Immunological complications of blood transfusions

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Summary

Most adverse blood transfusion (BT) events are immune-mediated and in the majority of severe reactions antibodies can be identified as causal factors. Alloimmunization not only causes symptomatic reactions, transfused cells can also be (silently) destroyed. Immunization by BT can contribute to hemolytic disease of the newborn as well as to allograft rejection after transplantation. Reversely, pregnancy and transplantation may evoke immunity hampering transfusion therapy. Besides causing mortality and morbidity, alloimmunization has a huge economic impact. Transfusion reactions prolong hospital stay, require diagnostic tests and complex donor selection procedures and create the need for typed donor registries. In the 1970s, Opeltz and colleagues described that pre-transplantation BT impaired rejection of renal transplants. Leukocytes were essential for this immunosuppressive BT effect that raised concern about negative effects on cancer growth and resistance against infections. Studies on the mechanism were however preliminary abandoned when calcineurin inhibitors for prevention of graft rejection became available and since all blood products underwent leukoreduction in most countries as precautionary measure against transmission of variant Creutzfeldt-Jacob disease. Whether current leukoreduced BT are immunosuppressive and for which patients or circumstances this may contribute to worse outcome, is unknown. The last decades of the previous century, leukoreduction of cellular blood products for leukemia patients significantly reduced the incidence of immunological platelet transfusion refractoriness. The first decade of this century the avoidance of plasma- and platelet-products from females, that may contain donor-derived leukocyte antibodies, decreased transfusion related acute lung injury (TRALI) by more than 30%. These were major achievements. Challenge for the near future is to further reduce alloimmunization in particular against red blood cells (RBC) as a cause of severe hemolytic transfusion reactions and problems to find compatible donors. This can be achieved by extended matching. Inventory limitations prevent to match all BT for clinically relevant RBC antigens. A (European-wide) registration of clinical and genetic risk factors associated with alloimmunization could support effective use of matched blood products.
Introduction

By exclusion of donors with risk behavior and mandatory testing of syphilis, HIV 0/1/2, HTLV 1/2, HBV and HCV, these transfusion transmitted infections (TTIs) became rare events in well-resourced countries with an incidence below 1 in > 100,000 transfusions. Most European member states have, voluntary or mandatory, a “blood transfusion surveillance” system in place (hemovigilance) and report severe adverse transfusion complications. Cumulative, non-infectious complications are reported in 1–5 per 1000 products and grossly outnumber infectious complications.

During pregnancy, there is natural blood exchange between mother and child associated with mutual tolerance, although exceptions exist leading to (antibody-mediated) fetal damage. Allogeneic transplantation and blood transfusions (BT) expose the recipient to multiple foreign antigens that can potentially evoke an immune response. Stored BT contain apoptotic and necrotic cells, which in vitro and in animal models impair the antigen presenting capacity of recipient macrophages and dendritic cells (DCs) and suppress the response against the donor and third party allo-antigens [1,2]. In the majority of patients transfusion-induced immune effects are not evaluated because there was no symptomatic transfusion reaction and the recipient was never challenged by subsequent transfusions, pregnancy or transplantation, which may have revealed sequels of immunization.

Tests for alloimmune responses

The generation of antibodies and of cytotoxic T effector cells requires help from CD4+ T/T follicular helper (Tfh) cells. Central in this process is recognition between the T cell receptor (TCR) of naive CD4+ T cells and a foreign peptide presented by class II antigens of the major histocompatibility complex (MHC II). DCs, macrophages and B cells constitutionally express MHC class II antigens and can act as antigen presenting cells (APCs), where DCs stimulate Th1 and B cells more the Th2 cytokine production. This indirect peptide presentation requires phagocytosis and fragmentation of the foreign proteins and transport of the fragmented peptides to the cell surface together with MHC. Another – direct – way of engagement of the TCR is unique for allogeneic viable APCs. The direct pathway is, compared to the indirect pathway, very effective as much more TCRs recognize foreign MHC (with or without a foreign peptide). Recently a third way of TCR engagement was described by so called cross-dressing through exosomes from apoptotic allogeneic cells complexing with host MHC on DCs and stimulating CD4+ T cells [3]. After formation of the TCR-peptide-CD4 tri-molecular complex (signal 1), a secondary, co-stimulatory signal is needed. This is provided by binding of molecules on the APC to ligands on the T cell, generating Th1, Th2 or Th17 cytokines leading to skewing of the subsequent T cell proliferation. Relevant in this context is that after 2 weeks storage of leukocyte-containing whole blood or red blood cells in the cold or platelets after three days room temperature, co-stimulatory molecules and class II antigens on the leukocytes are impaired and cannot be up-regulated in vivo to act as APCs [4].

Alloantibody assays

Natural alloantibodies may have been evoked by heterologous immunity with consumed plants or acquired infections. After an allogeneic contact, weak, low titer, or high-titer low avidity antibodies can be produced which are often harmless. IgG-3 and -1 antibodies can, if the cognate antigens are sufficiently dense expressed on the cell membrane, activate complement leading to either intravascular target cell lysis or to cell removal by Kuppfer cells in the liver via the C3b receptor. Cell bound IgG1 and 3 antibodies also bind to Fcγ-receptors on splenic macrophages leading to phagocytosis of (part of) the antibody coated cell. The binding of specific antibodies to their target and eventual subsequent complement activation generates several cytokines and chemokines having biological effects. Because of the short half-life of these bioactive substances, except the third complement factor fragment d (C3d) these are not routinely used for in vitro test.

An antibody can be demonstrated using native cells, antibody captured membrane fragments, cell eluates or synthetic/recombinant antigens coated onto solid phase microplates or beads. There are also several read-out methods. Agglutination is still applied to detect RBC antibodies. For decades, HLA antibodies were determined by the complement dependent cytotoxicity (CDC) of typed lymphocytes. Later, antibodies reacting with cells, solid phase or beads were visualized using enzymes (Enzyme-Linked ImmunoSorbent Assay or ELISA) or fluorescent...
dyes and flow cytometry. For high throughput screening and determination of the specificity of HLA-antibodies color-coded microspheres coated with multiple or single synthetic MHC I or II antigens are available in combination with Luminex technology. However, the clinical correlates found with older assays cannot simply translated to new tests. The Luminex-platform based technology to detect HLA antibodies, now available for over a decade, illustrates that it takes a long time to translate results of a new assay to the clinic [5].

For detection of antibodies against platelet- and granulocyte-specific antigens (respectively HPA and HGA) either typed native target cells are used or specific membrane antigens are immobilized by monoclonal antibodies on a solid phase (MAIPA or MAIGA). In the future, recombinant instead of cell-derived antigens for routine antibody testing will increase. High throughput detection of HPA-1 antibodies using recombinant HPA-1a antigen coated bead-based test giving results within three hours is already feasible [6].

Antigen typing
Antigen typing was traditionally performed with antibodies. Gradually, molecular typing of MHC and HPA antigens replaced serology. Molecular RBC antigen typing is restricted for special indications such as fetal typing, transfused patients with multiple antibodies or screening large numbers of donors to find a rare antigen type. The speed of transition to routine molecular typing of RBC depends on several uncertain factors such as choice of technology (microarray or next generation sequencing), the required levels of resolution for weak RhD antigens, discrepancies between genotype and predicted antigen type, costs for small and large scale application and extinction of skilled technicians performing classical serology [7,8].

Cellular immunity
Cellular immunity test are often home-made and/or used for specific clinical or research purpose. The direct (allo) APC-T cell stimulation can be measured in the mixed lymphocyte reaction (MLR) by co-culture of irradiated (donor) stimulator cells with recipient responder cells. Proliferation of responder T cells is quantified by release of $^{51}$Chromium, ethidium bromide or LDH. Anti-class I specific CTLs can still be detectable despite the corresponding HLA class I antibodies have disappeared below the detection level [9].

**Immune-mediated clinical transfusion effects**
Most adverse transfusion reactions are associated with antibodies. Natural antibodies, immune antibodies after allogeneic contact or passively acquired antibodies from donor plasma or produced by passenger donor B cells can all be harmful. Natural, passive or immune RBC antibodies can cause hemolytic transfusion reactions (HTR). HLA antibodies can destroy transfused platelets from random donors with or without a concomitant febrile non-hemolytic transfusion reaction (FNHTR). Passively acquired HLA antibodies from donor plasma are an important cause of transfusion related acute lung injury (TRALI). Antibodies to HPA are more important for pregnancy causing fetal and neonatal thrombocytopenia (FNAIT), although passively acquired anti-HPA antibodies can cause thrombocytopenia in recipients of plasma, or produced by grafts from donors possessing (memory) B cells. Hemovigilance in North West European (NWE) countries report HTR and TRALI as most frequent severe transfusion reactions, which require preventive measures. More rare and/or less severe are allergic reactions and FNHTR. These may not be harmful but require diagnostic tests, palliative treatment and can prolong the hospital stay (tables IA-IC). Consequently, the costs associated with alloimmunization are considerable.

**Erythrocyte antibodies**
The prevalence of RBC antibodies depends on the cohort studied from < 1% in blood donors to > 60% in chronic transfusion recipients and with large variations in-between. These proportions are mainly based on cross-sectional studies and have limited reliability, because some antibodies persist for decades and others quickly fall below the detection level [10]. Another factor that can explain discordance in published study results is the use of different intervals between transfusion and antibody screening. As example, in two studies the interval chosen to exclude a secondary booster (and inclusion for a primary response) was 10 and 28 days respectively [11,12]. Even less is known of the maximum interval for antibodies after primary immunization to reach a detectable level.

**Risk factors for red cell immunization**
The antibody response in first transfusion "users" was reported to be dependent on number of units exposed [11] and lower in patients with lymphocytic malignancy, immune deficiency, corticosteroid or other immunosuppressive treatment use [13,14].

Using a stochastic model, Higgins and Sloan pose that circa 13% of individuals are (multiple) RBC antibody responders having a high risk for additional new antibody formation. These respondents have in contrast just a weak association with transfusion numbers and not with patient age, diagnosis or treatment [15]. They hypothesize a genetic factor(s) for transfusion-induced RBC immunization. Their calculations confirm our observations that 20-25% of the patients who had produced a RBC
### TABLE IA

Adverse transfusion events caused by natural, immune or passive antibodies. Acute (AHTR) and delayed (DHTR) hemolytic transfusion reactions

<table>
<thead>
<tr>
<th>Cause</th>
<th>Reason/circumstances</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AHTR (&lt; 24 h)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural anti-A and/or B Ab</td>
<td>Major ABO-incompatible RBC</td>
<td>Clerical errors</td>
</tr>
<tr>
<td>Passive anti-A and/or B Ab</td>
<td>Minor ABO-incompatible PC</td>
<td>Often in children</td>
</tr>
<tr>
<td>Irregular RBC</td>
<td>False result compatibility test</td>
<td>Laboratory error/WBIT</td>
</tr>
<tr>
<td>Strong HLA antibodies</td>
<td>Extensive alloimmunization</td>
<td>HLA incompatible RBC/fresher RBC</td>
</tr>
<tr>
<td><strong>DHTR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary antibody boost</td>
<td>Evanescent antibody below detection level</td>
<td>Registration of clinically relevant antibodies</td>
</tr>
<tr>
<td>Passive B cells: anti-A and/or B, D or other anti- RBC</td>
<td>Passenger B cells in organ/stem cell grafts boosted by recipient Ag</td>
<td>15-20% in minor incompatible liver/kidney transplant</td>
</tr>
<tr>
<td><strong>Hyperhemolysis</strong></td>
<td>Unknown rare DTHR: ~50% new Ab; complement activation/NO generation</td>
<td>DTHR after BT or by passive B cells after stem cell transplantation After BT: often SCD patients 0 to A PBSC transplants</td>
</tr>
</tbody>
</table>

BT: blood transfusion; RBC: red blood cells; PBSC: peripheral blood stem cell; WBIT: wrong blood in tube; NO: nitric oxide; SCD: sickle cell disease.

### TABLE IB

Adverse transfusion events caused by HLA and HGA antibodies

<table>
<thead>
<tr>
<th>Cause</th>
<th>Reason/circumstances</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRALI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-recipient leukocyte Ab</td>
<td>Passive antibodies in (female) donor plasma</td>
<td>Probably responsible 30-35% TRALI</td>
</tr>
<tr>
<td>Ab cause pulmonary edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA antibodies recipient</td>
<td>Donor product contains much granulocytes</td>
<td>Described with PBSC and granulocytes</td>
</tr>
<tr>
<td><strong>Platelet transfusion refractoriness</strong></td>
<td>Prior pregnancies, RBC ± platelet transfusions</td>
<td>LR RBC/platelets still evoke HLA Ab</td>
</tr>
<tr>
<td>Broad (&gt; 60% PRA) HLA immunization (=HPA Ab)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FNHTR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA antibodies against leukocytes in blood product</td>
<td>Non-leukoreduced RBC and/or platelet-products</td>
<td>LR decreases ~50% FNHTR</td>
</tr>
<tr>
<td>Pro-inflammatory cytokines</td>
<td>Storage of WBC containing platelets (RBC)</td>
<td>Platelets stored at RT produce higher cytokine levels</td>
</tr>
</tbody>
</table>

Ab: antibodies; PBSC: peripheral blood stem cells; PRA: Panel Reactivity; HPA: human platelet antigens; RBC red blood cells; LR: leukocyte-reduced; FNHTR: febrile non-hemolytic transfusion reaction; RT: room temperature.

### TABLE IC

Adverse effects of HPA antibodies

<table>
<thead>
<tr>
<th>Cause</th>
<th>Reason/circumstances</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet transfusion refractoriness</strong></td>
<td>Mainly anti-HPA 5b/1b</td>
<td>Clinically relevant for patients dependent on HLA matched platelets</td>
</tr>
<tr>
<td>95% in the context of HLA immunization</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PTP</strong></td>
<td>Unknown; innocent bystander destruction autologous platelets</td>
<td>Most frequent anti-HPA-1a</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>Passive Ab in donorplasma (anti-5b)</td>
<td>Multiple plasma transfusions</td>
</tr>
</tbody>
</table>

HPA: human platelet antigens; Ab: antibodies; PTP: post-transfusion purpura; LR: leukocyte-reduce.
antibody respond to subsequent transfusion(s) with a second or multiple additional antibody response [16,17]. We found a similar proportion of these high antibody responders in surgical, medical and (onco)hematological patients and in women treated with intrauterine transfusions for HDFN. In these multiple responders an increased frequency (40%) of HLA DRB1*15 was found compared to 25% in single antibody responders and controls. This risk (OR: 1.7; 95% CI: 1.3-2.3) is modest and other risks for RBC immunization likely (co-)exist. DRB1*15 itself may not be a high responder locus, but other nearby located gene(s) such as encoding for TNF polymorphism could possibly predispose for multiple antibody formation [18].

The wide range (6-50%) of antibody responders observed in Hb-pathy patients relates to the intensity of transfusions (chronic monthly versus on demand) and on the occurrence of variant Rh alleles in the patient and donor populations [19-23]. The relationship between the development of antibodies in patients with chronic transfusion therapy is not necessarily caused by a transfusion dose effect. Patients on chronic transfusion treatment are frequently screened for antibodies and the chance is small that transient antibodies are missed. Only a few prospective studies, conducted in mainly surgical patients, are available and showed that 6-10% of the patients produce RBC antibodies after a single (first) transfusion episode receiving two to four RBC units [24-28]. These prospective studies also show that antibody screening 2-4 months after transfusion will detect most of the primary antibodies, although regular monthly follow-up until six months would be optimal. Clinical and product factors can create a danger (versus no danger) environment enhancing alloimmunization. Studies in a mouse model found that an inflammatory environment provided by infection, aged erythrocytes or the presence of contaminating leukocytes enhances formation and strength of RBC antibodies [29,30]. In humans however, leukocyte contamination and storage duration of RBC has not convincingly shown to enhance RBC immunization [31-34].

Prevention by extended RBC matching: which antigens and which patients?

More than 300 RBC antigens are molecular defined and new antigens are still discovered. The immunogenicity of Rh and – depending on the ethnic population studied – K antigens, is world-wide dominant, followed by antibodies against antigens of the Duffy, Kidd and MNS systems (and Mi and MUR in Asians) [35]. Studies in patients with SCD showed that progressive extended matching ultimately including Fy, Jk and K antigens drastically decreased the antibody incidence from over 40% to circa 7% of patients, corresponding with 0.001 antibody/unit transfused [20]. In many well-resourced countries patients with Hb-pathy and women in the (pre)fertile age receive preventive Rh and K1 matched transfusions. Broadening of the (patient and antigen) indications for extended matching requires more RBC typed donors. For this purpose a European consortium (BLOODgen) originally developed a microarray [36]. Results of high throughput genotyping of most common RBC antigens (Rh, Kell, Duffy, Kidd, MNS, Dombrock, Diego, Colton, Cartwright and Lutheran) and 18 HPA antigens using Luminex technology were recently reported [37]. Although this helps in finding compatible donors for broadly immunized patients, for several (inventory shortage, logistic, urgency, availability, costs) reasons not all patients can receive extended matched RBC and selection for patients with an increased risk is warranted. The occurrence of anti-K1, Fy-a, Jk-a, Mi-a and S is increased in patients possessing certain HLA restriction molecules. The odds varies between 2.3 (anti-Mi-a) and 12.9 (anti-Fy-a) (table II) [18,38-42]. RBC genotyping of patients with the aim of preventive extended matching of BT should include these identified HLA restriction genes and the HLA DRB1*15 as surrogate marker for high responders. Additional risk factors for immunization are preferentially collected from a European immuno-/hemovigilance registry, including (mixed) ethnic backgrounds. For non-Caucasians carrying D variants, the required RhD typing resolution level has to be determined [7,23].

<table>
<thead>
<tr>
<th>Antibody, reference</th>
<th>HLA DRB1</th>
<th>Patient vs. controls</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>K, [39]</td>
<td>11 and/or 13</td>
<td>83%/52%</td>
<td>4.5</td>
</tr>
<tr>
<td>Jk-a, [38]</td>
<td>01</td>
<td>55%/17%</td>
<td>7.7</td>
</tr>
<tr>
<td>Jk-a, [18]</td>
<td>01</td>
<td>46%/21%</td>
<td>3.1</td>
</tr>
<tr>
<td>Fy-a, [40]</td>
<td>04</td>
<td>59%/10%</td>
<td>12.9</td>
</tr>
<tr>
<td>Fy-a, [18]</td>
<td>04</td>
<td>75%/27.5%</td>
<td>7.9</td>
</tr>
<tr>
<td>Mi-a, [42]</td>
<td>09</td>
<td>30%/16%</td>
<td>2.3</td>
</tr>
<tr>
<td>S, [18]</td>
<td>07 and/or 08</td>
<td>73%/25%</td>
<td>8.3</td>
</tr>
</tbody>
</table>

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CURRENT QUESTIONS IN TRANSFUSION MEDICINE
Hyperhemolysis

Hyperhemolysis mostly presents as a delayed hemolytic transfusion reaction (DHTR) and is characterized by destruction of transfused and autologous erythrocytes, also referred to as bystander hemolysis. The complication occurs in approximately 1% of patients with hemoglobinopathy, but incidentally in patients with other transfusion indications and by passenger B cells in minor ABO-incompatible peripheral blood stem cell (PBSC) transplantation. In approximately half of the patients a new RBC alloantibody is detected, but as often no alloantibody, an auto-antibody or only reactivity with anti-complement immunoglobulin is found. HLA antibodies activating complement have also been suspected [43,44]. The mechanism of hyperhemolysis is speculative and presumed to be the result of acute onset severe hemolysis of transfused cells. This generates oxygen radicals, immune complexes and complement activation, priming autologous cells for phagocytosis, while there exists a clinical condition associated with a hyperactive phagocytic system [45]. The mortality is not well documented but assumed considerably. Besides withholding transfusions, immunosuppression with corticosteroids, HD-IVIG and rituximab are often used to dampen the process.

Leukocyte and platelet antibodies

Leukocyte antibodies comprise human leukocyte antigens (HLA)−, human granulocyte-specific (HGA−) and miscellaneous other antibodies reacting with white blood cells. HLA class I antigens, similar as ABO antigens, represent a histo-system and are with variable density present on all cells and in the soluble phase. Despite lacking a nucleus, platelets and RBC express HLA class I antigens. Originally named Bg (Bennett-Goodspeed)-antigens, HLA 7 (CREG), B17, A28 and A2 are clearly detectable on reticulocytes and young RBC. Despite the HLA class I expression on RBC is very low, strong HLA antibodies reduce red cell survival and can cause hemolytic transfusion reactions [46]. In contrast, the expression of HLA class I antigens on platelets is high [47] and platelets are very sensitive targets destroyed by HLA antibodies. Besides ABO and HLA class I antigens, platelets express human platelet specific antigens (HPA), some shared with endothelial cells. More than 30 HPA antigens, of which HPA 1-5 and HPA 15 are well-characterized bi-allelic systems, have different distributions dependent on the ethnic population. The formation of anti-HPA-1a antibodies, the most frequent cause of fetal/neonatal alloimmune thrombocytopenia (FNAIT) of pregnancy, is strongly associated with the presence of HLA DRB1*0103 as restriction antigen in the recipient. Anti-HPA-1a, followed by anti-5b are frequent causes of FNAIT in European, Arab and Indian descendents. In Asians, also other specificities (e.g. anti-HPA-4a, anti-CD36, anti-21bw) were identified [48].

HLA antibodies are the main cause of immunological platelet transfusion refractoriness. Antibodies against HPA and HGA play a secondary role in transfusion therapy, although HPA antibodies can complicate an optimal support with HLA matched platelet transfusions [49]. Donor-specific HLA antibodies are a strong contra-indication for hematopoietic stem cell and renal transplantation [50]. Most correlations between HLA antibodies and clinical effects were obtained in the past with less sensitive techniques than the currently used bead-based Luminex test. Although the latter is of indispensable value for quick identification of HLA antibody specificities and to unravel mixtures of HLA antibodies, the test is extremely sensitive. Depending on the cut-off level, even 9–> 50% of non-transfused male donors tested positive [50].

Risk factors for HLA immunization

BT and pregnancy are major causes of HLA immunization. Leukocyte-containing RBC and platelet transfusions caused immunization in over 60% of leukemia and Hb-pathy patients. Patients with HLA antibodies reacting (in the CDC test) with more than 60% of selected panel cells showed lack of increment of pooled random donor platelet transfusions [49]. The removal of leukocytes from platelet-products resulted in a dramatic reduction of HLA antibody formation and platelet transfusion refractoriness decreased to approximately 5% of the recipients. A meta-analysis conducted by Vamvakas, including the randomized studies performed between 1983 and 1997, showed a 70% reduction in HLA alloimmunization and transfusion refractoriness that could be attributed to leukocyte reduction [51]. Leukocyte removal of RBC products reduced the incidence of FNHTR by circa 50% [52], but resulted in less reduction of HLA immunization [27,53-55]. The benefit of leukoreduction of platelets is thus much larger than for RBC products. This may be explained by a difference in storage time between platelet and RBC products. (Prestorage) leukocyte removal depletes APCs expressing HLA class II and co-stimulatory molecules and thereby eliminates the direct pathway of CD4+ T cell activation. In the past (prior to the use of routine bacterial screening) platelet-products were not stored longer than three days and contained viable and effective donor APCs capable of direct recipient T cell activation. A substantial proportion of erythrocyte products were stored longer than 13 days and contained leukocytes that could not anymore provide co-stimulatory signals, making the effect of their removal from RBC products less impressive. After leukoreduction, residual HLA antibody formation must be initiated via the indirect pathway of T cell activation that requires processing of donor HLA antigens by recipient APCs. Despite their low HLA expression, the total content of HLA class I provided by RBC is as large as by leukocytes [47]. In addition, the long erythrocyte circulation time may prolong the period of antigen exposure. In contrast, platelets which carry a huge amount of HLA class I antigens, but have a short survival time, are apparently less capable to generate recipient APCs.
Immunological complications of blood transfusions

After multiple pregnancies up to 40% of women possess HLA antibodies. The number of amino acid differences (triplets or eplets) between mother and child MHC determine maternal HLA antibody formation [56]. HLA antibodies are considered to do no harm to the fetus, although exceptions exist. In combination with chooroamnioticitis HLA antibodies are suspected to play a role in preterm birth [57] and incidental cases of neonatal thrombocytopenia are associated with strong HLA antibodies in absence of other platelet-reactive antibodies. Often this involves anti-HLA-A2, but other specificities, e.g. anti-HLA-B56 caused fatal FNAIT [58]. HLA antibodies are more frequent than antibodies against RBC, HPA or HGA antigens, irrespective whether immunization was induced by pregnancy or transfusions. However, they more often occur simultaneously than expected by chance. RBC antibodies appeared a strong predictor of HLA immunization [59]. Patients with combined multiple RBC and HLA antibodies have an increased probability to carry the HLA DRB1*15 antigen [18].

Prevention of transfusion-induced HLA antibodies by pathogen reduction?

 Gamma-irradiation of leukocytes prevents cell division and transfusion associated graft versus host disease (TA-GVHD), but still induce HLA antibodies. Ultraviolet light (UV)-C and UVB irradiation combined with amotosalen or riboflavin, inactivate leukocytes in platelet-products and are considered a safe replacement of gamma-irradiation to prevent TA-GVHD [60,61]. UVB treated platelets (without photosensitizers) result in a similar reduction of HLA immunization as leukoreduction by filtration, likely by impairment of the direct donor APC-recipient T cell activation. Pathogen reduction techniques (PRT) may in addition also interfere with the indirect pathway because PRT generates more apoptosis associated with TGF-β and IL-10 generation, potentially suppressive for the recipient APC function [62]. Clinical effects must be awaited. In a murine model, extreme reduction of (class II bearing) leukocytes even enhanced antibody formation by platelets [63]. Considering the costs associated with PR it would be an important additional advantage when HLA immunization would be further decreased by inhibition of both direct and indirect CD4+ T cell activation. However, as discussed above, RBC transfusions remain a source of HLA immunization. Farther ahead lay other future options when large scale in vitro culture of red cells and hematopoietic progenitor cells. Except being pregnant the mother is healthy, in contrast to sick transfusion recipients receiving huge numbers of manipulated, stored and often fully mismatched cells. Despite these differences, for both types of immunization HLA, Rh and Kell represent the most frequent antibodies formed by both pregnant women and patients. Another similarity regards the 20–25% risk to form new additional RBC antibodies after a primary antibody response [16]. A major difference between pregnancy and BT induced antibodies is a difference in antibody evanescence. All HLA, RBC and HPA antibodies induced by pregnancy persist for decades, if not lifelong [67,68], whereas a large proportion of transfusion-induced HLA and RBC antibodies decrease below the detection level sometimes within six months [10,26,28,69]. An explanation could be that child-induced antibodies persist longer because of fetal microchimerism, which is demonstrated in a high proportion of women. In contrast, after blood transfusion donor chimerism is rare and only observed after transfusions for traumatic injury, which is associated with severe immunosuppression associated with impaired to absent T cell activation and proliferation in the MLR [70,71].

In 304 male military veterans, 48% of RBC antibodies induced by in-hospital transfusions had disappeared within six months and all antibodies were gone after 10 years. This was much faster than antibodies apparently elicited by transfusions given for combat-related trauma of which over 80% had persisted after more than 10 years [69]. We could perform a long-term follow-up study in 260 mothers of children with HDFN who had been treated with intrauterine transfusions and had produced 499 different antibodies. This enabled us to compare persistence of child and transfusion-induced antibodies after more or less simultaneous immunization. After a median interval of almost 9 years all (strong) antibodies causing HDFN had persisted, 70% of child-induced antibodies and 32% of non-child (transfusion-) induced antibodies had persisted and only 7% of antibodies elicited by irradiated intrauterine transfusions [68]. Obviously resulting in severe thrombocytopenia and bleeding, associated with mortality [65]. The destruction of autologus HPA-1a negative platelets shows similarities with hyperhemolysis, also the unraveled mechanism. Since the introduction of universal leukodepletion (removing also the majority of platelets) of cellular blood products, there are virtually no reported cases of PTP to the English hemovigilance system SHOT [66]. Besides withholding (random and HPA-1a matched) platelet transfusions, immunosuppression with HD-IVIG is recommended to dampen the process.

Pregnancy and BT induced immunity; antibody persistence and chimerism

From the 6–7 pregnancy week onwards, fetal cells enter the maternal circulation. This regards repeated maternal exposure to small amounts of viable semi-allogenic cells, including hematopoietic progenitor cells. Except being pregnant the mother is healthy, in contrast to sick transfusion recipients receiving huge numbers of manipulated, stored and often fully mismatched cells. Despite these differences, for both types of immunization HLA, Rh and Kell represent the most frequent antibodies formed by both pregnant women and patients. Another similarity regards the 20–25% risk to form new additional RBC antibodies after a primary antibody response [16]. A major difference between pregnancy and BT induced antibodies is a difference in antibody evanescence. All HLA, RBC and HPA antibodies induced by pregnancy persist for decades, if not lifelong [67,68], whereas a large proportion of transfusion-induced HLA and RBC antibodies decrease below the detection level sometimes within six months [10,26,28,69]. An explanation could be that child-induced antibodies persist longer because of fetal microchimerism, which is demonstrated in a high proportion of women. In contrast, after blood transfusion donor chimerism is rare and only observed after transfusions for traumatic injury, which is associated with severe immunosuppression associated with impaired to absent T cell activation and proliferation in the MLR [70,71].

In 304 male military veterans, 48% of RBC antibodies induced by in-hospital transfusions had disappeared within six months and all antibodies were gone after 10 years. This was much faster than antibodies apparently elicited by transfusions given for combat-related trauma of which over 80% had persisted after more than 10 years [69]. We could perform a long-term follow-up study in 260 mothers of children with HDFN who had been treated with intrauterine transfusions and had produced 499 different antibodies. This enabled us to compare persistence of child and transfusion-induced antibodies after more or less simultaneous immunization. After a median interval of almost 9 years all (strong) antibodies causing HDFN had persisted, 70% of child-induced antibodies and 32% of non-child (transfusion-) induced antibodies had persisted and only 7% of antibodies elicited by irradiated intrauterine transfusions [68]. Obviously
irradiated blood products do not establish chimerism. Whether fetal chimerism indeed sustains antibody production is difficult to prove and ideally requires demonstration of expression of cognate antigens by chimeric cells. Compared to males, females possess more antibodies because they are additionally exposed to fetal antigens. In general, females do not respond more frequent to BT than males. An exception is elderly post-menopausal females, a phenomenon probably related with fetal microchimerism acting as an additional immune system [72].

**Passive antibodies and passenger B cells**

**Hemolysis**

Compatible, non-identical plasma and platelet-products can contain ABO antibodies incompatible with the recipient. In large studies no signs of substantial hemolysis was observed after minor ABO-incompatible platelet transfusions. However case reports of severe, some even lethal, hemolytic transfusion reactions from minor ABO-incompatible platelets administered to children have been published [73]. One to three weeks after minor ABO-incompatible organ transplantation, up to 20% of liver and renal transplant recipients develop hemolysis due to anti-A and/or B (or D), produced by passenger (memory) B cells present in the graft [74]. After peripheral blood stem cell transplantation fatal (hyper) hemolysis is reported. This mostly happens after transplants from group O donors to blood group A and less often to B recipients, but anti-RhD and any other donor-derived irregular red cell antibody (anti-Jk-a, anti-Di-b) can be boosted in graft recipients, sometimes when it concerns kidneys in both recipients [75]. Besides hemolysis, incompatible ABO antibodies may react with A and/or B antigens expressed on other tissues. In combination with chemotherapy, ABO non-identical platelet transfusions were in multivariate analysis associated with veno-occlusive disease after stem cell transplantation in children [76].

**Transfusion related lung injury (TRALI)**

TRALI results from increased vascular permeability of pulmonary endothelium leading to non-cardiogenic pulmonary edema. The endothelial damage results from bioactive substances released through binding of leukocyte antibodies with pulmonary granulocytes. The marginal granulocyte pool in the lungs is very large. HLA and HGA antibodies present in plasma-containing products are the major cause of alloimmune TRALI. Exclusion of plasma products from female donors resulted in a more than 30% absolute reduction of TRALI [77]. Non-alloimmune TRALI attributed to transfusion still occurs in the context of primed granulocytes in ill or septic patients or after transfusion of multiple (stored) products of RBC, platelets and/or plasma [77,78]. In particular stored platelets contain soluble and platelet bound CD40L (CD154) and huge amounts of CD40L have been associated with TRALI [78]. The pathophysiology of non-alloimmune TRALI is complex and the clinical picture may be difficult to distinguish from TACO (Transfusion Associated Circulatory Overload) and ARDS (Acute Respiratory Distress Syndrome).

**Thrombocytopenia and bleeding**

Donor-derived anti-HPA antibodies can cause thrombocytopenia in a recipient. When this occurs in a patient who also receives (unfractionated) heparin or other drugs that can cause drug-induced thrombocytopenia, it can be very difficult to unravel the cause of thrombocytopenia. Differential diagnosis includes post-transfusion purpura (PTP), heparin-induced thrombocytopenia (HIT) and drug-induced thrombocytopenia [65,79]. Passive transfer of (memory) B cells by stem cell and organ grafts producing platelet antibodies can cause thrombocytopenia in the recipient(s), simulating non-engraftment after stem cell transplantation.

**Soluble ABO antigens**

A, B or AB individuals possess soluble A and/or B antigens forming immune complexes after encounter with anti-A and/or B antibodies. This situation occurs after compatible, non-identical platelets and/or plasma transfusions. Enhancement of thrombocytopenia as well as platelet activation leading to thrombosis were both attributed to incompatible platelet transfusions [80]. The ABO-Ig immune complexes can bind to the FcγRIIA [80,81]. This FcR is expressed on monocytes, neutrophils, DCs and also on platelets. Engagement of the receptor on platelets can lead to platelet activation, dysfunction, thrombocytopenia and sometimes thrombosis [81].

**Transfusion related immuno-modulation (TRIM)**

The beneficial effect of pre-transplantation BT associated with better renal graft outcome raised questions on the consequences of this immune suppression. Gantt suggested that this effect might allow cancer growth, in particular enhancing metastasis after surgery with curative intent [82]. Three randomized clinical trials (RCT) in colorectal cancer, one evaluating leukodepletion, the two others comparing autologous with allogeneic erythrocyte transfusions, found no difference in cancer recurrence [83]. The immunsuppressive effect was attributed to three possible factors: first the presence of leukocytes in blood products, second to WBC-derived soluble mediators and third soluble HLA molecules. Only one three-arm study compared WBC with WBC-derived soluble mediators and prestorage WBC reduction generating no WBC soluble mediators. Only the control population receiving WBC containing RBC showed more postoperative infections and higher mortality compared to the other two groups, indicating no deleterious role of during storage produced leukocyte mediators [27]. Over 20 RCTs compared standard with leukoreduced RBC in various types of surgery. A negative effect of leukocyte-containing blood on postoperative
complications was only present in cardiac surgery [83]. In a meta-analysis of the five RCTs performed in cardiac surgery, mortality at three months was 72% higher (summary: OR: 1.72; 95% CI: 1.05-2.81) using non-leukocyte depleted blood [83]. Analysis of the causes of excess death after leukocyte-containing transfusions identified a combination of multi-organ dysfunction syndrome (MODS) with infection; all other causes of mortality such as bleeding, cardiac, MODS or infection alone were equal and not affected by the type of transfused blood [84]. It will probably remain unknown whether the "TRIM" effect observed in cardiac surgery resulted from a non-antigen specific effect of leukocyte-containing transfusions via the innate immune system. A pro-inflammatory signature was observed in particular in patients who received more than four units leukocyte-containing RBC [84].

A TRIM effect in the era of universal leukoreduction has hardly been studied. The widely observed trend favoring a more restrictive transfusion policy instead of a more liberal policy may have a relationship with storage of products, storage-dependent bioactive substances, residual plasma in products or number of units transfused. In vitro studies revealed that during storage, leukocyte depleted RBC and platelets still release immunomodulatory mediators [85,86]. In vitro, the induction of regulatory T cells by stored RBC appeared dependent on the amount of plasma in the product and less on storage interval or leukoreduction [87]. In vitro studies on immunomodulatory transfusion effect are difficult to standardize and to extrapolate to clinical effects. Two large RCTs, one in cardiac surgery and one in critical ill intensive care patients, found similar effects on mortality in patients who received fresher red cells or standard stored or deliberately older red cells [88,89].

**Mild adverse transfusion events**

Febrile non-hemolytic febrile reactions (FNHTR) are frequent events in particular after platelet transfusions. FNHTR result from HLA antibodies reacting with leukocytes contaminating the blood products. Also cytokines released and produced by WBC during storage of platelet transfusions proved in an RCT as cause of FNHTR [90]. Prestorage leukoreduction of blood reduces FNHTR by leukocyte antibodies as well as reactions resulting from WBC-mediated bioactive factors that accumulated during storage [52]. Despite prestorage leukodepletion reduces FNHTR by 40-70%, a considerable incidence of 0.1-0.4%/product of these inconvenient reactions remain [52]. They form a frequent cause of longer hospital stay because of tests requiring exclusion of a hemolytic reaction or bacterial infection. Whether and how these FNHTRs are related to bioactive substance derived from platelets itself during storage needs further studies, important for agreement on an acceptable storage interval for platelet transfusions [91].

Allergic reactions in particular urticarial skin manifestations are the most frequent transfusion reactions occurring up to 3% per transfusion episode. These mainly occur in patients with allergic constitutions with a small contribution of (allergens) of donor or product factors.

**Conclusions**

The prevalence of alloimmunization relevant for transfusion therapy is unknown as is the economic impact to provide diagnostic test and treatment for immunized patients needing blood, transplants or management of pregnancy. Despite universal leukoreduction of blood, HLA immunization is not completely abolished and can hamper optimal platelet supportive care and transplantation treatment. It remains to be seen whether pathogen reduction methods of platelets can further decrease HLA immunization and how residual HLA immunization by red cell transfusions can be diminished. Besides the challenge to further reduce HLA immunization, hemolytic transfusion reactions, in particular hyperhemolysis, due to erythrocyte antibodies requires prevention by extended RBC matching. This can only be achieved when the typed donor file is enlarged and because the inventory will not be able to support all patients with extended matched transfusions, patients at increased risk for alloimmunization must be selected. Large scale donor typing needs high throughput techniques. Patient selection needs besides patient (geno)typing, identification of risk factors.

Molecular genotyping of HLA and HPA is replacing serology. For stem cell and organ transplantation and donor selection for platelet transfusions matching programs are available to select donors from large (international) registries. New matching strategies focus on matching for relevant antibody epitopes rather than matching for the HLA alleles. Red cell genotyping is possible for most blood group antigens but not yet routinely used and restricted to find rare blood donors, selected patients or to detect fetal (Rh) antigens in maternal blood of immunized pregnant women.

An (European) immunovigilance system could answer several above raised questions regarding costs, incidence and definition of genetic and environmental risk factors for alloimmunization. At last, storage and manipulation of blood products to enhance infectious safety, prolonging storage time and other logistic improvements, may interfere with cell activation and release of biological response modifiers. Clinical studies are needed to identify immunomodulatory TRIM effects.

**Disclosure of interest:** the author declares that she has no competing interest.
References


