LETTER TO THE EDITORS

Evaluation of human leukocyte antigen sensitization in burn patients after treatment with skin allografts and transfusion of blood products

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Dear Editors,

Treatment for extensive burns may sensitize patients to antibodies specific for human leukocyte antigens (HLA), thus endangering their eligibility for organ and vascularized composite allotransplantation (VCA) recipients. Blood transfusions and allograft skin use are the most common reasons for HLA sensitization in burn patients [1]. The risk of forming alloantibodies against donor red blood cell (RBC) antigens is most likely linked to the number of transfusions [2], genetic predisposition, and immune response [3]. HLA alloimmunization in RBC transfusions is probably mediated via the remaining leukocytes, white cell fragments, DNA, HLA peptides, cell debris, and pro-inflammatory cytokines during storage [4]. Leukoreduction of blood products has probably reduced the HLA alloimmunization rate [4]. Allograft skin, and especially cryopreserved allograft skin, may be associated with a more frequent occurrence of anti-HLA antibodies [5].

This study aimed to prospectively assess both HLA sensitization of burn patients treated with skin allografts and sensitization to RBC antigens by prospective and retrospective analysis. Inclusion criteria consisted of: age ≥18 years, ≥20% total body surface area (TBSA) burn injury requiring admission to the Helsinki Burn Centre, and receiving glycerol-preserved allograft skin during 2015–2016. Medical records were reviewed for number of blood transfusions and other potential sensitizing events. Cross-sectional analysis involved the identification of allograft skin donor HLA antigens and donor-specific HLA antibodies (DSA) via a single blood sample during the study period. To assess the prevalence of sensitization to RBC antigens, files of 50 consecutive burn patients treated for burns >20% TBSA were retrospectively reviewed. Prior immunization status was unknown.

RBC antibodies were screened using a gel-based test with the ID Gelstation analyzer (Bio-Rad Laboratories, 1785 Cressier, Switzerland). Luminex® with One Lambda Labscreen® mixed beads followed by single antigen beads in positive cases were used for HLA antibody screening and identification, respectively. HLA FUSION 3.0 software (One Lambda Inc., A Thermo Fisher Brand, Canoga Park, CA, USA) was used. A normalized Mean Fluorescence Intensity (MFI) cut-off point of 1000 was used for positivity in single antigen analyses. Antibodies against HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ, and HLA-DP were analyzed. MFI levels of donor-specific HLA antibodies were recorded. Donors were HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ typed with the complement-mediated lymphocytotoxicity test (CDC) and low-resolution sequence-specific primers (PCR-SSP). DRB3-5 antigens of the donors were determined according to the known DRB1-DQ haplotypes. DSA analysis was performed except for DP antibodies due to lack of donor HLA-DP typing. Two patients had antibodies against HLA-DP antigens, and all antibodies had MFI levels less than 2000.

Ten patients (eight males) comprised the prospective study cohort with a mean age of 54.8 years and a mean %TBSA burn of 33.5 (Table 1). Blood samples were collected at a mean 3.36 months (range 1–7 months) following burn injury. An average of 26 units of packed RBC, 23 units of fresh frozen plasma, and six units of platelets were transfused per patient.

Analysis revealed that 7 of 10 patients developed anti-HLA antibodies to HLA class I and/or II antigens, and their mean panel reactive antibody (PRA) value was 78. HLA sensitization was linked to the allograft skin donor HLA antigen type in six of the seven

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Table 1. Demographic, burn injury-related data, and HLA sensitisation of the study population.

Patient number	Age	Gender	Mechanism	TBSA % burn	Length of stay (days)	Red blood cell components, units	Fresh frozen plasma, units	Platelet component, units	Previous risk factors for sensitization	HLA-sensitized Yes/No	Allograft Skin HLA-sensitized Yes/No	Number of donor antigens	Mean DSA MFI level
1	68	Male	Flame	28	43	22	30	4	No	Yes	Yes	5	9256
2	49	Male	Flame	24	53	27	45	28	No	Yes	Yes	4	4187
3	78	Female	Contact-sauna	24	68	3	3	0	Pregnancy	Yes	Yes	4	3671
4	62	Male	Flame	45	139	11	8	0	No	No	No	NA	NA
5	22	Male	Flame	50	54	31	24	8	No	No	No	NA	NA
6	51	Male	Flame	47	64	44	32	4	No	Yes	Yes	6	14 070
7	45	Male	Flame	50	46	38	32	7	No	Yes	No	NA	NA
8	39	Male	Flame	27	60	58	44	3	Pancreatectomy, subtotal colectomy for pancreatitis	No	No	NA	NA
9	57	Female	Flame	28	30	12	0	3	No	Yes	Yes	9	10 914
10	77	Male	Flame	22	54	16	16	2	Hip replacement	Yes	Yes	1	8422

TBSA, total body surface area; HLA, human leukocyte antigen; DSA, donor-specific antibody; MFI, mean fluorescence intensity; NA, not applicable.

sensitized patients. Most of these DSA were detected at rather high MFI levels with a mean of 9268 (Table 1) and were thus considered clinically relevant. There were no cases of allosensitization to RBC antigens in the prospective or retrospective group.

We established that HLA sensitization was linked to the allograft skin donor HLA antigen type in the majority of cases. However, no firm conclusions can be drawn as we analyzed only one time point. Concerning RBC alloimmunization, patients in neither the prospective nor retrospective cohort tested positive. Based on previous studies, we would have expected at least a small percentage of patients to be immunized [5] as antibodies were screened at a range 1–6 months after transfusion.

Preformed, donor-specific recipient HLA antibodies (DSA) have been recognized as a major risk factor for hyperacute and acute transplant rejection [6]. The presence of HLA antibodies can be determined by testing patient sera against cells from a panel of HLA-typed donors, and results can be used to estimate the panel reactive antibodies (PRA) or percentage of likely crossmatch-incompatible donors. A higher value implies a greater risk for a positive crossmatch. Thus, sensitized patients have to wait significantly longer (or may even be excluded) and once transplanted have a greater risk of graft loss from rejection [7]. Burn patients with severe facial injury might otherwise be possible candidates for facial VCA, but may have become sensitized during their early burn management. Chandraker *et al.* [8] recently described antibody-mediated rejection within 5 days after face transplantation in a sensitized burn patient.

In vitro and animal studies have suggested that glycerol preservation might result in a weaker immune response than cryopreservation [9,10]. Duhamel *et al.* [11] found that glycerol-preserved skin may be less likely to promote anti-HLA antibody production than cryopreserved skin. However, their study lacked information on the donor skin HLA type and therefore they were not able to determine whether HLA sensitization was due to skin allografting or blood transfusion. Another recent report by Win *et al.* correspondingly showed that burn patients receiving cryopreserved allograft skin grafts had high levels of anti-HLA antibodies. They also demonstrated that neither the amount of allograft skin nor the number of donors per recipient correlated with the level of sensitization [12]. However, their study also lacked information on the allograft donor patient HLA type. To date, there are no studies on humans comparing

immunological responses to glycerol-preserved and cryopreserved allografts.

Limitations of our study include the small number of patients and only a single antibody screening blood test obtained within a relatively short time span following the burn injury. However, an earlier study has shown that sensitization continues for a mean of 4.36 years following cryopreserved allograft skin use [12]. The strengths of our study include the prospective nature of patient recruitment and direct tracing of the HLA antibodies to the allograft skin donor patients' HLA antigen type. In conclusion, glycerol-preserved allograft skin may have a greater potential to sensitize burn patients than transfusion of blood products.

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