A

Chronic Rejection in Vascularized Composite Allotransplantation

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Classification of rejection in VCA

- According to time post-Tx: early/acute vs late/chronic
- According to mechanism: Cell-mediated vs Antibody-mediated
- According to Severity (grades)
- According to main tissue target: Cutaneous vs Vascular/other
- According to outcome: Reversible vs Irreversible

Presently no totally comprehensive/satisfying classification of VCA rejection exists

- **Acute rejection (AR) in VCA:** very common (>80% of VCA recipients), within some weeks/months after grafting
- Clinically and histologically rather well characterized, although the clinicopathological manifestations are not very specific – the microscopic skin changes form the basis of the Banff 2007 score of VCA rejection (grades 0-IV)

The Banff 2007 Working Classification of Skin-Containing Composite Tissue Allograft Pathology

Am J Transplant 2008; 8: 1396-400

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sus discussion session attended by the first authors of three published classification systems, pathologists and researchers from international centers where clinical CTA has been performed. It was open to all attendees to the Banff conference. To the extent possible, the format followed the established National Institutes of Health (NIH) guidelines on Consensus Development Programs. By consensus, the defining features to diagnose acute skin rejection include inflammatory cell infiltration with involvement of epidermis and/or adnexal structures, epithelial apoptosis, dyskeratosis and necrosis. Five grades of severity of rejection are defined. This classification refines proposed schemas, represents international consensus on this topic, and establishes a working collective classification system for CTA reporting of rejection in skin-containing CTAs.

Key words: Anitbody-mediated rejection, Banff, Banff schema, chronic rejection, composite tissue allograft, humoral rejection, rejection, skin allograft, transplant

Acute Rejection in VCA: skin findings

- Clinical

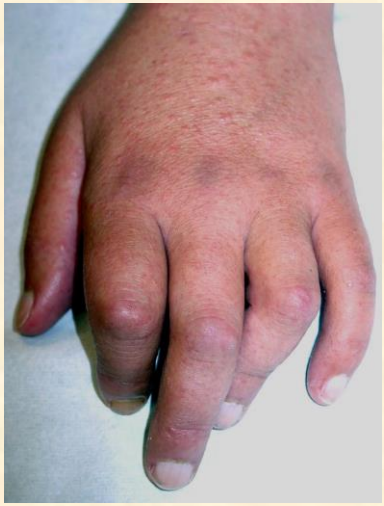
• Erythematous macules/papules, diffuse erythema, edema

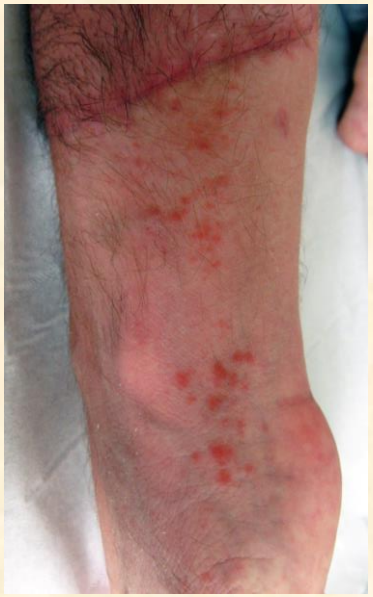
- Pathological

• Perivascular or diffuse dermal lymphocytic infiltrate (mainly CD3+/CD4+ T-cells, very rare B-cells)

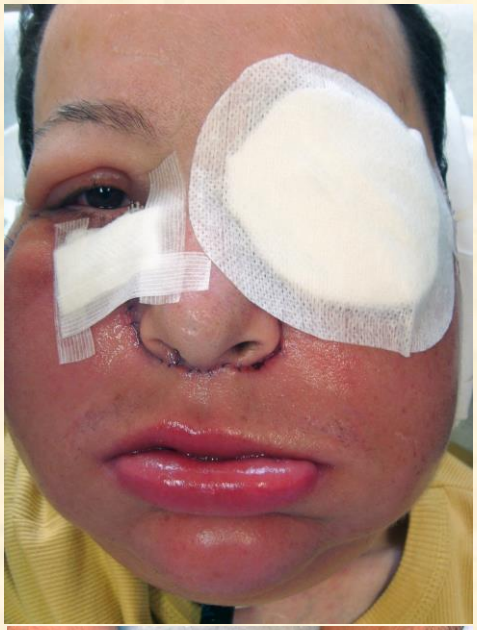
• Epidermal lymphocytic exocytosis (± spongiosis), basal cell vacuolization, ketatinocyte necrosis, epidermal hyperplasia (hyper-orthokeratosis, hypergranulosis, acanthosis: lichenoid aspect, GVHD-like), epidermal/adnexal necrosis

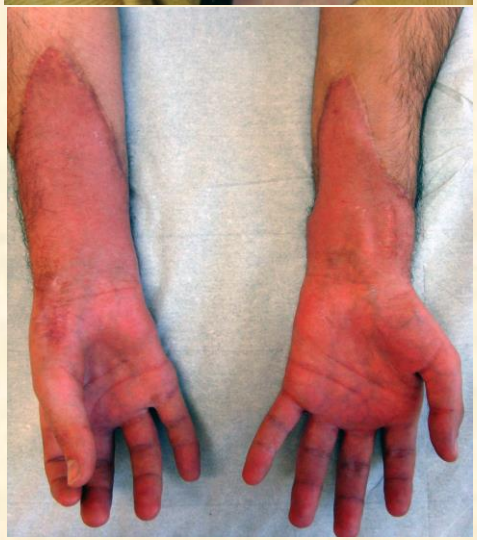
Acute Rejection in VCA: clinical appearance

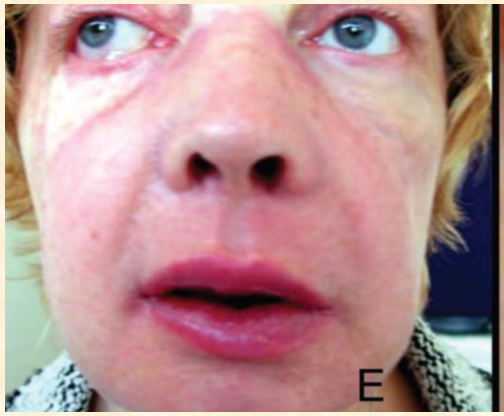


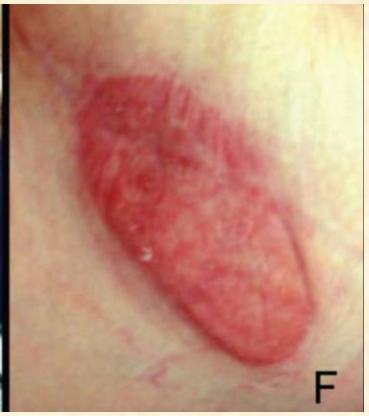


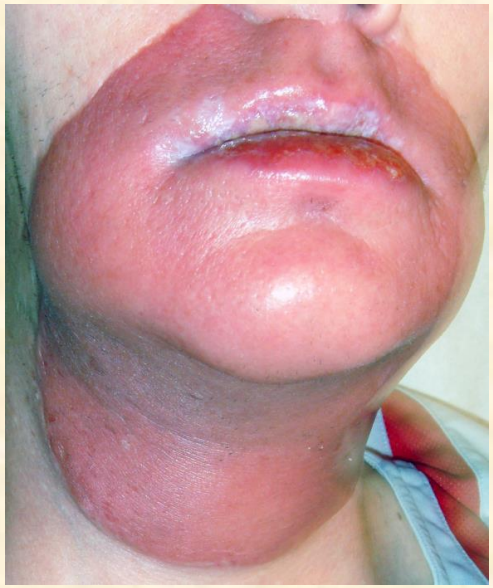
Erythematous maculopapules

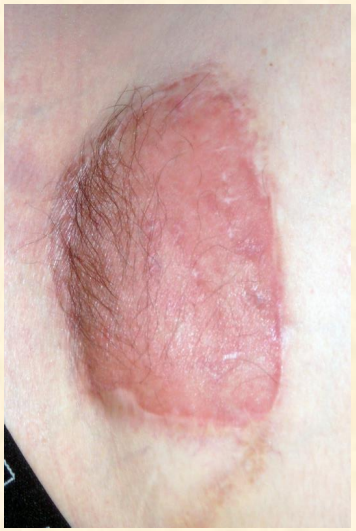






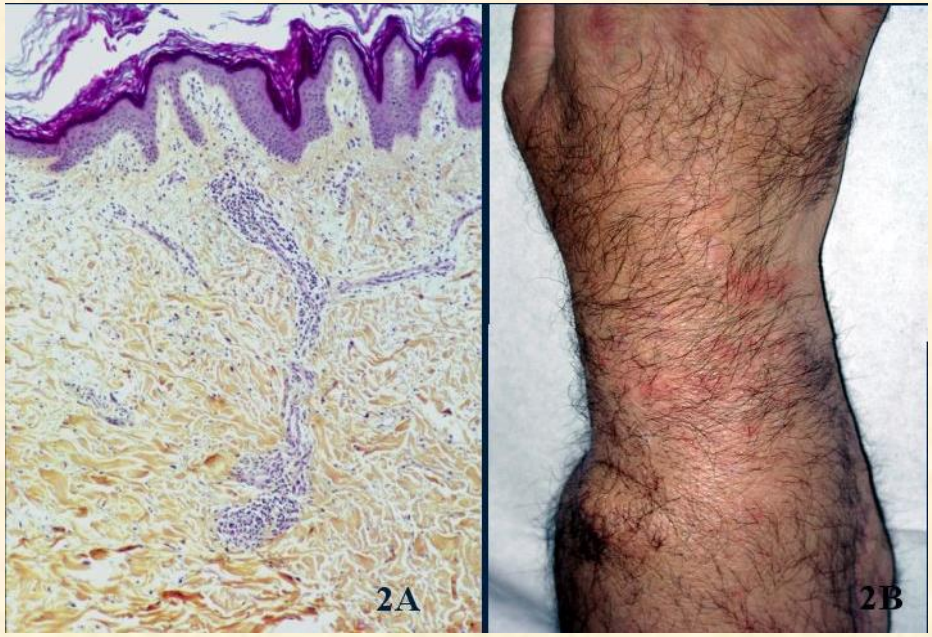




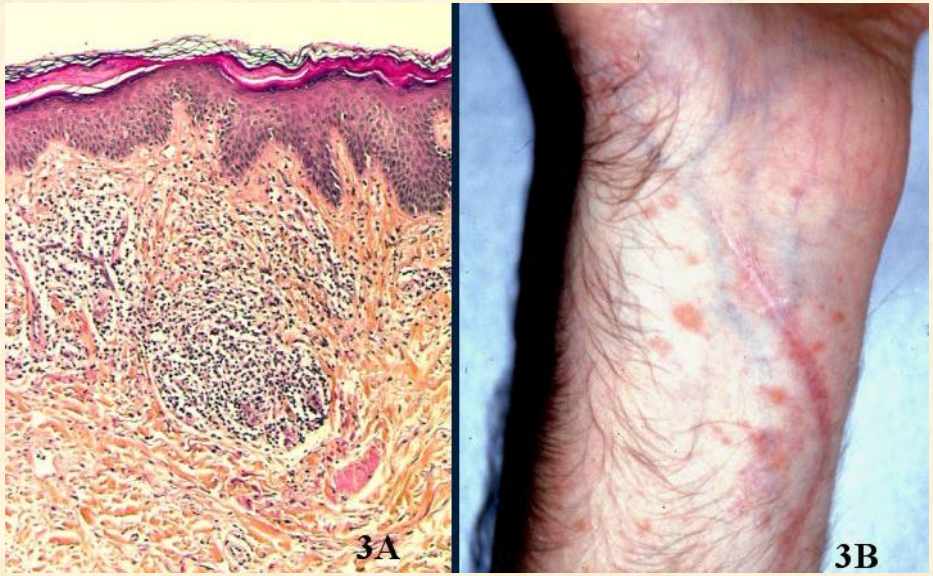


Diffuse erythema ± edema

Acute Rejection in VCA: skin findings

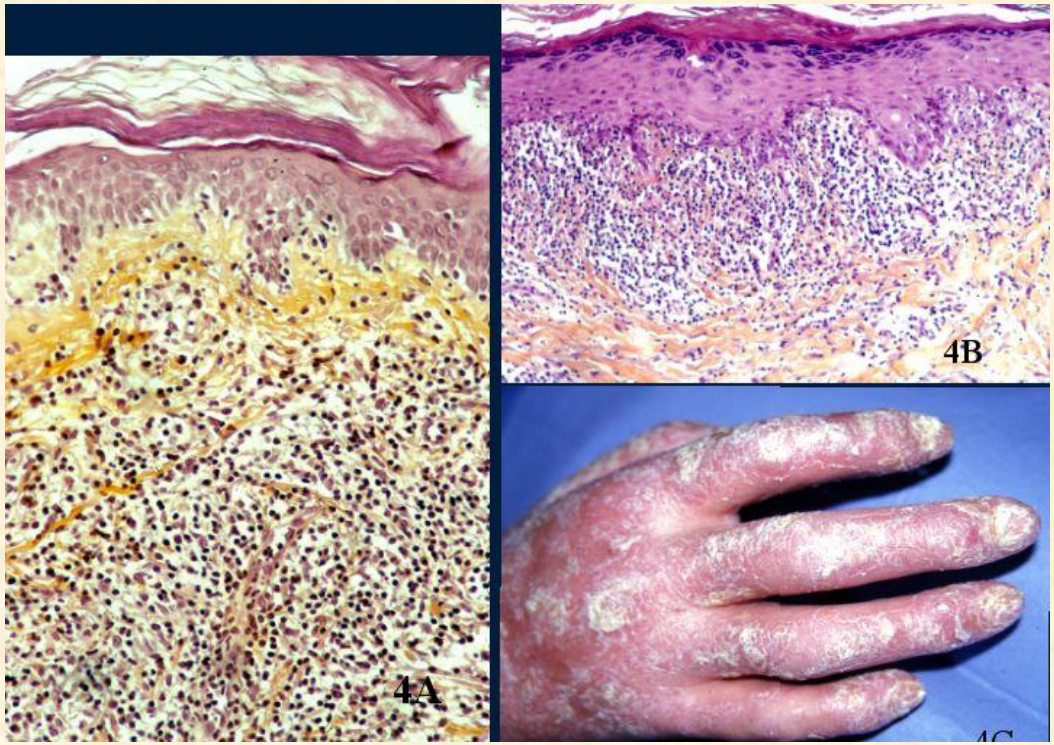


Grade I



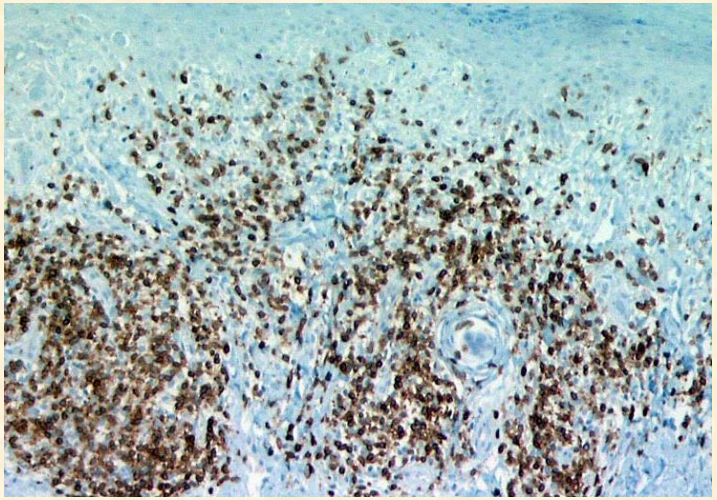
Grade II

Acute Rejection in VCA: skin findings



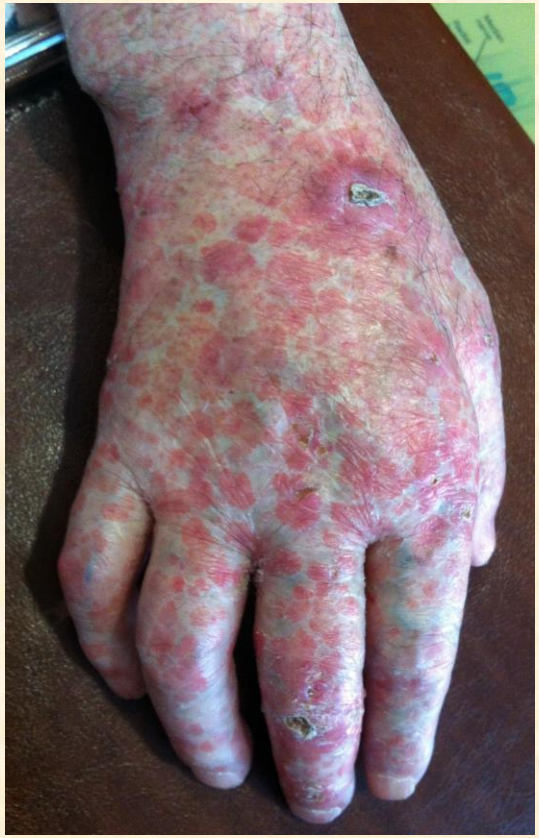
Grade III

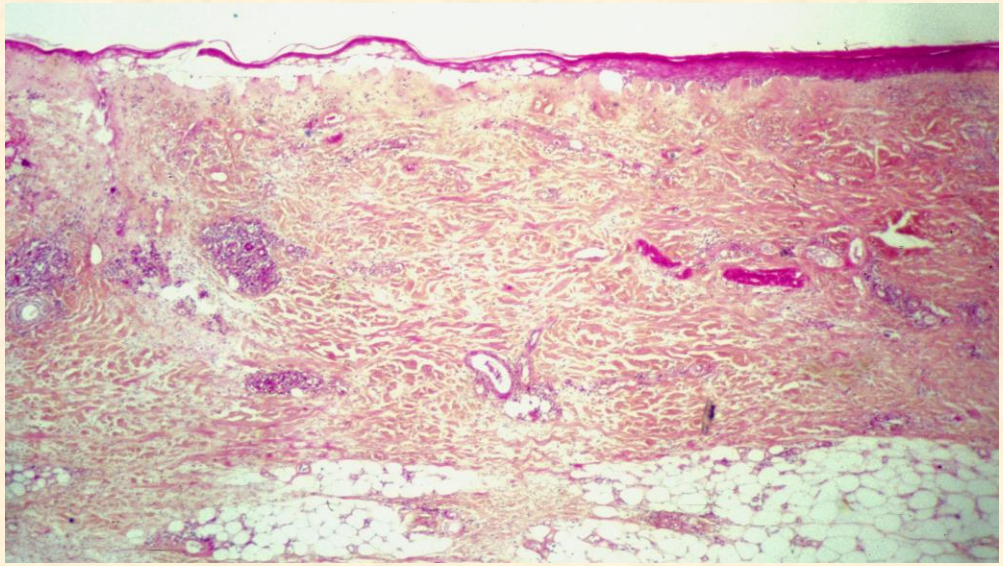
lichenoid (GVHD-like)



Predominant CD3+/CD4+ dermal infiltrate

Acute Rejection in VCA: skin findings





grade IV – necrotic changes
(more likely chronic rejection)

The Banff 2007 Working Classification of Skin-Containing Composite Tissue Allograft Pathology

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Chronic Rejection

Currently, insufficient data are available to define specific changes of chronic rejection in a CTA. Chronic changes and injury to an allograft evolve over time with persistent immune insult and are likely to be altered in tempo and character by concomitant treatment. Fibrosing changes can also be caused by non-immune events, and in certain circumstances both can overlap. Histologic and clinical features highlighted as indicative of chronic injury in a CTA include vascular narrowing, loss of adnexa, skin and muscle atrophy, fibrosis of deep tissue, myointimal proliferation and nail changes. As with other solid organs, it is likely that chronic/persistent injury begets a common histological phenotype through a variety of nonexclusive mechanisms. A possible correlation between graft-versus-host disease (GVHD) and CTA-skin was noted.

Chronic Rejection in VCA

- no formal definition - poorly-studied, few cases reported

- possible reasons for low CR frequency: really low frequency (despite the high frequency of AR), limited n° of VCA recipients, rather short follow-up, early reversal of AR episodes thanks to early diagnosis by skin inspection/biopsy

- CR may lead to irreversible graft dysfunction and loss

→ important to detect early and understand the underlying pathomecanisms so that adequate prevention/treatment can be applied in order to avoid graft loss

Animal models of CR in VCA (rodents, primates):

following mismatch alloTx, immunosuppression either missing or repeatedly withdrawn, leading to multiple AR episodes

- graft vasculopathy, lumen occlusion, hair atrophy, dermal thinning, sebaceous gland fibrosis *Unadkat J et al. Am J Transplant 2009;10:251*

- myointimal hyperplasia, 3y lymphoid follicle development, graft fibrosis

Mundinger G et al. Transplantation 2013;95:1204 Plast Recontr Surg 2011;128:1193

Chronic Rejection in human VCA

Graft vasculopathy: has been observed in deep arteries[1-4] and medium-sized cutaneous vessels[3]

Pathologically similar to vasculopathy of animal and other human allografts (heart, kidney) during CR:

vascular myo-intimal proliferation & thickening, fibrosis,

vascular obliteration leading to graft ischemia & loss

1 Diefenbeck M et al. Transpl Int 2011; 24: e1

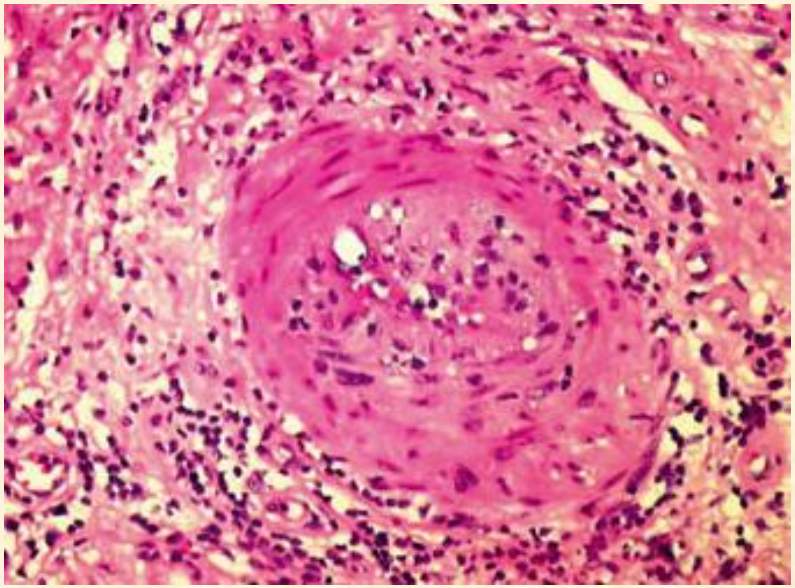
2 Kaufman C et al. Am J Transplant 2012;12:1004

3 Kanitakis J et al. Transpl Int 2014; 27: e118

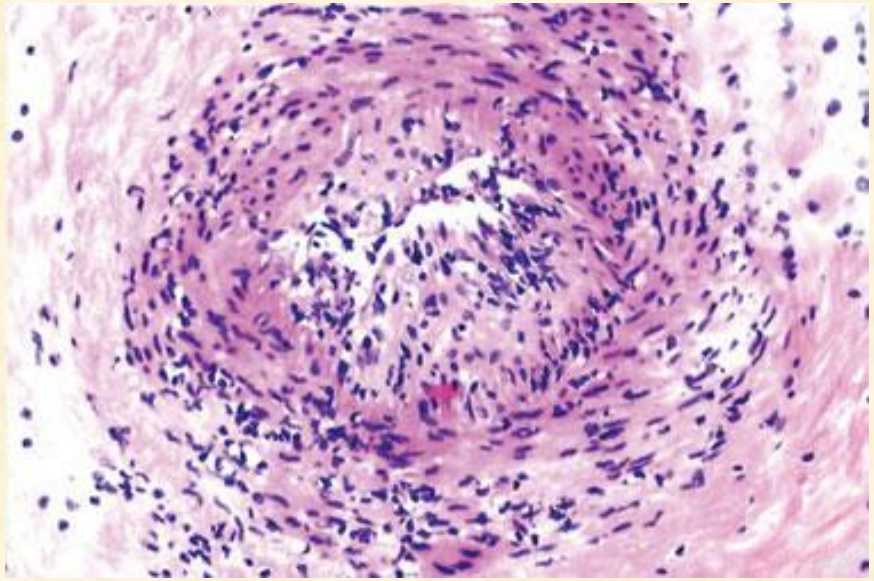
4 Morelon E et al Am J Transplant 2017 doi: 10.1111/ajt.14218 e-pub Jan 31)

Allograft vasculopathy after allogeneic vascularized knee transplantation

Diefenbeck M et al. Transpl Int 2011; 24: e1-e5



Sentinel skin graft 36 mo post-Tx: concentric narrowing of small arteries by fibrotic proliferation of the intima

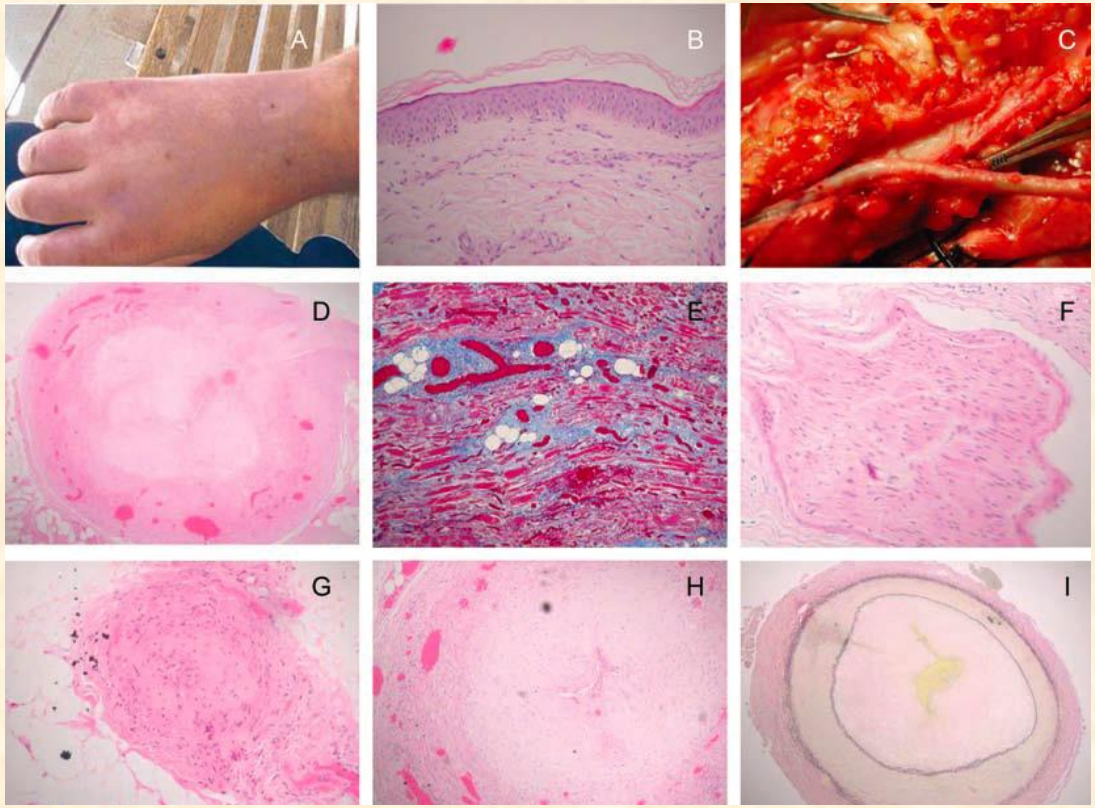


Synovial biopsy 50 months post-Tx: Concentric fibroses of the intima, subtotal occlusion of the lumen

Loss of knee range of motion – allograft loss at 56 mo due to infection

Graft Vasculopathy in Clinical Hand Transplantation

Kaufman C et al Am J Transplant 2012;12:1004



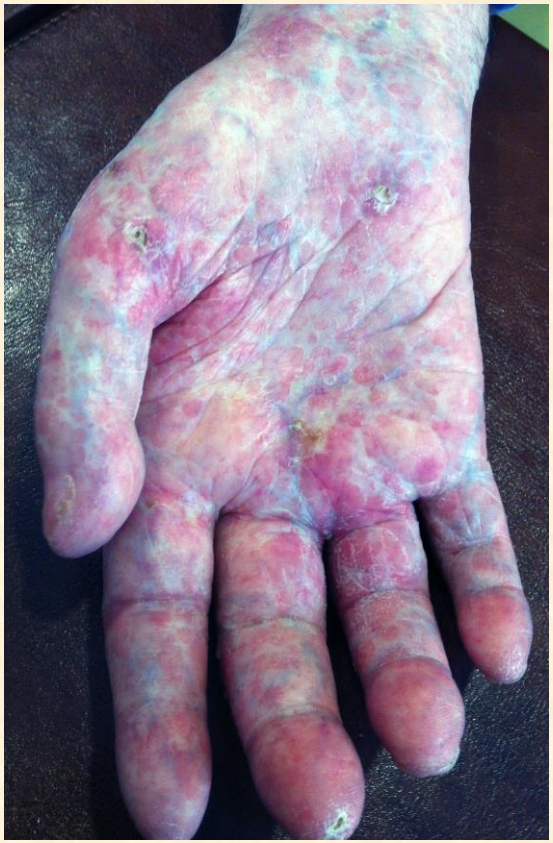
A: Bruising on wrist/hand pod 269; B: punch skin biopsy (gr. 0); C: thickened stiff arteries; D: radial artery: massive intimal hyperplasia, vascular leaking within the medial layer E: trichrome stain on thenar eminence muscle: mild fibrosis & muscle atrophy, secondary to ischemia; F: H&E stain of donor medial nerve (relatively spared; G: index finger digital artery: severe intimal hyperplasia; H: donor radial artery, showing similar changes as ulnar artery; I: elastin stain of radial artery: flattening and duplication of elastic lamina

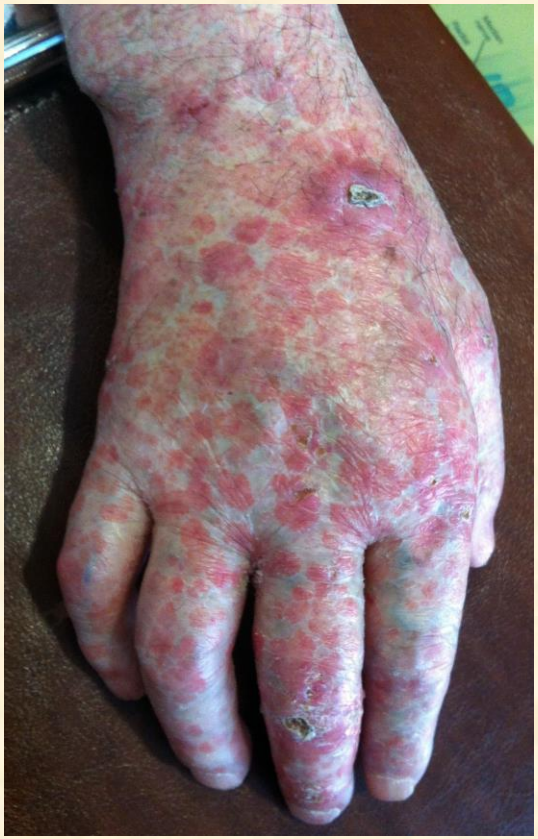
Graft vasculopathy in the skin of a human hand allograft: implications for diagnosis of rejection of vascularized composite allografts

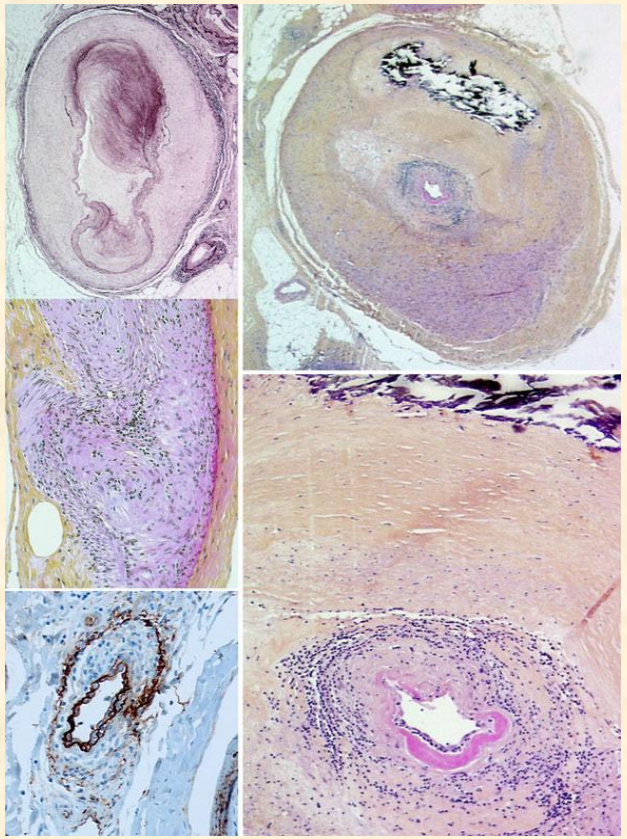
Transpl Int 2014; 27: e118-23

Jean Kanitakis,[1,2] Georgia Karayannopoulou,[3] Marco Lanzetta[4] and Palmina Petruzzo[5,6]

Patient WV







Diffuse violaceous maculopapules, focal skin necrosis

Radial artery

Graft vasculopathy in the skin of a human hand allograft: implications for diagnosis of rejection of vascularized composite allografts

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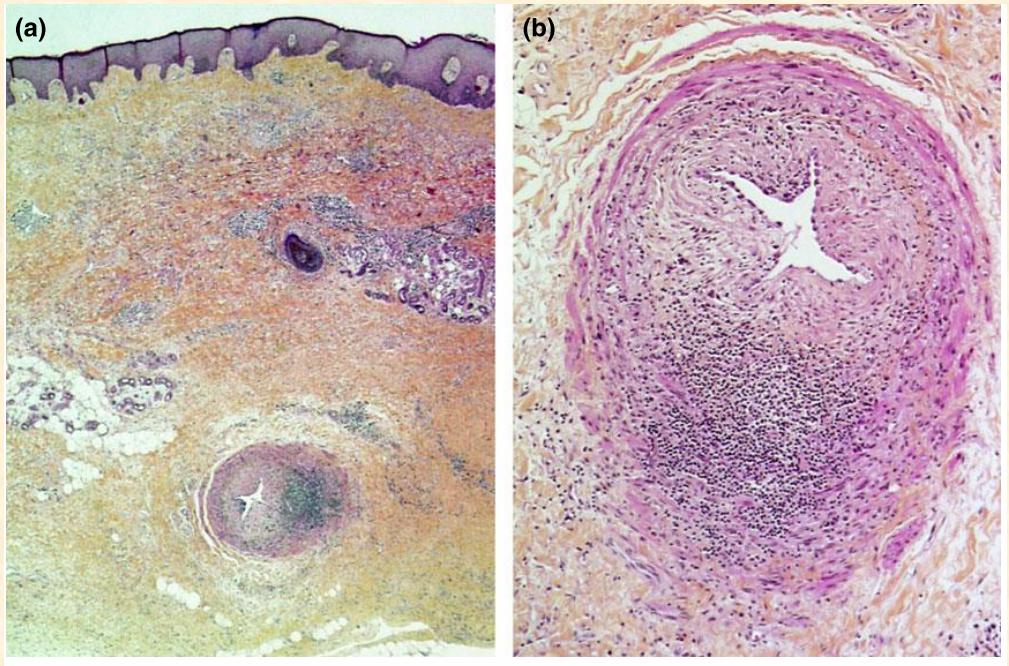
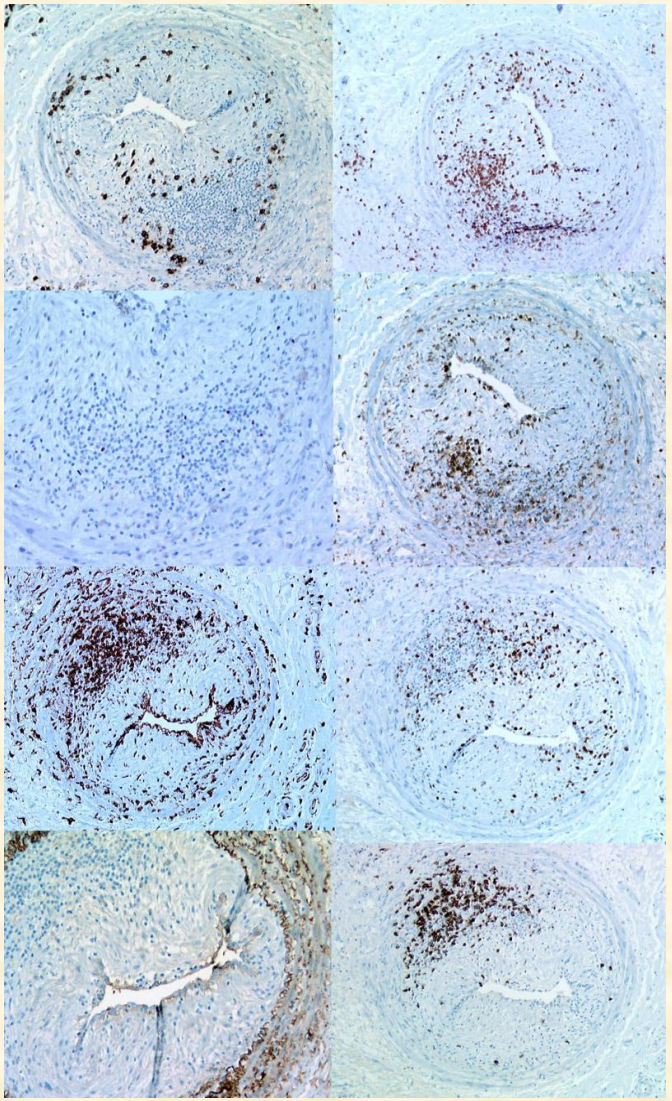
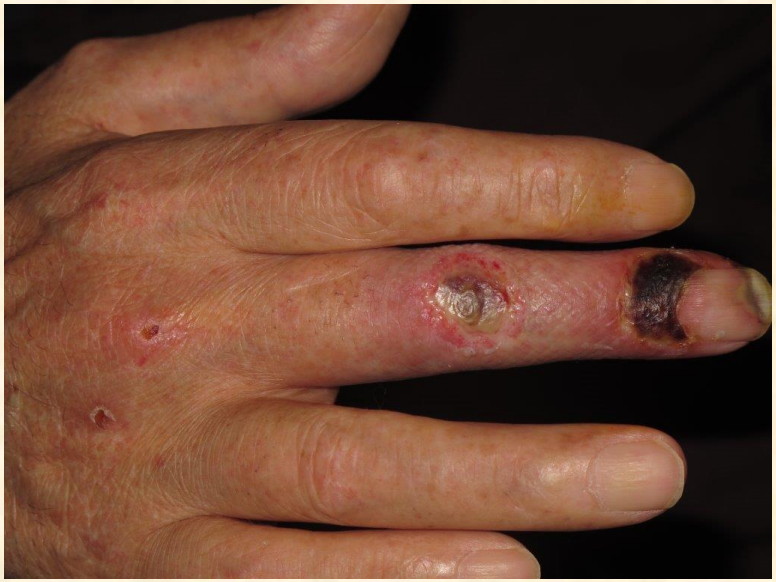


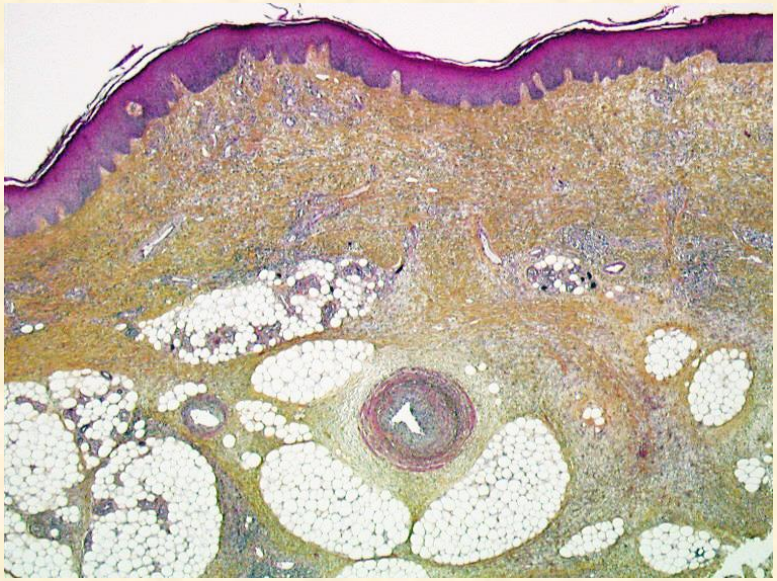
skin biopsy: graft vasculopathy in cutaneous aterioles

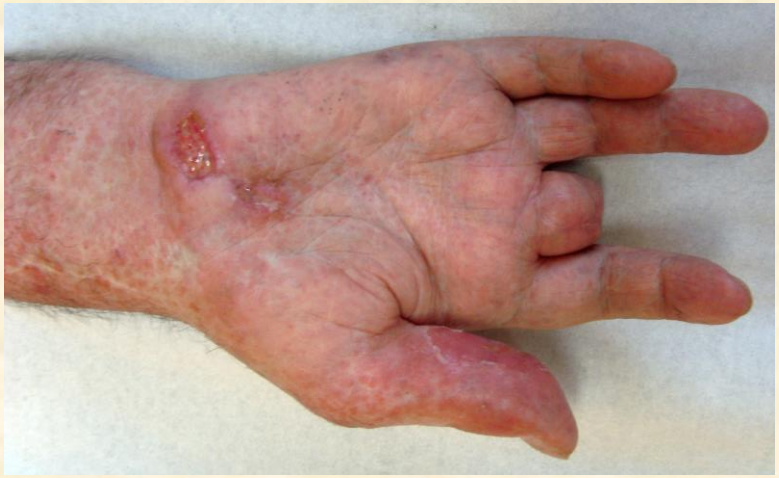


Graft Vasculopathy in the skin of a Human Hand Allograft 11 yrs post-Txading to amputation, despite low n° of preceeding AR episodes

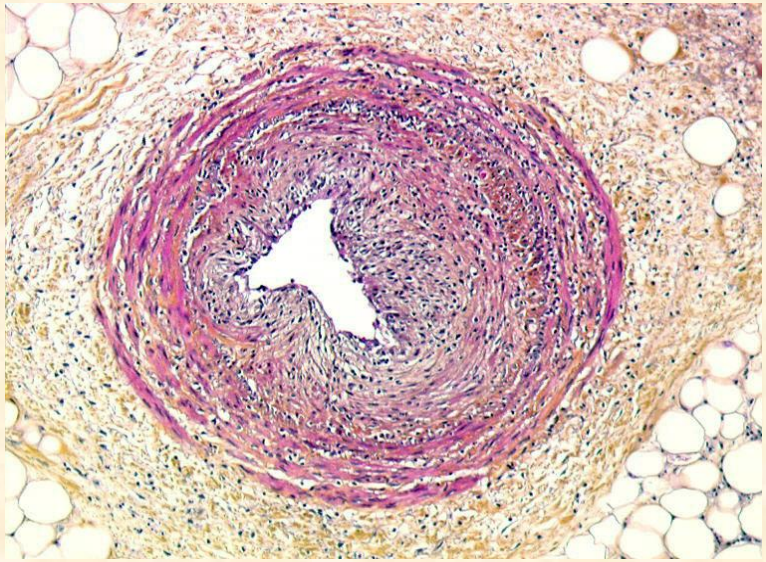
Patient BY



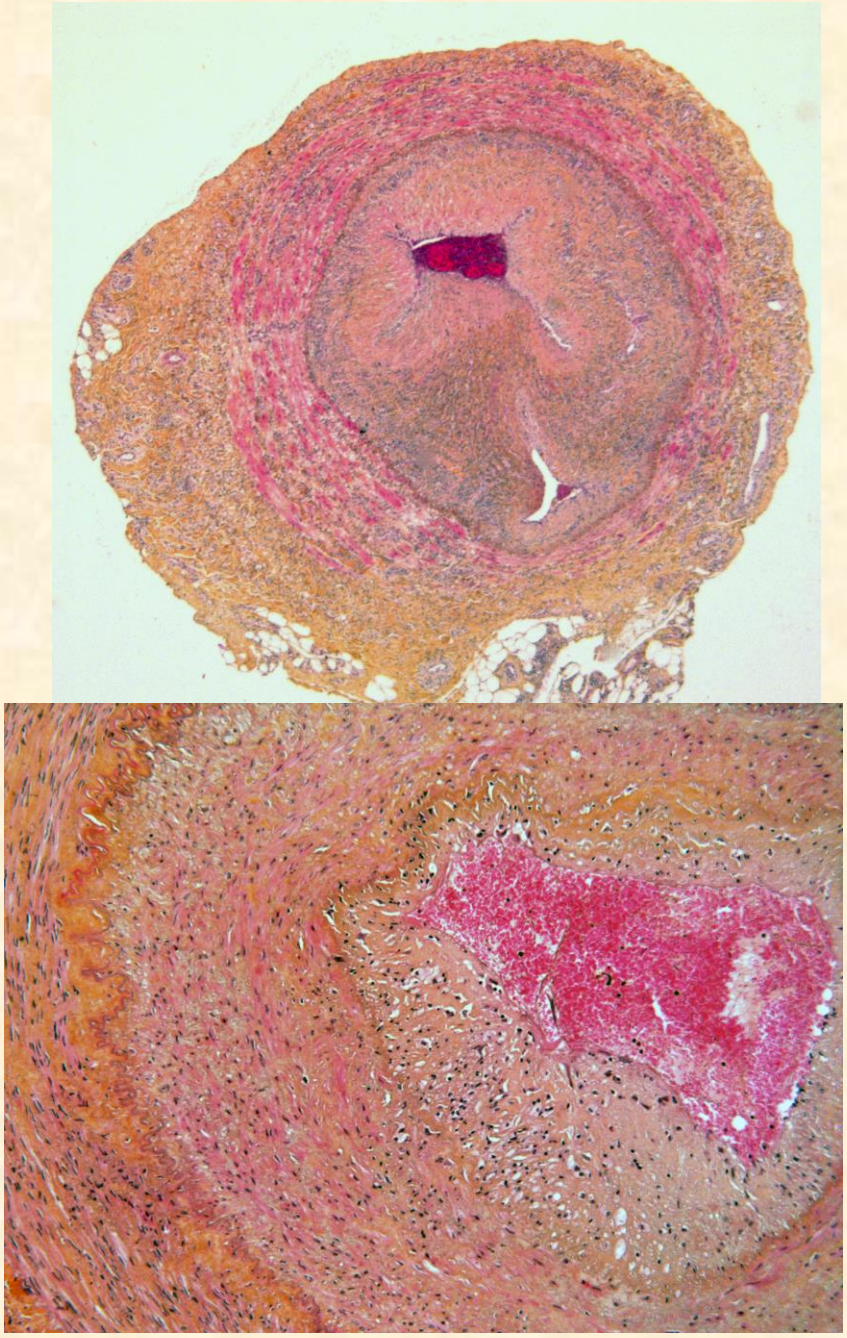




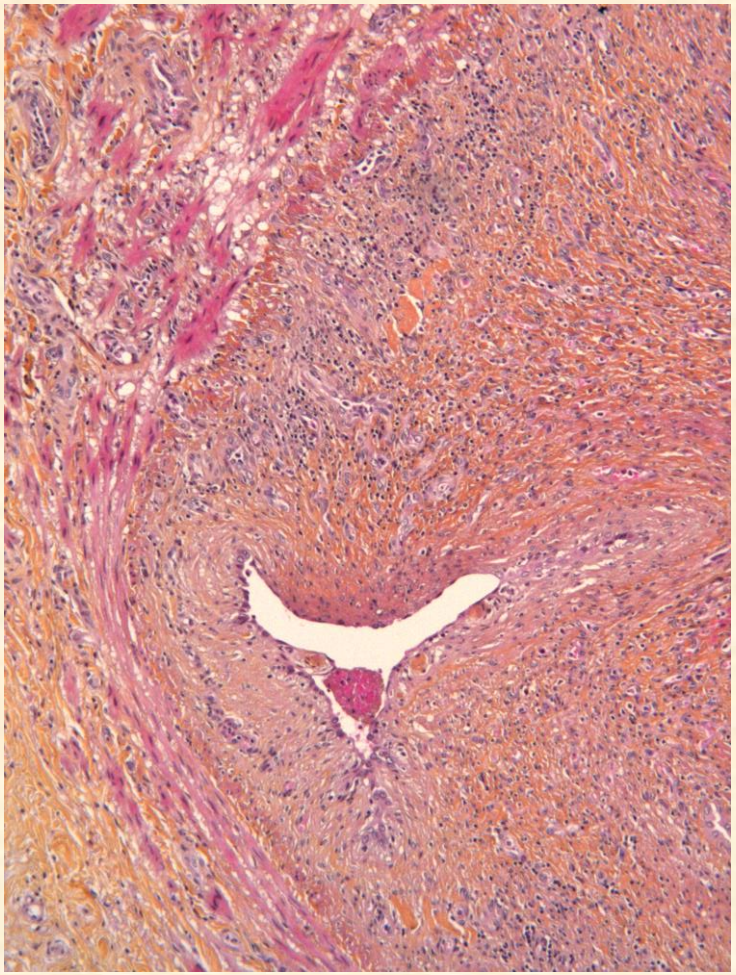
areas of ischemic skin necrosis, pain, decrease of graft function



Patient BY
Graft vasculopathy affecting arteries & veins
(post-amputation findings)



artery



vein

Clinicopathological Findings of Chronic Rejection in a Face Grafted Patient

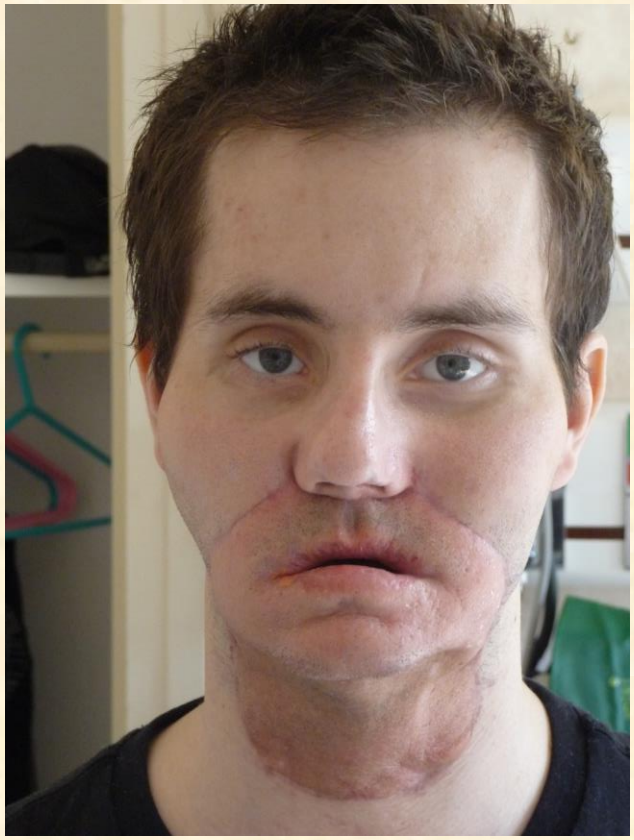
Palmina Petruzzo, MD,[1,2] Jean Kanitakis, MD,[3] Sylvie Testelin, MD,[4] Jean-Baptiste Pialat, MD,[5] Fanny Buron, MD,[1] Lionel Badet, MD,[1] Olivier Thaunat, MD,[1,6] Bernard Devauchelle, MD,[4] and Emmanuel Morelon, MD[1,6]

Background. Skin chronic rejection (CR) in vascularized composite allotransplantation has not been included in the Banff classification yet. We report a face-transplant patient who developed cutaneous clinicopathologic changes suggestive of CR. **Methods.** The recipient was a 27-year-old man with severe disfigurement of the lower face due to a pyrotechnic explosion. He received a facial allograft, including mandible, cheeks, lips, and chin, in November 2009. Immunosuppression included antithymocyte globulins and bone-marrow infusion then steroids, tacrolimus, and mycophenolate mofetil. **Results.** During the first posttransplant year the acute rejection episodes were characterized by reversible oedema and erythema of the graft. Subsequently, the patient developed primary asymptomatic Epstein-Barr virus (EBV) infection, followed by EBV+ B-cell lymphoma and hepatic EBV-associated posttransplant smooth muscle tumors; therefore, the immunosuppressive treatment was greatly reduced. Since the second posttransplant year, the allografted facial skin became progressively sclerotic and presented pigmented macules on a background of hypopigmentation and teleangiectasias, resulting in a poikilodermatous aspect. Skin biopsies showed epidermal atrophy, basal cell vacuolization, and diffuse dermal sclerosis in the absence of significant dermal cell infiltration. The dermal capillaries showed thickened walls and narrowed lumina, whereas the large vessels did not show significant alterations. Neither donor-specific antibodies nor vascular Cd4 deposits were detected. A dysfunction of the graft functions occurred. It was evidenced by a decrease in mouth opening and modification of some phonemes although lip closure was still possible allowing food intake. **Conclusions.** This is the first report suggestive of CR in a face allotransplantation after immunosuppression minimization.

(*Transplantation* 2015;99: 2644–2650)

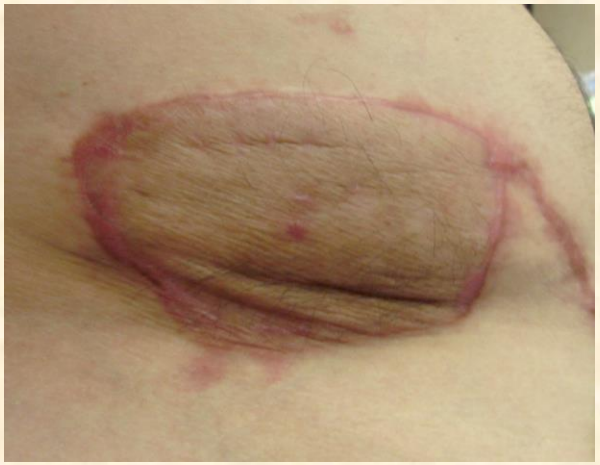
Clinicopathological findings of chronic rejection in a face-grafted patient

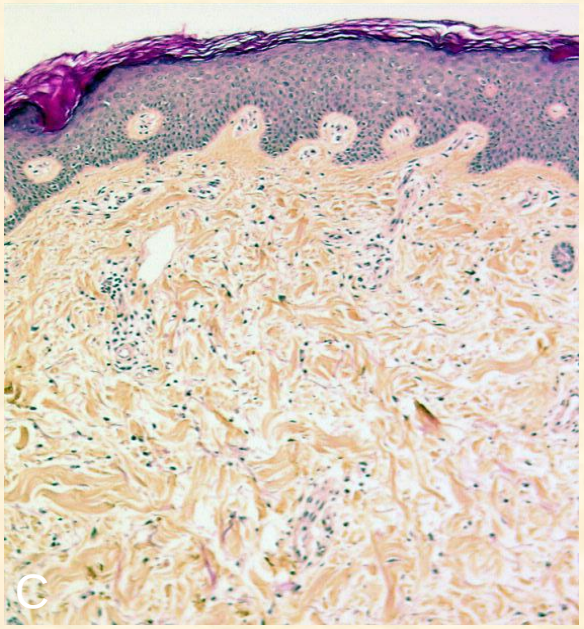
Petruzzo P et al. Transplantation 2015;99:2644



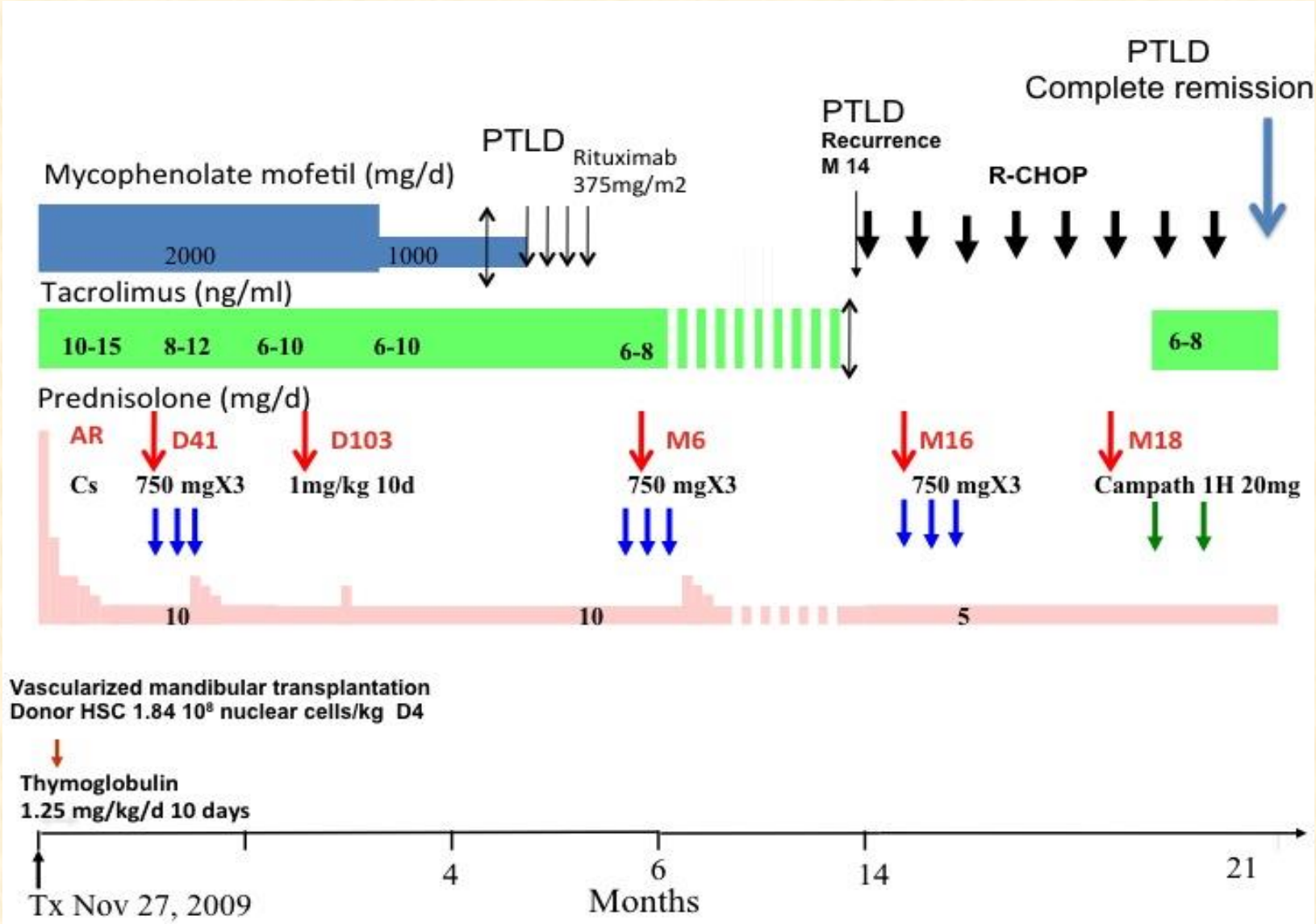
Face alloTx Nov 2009

PTLD 6 mo post-Tx treated with chemotherapy & IST decrease



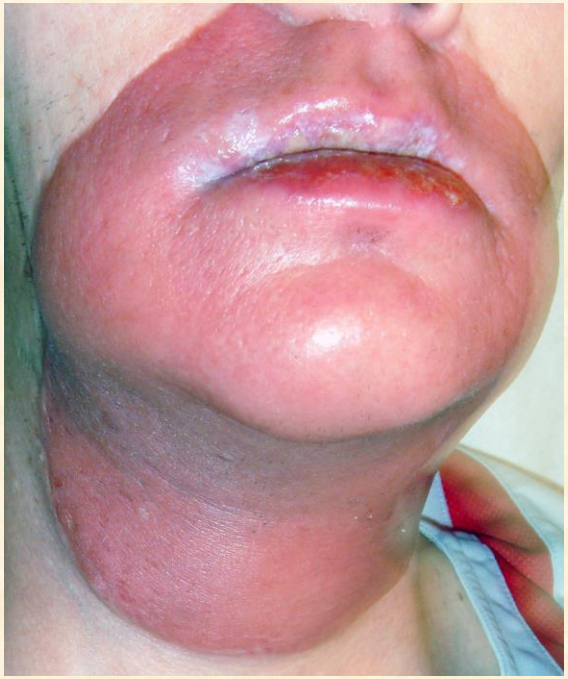


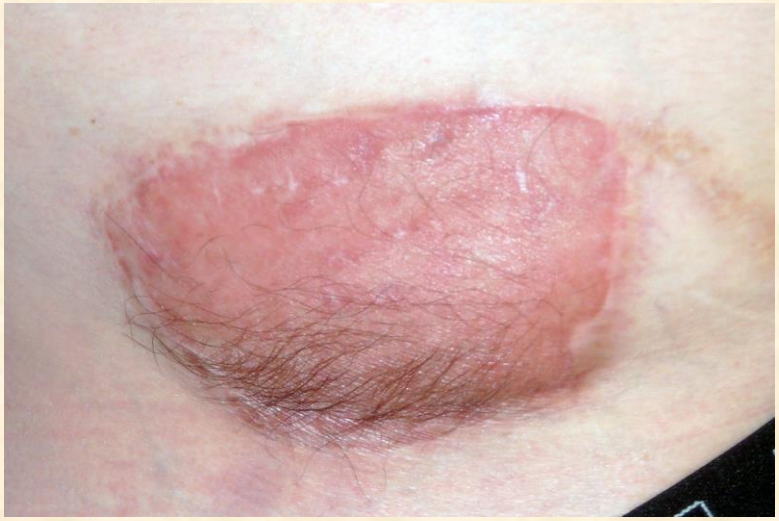
Face alloTx Nov 2009

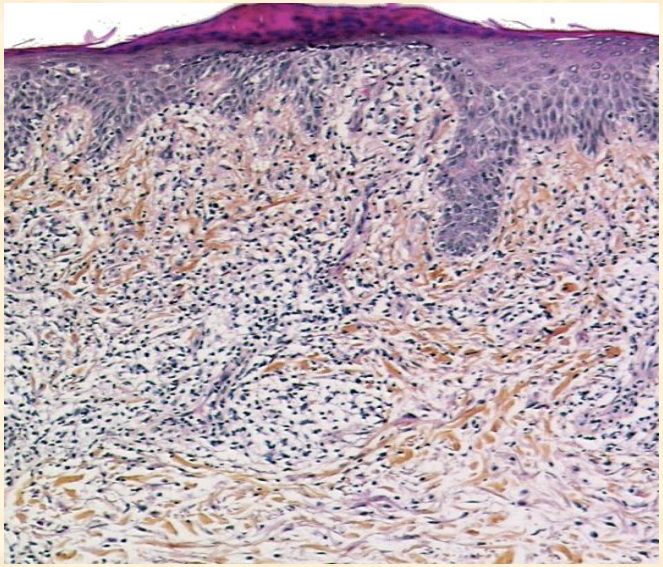


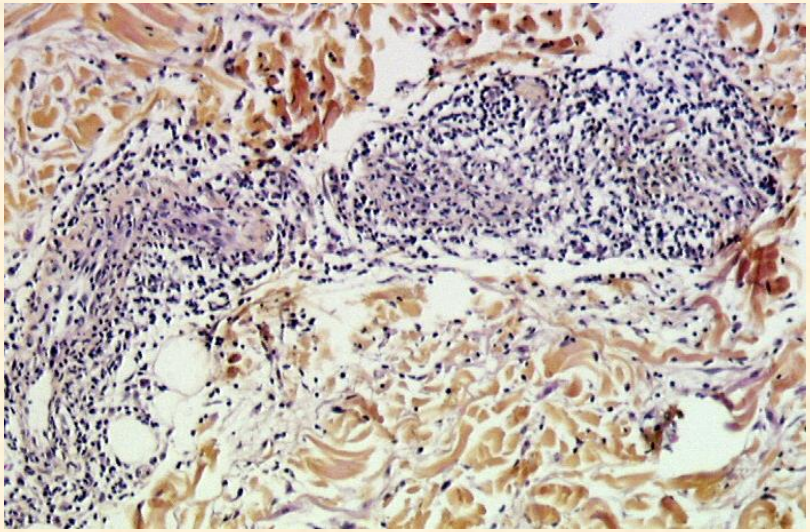
Petruzzo P, Kanitakis J, Testelin S et al. Transplantation 2015;99:2644

MT: repeated AR episodes during f/u



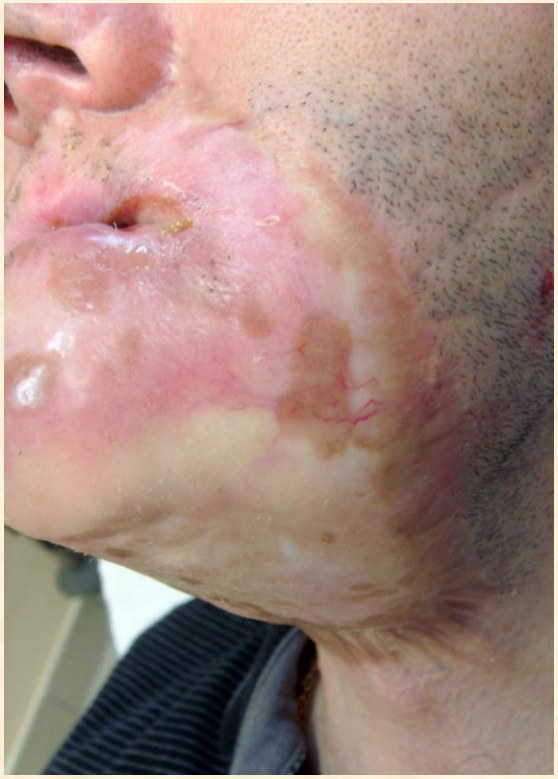


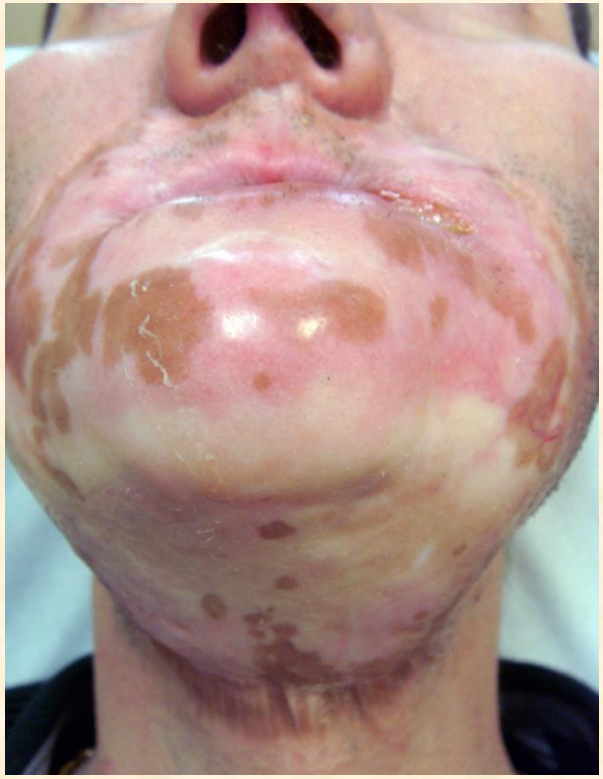


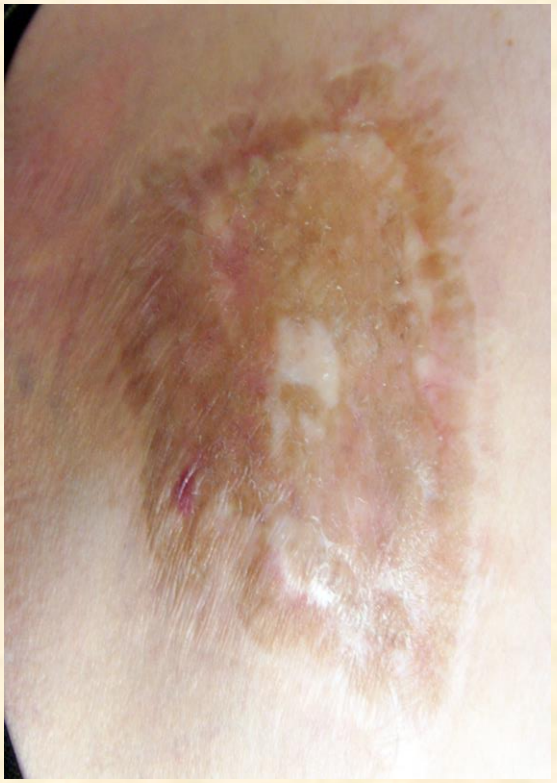


C D

Sclerotic & poikilodermic skin changes (dyschromy, telangiectases) in chronic face alloTx rejection (≈ chronic GVHD)



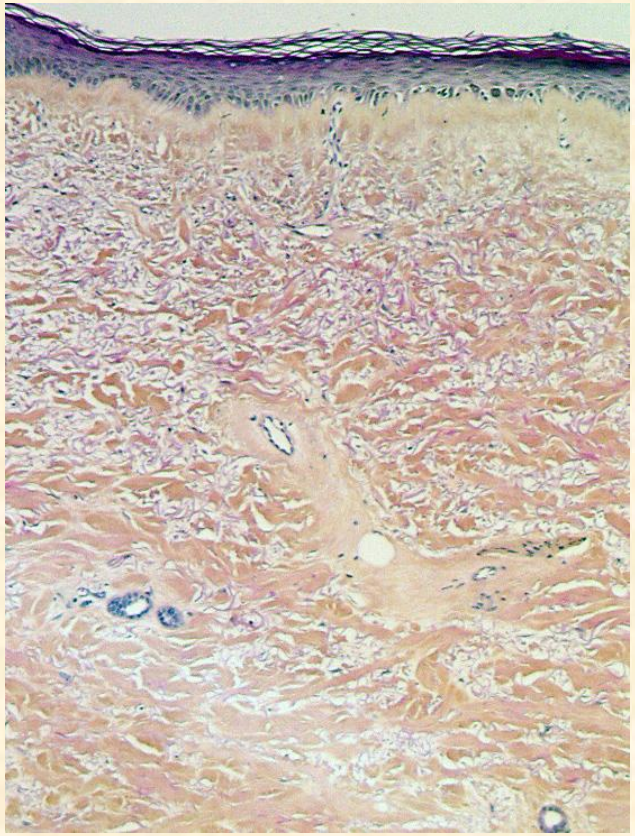


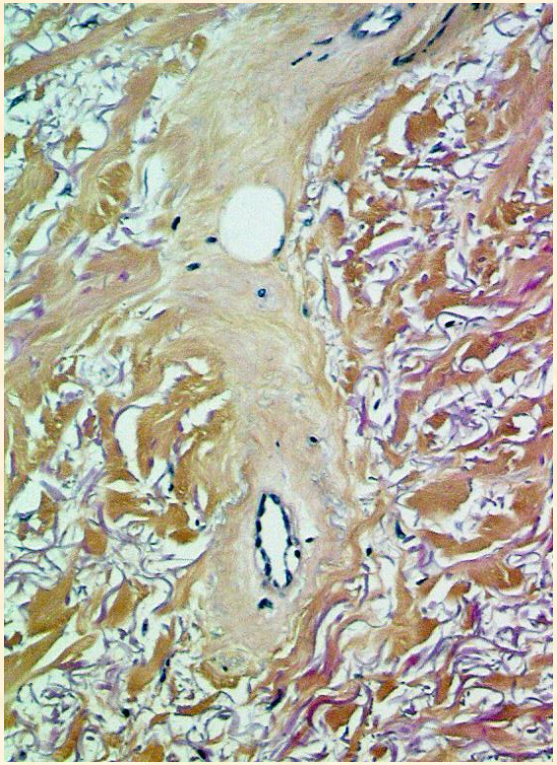


large vessel (facial arteries) poorly involved

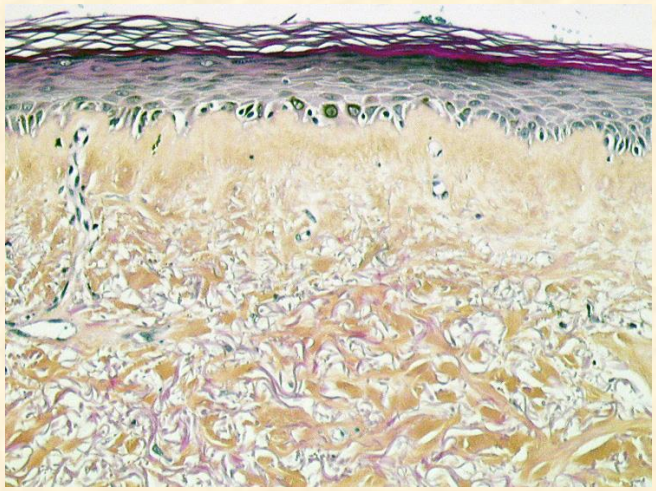
- decrease in esthetic & functional recovery

Sclerotic (chronic GVHD-like) changes in CR

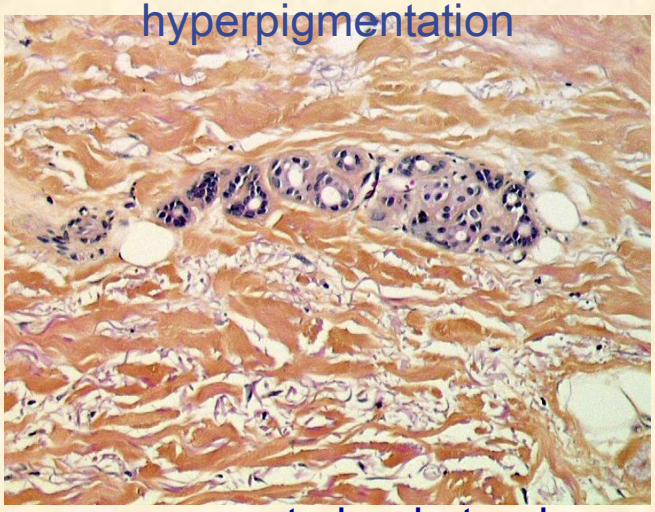




Dermal sclerosis/narrowing
of dermal capillaries - no cell infiltrates



dermal sclerosis/
basal cell
vacuolization/melanin
hyperpigmentation



sweat gland atrophy

Petruzzo P et al. Transplantation 2015;99:2644

American Journal of Transplantation 2017; XX: 1–6
Wiley Periodicals Inc.

doi: 10.1111/ajt.14218

Case Report

Face Transplantation: Partial Graft Loss of the First Case 10 Years Later

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**Corresponding author: Palmina Petruzzo, palmina.petruzzo@chu-lyon.fr*

Abbreviations: AMR, antibody-mediated rejection; AR, acute rejection; DSA, donor-specific antibody; GV, graft vasculopathy; IVIG, intravenous immunoglobulins; MMF, mycophenolate mofetil; VCA, vascularized composite allotransplantation

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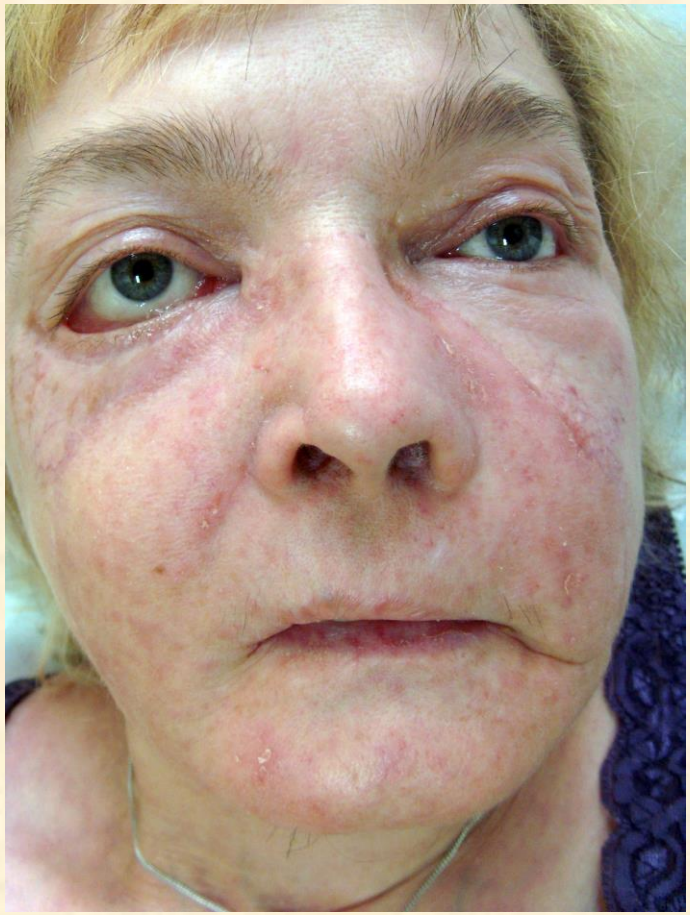
Introduction

The first face allotransplantation, performed in November 2005, showed the feasibility of this complex surgery, the ability of a triple immunosuppressive regimen to control acute rejection (AR) episodes, as well as the possibility to achieve a satisfactory esthetic and functional recovery.

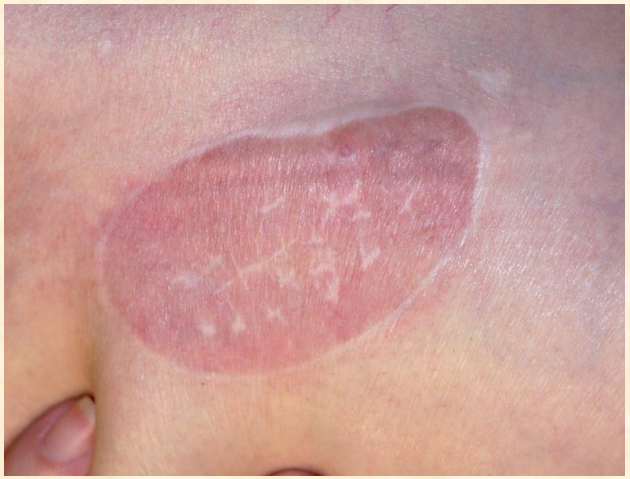
Face allotransplantation, similar to other vascularized composite allotransplantations (VCAs), shows a high rate of AR episodes, which are almost always limited to the skin and fully respond to prompt intensification of the

Face alloTx Nov 2005 - development of DSA 9 yrs post_Tx

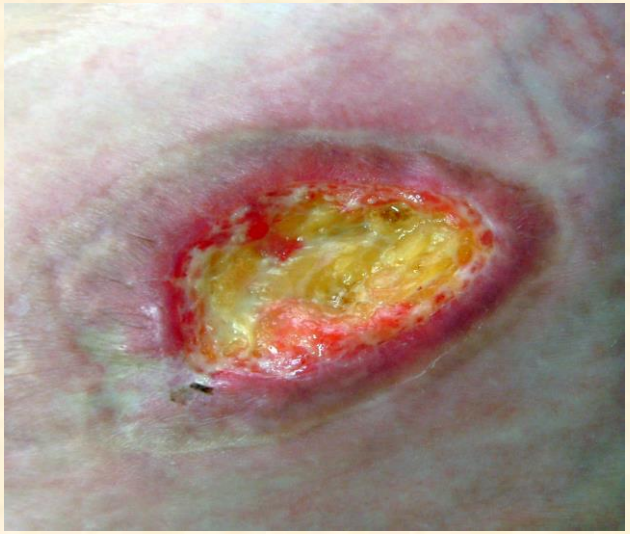


Scaly erythematous maculopapules
9 years postTx

SSG

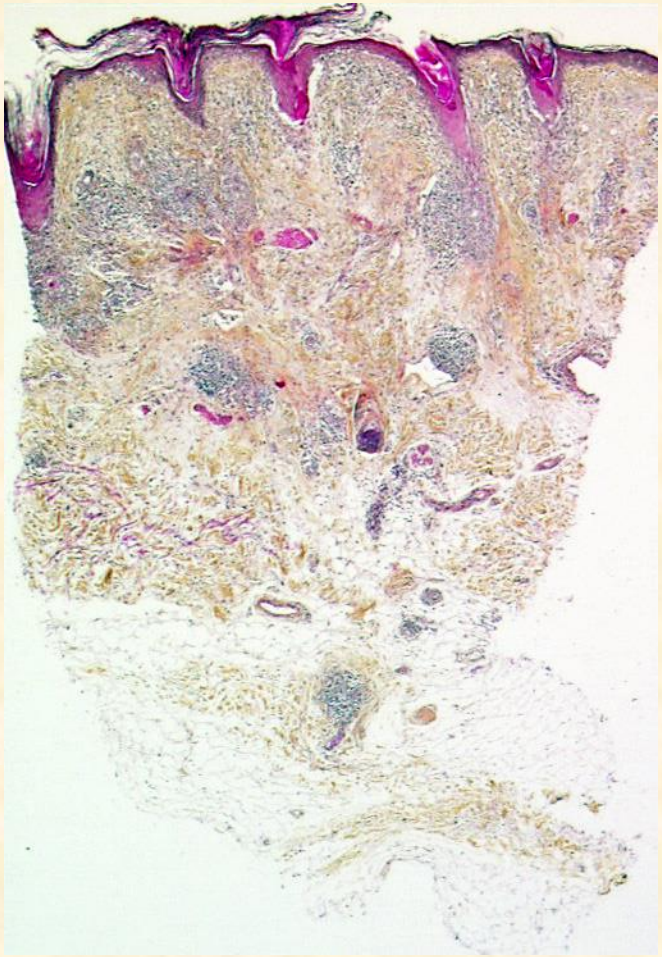


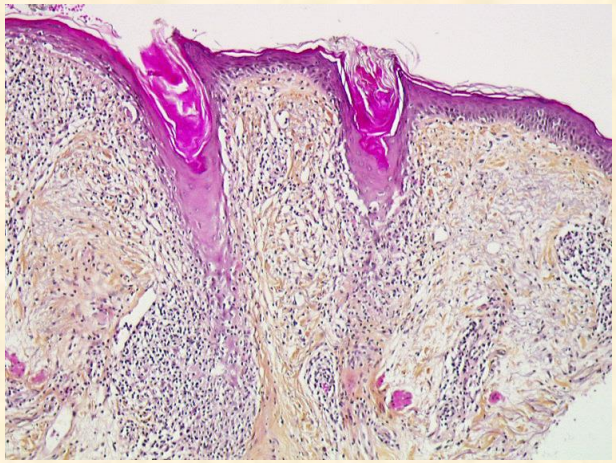
initial aspect

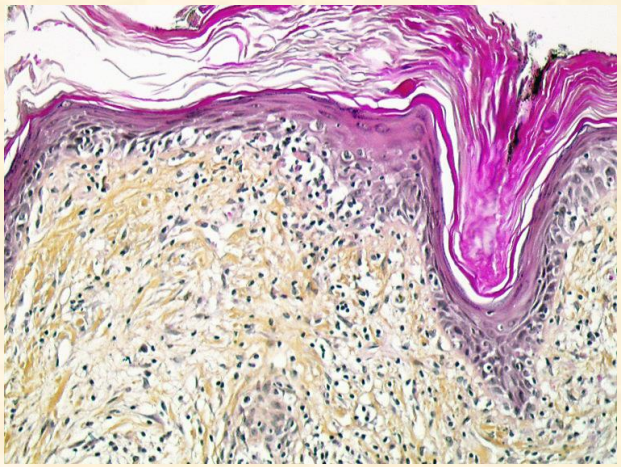


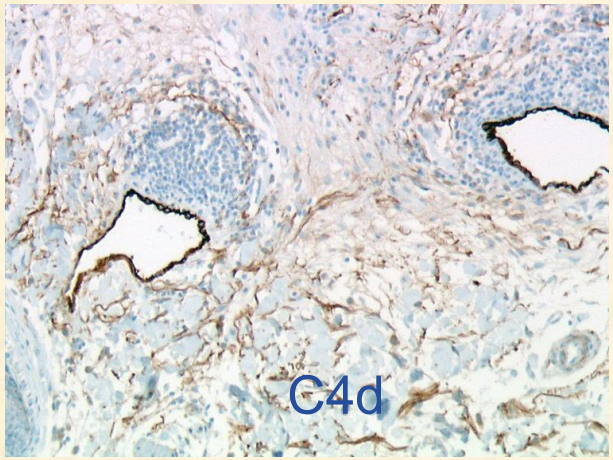
Necrotic ulceration 9 years postTx

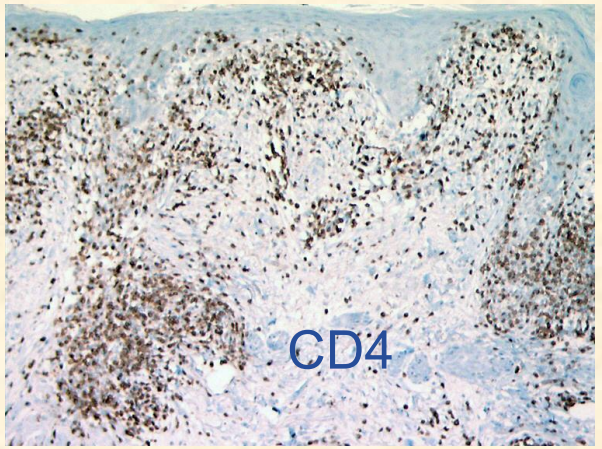
ID – 9 years post alloTx – facial skin

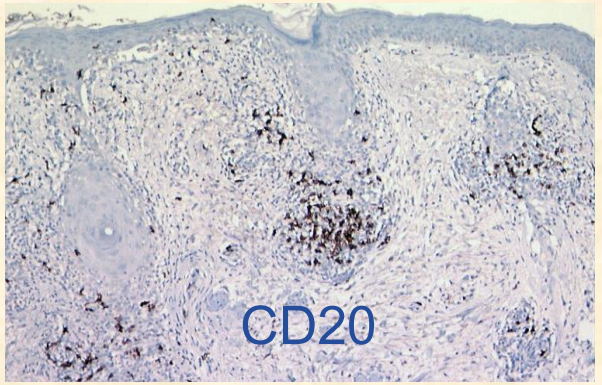








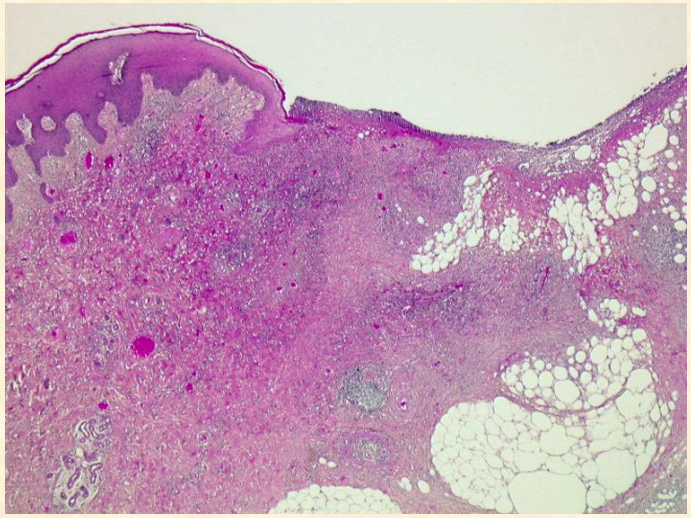




Banff grade III (lupus/lichen-like)

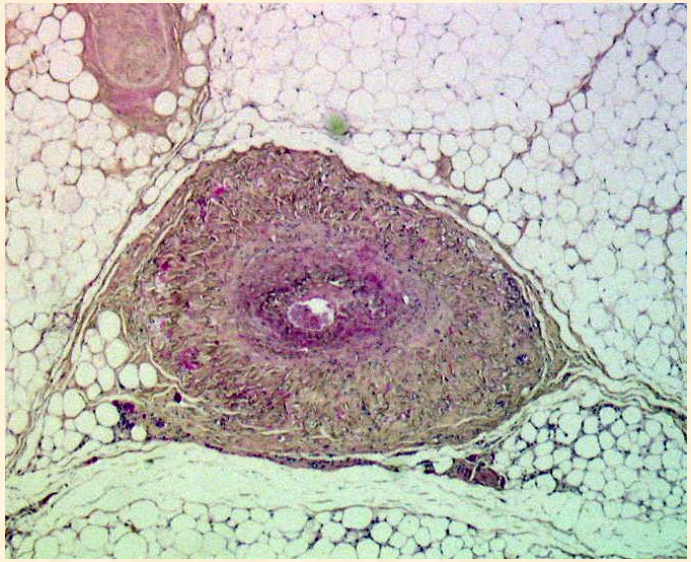
SSG ulceration

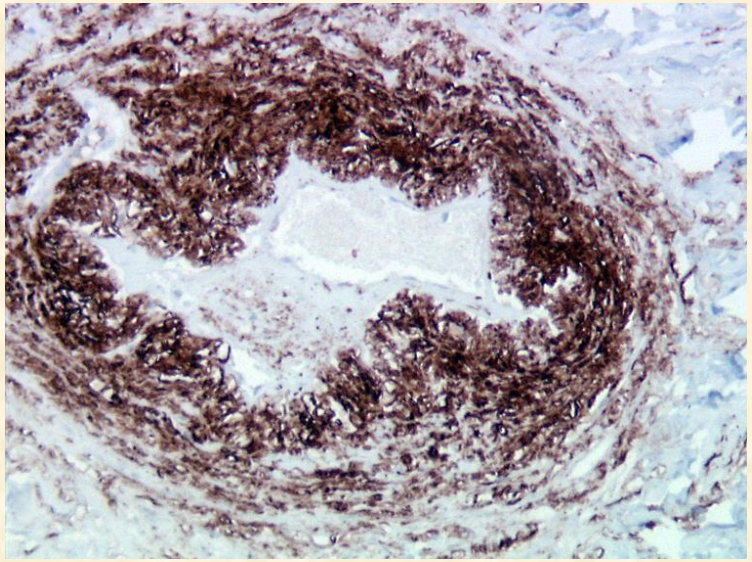
ID – 9 years post alloTx - SSG



Grade IV/necrotic skin rejection

Vascular rejection (SSG)



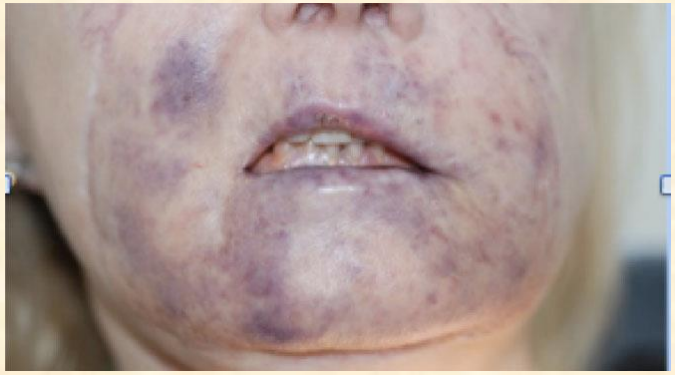


C4d-

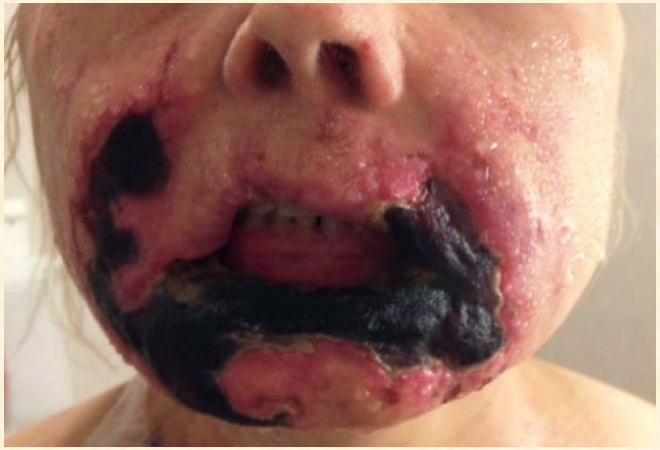
Wall thickening/luminal obstruction of the nutrient flap artery

≈ graft vasculopathy

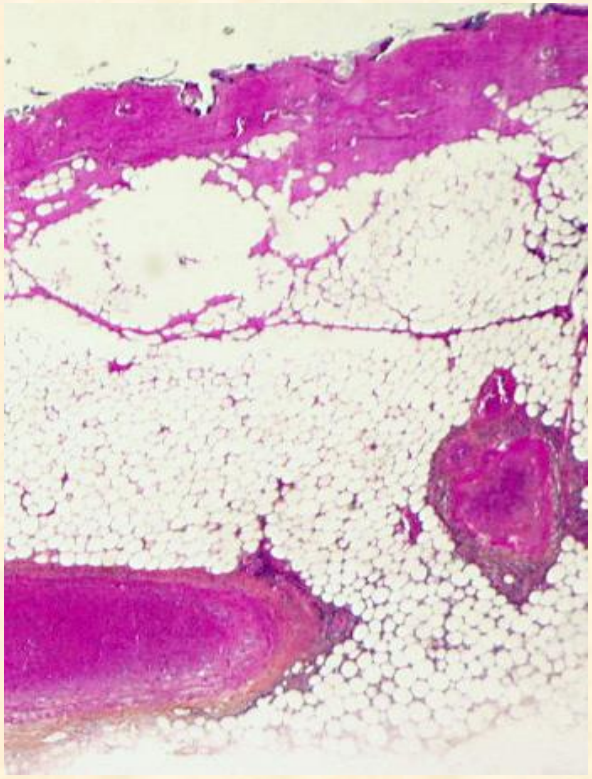
Patient ID – Face alloTx (Nov 2005) 10 years post-Tx: evidence favoring chronic, antibody-mediated rejection

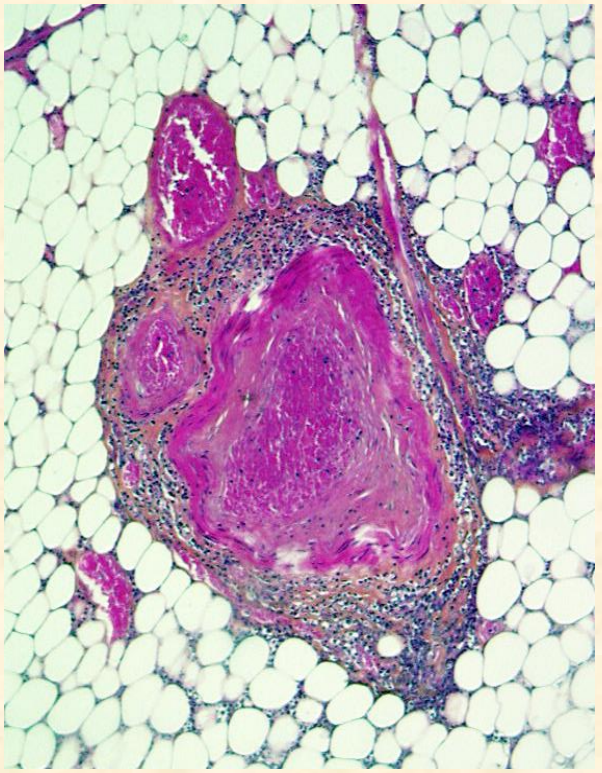


Cyanotic-ischemic facial lesions



Necrotic lesions
in part of the graft





Grade IV/necrotic rejection,
with graft vasculopathy & thrombosis
in facial (dermal) vessels

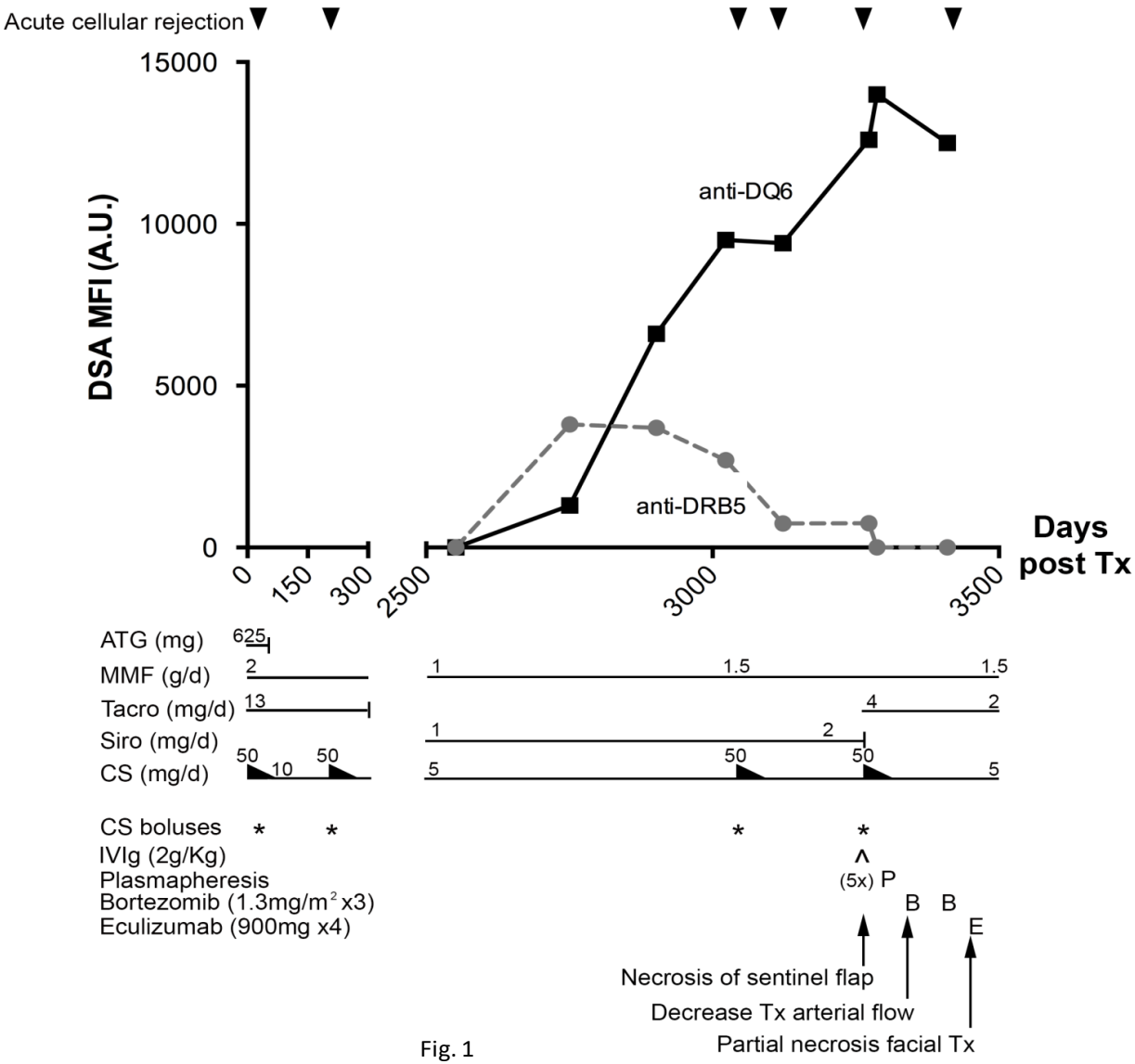


Fig. 1

Morelon E, Petruzzo P, Kanitakis J et al.

Am J Transplant epub 2017 doi: 10.1111/ajt.14218

Capillary Thrombosis in the Skin: A Pathologic Hallmark of Severe/Chronic Rejection of Human Vascularized Composite Tissue Allografts?

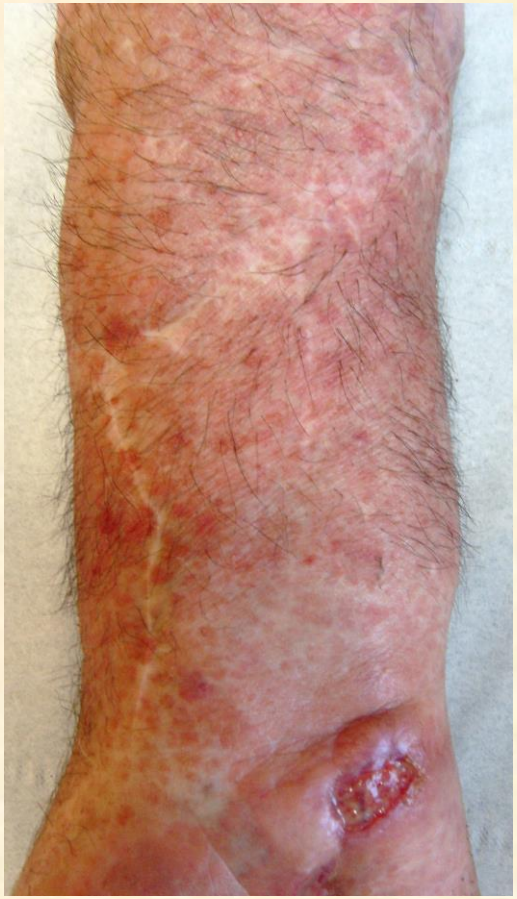
Jean Kanitakis, MD,[1] Palmina Petruzzo, MD,[2] Aram Gazarian, MD,[3] Georgia Karayannopoulou, MD,[4] Fannie Buron, MD,[2] Valérie Dubois, MD,[5] Olivier Thaunat, MD,[2] Lionel Badet, MD,[2] and Emmanuel Morelon, MD[2]

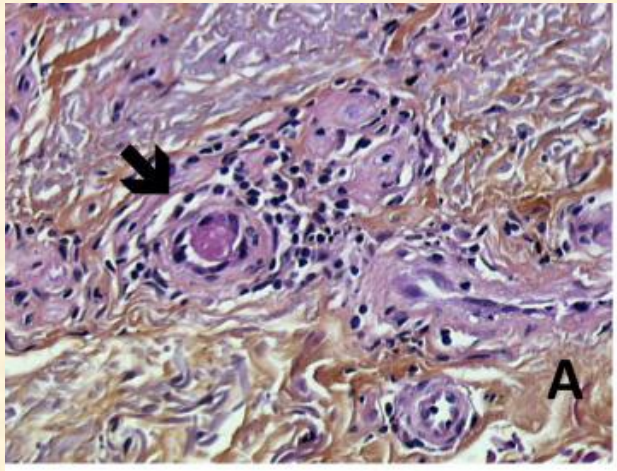
Background. Vascularized composite tissue allografts (VCA) can undergo rejection, manifesting pathologically with skin changes that form the basis of the Banff 2007 classification of VCA rejection. **Methods.** We have followed 10 human VCA recipients (7 with hand allografts, 3 with face allografts) for pathological signs of rejection. All of them developed episodes of acute rejection. Two patients with hand allografts presented in some of their skin biopsies an as yet unreported pathological finding in human VCA, consisting of capillary thromboses (CT) in the upper dermis. **Results.** Capillary thrombosis was associated with other typical changes of grade II to III VCA rejection, namely, perivascular T cell infiltrates, but not with vascular C4d deposits (in formalin-fixed tissue). Clinically, the lesions presented as red or violaceous (lichenoid) cutaneous maculopapules. The first patient had several episodes of acute rejection during the 7-year follow-up. The second patient developed donor-specific antibodies; some months after CT were first observed, he developed chronic rejection leading to partial amputation of the allograft. Pathological examination of the skin showed graft vasculopathy and occasional C4d deposits in cutaneous capillaries. **Conclusions.** Capillary thrombosis seems to be a novel pathologic finding associated with human VCA rejection. Although its mechanism (immunologic vs nonimmunologic) remains unclear, this finding could carry an unfavorable prognostic significance, prompting close monitoring of the patients for severe/chronic rejection.

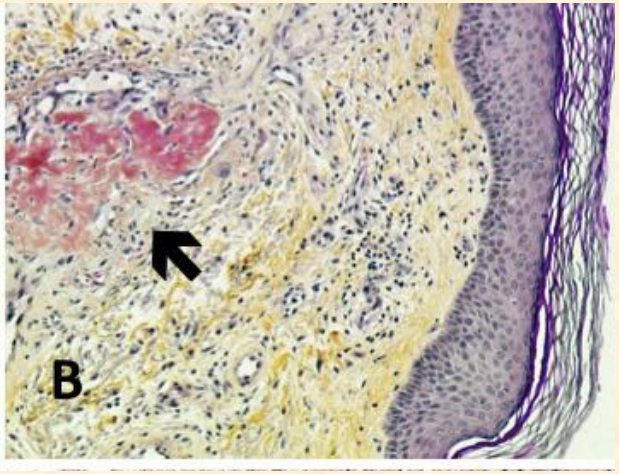
(*Transplantation* 2016;100: 954–957)

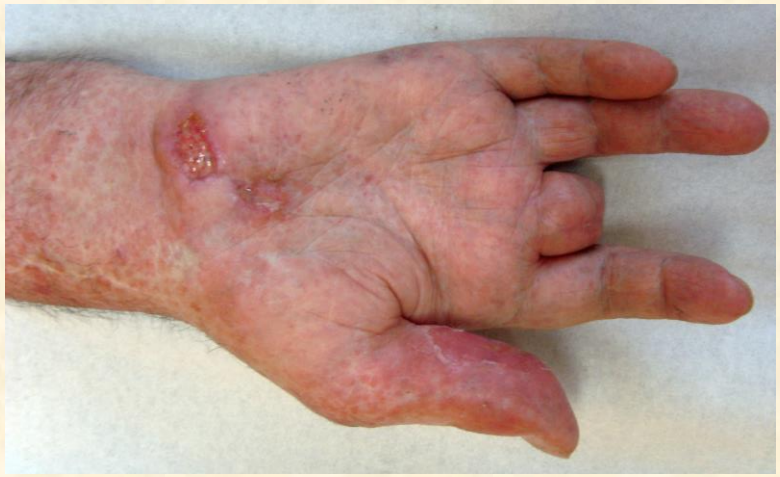
Transplantation 2016;100:954-7

Patient YB. Skin capillary thromboses during rejection (grade I) preceding the development of chronic rejection leading to non-functional graft and amputation

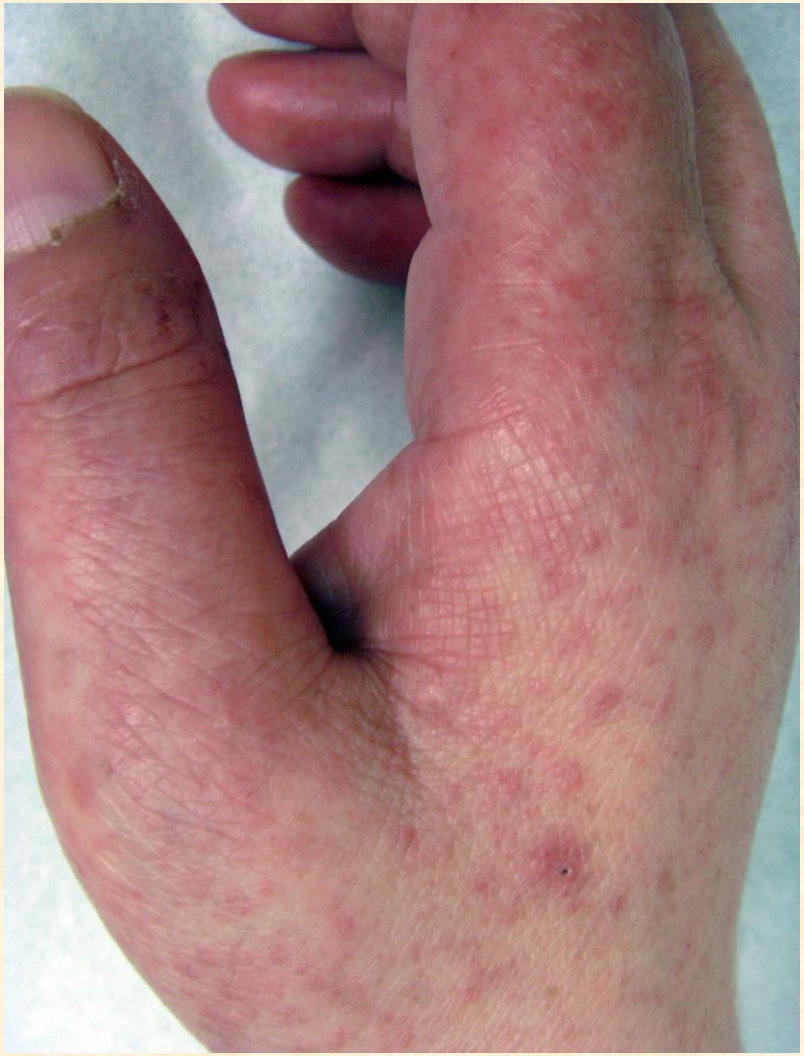




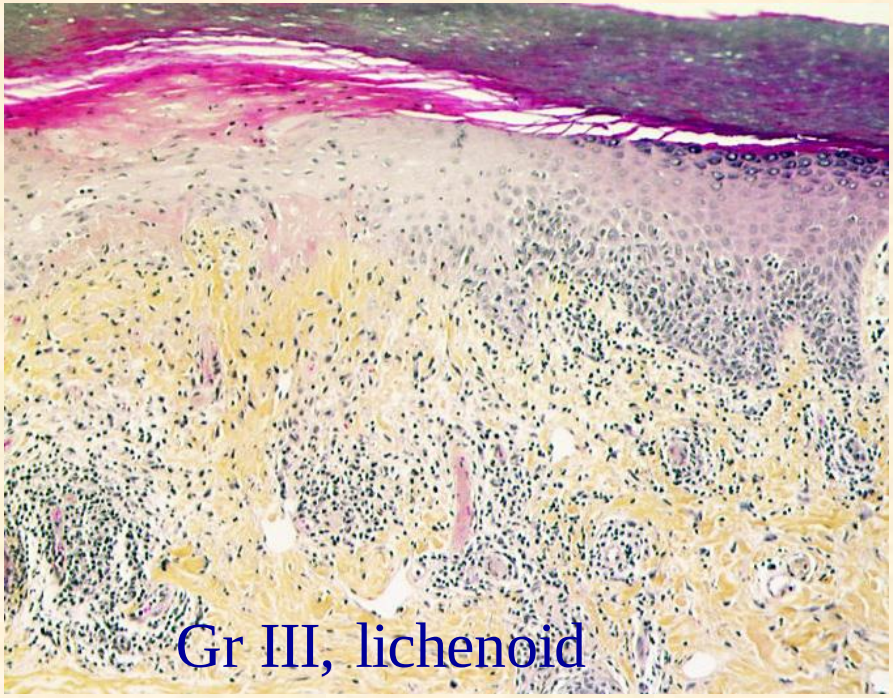


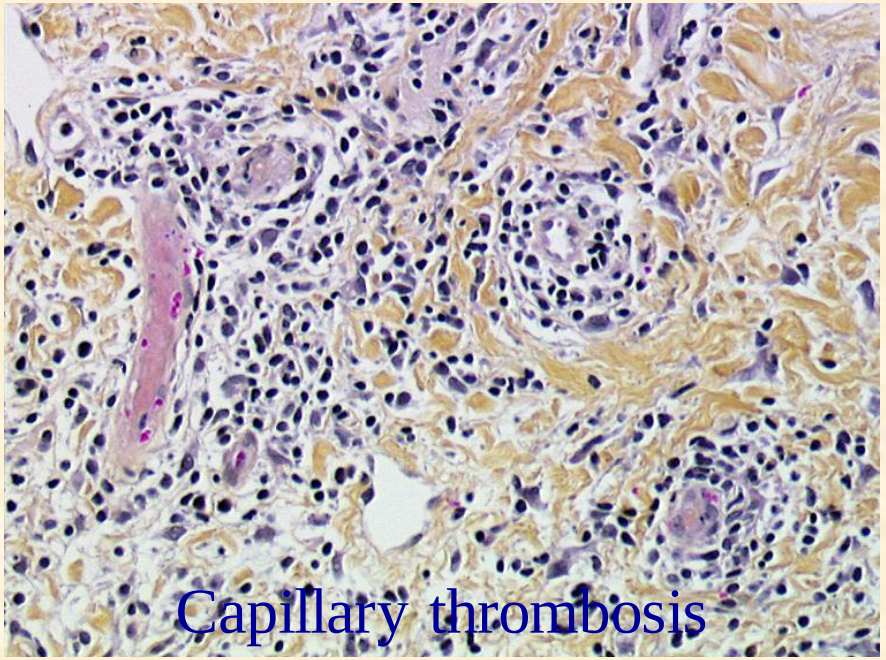


Patient ASLD



violaceous lichenoid maculopapules





Antibody-mediated rejection in hand transplantation

Weissenbacher A et al. Transpl Int 2014

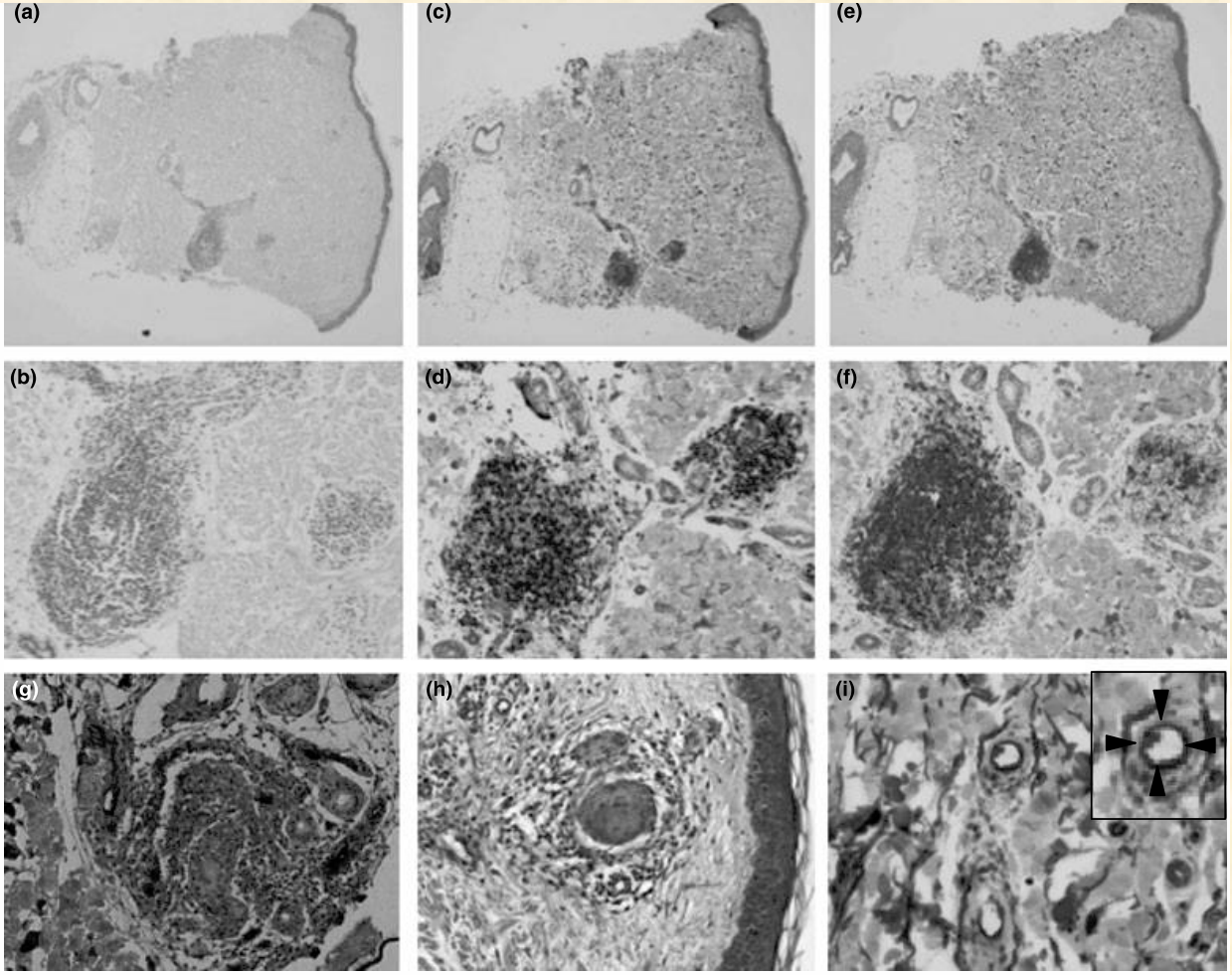


Figure 1 (a–i) Histology and immunohistochemistry of an allograft skin biopsy at the time of diagnosis: H&E histology revealed Banff grade II rejection with prominent perivascular cell infiltrates and mild epidermal involvement (a) together with a cellular accumulation resembling a nodular lymphoid aggregate (lymph follicle-like feature) in the deep dermis (b). The overall cell infiltrate was dominated by CD3 + T cells (c + d), whereas the cel accumulation mainly consisted of B cells, CD20 + (e + f). PNAd+ HEV-like vessels were found within the cell accumulation (g). The majority of infiltrating cells were positive for BAFF (h). Vessels were highly positive for C4d (i); arrows in the high power inset mark specific C4d staining of vascular endothelium. C4d staining for elastic fibers was considered unspecific.

lymphoid aggregate (lymph follicle-like feature, TLOs)

CD20 + B-cells

PNAd+ HEV-like

BAFF+ cells

Cd4+ vessels

Chronic Rejection in human VCA

Clinical skin findings

Scaly erythematous (lichenoid) papules/plaqus,

skin sclerosis, dyschromia, ischemic skin necroses

Pathological findings

- graft vasculopathy
- dermal sclerosis, epidermal and adnexal atrophy
- ? capillary thrombosis
- ? lymphoid follicles/TLO

Koenig A, Thaunat O. Front Immunol epub Dec 27, 2016 doi: 10.3389/fimmu.2016.00646

Mechanisms of CR in VCA: poorly-known

- Non-immunologic: chronic trauma, thermal injury, revisional surgical procedures ?

- Immunologic: DSA - other (non anti-HLA) antibodies? T & B-cells? Role of innate immunity?

Gr. II rejection

Erythema multiforme

Role of Antibody-mediated rejection

- contributive to the development of graft vasculopathy in heart Tx
- in VCA (animal/human), CR not always correlated with circulating DSA – however these could be retained in the graft and therefore undetectable in the serum
- C4d vascular deposits rarely reported, not always correlated with DSA (but C4d- AMR possible!)

Weissenbacher AM et al. Curr Opin Organ Transplant 2016;21:510

Rejection in VCA

- Acute: frequent, T-cell-mediated, mainly skin-directed, reversible with increase of the usual IST (steroids, MMF, CNI)
- Chronic: rarer, mainly vessel-directed, irreversible, mechanism poorly-known (probably AMR, ± cell-mediated)

Morelon E et al. Immunological challenges in VCA Curr Transpl Rep 2015;2:276

Chronic Rejection in Human VCA

Facts:

- Exists, rarer than AR (preventive role of early AR treatment ?) – could be encountered more frequently with longer f/u and more patients with VCA
- Main pathological expression: graft vasculopathy in deep and cutaneous vessels - dermal sclerosis, adnexal atrophy

Kanitakis J et al. Transplantation 2016;100:2053

Chronic Rejection in Human VCA

Questions:

- precise definition, diagnostic criteria, grading/classification?
- best diagnostic test: deep skin biopsy? non-invasive methods for vascular monitoring (eg. ultrasound biomicroscopy)? newer techniques (gene expression, proteomics, nanoengineering/nanotheranostics)?
- Relation with AR?
- Pathogenetic mechanisms: AMR (± cellular) ? role of DSA, lymphoid neogenesis in the graft? non-immunologic triggers?
- Best prevention/treatment?

Kanitakis J et al. Transplantation 2016;100:2053

Special thanks to colleagues from…

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Miss C. Dagot

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Dr V. Dubois

Dr A. Gazarian

Pr E. Morelon

Pr P. Petruzzo

Pr O. Thaunat

… and elsewhere

Pr B. Devauchelle, Amiens

Pr M. Lanzetta, Milan

Pr S. Testelin, Amiens

Thank you for your attention!