations have now been performed globally and 18 offspring have been reported in the media to have been delivered following successful procedures .




***(Figure 8)***

Etiologies of AUFI where the uterus is absent, can be categorized into congenital, such as Mayer–Rokitansky–Küster–Hauser syndrome or complete androgen insensitivity syndrome, and acquired, following hysterectomy to treat postpartum hemorrhage, benign gynecological disease, or gynecological cancer. Causes of AUFI characterized by a nonfunctioning uterus include Asherman's syndrome, severe inoperable fibroids, adenomyosis, radiation damage, and congenital structural abnormalities (70).

In UTx procedures performed to date, the vast majority (88.9%) have been undertaken in women with Mayer–Rokitansky–Küster–Hauser syndrome. The mean age of the recipients in the UTx procedures performed to date is 27.8 years (range 20–38; SD 4.5). Of the UTx procedures reported so far, 80% have been performed using living donors. Twenty percent UTx procedures have used deceased donors. The mean age at donation is 44 years (range 20–62; SD 10.2). Where the parity of the donor is reported, most have been multiparous (93.2%) (72). The mean cold ischemia time (CIT) in UTx involving deceased donors is double that seen for living donors (5 hours 42 minutes ± 2 hours 7 minutes versus 2 hours 50 minutes ± 1 hour 47 minutes) (72). However, UTx grafts have been demonstrated to tolerate CITs of up to 24 hours in both animal and human models (73,74), so the extended CIT associated with the use of deceased donors is of uncertain clinical significance. Approximately half of living donors so far have been directed donations from biological relatives (52.8%), with either first-degree (mother, sister) or second-degree (aunt) relation. Fourty seven percent donors were unrelated, with either directed donation from close friends or extended family, or non-directed donation from women with no pre-existing genetic or emotional relationship to the recipient (8). The average age in living donors is

45.4 ± 8.6 (range 30–62) years, compared with deceased donors, who are aged 38.3 ± 14.3 (range 20–57) years (8).

The major disadvantage of using living donors is the associated risk. Regarding donor surgical morbidity in the cases performed so far, as per the Clavien-Dindo classification of surgical complications, 11.1% of living donors suffered from Grade IIIb complications whereas 27.8% experienced Grade I–II. The mean donor blood loss in reported cases so far has been 600 ± 581 ml (range 100–2400 ml) (70).

*4.1 Donation after brainstem death*

Donation after brainstem death (DBD) refers to the retrieval of the uterus during a multi-organ retrieval. In humans, the uterus has been successfully retrieved at the beginning of a multi-organ retrieval (70, 71) and after the retrieval of the other solid organs (73, 77), with no negative impact upon other organs retrieved or the retrieval process. The first live birth following UTx after DBD was achieved in Brazil in 2017 (78), which demonstrates the feasibility of UTx using deceased donors. The major advantage of DBD is the elimination of donor risk. However, the associated logistical difficulties can be difficult to overcome, and can compromise the viability of DBD UTx programs (79).

Another issue for DBD is the number of organs that may become available to meet demand. AUFI has been estimated to affect one in 500 women of reproductive age (80). Whereas 955 DBD retrievals were undertaken between 2017 and 2018, only 47% were undertaken in female donors, and only 37% were between the ages of 18 and 50 years (72). Therefore, it is anticipated that between 150 and 175 donors will be broadly eligible for uterine retrieval each year. On the basis of current selection

criteria, including necessity for being parous, having an uncomplicated obstetric history, and lack of significant medical problems (81), this number will decrease significantly further.

*4.2 Outcomes*

The mean operative time for the recorded implantations performed to date is 5 hours 5 minutes (range 3 hours 30 minutes to 11 hours; SD 1 hour 25 minutes). The mean blood loss at implantation is 632 ± 397 ml (200–2000 ml). With regard to graft survival, of the 45 procedures reported in detail so far, 13 (28.6%) have required emergency hysterectomy, mostly because of graft thrombosis (53.8%), with three (23.1%) due to infection (pelvic bacterial infection/abscess, candidal vasculitis of the arterial anastomosis, herpes simplex virus infection of the graft) and two (15.4%) secondary to unspecified graft ischemia. One hysterectomy (7.7%) was also undertaken following postoperative haemorrhage from a branch of the internal iliac, which probably led to uterine hypoperfusion and resultant graft failure. The majority of emergency hysterectomies were undertaken in the first 15 days postoperatively (76.9%), whereas two were undertaken during months 3–4 (15.4%) and one during month 7 (7.7%). Seven (15.6%) women have since had planned completion hysterectomies, six of whom (85.7%) achieved successful live births. Twenty-five (55.4%) continue to have functioning grafts. More than half of recipients have not suffered any surgical or medical complications in the postoperative period (55.6%). Other complications, in addition to the emergency hysterectomies, include six (13.3%) grade IIIb complications as per the Clavien-Dindo classification. Five (11.1%) recipients suffered grade I–II complications (72).

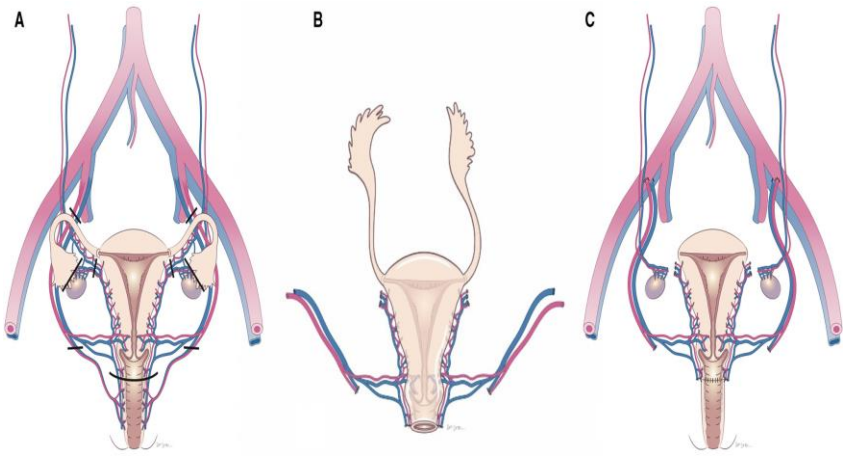
Following the successful live births of 18 infants following UTx, it appears to be a viable option for women with AUFI. Regarding neonatal outcomes, all infants

born following UTx are healthy with no evidence of congenital abnormality. Apgar scores at 10 minutes were normal (>7) in all offspring.

*4.3 Immunosuppression and rejection*

The polyclonal antibody anti-thymocyte globulin has been most commonly used for induction of immunosuppression (93.3%), with or without the addition of methylprednisolone. One (2.2%) procedure used cyclosporin and two (4.4%) received the monoclonal antibody basiliximab, all in combination with methylprednisolone. Although the first human case used cyclosporin maintenance (82), the majority since have used tacrolimus in combination with mycophenolate mofetil (MMF; 97.1%). Four cases have also used everolimus, in addition to tacrolimus, to reduce the dose of tacrolimus being administered (83). Steroids were usually commenced and weaned, and reintroduced in cases complicated by rejection. MMF is stopped before embryo transfer owing to its teratogenic nature (84), when it is often replaced with azathioprine (31.8%), or tacrolimus monotherapy is attempted (68.2%).

In the available data from human cases to date, 45.9% have experienced a total of 26 episodes of rejection, but 54.1% have not had any episodes. The majority were proven on histology (24 episodes), whereas two were diagnosed based on clinical findings and raised serum lymphocyte subpopulations. In those diagnosed histologically, 62.5% were categorized as grade 1 (Mild), 25% were grade 2 (Moderate), and 13.5% were grade 3 (Severe). Grade 1–2 episodes of rejection were treated with 3-day courses of intravenous methylprednisolone and escalation of maintenance immunosuppression. Two episodes of grade 3 rejection were successfully treated with the addition of anti-thymocyte globulin, and one case resolved with methylprednisolone.




***( Figure 9)***

### *5. VCA and Immunosuppression Strategies*

An important element for the clinical advancement of VCA is the development of immunosuppression regimens that prevent rejection by targeting specific immune mechanisms known to influence the tissues transplanted in a VCA, while avoiding major toxicities of immunosuppressants (85). Current standard immunotherapies in VCA rely on agents such as calcineurin inhibitors (CNIs) and steroids which are known to cause side effects including nephrotoxicity, hypertension, diabetes (86). Belatacept is a high-affinity fusion protein that targets the CD80/CD86 costimulation pathway, which is the best recognized pathway for immune cell activation and proliferation. Prior work has shown that belatacept is able to prolong

renal allograft survival in non-human primates (87). Clinically, this agent provides benefits not provided by other agents such as less nephrotoxicity compared to calcineurin inhibitors. However, belatacept is less efficacious than tacrolimus at preventing acute rejection (88). Due to the drug mechanism of action, belatacept is unable to prevent rejection by memory T-cells, which do not require costimulation for activation. Previous work in non-human primates (NHP) suggests that drugs designed to treat psoriasis can prolong costimulation blockade-based allograft survival and prevent belatacept resistant rejection (89,90). This is important since skin is the most immunogeneic of the tissues transplanted and serves as a harbinger of rejection in a VCA (91). Belatacept has been extensively studied by our group in vitro, in NHPs, and in clinical transplantation, showing a delayed onset of rejection in transplant recipients (92,93). In clinical hand transplantation, we have previously reported that conversion from a standard maintenance regimen (tacrolimus, mycophenolate mofetil and steroids) to belatacept and sirolimus led to improving renal function in the absence of calcineurin inhibitors long term (94). In a stepwise progression, we have also shown that hand transplantation can be performed using a de novo belatacept-based treatment without CNIs long-term, resulting in sufficient prophylaxis from rejection, reversible rejection when occurred, and reduced side effects (95).

## 6. VCA and Legal Frame

Vascularized composite allotransplantation now falls under the scope of organ transplant legislation in Europe and the United States. While in the USA, VCA has been considered as standard care since 2014, VCA in Europe is still performed through clinical research trials, except in United Kingdom. However, after two

decades of favorable experience with upper extremity transplantation, professionals in Europe are proposing hand allotransplantation as "controlled standard" care, as opposed to face transplantation, which is still a challenging activity (96). The peculiarity of VCA is that it combines elements of both organ and tissue donation and transplantation, each of which is usually separately regulated. As a result, VCAs did not fit squarely under the existing regulatory frameworks.

In the US, VCAs were not included in the definition of “organs” as defined and regulated under the National Organ Transplant Act (NOTA) 4 and the Organ Procurement and Transplant Network (OPTN) Final Rule 5. In June 2014, the OPTN Board of Directors unanimously approved the first set of national policies and standards for VCA transplantation in the US (97). Because the federal regulations and OPTN policies have been in place for such a short period of time, it is premature to determine the benefits and challenges, but there are several aspects including program requirements, donor authorization, allocation, and living donation of critical importance to the future development of VCA transplantation.

## 7. Conclusions and future perspectives

The field of vascularized composite allotransplantation is no longer in its infancy. Many transplants have been performed world-wide, including abdominal wall, lower extremity, knee, larynx, uterus, and penis transplants have been reported. However, despite expanding numbers of VCA centers (in the United States and abroad) and the slowly expanding volume of procedures, the field is far from achieving widespread adoption that signals maturity. Although VCA has established itself as independent from, it remains incompletely defined. The field is encouraged by early successes and promising graft survival rates that suggest that VCA may be a

realistic solution for catastrophic injuries or other disfiguring conditions for which conventional reconstruction is insufficient.

Moreover, more recent approaches such as UTx have provided a first-time successful treatment for conditions such as uterine infertility. However, for all its promise, the number of candidates who have undergone VCA has been very small, and most have been transplanted in the last 10 years. Therefore, robust assessment of long-term outcomes is not available. The successful live births of 18 infants following UTx, it appears to be a viable option for women with AUFI. Teams undertaking UTx are now established globally, with performance of UTx procedures expected to continue to increase exponentially into the future. However, the process is associated with significant risk, with the potential for complications in both donors and recipients, and a considerable risk of graft failure. The long-term future of UTx will undoubtedly focus around the development of a bioengineered uterine graft, which although out of the scope of this review, would remove donor risk and negate the requirement for immunosuppression in the recipient.

## 8. Figure Legends

**Figure 1.** Iconic painting from de Sedano. It depicts St. Cosmas and St. Damian appearing in a dream to physicians who have removed a cancerous leg from a patient and are replacing it with the leg of a cadaver.

**Figure 2.** Clockwise from left: first live birth after deceased donor uterus transplant in Brazil (University of Sao Paulo, Dec. 2018, published in Lancet); first US penis transplant (Mass Gen, 2016); larynx transplant at UC Davis (2011); first bilateral pediatric hand tx (Penn, 2015); total face transplant w/ eyelids (NYU, 2015).

**Figure 3.** Acute rejection typically presents clinically with mainly skin changes including a maculopapular variably erythematous rash with edema and with histologic findings of perivascular or interstitial mononuclear cell infiltration with epidermal and/or adnexal involvement dependent on the grade of rejection.

**Figures 4 and 5.** Clinicopathologic signs felt to be indicative of chronic rejection included vascular narrowing, loss of adnexa, skin and muscle atrophy, fibrosis of deep tissue, myointimal proliferation and nail changes.

**Figure 6.** Clinical vasculopathy is thought to be caused by low-grade inflammation-induced endothelial damage and chronic remodeling of smooth muscle cells in an attempt to heal this damage. This induces neointimal thickening from both chronic adventitial inflammation and excess deposit of perivascular collagen.

**Figure 7.** Banff Classification. Grade I includes mainly lymphocytic perivascular aggregates in the dermis. In mild rejection stages, the inflammatory infiltrate is found in the interstitium and interphase between dermis and epidermis and/or adnexal structures. Moderate rejection is characterized by cellular infiltrate in the epidermis. Advanced stages are characterized by necrosis of keratinocytes resulting in focal dermal-epidermal separation, and finally necrosis with loss of the epidermis

**Figure 8.** More than 60 uterus transplantation operations have been performed globally and 18 offspring have been reported in the media to have been delivered following successful procedures.

**Figure 9.** Diagrammatic representation of (A) uterine transplant retrieval technique including bilateral long internal iliac arteriovenous pedicles, (B) uterine transplant

graft following retrieval, and (C) recipient anatomy following implantation, demonstrating bilateral internal iliac to external iliac arterial and venous anastomoses.

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