

ORIGINAL ARTICLE

Long-term outcome after hand and forearm transplantation – a retrospective study

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SUMMARY

Between 2000 and 2014, five patients received bilateral hand ($n = 3$), bilateral forearm ($n = 1$), and unilateral hand ($n = 1$) transplants at the Innsbruck Medical University Hospital. We provide a comprehensive report of the long-term results at 20 years. During the 6–20 years follow-up, 43 rejection episodes were recorded in total. Of these, 27.9% were antibody-related with serum donor-specific alloantibodies (DSA) and skin-infiltrating B-cells. The cell phenotype in rejecting skin biopsies changed and C4d-staining increased with time post-transplantation. In the long-term, a change in hand appearance was observed. The functional outcome was highly depending on the level of amputation. The number and severity of rejections did not correlate with hand function, but negatively impacted on the patients' well-being and quality of life. Patient satisfaction significantly correlated with upper limb function. One hand allograft eventually developed severe allograft vasculopathy and was amputated at 7 years. The patient later died due to progressive gastric cancer. The other four patients are currently rejection-free with moderate levels of immunosuppression. Hand transplantation remains a therapeutic option for carefully selected patients. A stable immunologic situation with optimized and individually adopted immunosuppression favors good compliance and patient satisfaction and may prevent development of DSA.

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Key words

complications, donor-specific antibodies, hand and forearm transplantation, immunosuppression, rejection, vascularized composite allotransplantation

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Introduction

Hand and forearm transplantation are therapeutic options for patients suffering from amputation. While the first successful case was performed 21 years ago [1], the total number of upper limb transplantations remains small and the progress of the field slow. This is sobering since conceptually, the idea to replace a missing limb with an allograft is attractive and the early functional results were very good [2–4]. The long-term outcomes are only slowly emerging and include early and late graft losses [5–7], development of chronic rejection and graft vasculopathy [6,8–10], donor-specific alloantibodies (DSA), antibody-mediated rejection (ABMR) [11,12], and side effects of long-term immunosuppression (IS). Despite immunologic problems, psychological well-being, psychosocial factors, compliance, and a more selective evaluation process to determine the “ideal” candidate for a vascularized composite allograft (VCA) have come into focus and require attention in the field.

Close patient monitoring and detailed, transparent reporting of the outcome remain key for the field and

may help to eventually make this procedure safe and successful in the long run. We herein report and critically reflect the outcomes (mean 13 years) of nine limbs transplanted in five patients at the Innsbruck Medical University Hospital between March 2000 and March 2014. This report focuses on the occurrences and challenges in the long-term.

Materials and methods

Patients

Earlier reports describe the patient details and the early post-transplant course [13–18]. Patient characteristics and the immunologic risk profile are provided in Table 1. Five male patients were given a bilateral ($n = 4$) or unilateral ($n = 1$) hand or forearm transplantation following traumatic hand loss. Time from amputation to transplantation was 5.0 ± 1.16 years (mean \pm standard deviation) and time on the waiting list was 10.0 ± 4.7 months. No IRB approval was required since this is a retrospective data analysis.

Table 1. Patient characteristics.

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	M	M	M	M	M
Amputation year	1994	2000	2000	2004	2009
Amputation cause	Explosion	Electric current accident	Explosion	Timber machine accident	Car accident
Age at Tx (year)	47	41	23	55	55
Tx date	07.03.2000	17.02.2003	29.05.2006	22.07.2009	26.03.2014
Type	Bilateral	Bilateral	Bilateral	Unilateral [®]	Bilateral
Level	Distal forearm	Proximal forearm	Mid forearm	Wrist	r: metacarpal, l: wrist
Use of myoelectric prostheses	Yes	Yes	No	Yes	No
CMV status (rec/don)	neg/pos	pos/pos	pos/pos	neg/neg	neg/neg
PRA pre-Tx (%)	5	0	0	0	0
HLA mismatch (A/B/DR)	2/2/2	1/2/1	1/1/1	2/2/2	2/2/2
Lymphocytotoxic crossmatch	neg	neg	neg	neg	neg
Perfusion solution	UW	UW	HTK	HKT	HTK
Cold ischemia time (min)	r:150, l:170	r: 155, l: 153	r: 183, l: 195	200	r: 368, l: 399
Induction therapy	ATG	ATG	Alemtuzumab	Alemtuzumab	Alemtuzumab
Early maintenance IS	tac/MMF/ster	tac/MMF/ster	tac/MMF/ster	tac/MMF	tac/MMF/ster
Follow-up (year)	19	16	13	7*	5

ATG, antithymocyte globulin; CMV, cytomegalovirus; HLA, human leukocyte antigens; HTK, histidine-tryptophan-ketoglutarate solution; IS, immunosuppression; l, left; M, male; MMF, mycophenolate mofetil; PRA, panel reactive antibodies; r, right; ster, steroids; tac, tacrolimus; Tx, transplantation; UW, University of Wisconsin solution.

*Graft had to be amputated 7 years post-transplantation.

Surgery and immunosuppression

Details of surgical techniques have been reported earlier [3,13–15,19]. Intra- and postoperative IS consisted of induction and maintenance treatment (Table 1). Reduction of overall IS including steroid withdrawal, reduction of tacrolimus trough levels and conversion from tacrolimus to mTOR-inhibitors sirolimus or everolimus was cautiously aimed for in all patients. In patient 5, belatacept (Nulojix) has been successfully introduced to the IS protocol [5], while in patients 2 and 3 balatacept was only added transiently and discontinued after 3 years. IS was adjusted under close surveillance of skin appearance, skin histology, metabolic, and kidney function parameters.

Histology and immunohistochemistry

Skin biopsies were collected according to a previously published protocol [20]. Paraffin embedded and hematoxylin&eosin (H&E)-stained sections were graded according to the Banff 2007-guidelines [21] with particular attention to luminal narrowing/occlusion. Immunohistochemical staining for CD3, CD4, CD8, CD20, CD68, Foxp3, and C4d was routinely performed. CD3, CD4, CD8, CD20, and CD68-stainings were read as percentage of the cellular infiltrate. Foxp3-staining was graded as 0 (0–<2%), 1 (2–10%), and 2 (>10% positive stained infiltrating cells). Endothelial C4d-staining was graded as follows: 0 (no/unspecific staining), 1 (mild/noncircumferential staining in some vessels), 2 (intense/circumferential staining in most vessels).

Imaging modalities

To monitor bone healing and integrity, vessel patency and muscle texture, X-ray and ultrasound were performed at close intervals during the first year and annually thereafter. Angiography and computed tomography angiography with three-dimensional reconstruction of graft vessels were undertaken annually after year 1. Functional magnetic resonance imaging (fMRI) was performed in patient 2 at 5-, 9-, and 15-year post-transplant.

Rehabilitation program and evaluation of hand function

Details on the specific rehabilitation program were published earlier [18,22]. Among others, the following tests were applied to evaluate and document hand function:

Thumb opposition – Kapandji score, key pinch strength, grip strength, static 2-point discrimination (s-2PD) test, and total active range of motion (TAROM) measurement. Functional results and subjective assessment were evaluated utilizing the disabilities of the arm, shoulder and hand (DASH) score, the action research arm test (ARAT) and the hand transplant score system (HTSS) score.

Electrophysiological studies

Motor and sensory nerve conduction studies were performed regularly according to standard procedures. For measurement of compound motor action potentials (CMAP) disposable surface electrodes were placed on the abductor pollicis brevis and abductor digiti minimi muscle after stimulation of the median and ulnar nerve proximal to the level of hand/forearm transplantation. Compound sensory action potentials (CSAP) were recorded from the index and the fifth finger with band electrodes.

Psychological evaluation

The “Innsbruck Psychological Screening Program for Reconstructive Transplantation (iRT-PSP)” [23] was applied for evaluation and follow-up. The iRT-PSP protocol highlights areas that are specific for hand transplantation and is a useful tool for the development of interventions that help patients to enhance coping strategies, manage life stress, and support their innate resilience to best adapt to life post-transplant [23,24].

Statistical analysis

Immunohistochemical data are expressed as mean \pm standard error of the mean (SEM). The comparisons of the phenotype at three time-periods post-transplant (early: 0–3 years, $n = 54$ skin samples; late: 3–10 years, $n = 88$; very late: after year 10, $n = 12$) were carried out using one-way analysis of variance with the Bonferroni post hoc test. Wilcoxon rank sum test was used to assess for differences in hand function and psychological parameters/scores during (value minus patient side median centered no rejection) and in the absence of rejection (median). Spearman’s rank correlation rho or Kendall’s tau b was used for correlation analysis. P -values were adjusted based on the false discovery rate (FDR) according to the Benjamini–Hochberg method [25]. Loess (locally weighted scatterplot smoothing) plots were used to depict trends in hand function over time, values were related to the 5-year results. A P -value < 0.05 was considered as statistically significant.

Table 2. Secondary surgical interventions.

Secondary surgical procedures	Patient (time post Tx)
Decompression of hematoma and tissue swelling	3 (day 7), 5 (days 1, 5, 10, 13)
Transplantation of skin autografts for wound closure	1 (day 2), 3 (day 2)
Coverage of a skin defect with a split-thickness skin graft	2 (day 3)
Resection of arteriovenous fistulas	1 (6 months), 5 (3.5 years)
Skin graft resection	3 (1 year)
Cosmetic scar surgery, scar correction	2 (2 years), 3 (4 years)
Arthroplasty MR II-V dextra, and tenolysis extens	5 (1.5 years)
Opponensplasty (IV)	3 (2 years)
Finger amputation	4 (7 years)

Tx, transplantation.

Statistical analyses were performed using the statistical software environment R (version 3.5.2; R Foundation for Statistical Computing; <http://www.R-project.org>).

Results

Surgery

Early postoperative surgical interventions were needed in four patients due to immediate postoperative swelling/hematoma and to cover skin defects. Other surgical procedures included resection of arteriovenous fistulas, scar correction, and interventions for improvement of hand function (Table 2). The esthetic outcome is shown in SDC1.

Rejection and immunosuppression

All patients experienced acute rejections during their follow-up. Detailed reports were published elsewhere [12–14,16–19,26,27]. Most often macroscopic skin lesions were indicative for rejection with or without tingling or burning sensations. Out of 43 rejection episodes, the majority were T-cell mediated rejections (TCMR), 12 were classified as ABMR, one as B-cell mediated rejection (BCMR), and one as chronic rejection (Fig. 1a, Table 3). Rejections were observed more frequently early after transplantation (Fig. 1b). ABMR was noticed first at 1.5 years. 2/5 patients developed serum DSA (anti-HLA class II) and 2/5 anti-HLA-class I, as assessed by Luminex® 200™ (Table 3).

Banff grading [21] of rejection in punch skin biopsies revealed 51.28% rejections as moderate (grade II, Fig. 1c,d). However, the histopathologic appearance of ABMR as indicated by a predominance of B-cells coinciding with the presence of DSA was found to be rather heterogenous and varied with regard to localization, extent, and dynamics of the cell infiltrate (Fig. 2a–h). This makes it difficult to precisely classify such rejections using the Banff-scheme 21, which currently lacks a classification for ABMR and chronic rejection. Immunohistochemistry in rejecting skin biopsies revealed a significantly increased proportion of CD3+ T-cells after year 3 (67.57 ± 5.34 vs. 82.47 ± 1.78 vs. 81.08 ± 5.02 , $P < 0.001$). The highest proportion of CD20+ B-cells was observed at 4–10 years (8.40 ± 1.27 , $P = 0.012$), while CD68+ macrophages were more dominant early after transplantation (15.48 ± 2.59 , $P = 0.022$). No significant change in Foxp3+ T-regulatory-cells was found over time. Endothelial C4d expression significantly increased with time post-transplant ($P < 0.05$, Fig. 2i,j).

The unilateral transplant recipient (patient 4) developed DSA at 1.5 years and underwent multiple ABMRs with levels of DSA ranging between 8000 and 19 000 MFI (mean fluorescent intensity). Histopathology revealed sparse infiltrates in the superficial dermis without epidermal involvement, even in the presence of macroscopic changes. A B-cell-dominated infiltrate was organized in cell aggregates in some cases and mainly located perivascular in the mid-dermis (Fig. 2e–h). Also, vascular changes consistent with vasculitis were observed. Overall, histopathologic changes were mild or even absent in this patient and did not correlate with rejection severity, including macroscopic skin changes, DSA and challenging rejection treatment. Development of skin ulcers/necrosis leads to amputation of the hand allograft at approximately 7 years (Fig. 3a–c). Histopathology showed severe graft vasculopathy by then, indicating chronic rejection (Fig. 3d–l).

In all other cases, rejection had been successfully managed. Most often, rejection responded to a steroid bolus and transient increase of IS ± topical treatment. More aggressive rejections during the early postoperative period were treated with thymoglobulin and/or alemtuzumab. ABMR in general did not respond to conventional treatment (steroids + tacrolimus dose increase); however, a rapid effect of rituximab treatment was observed. Immunoabsorption had been applied successfully to treat ABMR at 14 years in patient 2. Unfortunately, none of these treatment strategies including plasmapheresis were able to prevent graft loss in patient 4.

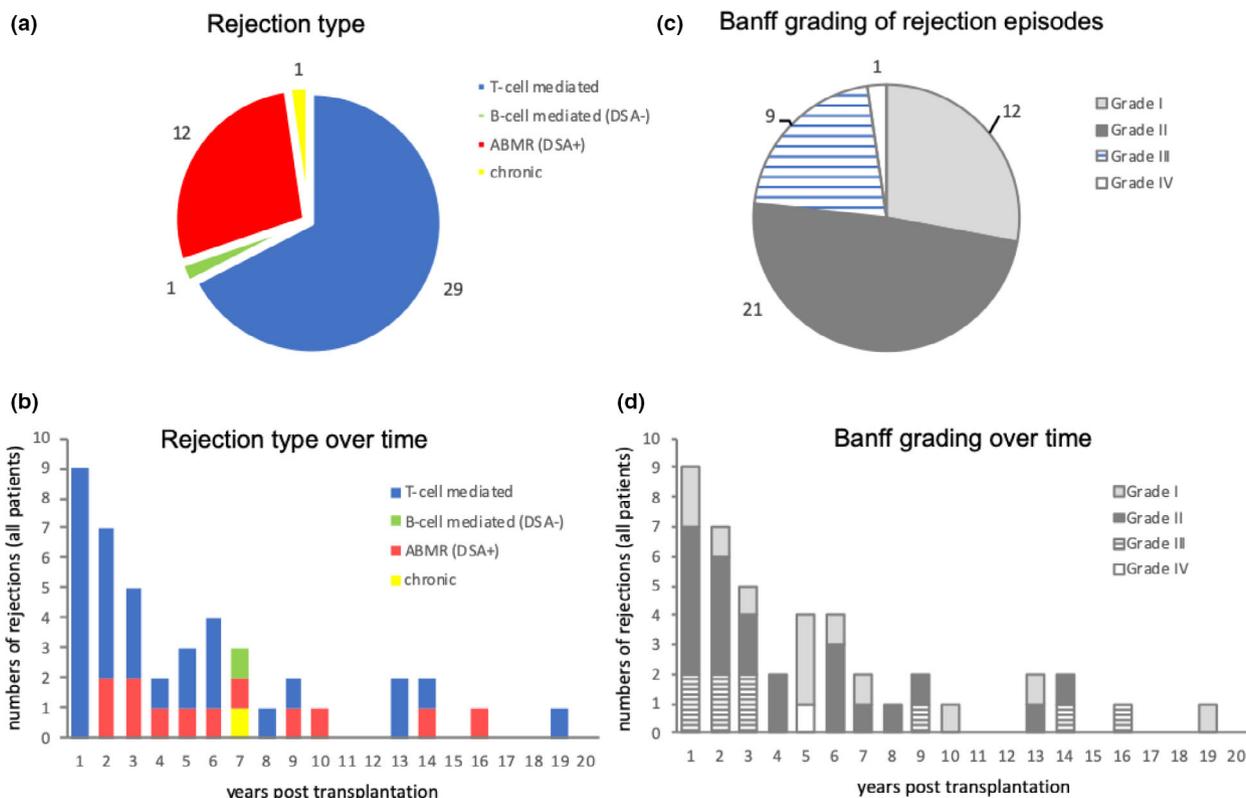


Figure 1 Characteristics of rejection episodes. Characteristics of rejection overall (a,c) and over time (b,d) with regard to rejection type and rejection severity according to Banff 2007 guidelines, observed in patients 1–5 during a 6–20-year follow-up after hand or forearm transplantation. Antibody-mediated rejection (ABMR), donor-specific alloantibodies (DSA).

The remaining patients are free of rejection (Banff 0 or 0-I) and negative for DSA at this point. No histopathologic signs for chronic rejection (as per the description by Morelon *et al.* [28]) were observed in skin biopsies. However, two patients have developed skin and nail changes over time (Table 3), which may be indicative for an early stage of chronic rejection.

Infectious complications and side effects

All patients experienced infections and metabolic side effects (Table 4). The majority of these complications evolved within the first and second postoperative year and are most likely attributable to high-dose IS during the early postoperative period. In the long run, autoimmune and proliferative diseases were observed. Details on the specific therapy are published elsewhere [17,18,29–31]. To keep calcineurin inhibitor (CNI)-induced side effects low, reduction of tacrolimus trough levels and conversion from tacrolimus to mTOR-inhibitors or to Belatacept was cautiously aimed for in all patients. No neurologic side effects caused by CNI were recorded in our cohort.

Introduction of Belatacept led to a stabilization of creatinine plasma levels in our patients [5]. Patient 5 was transplanted a donor kidney 191 days after bilateral hand transplantation due to preexisting but undetected kidney disease that did not recover even after conversion to Belatacept [5]. Patient 4 developed a progressive CNI-induced renal insufficiency/nephropathy at 3 years after unilateral hand transplantation. All attempts to convert his IS to the aforementioned substances failed and the hand allograft had to be amputated due to severe rejection at year seven. At 1 year after hand transplant removal, gastric cancer was diagnosed. Magnetic resonance and computed tomography scans revealed metastasis and peritoneal carcinosis at time of diagnosis. Chemotherapy with Leucovorin (200 ml/m²) and 5-Fluorouracil (2600 mg/m²) was initiated but failed to significantly decelerate progression of the disease. The patient eventually succumbed to this disease. While gastric cancer is not typically associated with IS and cancer occurred after graft removal and cessation of IS, a relation between the intense and multi-year IS and the cancer cannot be ruled out.

Table 3. Immunologic complications and immunosuppression.

	Overall	Patient 1	Patient 2	Patient 3	Patient 4*	Patient 5
Follow-up (year)	Mean 13	20	17	14	7	6
Rejection episodes (n)	43	4	16	10	10	3
TCMR (n)	29 (67.44%)	4	12	9	1	3
BCMR (n)	1 (2.33%)	0	0	1	0	0
ABMR (n)	12 (27.90%)	0	4	0	8	0
Chronic rejection (n)	1 (2.33%)	0	0	0	1	0
Graft loss	1/9 hands	No	No	No	Yes (at 7 years)	No
Development of anti-HLA class II	3/5	No	Yes (donor-specific, initially at 9 years)	Yes (not donor-specific, initially at 6.5 years)	Yes (donor-specific, initially at 1.5 years)	No
Development of anti-HLA class I	1/5	No	Yes (initially at 12 years)	No	No	No
Skin changes	2/5	No	Abnormal skin color and texture, diminished hair growth (since 12 years)	Abnormal skin color, texture, vascularization, and hair growth (since 7–9 years)	No	No
Nail changes	2/5	No	Abnormal nail growth (since 13 years)	Diminished nail growth (since 7 years)	No	No
Early steroid-sparing IS protocol	1/5	No	No	No	Yes	No
Belatacept treatment	3/5	No	Transient conversion (~13–16 years)	Transient conversion (~9–12.5 years)	Attempt (at 6 years, but failed)	Yes (start POD 100)
Current IS		Sirolimus (4–6 ng/ml) prednisolone (5 mg/day)	Tacrolimus (10–12 ng/ml), mycophenolic acid 500 mg/day, prednisolone 10 mg/day	Tacrolimus (8 ng/ml), sirolimus (8 ng/ml), prednisolone 5 mg/day, protopic + advantan creme		Tacrolimus (8 ng/ml), belatacept (5 mg/kg/6 weeks)

ABMR, antibody-mediated-rejection; BCMR, B-cell-mediated rejection; DSA, donor-specific antibodies; HLA, human leukocyte antigen; IS (-), targeted trough levels; IS, immunosuppression; n, number; POD, postoperative day; TCMR, T-cell-mediated rejection.

*Graft had to be amputated 7 years post-transplantation.

Imaging

At the most recent follow-up, radio-morphological studies demonstrated intact osteosynthesis in all hands/forearms without signs of osteodestructive/proliferative alterations (SDC2 a–d). Angiography and computed tomography angiography revealed patent blood flow and consistent perfusion of tissues without irregularities (SDC2). Duplex ultrasound constantly indicated an increased flow resistance in the radial and ulnar artery of patients 1, 2, and 3 (RI ranging between 0.9 and 1), and fatty degeneration of the allograft forearm muscles in patient 2. Importantly, no vessel wall thickening as a signal for emerging vasculopathy was seen in any of the four patients. FMRI revealed typical activation in the

motor and somatosensory cortex at 5 years after bilateral forearm transplantation. At 15 years, a broad activation in the respective areas was observed when stimulated by finger tapping (Fig. 4), comparable to healthy, nontransplanted individuals. This observation indicates fully reorganization of the motor and somatosensory cortex after limb transplantation.

Functional outcome

Hand function and sensitivity continuously improved during the first years in all patients (Fig. 5, SDC3). In hands transplanted at wrist level or above recovery of intrinsic muscle activity was observed at year 1, while the one hand transplanted at the metacarpal level

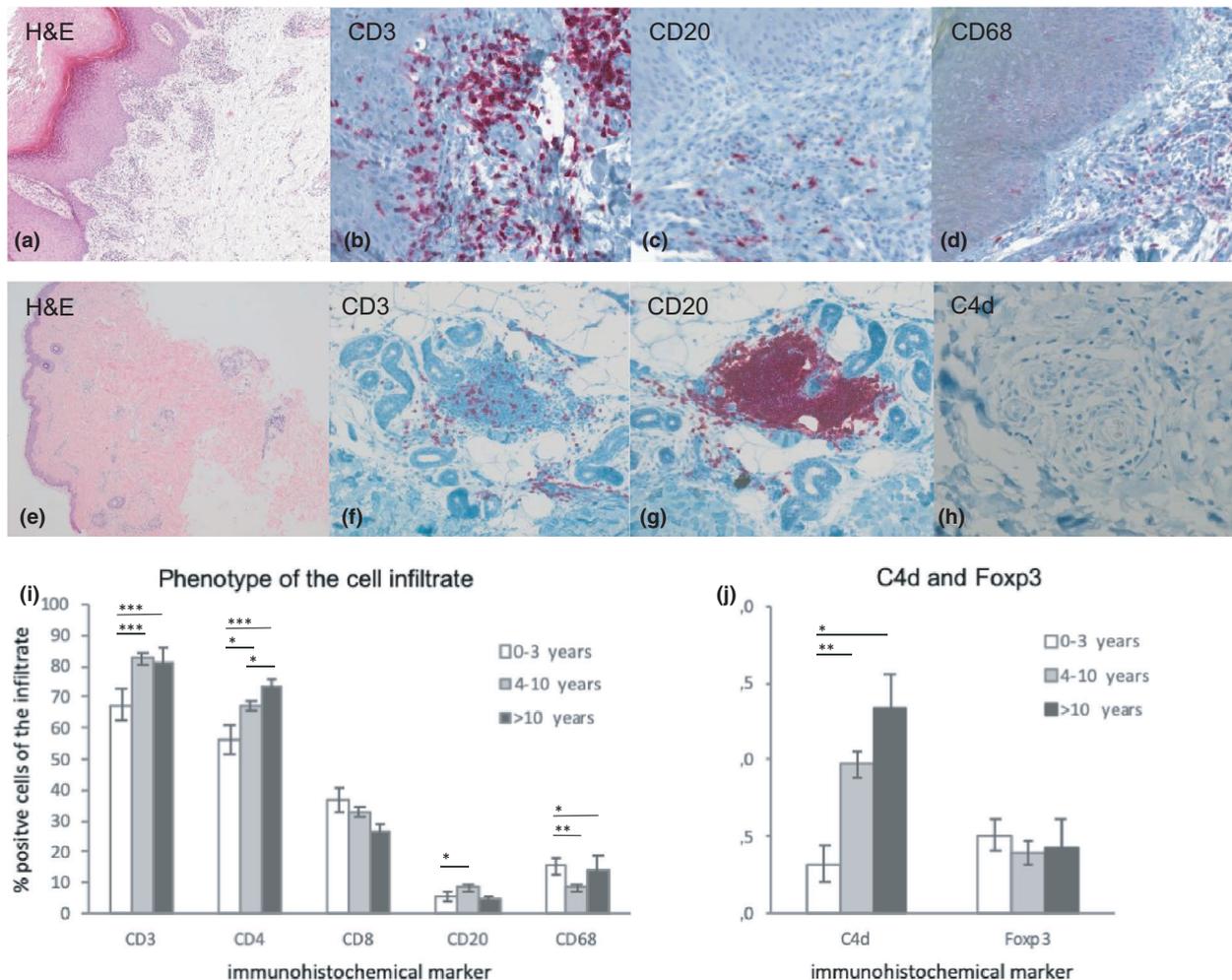


Figure 2 Histology and immunohistochemistry of rejection. Skin biopsies revealed a heterogeneous histopathologic appearance of ABMR (DSA+, a–h). During ABMR with high levels of DSAs observed in patient 2 at 14 years, Banff rejection grade III (a), infiltrating cells consisted of T-cells (b), B-cells (c), and macrophages (d), which were mainly located in the superficial dermis. ABMR, DSA+ in patient 4 at 2.5 years histopathologic revealed aggregates of cell infiltrates located perivascular in the deep dermis, while the superficial dermis was spared (e). These infiltrates were dominated by B-cells (g) with few T-cells (f), while endothelial C4d was negative (h). Analysis of immunohistochemical markers found in the skin during rejection early, late, and very late after transplantation (i,j). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

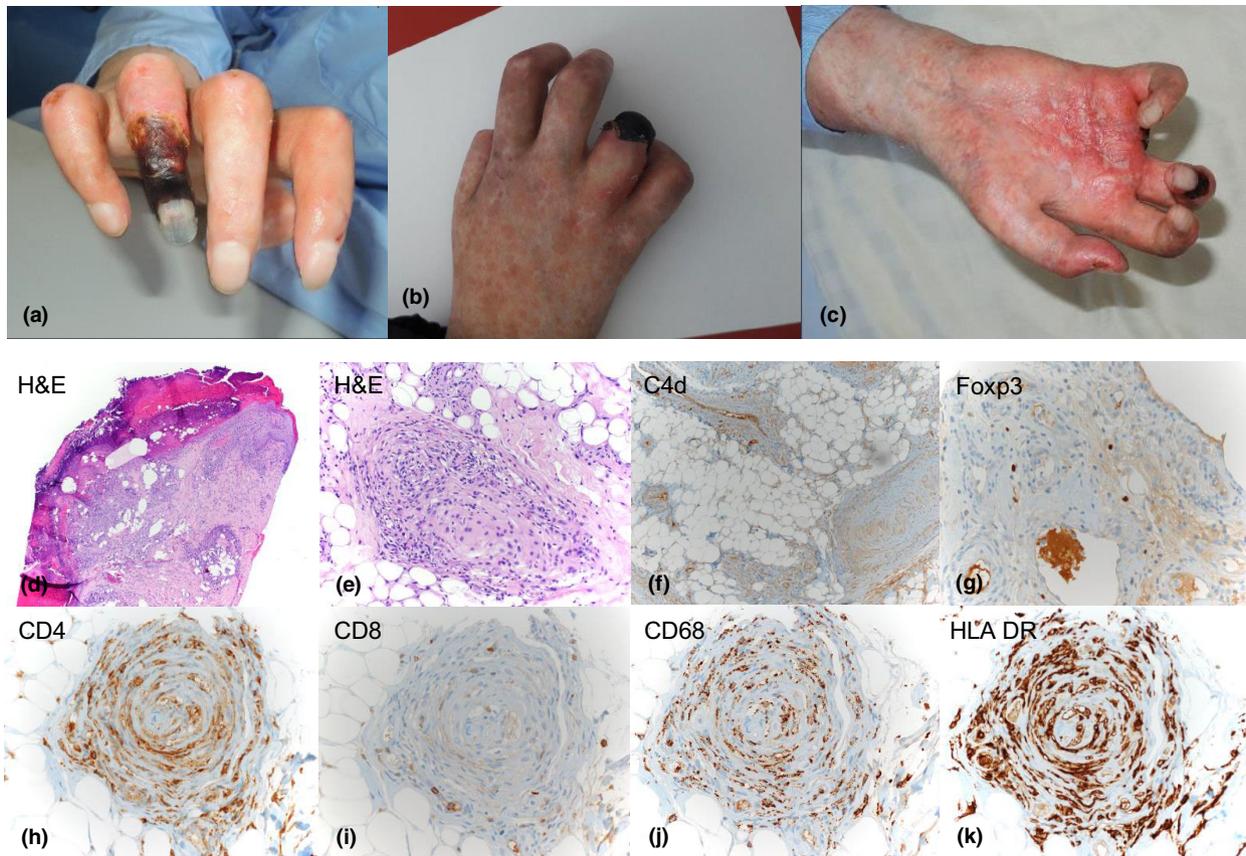


Figure 3 Graft loss in the unilateral hand transplant recipient (patient 4) due to chronic rejection at 7 years. History of amputation of dig. IV and skin changes one month before graft amputation (a–c). Histopathology of the amputated hand allograft at 7 years revealed severe skin necrosis (Banff grade IV) and cell infiltration (d). Myointimal hyperplasia and occlusion of the lumen (e) were observed in superficial dermal as well as deeply located large vessels, which stained highly positive for C4d (f). Further immunohistochemical analysis (g–k) revealed the perivascular cell infiltrate highly positive for CD4 (h) and HLA DR (k).

regained intrinsic hand function not before year 2. Protective sensation was found in all patients within the first year. Specifically, patient 1 regained outstanding hand function, performing all activities of daily living (ADLs) [27]. Functional recovery after forearm transplantation (patient 2) was slower compared to distal hand transplantation, but eventually a satisfying functional outcome was achieved. The return of function is superior to what the patient had experienced with myoelectrical prostheses. An improvement in the performance of ADLs, the patient's independence and satisfaction as assessed by DASH questionnaire, ARAT and HTSS was observed in all patients (Fig. 6).

In the long run, intermittent improvement or deteriorations of hand function were observed (Fig. 5 + SDC3). A tendency toward an overall slow deterioration, especially late after transplantation, was found for grip strength, key pinch strength, and thumb opposition (Fig. 5d–f). No association of a concurrent

decrease in function and rejection could be observed (Table 5). In fact, hand function remained stable also during rejection episodes (Fig. 7a). However, a negative impact of rejection on patients' well-being – according to the score indicative for presence of side effects due to intensified IS requiring pharmacological treatment - and quality of life (QOL) was found (Table 5). QOL scores were significantly lower during rejection, when compared to QOL scores in the absence of rejection (Fig. 7b). Patient satisfaction significantly correlated with an overall acceptable upper limb function expressed by a high ARAT and HTSS and a low DASH, a flexible, movable index finger and good sensitivity, as indicated by a low s-2PD (Table 5).

Electrophysiological studies

Nerve conduction studies demonstrated motor reinnervation for the median and ulnar nerve in all patients.

Table 4. Side effects.

Side effects	Patient
Recurrent CMV infection	1, 2, 3
HPV associated skin warts	2, 5
Invasive fungal infection with <i>Alternaria</i>	2
Oral aphthous lesions	2
Epidermatitis/eczema (recipient skin)	2
Condyloma	3
Myocarditis	3
Pangastritis	3
Hypertension	2, 3, 4, 5
Hyperlipidemia	1, 3, 4
Diabetes mellitus (noninsulin-dependent)	1
Headache	2
Leukocytopenia	2
Radius fracture (graft)	1
Subdural hematoma	5
Bullous pemphigoid	1
Nasolabial and frontal basal cell carcinoma	1
Nasal squamous cell carcinoma (Keratoacanthoma)	1
Acute prerenal kidney failure due to gastroenteritis	3
CNI-induced renal insufficiency/nephropathy	4
Acute kidney failure necessitating kidney transplantation	5
M-TOR intolerance	4
Lung emphysema	4
Urosepsis (<i>E. faecium</i>)	5
Sepsis with multi-organ failure due to influenza virus infection	3
Gastric cancer	4

CMV, cytomegalovirus; CNI, calcineurin inhibitor; m-TOR, mammalian target of rapamycin; HPV, human papilloma virus.

Motor action potentials steadily increased starting at year one until year five and remained stable thereafter with the exception of patient 4, where deterioration was timely related to repeated, severe rejection after year three (SDC4 a–e). Sensory reinnervation was observed later than motor reinnervation (SDC4 f–j). In general, amplitudes of compound motor and sensory action potentials remained lower when compared to healthy individuals.

Psychological outcomes

Although the patients had a different history of hand loss and showed diverse psychological conditions, all had one common aim: being “whole” again [23]. For evaluation of the first three patients, the standardized psychosocial evaluation and follow-up protocol (iRT-PSP) was not yet in place and psychosocial outcomes

are descriptive. Table 6 summarizes the main psychosocial outcomes with respect to psychopathology, depression, anxiety, psychological well-being, and QOL, gathered by recent post-transplant follow-up ratings.

All patients successfully assimilated the transplanted hand(s) into their body-/self-image and were able to develop a sense of “ownership”. They reported a high degree of satisfaction and improved confidence in appearance and social situation. No psychiatric disorders have been recorded in the post-transplant course and all patients described average levels of psychological distress. Specifically, no severe depression and/or anxiety have been evaluated post-transplantation. Patients unanimously observed improvements in QOL, psychological well-being, and ADLs, as stated above. Multiple rejections and difficulties with rehabilitation caused psychological distress in the unilateral hand transplanted patient.

Discussion

Good to excellent functional results with a high degree of patient satisfaction can be achieved after hand/forearm transplantation, however, immunologic complications including acute and chronic rejection, and side effects burdened the postoperative courses to various degrees in our cohort and remain the main challenges. Adverse effects were manageable with specific therapy or interventions, except for the gastric cancer where the disease was advanced when diagnosed and progressed rapidly despite therapy. Even if the number of acute rejections decreased after the early postoperative phase in our patients, events do occur years after hand/forearm transplantation. In comparison to the first decade of our experience, an increased number of rejections concomitant to presence of DSA were observed early and late post-transplantation. While patients 1 and 5 with uneventful follow-ups were never positive for DSA, patients with more complicated immunologic courses developed DSA between 1.5 and 9 years. The relevance of DSA in the context of VCA remains poorly understood. In solid organ transplantation, DSA have been shown to exhibit a detrimental impact on risk of rejection and graft survival [32,33]. When the first VCA cases were performed 20 years ago, methods for DSA assessment were not as advanced and routine clinical use was not established in most centers. Our unilateral hand transplant recipient experienced multiple ABMR with high levels of DSA over a period of 4 years [5], resulting in chronic rejection and graft loss. In reference, the French group reported development of de novo DSA and several rejection episodes, resulting in

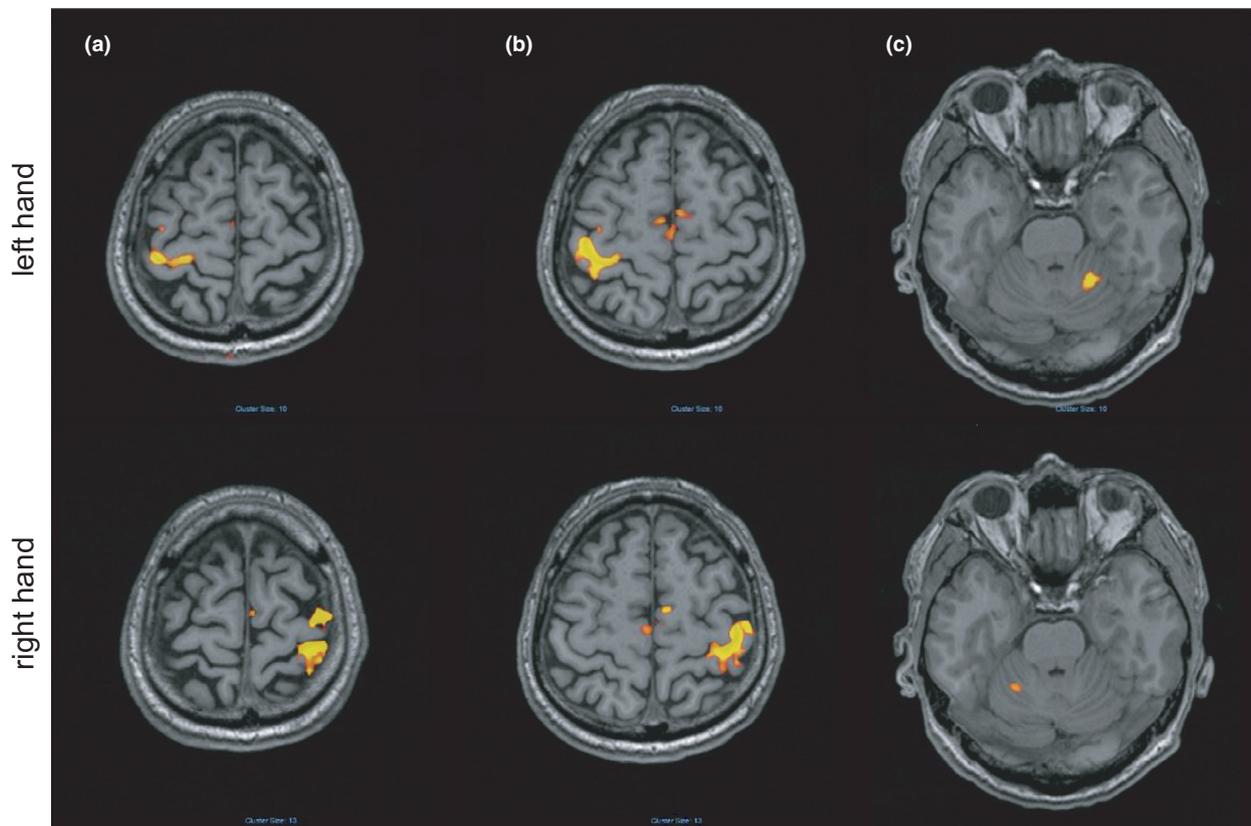


Figure 4 Functional magnetic resonance imaging (fMRI). fMRI was performed using a 1.5 Tesla magnetic resonance scanner and a head coil with eight arrays. Four consecutive fMRI measurements were performed with rest and motion paradigms including finger typing, softball compression, and fist clenching of both hands. 15 years after bilateral forearm transplantation motoric activation after finger tapping of the left (upper row) and right hand (lower row) appeared to be normal and was comparable to healthy, nontransplanted individuals. Activation of the primary motor and somatosensory cortex as well as the supplementary motor areas (SMA, a,b). In both hands, an adequate sensomotoric activation was achieved. Ipsilateral cerebellar activation after finger tapping (c).

partial graft loss of the first face allograft [7]. Based on our observations and the reports by others [34], we speculate that DSA and repetitive ABMR may result – or be the result of – a complicated immunologic course and indicative for the risk of developing vasculopathy and chronic rejection in VCA. Such patients should be evaluated at close intervals with particular attention to DSA levels and vascular changes [35]. We hypothesize, that fluctuations of trough levels of IS may have contributed to the challenging immunologic courses in our patients 2 and 4 and facilitated the repeat/chronic activation of the immune response with development of DSA. Patient nonadherence is often difficult to prove but correlates well with DSA development and graft loss in solid organ and VCA [9,28,36–38]. Minimizing the overall level of IS to the level required to prevent rejection remains an important factor in VCA patients. However, according to our experience a slow and cautious adoption of IS over years may help to avoid development of DSA, ABMR, and possibly graft loss. Our

observations are based on a relatively small number of patients, but these findings together with a systematic evaluation of the phenomenon of DSA and ABMR in VCA [34] supports the relevance for the immunologic course on graft survival.

Currently, diagnosis of rejection is based on histopathologic examination of a skin biopsy [20] using a 5-graded classification system introduced in 2007 [21]. Severity of ABMR in our patients was difficult to classify per the existing Banff criteria since criteria for ABMR have not been formally publicized. In our cohort, cases classified as ABMR histopathologically showed a considerable number of B-cells, which were most often organized in aggregates. The bulk of the infiltrate was located perivascular in the deep dermis, while the superficial dermis was often spared and the epidermis hardly ever involved. In some, but not all ABMR endothelial C4d and peripheral node addressin (PNAd)-staining were detected. Vascular alterations have been observed in severe cases. As the number of reports on this specific type

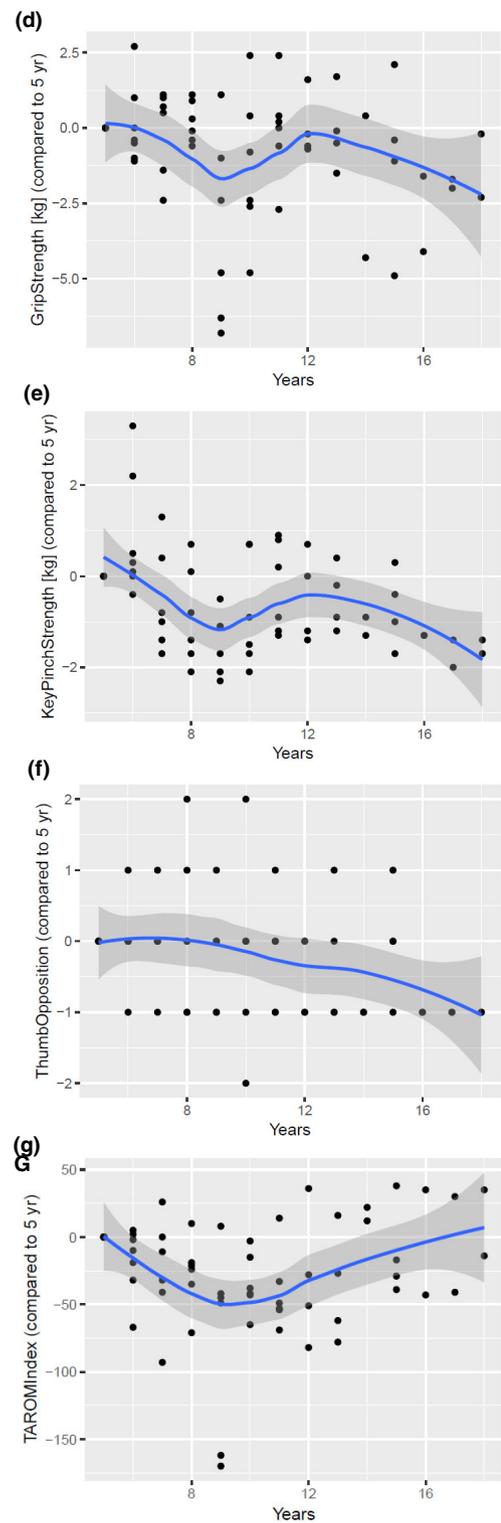
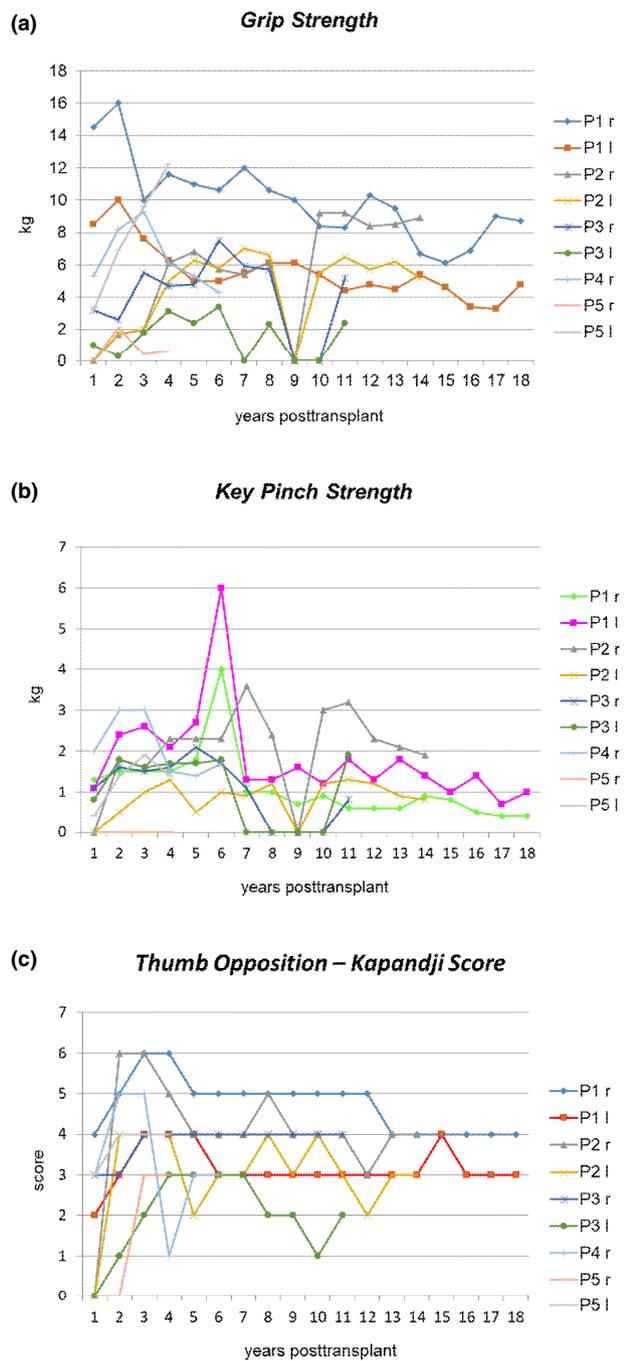


Figure 5 Functional outcomes and trends. Grip strength (a) and key pinch strength (b) evolution for the right (r) and the left (l) transplanted hand of patients 1–5 in kilograms (kg). Evolution of thumb opposition measured by the Kapandji Score for the right (r) and left (l) transplanted hand of patients 1–5 (c). Trends for functional parameters over time (d–g). Values were related to the 5-years outcome for each patient.

of rejection in VCA have increased over the past years [5,7,12,39], an update of the existing Banff classification is urgently warranted. Our findings further fuel the need

to perform deep skin tissue biopsies since the manifestations of rejection are most prominent in the deeper dermis during ABMR.

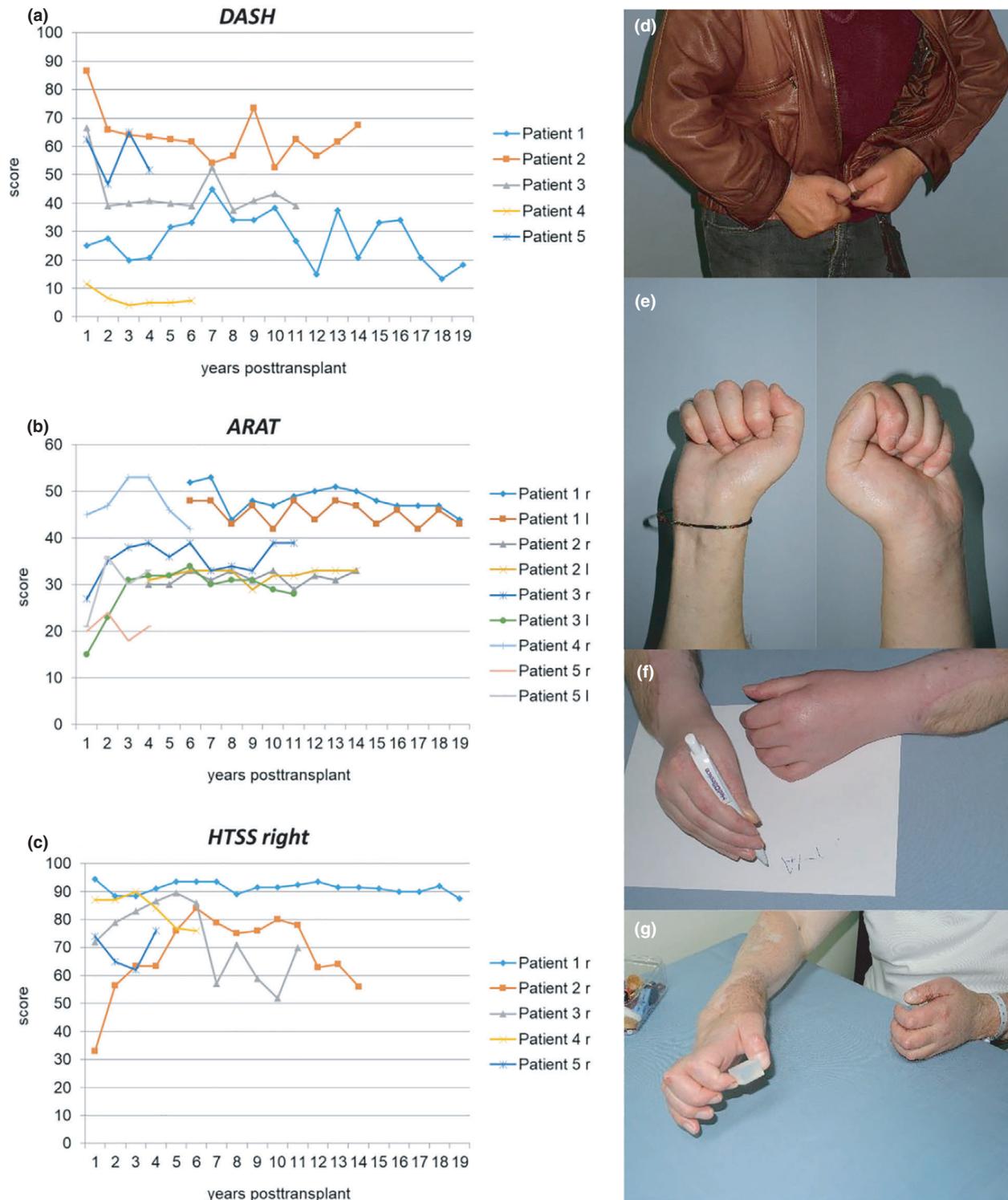


Figure 6 Functional outcomes. Disabilities of the arm, shoulder and hand (DASH) score evolution (a). Action research arm test (ARAT) evolution recorded for the right (r) and left (l) arm of patients 1–5 (b). Hand transplantation score system (HTSS) score evolution (c). Activities of daily living (ADL) presented by patient 1 (d), patient 2 (e), patient 3 (f), and patient 5 (g).

Overall, the phenotype of the infiltrate during rejection significantly changed over the observation time-period of 19 years. This phenomenon may be explained in

part by differences in rejection severity. While six grade III rejections were observed early (until year 3), only one grade III and one grade IV rejection were recorded

Table 5. Correlation of functional and psychological parameter and scores with rejection and patient satisfaction.

	Rejection			Patient satisfaction		
	COR	<i>P</i>	Adj. <i>P</i> (FDR)	COR	<i>P</i>	Adj. <i>P</i> (FDR)
Grip strength	0.107	0.288	0.550	0.183	0.114	0.156
Key pinch strength	0.094	0.350	0.550	0.126	0.279	0.307
Thumb opposition*	0.390	2.1 × 10⁻⁵	2.3 × 10⁻⁴	0.124	0.244	0.298
DASH	-0.007	0.946	0.952	-0.521	1.4 × 10⁻⁶	7.7 × 10⁻⁶
ARAT	0.158	0.152	0.550	0.587	2.5 × 10⁻⁸	2.8 × 10⁻⁷
HTSS	0.171	0.221	0.550	0.510	6.6 × 10⁻⁴	2.3 × 10⁻³
TAROM thumb	0.011	0.910	0.952	0.119	0.308	0.308
TAROM index	0.006	0.952	0.952	0.375	8.5 × 10⁻⁴	2.3 × 10⁻³
s-2PD thumb	-0.052	0.610	0.838	-0.350	2.3 × 10⁻³	5.0 × 10⁻³
s-2PD middle	-0.098	0.339	0.550	-0.292	0.011	0.018
s-2PD little	-0.101	0.345	0.550	-0.323	5.0 × 10⁻³	9.1 × 10⁻³
Patient satisfaction*	-0.142	0.219	0.219			
Well-being*	-0.232	0.039	0.058			
QoL*	-0.278	0.015	0.045			

Adj. *P* (FDR), adjusted *P*-value based on the false discovery rate (FDR) according to the Benjamini–Hochberg method; COR, correlation coefficient (Spearman's rank correlation rho or Kendall's tau *b*); *P*, *P*-value.

Significant *P*-values (<0.05) are shown in bold.

*For ordinal data Kendall's tau *b* was used.

between years 4–10. The highest number of ABMRs was seen between years 4–10, which correlates with a significant increase in CD20+ B-cells. Contrary to previous observations by our group [40], the amount of Foxp3+ cells during rejection was not increased at later time-points. The increase in endothelial C4d expression during rejections between years 4–10 correlates with the high number of ABMR during this time-period. However, a further significant increase of C4d-staining during rejections after year 10 does not correlate with a higher number of ABMR, suggesting that this marker might not be specifically indicative for ABMR in VCA.

Two patients have developed macroscopic features suggestive for a mild stage of chronic rejection over the past years, including lichenoid skin changes, dyschromia, nail changes, and finger thinning, accompanied by recurrent pain. Both patients had also experienced ABMR and/or developed anti-HLA antibodies, and showed a rather complicated immunologic follow-up. While histopathologic skin or vascular changes as per ultrasound/computed tomography hinting on chronic rejection were not evident, a mild stage of chronic rejection cannot be ruled out and both patients are under close surveillance.

Interestingly, regain of intrinsic musculature took longest in the very recent case with a transplantation level at the metacarpal level at the right side. This was surprising as this hand allograft revealed the lowest

reinnervation distance of all. The exceptional long ischemia time (Table 1) may in part be accountable for this. While in general hand function remained relatively stable with intermittent fluctuations after year 5, a slow but remarkable trend toward deterioration of hand function, especially of the intrinsic musculature, was observed late after transplantation. However, it should be taken into account that a mild decrease of hand function may also be attributable to the advanced age of patient 1. Acute rejection did not show an immediate negative impact on hand function; however, a long-term effect cannot be ruled out at this point. In the unilateral hand transplant recipient, a slight, but continuous decrease of all functional parameters was recorded three years before graft amputation, suggesting that repetitive rejections over years may negatively affect the functional outcome. Rather than a decrease of hand function, concurrent rejection negatively influenced patients' well-being and QOL, which may be explained by intensified IS, hospitalization and concern by then. Moreover, our data indicate that besides restoration of functionality regain of sensitivity probably is the most significant factor for patient satisfaction and happiness after hand transplantation.

Indication and patient selection remain challenging due to the inherited health risks of the procedure. Successful outcome appears to be highly dependent on the motivation and reasonable expectations of the patients.

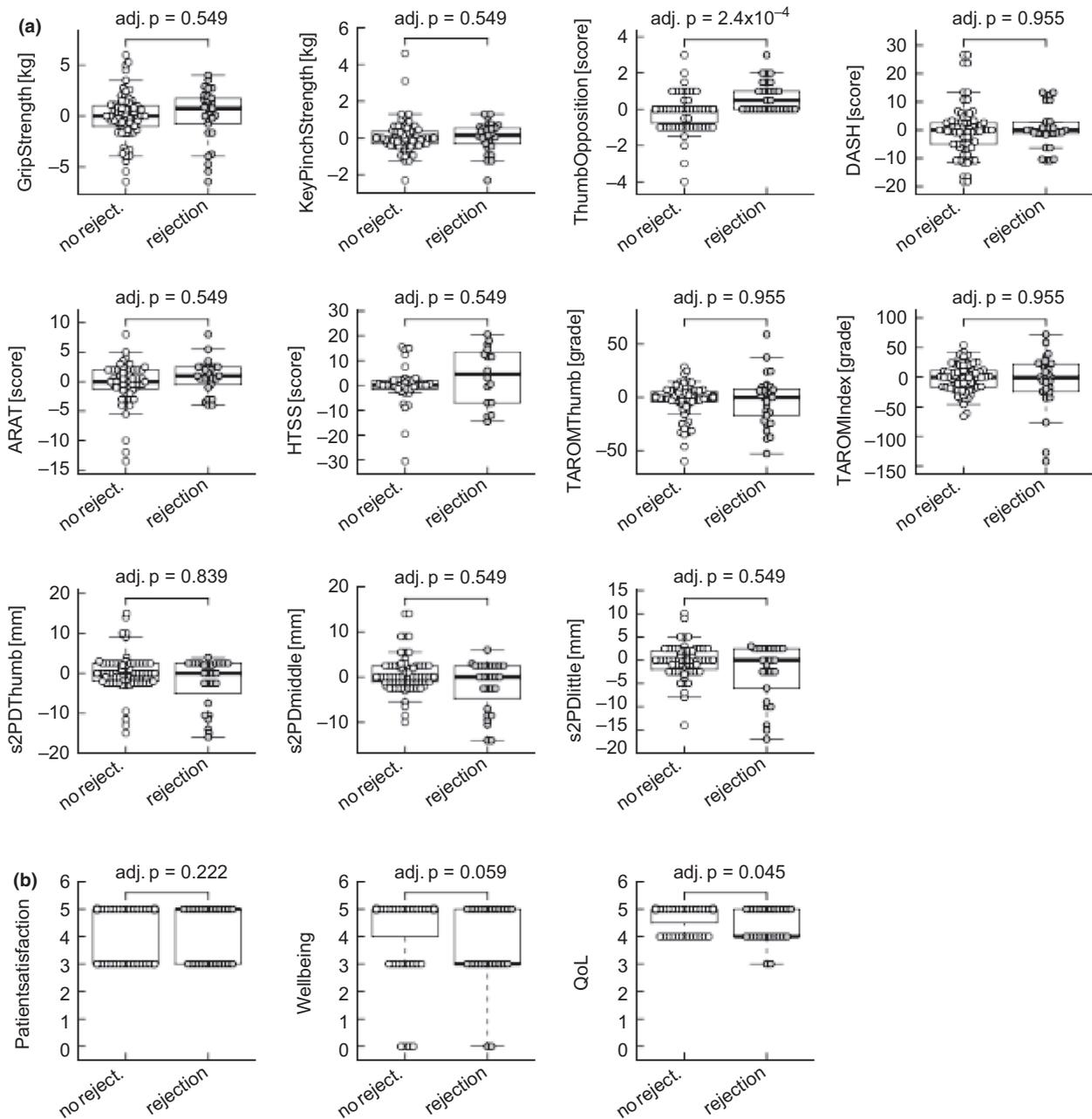


Figure 7 Functional outcome related to rejection. Functional parameters/scores (a) and psychological scores (b) in the absence and during rejection.

Hence, patient selection and informed consent remain critical and have become the center of attention. We felt that the iRT-PSP [23] helps with this process since it ensures a structured approach tailored to the specific criteria relevant in patients who suffer from limb loss and consider transplantation. Another important immediate goal in the field is the definition of endpoints for the assessment of the outcome. In solid organ transplantation, an established and robust primary endpoint is graft survival. In hand transplantation, however, graft

survival alone may not be considered as a veritable primary endpoint since “survival” of a graft does not necessarily imply good function. Ultimately, hand transplantation is performed with the intention to improve a patients’ QOL. This would imply QOL-measures as endpoints. The assessment of QOL, however, remains inconsistent and depending on the status prior to the intervention. The International Hand and Composite Tissue Allotransplantation Society (IRHCTA) score established in 2005 [2] was a commendable first

Table 6. Psychosocial outcomes of annual follow-up screenings (IRT-PSP protocol).

Psychometric iRT-PSP results	Patient 1	Patient 2	Patient 3	Patient 4*	Patient 5
Brief Symptom Inventory (BSI)* by Derogatis et al. [41]					
	T-values (cutoff score >65)				
Hostility	55 ^A	38^{BA}	38^{BA}	40 ^A	38 ^A
Anxiety	38 ^A	48 ^A	38^{BA}	48 ^A	38 ^A
Depression	41 ^A	41 ^A	41 ^A	43 ^A	41 ^A
Paranoid ideation	41 ^A	41 ^A	41 ^A	41 ^A	41 ^A
Phobic anxiety	45 ^A	45 ^A	45 ^A	45 ^A	45 ^A
Psychoticism	44 ^A	44 ^A	44 ^A	44 ^A	44 ^A
Somatization	57 ^A	61 ^A	40 ^A	56 ^A	40 ^A
Obsessive–compulsive	43 ^A	35^{BA}	35^{BA}	36^{BA}	35 ^A
Interpersonal sensitivity	48 ^A	40 ^A	40 ^A	41 ^A	40 ^A
PSDI positive symptom distress index	48 ^A	55 ^A	26^{BA}	26^{BA}	26^{BA}
PST positive symptom total	45 ^A	40 ^A	30^{BA}	40 ^A	20^{BA}
GSI global severity index	44 ^A	44 ^A	31^{BA}	38 ^A	26^{BA}
Patient Health Questionnaire (PHQ) by Spitzer et al. [42]					
	Depression & anxiety index				
PHQ-9 depression scale	None-minimal	None-minimal	None-minimal	None-minimal	None-minimal
GAD-7 anxiety scale	None-minimal	None-minimal	None-minimal	None-minimal	None-minimal
Scales of psychological well-being (PWB) by Ryff and Keyes [43]					
	Psychological well-being				
PWB total score	89	96	90	81	79
SF-36 health survey by Ware et al. [44]					
	T-values (cutoff score >65)				
Physical functioning	40 ^A	50 ^A	53 ^A	58 ^A	33^{BA}
Role-physical	58 ^A	56 ^A	56 ^A	58 ^A	52 ^A
Bodily pain	51 ^A	55 ^A	45 ^A	51 ^A	55 ^A
General health	52 ^A	49 ^A	45 ^A	43 ^A	53 ^A
Vitality	49 ^A	66^{AA}	51 ^A	59 ^A	48 ^A
Social functioning	42 ^A	57 ^A	57 ^A	45 ^A	42 ^A
Role-emotional	54 ^A	54 ^A	54 ^A	54 ^A	53 ^A
Mental health	46 ^A	65^{AA}	56 ^A	64 ^A	43 ^A

Selection of psychometric instruments of the iRT-PSP evaluation and follow-up protocol. *T*-values have been calculated to compare the iRT-PSP results of evaluated patients with norm samples.

Severity index (compared to norm samples): ^{BA}below average; ^Aaverage; ^{AA}above average.

*Psychosocial outcomes of patient 4 have been collected before chronic graft rejection and amputation.

step. While this tool considers the situation of hand amputation as a starting point and addresses not only functional but also psychological and patients' subjective parameters on satisfaction, the design may make it relatively easy to provide very high scores and come short in effectively identifying less satisfactory outcomes. Value and significance, may vary within individuals, making it more difficult to objectively evaluate the outcome in a standardized fashion and define a certain level of success. An interdisciplinary consensus is warranted as the field is advancing.

In conclusion, complicated immunologic courses after hand transplantation may favor development of DSA and ABMR and eventually result in chronic

rejection and graft loss. Aiming for a most stable immunologic situation with optimized and individually carefully adopted IS may be the key to prevent rejection, deterioration of graft function, and graft loss, and thereby guarantee good compliance and patient satisfaction.

Authorship

TH: participated in performance of the study, data analysis, writing of the paper. FM: participated in performance of the study, writing of the paper. AW: participated in performance of the study. HH: participated in data analysis. MN: participated in study

design, performance of the study, data analysis. VB: participated in performance of the study. JK: participated in performance of the study, data analysis, writing of the paper. BGZ: participated in data analysis. BZ: participated in data analysis. DW: participated in performance of the study. GP: participated in study design, performance of the study. WNL: participated in performance of the study, data analysis. RZ: participated in performance of the study. MG: participated in study design, performance of the study. RA: participated in performance of the study. GB: participated in study design, performance of the study. RM: participated in study design, performance of the study. DÖ: participated in study design, performance of the study. SS: participated in study design, performance of the study, writing of the paper.

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Conflicts of interest

The authors have declared no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Aesthetic outcome after bilateral hand (patients 1, 3 and 5) and forearm (patient 2) transplantation at the most recent follow-up.

Figure S2. Computed tomography-angiography with 3D-reconstruction of graft vessels (a–d, pronation; h, supination) and conventional angiography (e–g) of patients 1, 2, 3 and 5 at the most recent follow-up.

Figure S3. Results of total active range of motion (TAROM, a–e) and static 2-point discrimination (s-2PD, f–j) recorded at the annual follow-up are shown for patients 1–5 individually (blue arrow: T-cell-mediated rejection; red arrow: antibody-mediated rejection; green arrow: B-cell-mediated rejection).

Figure S4. Evolution of amplitudes of compound motor action potentials (CMAP, right column, a–e) and compound sensory action potentials (CSAP, left column, f–j) recorded from m. abductor pollicis brevis (APB, median nerve) and m. abductor digiti minimi (ADM, ulnar nerve) for the right (r) and left (l) hand allograft of patients 1–5. *x*-axis: years post-transplant.

REFERENCES

- Dubernard JM, Owen E, Herzberg G, *et al.* Human hand allograft: report on first 6 months. *Lancet* 1999; **353**: 1315.
- Lanzetta M, Petruzzo P, Margreiter R, *et al.* The international registry on hand and composite tissue transplantation. *Transplantation* 2005; **79**: 1210.
- Margreiter R, Brandacher G, Ninkovic M, Steurer W, Kreczy A, Schneeberger S. A double-hand transplant can be worth the effort!. *Transplantation* 2002; **74**: 85.
- Petruzzo P, Badet L, Gazarian A, *et al.* Bilateral hand transplantation: six years after the first case. *Am J Transplant* 2006; **6**: 1718.
- Grahammer J, Weissenbacher A, Zelger BG, *et al.* Benefits and limitations of belatacept in 4 hand-transplanted patients. *Am J Transplant* 2017; **17**: 3228.
- Kaufman CL, Ouseph R, Blair B, *et al.* Graft vasculopathy in clinical hand transplantation. *Am J Transplant* 2012; **12**: 1004.
- Morelon E, Petruzzo P, Kanitakis J, *et al.* Face transplantation: partial graft loss of the first case 10 years later. *Am J Transplant* 2017; **17**: 1935.
- Diefenbeck M, Nerlich A, Schneeberger S, Wagner F, Hofmann GO. Allograft vasculopathy after allogeneic vascularized knee transplantation. *Transpl Int* 2011; **24**: e1.
- Kanitakis J, Karayannopoulou G, Lanzetta M, Petruzzo P. Graft vasculopathy in the skin of a human hand allograft: implications for diagnosis of rejection of vascularized composite allografts. *Transpl Int* 2014; **27**: e118.
- Petruzzo P, Kanitakis J, Testelin S, *et al.* Clinicopathological findings of chronic rejection in a face grafted patient. *Transplantation* 2015; **99**: 2644.
- Chandraker A, Arscott R, Murphy GF, *et al.* The management of antibody-mediated rejection in the first presensitized recipient of a full-face allotransplant. *Am J Transplant* 2014; **14**: 1446.
- Weissenbacher A, Hautz T, Zelger B, *et al.* Antibody-mediated rejection in hand transplantation. *Transpl Int* 2014; **27**: e13.
- Brandacher G, Ninkovic M, Piza-Katzer H, *et al.* The Innsbruck hand transplant program: update at 8 years after the first transplant. *Transplant Proc* 2009; **41**: 491.
- Hautz T, Engelhardt TO, Weissenbacher A, *et al.* World experience after more than a decade of clinical hand transplantation: update on the Innsbruck program. *Hand Clin* 2011; **27**: 423, viii.
- Piza-Katzer H, Ninkovic M, Pechlaner S, Gabl M, Ninkovic M, Hussl H. Double hand transplantation: functional outcome after 18 months. *J Hand Surg Br* 2002; **27**: 385.
- Schneeberger S, Kreczy A, Brandacher G, Steurer W, Margreiter R. Steroid- and ATG-resistant rejection after double forearm transplantation responds to Campath-1H. *Am J Transplant* 2004; **4**: 1372.
- Schneeberger S, Ninkovic M, Gabl M, *et al.* First forearm transplantation: outcome at 3 years. *Am J Transplant* 2007; **7**: 1753.
- Schneeberger S, Ninkovic M, Piza-Katzer H, *et al.* Status 5 years after

- bilateral hand transplantation. *Am J Transplant* 2006; **6**: 834.
19. Weissenbacher A. Hand transplantation in its fourteenth year: the innsbruck experience. *Vasc Compos Allotransplant* 2014; **1**: 11.
 20. Hautz T, Zelger BG, Weissenbacher A, et al. Standardizing skin biopsy sampling to assess rejection in vascularized composite allotransplantation. *Clin Transplant* 2013; **27**: E81.
 21. Cendales LC, Kanitakis J, Schneeberger S, et al. The Banff 2007 working classification of skin-containing composite tissue allograft pathology. *Am J Transplant* 2008; **8**: 1396.
 22. Ninkovic M, Weissenbacher A, Gabl M, et al. Functional outcome after hand and forearm transplantation: what can be achieved? *Hand Clin* 2011; **27**(4): 455, viii–ix.
 23. Kumnig M, Jowsey SG, Rumpold G, et al. The psychological assessment of candidates for reconstructive hand transplantation. *Transpl Int* 2012; **25**: 573.
 24. Kumnig M, Jowsey SG, DiMartini AF. Psychological aspects of hand transplantation. *Curr Opin Organ Transplant* 2014; **19**: 188.
 25. Benjamini Y, Hochberg Y. Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J R Stat Soc Ser B-Stat Methodol* 1995; **57**: 289.
 26. Schneeberger S, Gorantla VS, van Riet RP, et al. Atypical acute rejection after hand transplantation. *Am J Transplant* 2008; **8**: 688.
 27. Schneeberger S, Petruzzo P, Morelon E, et al. The first two cases of bilateral hand transplantation: 20 years follow-up. *N Engl J Med* 2020. (in press).
 28. Morelon E, Petruzzo P, Kanitakis J. Chronic rejection in vascularized composite allotransplantation. *Curr Opin Organ Transplant* 2018; **23**: 582.
 29. Bonatti H, Aigner F, De Clercq E, et al. Local administration of cidofovir for human papilloma virus associated skin lesions in transplant recipients. *Transpl Int* 2007; **20**: 238.
 30. Bonatti H, Lass-Flörl C, Zelger B, et al. *Alternaria alternata* soft tissue infection in a forearm transplant recipient. *Surg Infect (Larchmt)* 2007; **8**: 539.
 31. Weissenbacher A, Hautz T, Zelger B, et al. Bullous pemphigoid eleven years after bilateral hand transplantation. *Am J Transplant* 2012; **12**: 1064.
 32. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969; **280**: 735.
 33. Bouquegneau A, Loheac C, Aubert O, et al. Complement-activating donor-specific anti-HLA antibodies and solid organ transplant survival: a systematic review and meta-analysis. *PLoS Med* 2018; **15**: e1002572.
 34. Berglund E, Andersen Ljungdahl M, Bogdanovic D, et al. Clinical significance of alloantibodies in hand transplantation – a multicenter study. *Transplantation* 2019; **103**: 2173.
 35. Kueckelhaus M, Imanzadeh A, Fischer S, et al. Noninvasive monitoring of immune rejection in face transplant recipients. *Plast Reconstr Surg* 2015; **136**: 1082.
 36. Kanitakis J, Jullien D, Petruzzo P, et al. Clinicopathologic features of graft rejection of the first human hand allograft. *Transplantation* 2003; **76**: 688.
 37. Kanitakis J, Petruzzo P, Badet L, et al. Chronic rejection in human vascularized composite allotransplantation (hand and face recipients): an update. *Transplantation* 2016; **100**: 2053.
 38. Pei G, Xiang D, Gu L, et al. A report of 15 hand allotransplantations in 12 patients and their outcomes in China. *Transplantation* 2012; **94**: 1052.
 39. Schneeberger S, Gorantla VS, Brandacher G, et al. Upper-extremity transplantation using a cell-based protocol to minimize immunosuppression. *Ann Surg* 2013; **257**: 345.
 40. Hautz T, Brandacher G, Zelger B, et al. Indoleamine 2,3-dioxygenase and foxp3 expression in skin rejection of human hand allografts. *Transplant Proc* 2009; **41**: 509.
 41. Derogatis LR. *Brief Symptom Inventory (BSI): Administration, Scoring, and Procedures Manual*. Minneapolis, MN: National Computer Services, 1993.
 42. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. *JAMA* 1999; **282**: 1737.
 43. Ryff CD, Keyes CL. The structure of psychological well-being revisited. *J Pers Soc Psychol* 1995; **69**: 719.
 44. Ware JE, Snow KK, Kosinski M, Gandek B. *SF-36 Health Survey Manual and Interpretation Guide*. Boston, MA: New England Medical Center, The Health Institute, 1993.