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Systemic Lupus Erythematosus Leading to Terminal Renal Failure and Excluding Patients from Kidney Allocation Due to Inadequate CDC-based Cross-Matching: Is There a Diagnostic Way out?

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Abstract

Systemic lupus erythematosus (SLE) is known to proceed to clinically relevant nephritis in more than 50% of patients and, in about 20% of these patients, to terminal renal failure. Thus, renal replacement therapy including kidney allografting is required for a considerable number of SLE-patients. For allografting patients' donor-specific antibodies against HLA molecules of given donors (DSA) have to be excluded as preformed antibodies against these molecules represent the main cause for hyper-acute or acute rejections. In order to select recipients without these deleterious antibodies the complement-dependent cytotoxicity crossmatch (CDC-XM) assay was developed about forty years ago. Its negative pre-transplant outcome is currently regarded as the most important requirement for a successful kidney graft survival. During the last years several disadvantages of the CDC-based procedure have increasingly been discussed with respect to this assay's high susceptibility to disruptive factors mainly leading to false positive outcomes. As is clearly shown by our case series this holds also true for the underlying disease SLE. We here present the data of SLE-patients initially destined for cadaver kidney donations. They all exhibited positive CDC-XM outcomes for the most part without known historical immunizing events. Furthermore, solid phase-based antibody screening and specification analyses did generally not show anti-HLA antibodies or antibodies directed against HLA-phenotypes of the corresponding donors thus leading to negative results of the so-called virtual crossmatch. Since exclusive virtual cross-matching is not allowed for the acceptance of allografting all of the positive CDC-XM assays were performed as reruns using alternative solid phase- (ELISA-) based crossmatch assays. In best accordance with virtual cross-matching solid phase-based cross-matching did not exhibit DSA. Our data clearly demonstrate the benefit of alternative ELISA-based cross-matching to circumvent CDC-based artefacts and point on the urgent requirement to substitute the historical CDC-based procedure at least for this group of patients.

Keywords: Allo-grafting; Complement-dependent cytotoxicity assay; Crossmatch; Donor-specific antibodies; Human leukocyte antigens; Kidney; Lupus; Rejection; Renal replacement therapy; Terminal renal failure

Introduction

As a type III immune complex disease Systemic Lupus Erythematosus (SLE) leads to clinically relevant nephritis in about 50% of patients during the first years of this disease [1]. In about one fifth of these 50% the renal damage proceeds to terminal renal failure/end stage renal disease thus requiring long term dialysis or kidney allo-grafting [2-4]. Consequently a considerable number of SLE-patients appear on the list of patients waiting for a kidney allograft. According to the transplantation guidelines of nearly all countries and or supranational societies supervising the allocation of kidneys such as Eurotransplant Foundation (ET) the verification of donor-specific antibodies directed against HLA-molecules (DSA) of a given donor is regarded as a clear contraindication for grafting in order to avoid highly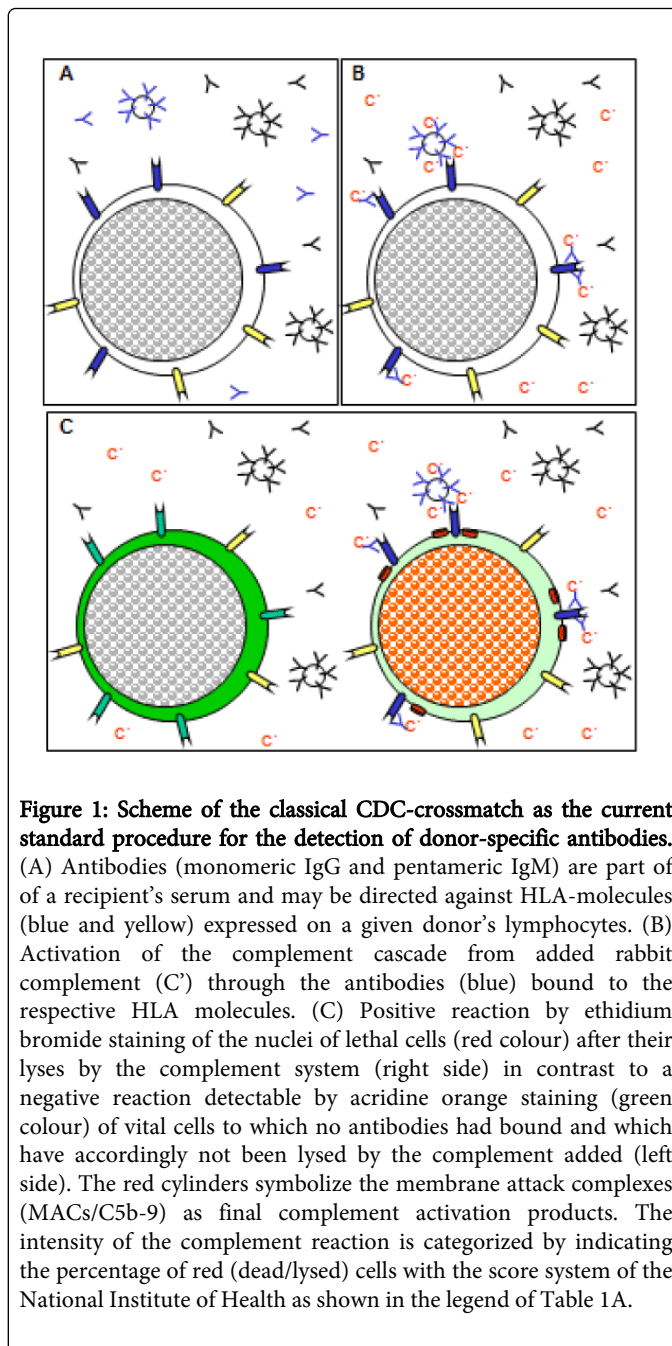 deleterious (hyper-) acute rejections by preformed DSA. Of course this holds true for cadaver as well as living kidney donations requiring the procedure of reliable pre-transplant cross-matching. In this regard especially patients with previous exposures to non-self HLA antigens have i) to be screened very carefully for anti-HLA antibodies and ii) carefully to undergo the crossmatch procedure against a prospective kidney donor with a clearly negative outcome. To avoid grafting in spite of demonstrable DSA the complement-dependent cytotoxicity crossmatch (CDC-CM) was established in the late sixties of the last century as standard technique. Donors' lymphocytes are incubated with the sera of potential recipients in the presence of rabbit complement using this functional "microdroplet assay" [5]. However, as a functional assay the CDC-CM indicates only those antibodies which potentially attack allogeneic HLA target structures via the activation of the complement system thus leading to the lyses of donor cells. An alternative approach, indicating both complement-activating and complement-independent DSA was introduced with the flow cytometric crossmatch (FACS-CM) in the early eighties of the last century [6-8]. A striking disadvantage highly limiting the value of both the CDC- and the FACS-based crossmatch assays is the fact that valid results are not obtained if only cells of poor quality/vitality are available. To circumvent these drawbacks solid phase-based (i.e. in the design of an ELISA or using the Luminex-

microsphere system) have been established in an increasing number of tissue typing laboratories for the last seven years [9-13]. As one of these assays, the Antibody Monitoring System (AMS) HLA class I & II ELISA (GTI, Waukesha, USA) had been implemented by us already in the year 2005 and was successfully used for special cases of patients not resulting in reliable and valid outcomes for various reasons [2,3,9] until its discontinuation by the manufacturer in 2013. However, this assay has afterwards been replaced by the alternative AbCross crossmatch ELISA (Biotest/Biorad, Dreieich, Germany) also leading to valid results. However, the negative results of ELISA-based cross-matching did not correspond with the positive CDC-CM-based outcomes of sixteen prospective recipients suffering from SLE as shown in this report. In the context of these differences we discuss the impossibility to allocate a kidney to these patients if the CDC-based assay is regarded as "gold standard" and implemented as current mandatory standard procedure despite its susceptibility to disruptive factors such as immune-complexes.

Patients and Methods

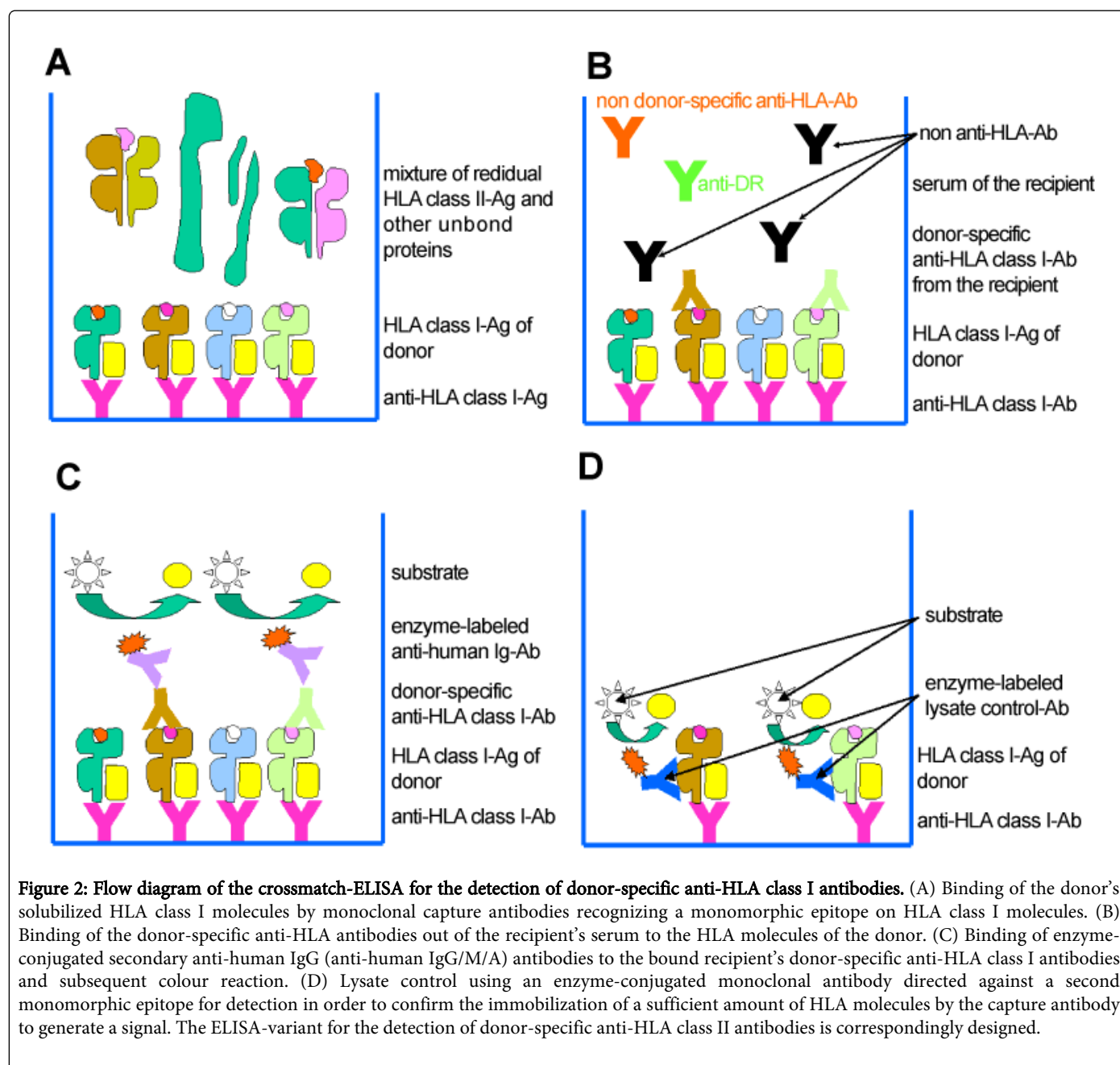### Detection of donor-specific anti-HLA antibodies (DSA) / Cross-matching

All of the SLE-patients (n=16) presented here were examined for the purpose of routine diagnostics in the tissue typing laboratory Halle/Germany (GHATT) between 2010 and 2013. DSA were initially analyzed by cross-matching using the standard CDC-CM procedure the workflow of which is shown in Figure 1. Although characterized by several disadvantages the CDC-CM has been accepted for years and also currently represents the standard procedure for the selection of recipients without DSA. In accordance with most laboratories the test is generally performed by us using not only the whole fraction of peripheral blood lymphocytes (PBL) but also using separated T- or B-cells. The cell isolation is performed using tetrameric antibody technique which crosslinks unwanted cells to red blood cells and subsequently eliminates them via density gradient centrifugation (System RosetteSep, Stem Cell Technologies, via CellSystems Biotechnology GmbH, St. Katharinen, Germany). After their initial isolation the cells are incubated with serum of a chosen recipient prior to adding complement proteins from rabbit. In this assay the complement system is activated via the classical activation pathway only by those antibodies which have been bound to the cells in the first incubation step (Figure 1B). Furthermore, the antibodies must belong to the so-called cytotoxic isotypes IgM, IgG3 and IgG1 which are capable of activating this humoral immune system. The result of this procedure is determined by two-colour fluorescence microscopy. Cells that have been recognized by DSA are dead due to their lyses by the complement proteins and are consequently stained red by the DNA-intercalating agent ethidium bromide (Figure 1C, right), whereas vital cells which have not been recognized by antibodies exhibit a green staining pattern due to the active uptake of acridine orange (Figure 1C, left). The intensity of the complement reaction is categorized by indicating the number of dead (red) cells with a score system of the National Institute of Health (Washington, USA) which is described in the legend of Table 1. It is noteworthy that the dead cell background of the CDC-CM should not exceed 10% to get reliable results of faint antibody-mediated reactions.

Figure 1: Scheme of the classical CDC-crossmatch as the current standard procedure for the detection of donor-specific antibodies. (A) Antibodies (monomeric IgG and pentameric IgM) are part of of a recipient's serum and may be directed against HLA-molecules (blue and yellow) expressed on a given donor's lymphocytes. (B) Activation of the complement cascade from added rabbit complement (C') through the antibodies (blue) bound to the respective HLA molecules. (C) Positive reaction by ethidium bromide staining of the nuclei of lethal cells (red colour) after their lyses by the complement system (right side) in contrast to a negative reaction detectable by acridine orange staining (green colour) of vital cells to which no antibodies had bound and which have accordingly not been lysed by the complement added (left side). The red cylinders symbolize the membrane attack complexes (MACs/C5b-9) as final complement activation products. The intensity of the complement reaction is categorized by indicating the percentage of red (dead/lysed) cells with the score system of the National Institute of Health as shown in the legend of Table 1A.

The alternative procedure of ELISA-based cross-matching was first implemented through the use of the Antibody Monitoring System (AMS)-class I/II ELISA (GTI, Waukesha, USA; FDA-No. BK060038 given in June 26th, 2006) already in the year 2005. This assay was used until its discontinuation in 2013 when it had to be replaced by the AbCross HLA classI/II ELISA (Biotest/BioRad, Dreieich, Germany). However, the laborious lead-through as presented in the manufacturer's manual was completely modified in our laboratory thus resulting in the assay's higher sensitivity and considerably faster results. Both ELISA-based crossmatch assays allow the direct detection of DSAs by immobilizing extracted HLA molecules of selected donors onto pre-coated capture antibodies to which in a consecutive step only donor-specific but not anti-HLA antibodies in general bind. As shown

in the workflow scheme (Figure 2) detergent lysate of a given donor's leukocytes/tissue comprising HLA class I and class II molecules has to be pipetted into the wells of ELISA-strips (GTI) or Terasaki-microtest plates (BioRad), respectively, pre-coated with monoclonal capture antibodies (Figure 2A). These are directed against a monomorphic epitope available on all HLA class I or class II molecules, respectively. After this first incubation and subsequent washing the recipient's sera are pipetted onto the immobilized HLA molecules and, in case of recognizing them, serve as detection antibodies in this sandwich assay (Figure 2B). Upon consecutive washing steps the samples are incubated with enzyme-conjugated secondary anti-human IgG (alternatively anti-human IgG/M/A) antibodies provided by the manufacturer which induce the final substrate reaction (Figure 2C). Of high relevance are the so-called lysate controls of both crossmatch ELISAs, which consist of a second enzyme-labeled monoclonal antibody thus providing evidence that a sufficient quantity of the donor's HLA molecules have been immobilized to achieve a signal (Figure 2D). The value of a given recipient's serum sample under investigation has to exceed two-fold the value of the negative control serum to be classified as positive. ELISA-based cross-matching was established in our tissue typing laboratory more than seven years ago and has been employed for nearly all samples that were characterized by special problems arising from invalid or doubtful results of the conventional CDC-CM.

Figure 2: Flow diagram of the crossmatch-ELISA for the detection of donor-specific anti-HLA class I antibodies. (A) Binding of the donor's solubilized HLA class I molecules by monoclonal capture antibodies recognizing a monomorphic epitope on HLA class I molecules. (B) Binding of the donor-specific anti-HLA antibodies out of the recipient's serum to the HLA molecules of the donor. (C) Binding of enzyme-conjugated secondary anti-human IgG (anti-human IgG/M/A) antibodies to the bound recipient's donor-specific anti-HLA class I antibodies and subsequent colour reaction. (D) Lysate control using an enzyme-conjugated monoclonal antibody directed against a second monomorphic epitope for detection in order to confirm the immobilization of a sufficient amount of HLA molecules by the capture antibody to generate a signal. The ELISA-variant for the detection of donor-specific anti-HLA class II antibodies is correspondingly designed.

Determination of the anti-HLA antibody status (antibody monitoring) for virtual cross-matching as plausibility check for CDC-or ELISA-based *de facto* cross-matching

For the general detection of anti-HLA class I antibodies the sera were screened using the QuikScreen ELISA (GTI, Waukesha, USA). For the screening of anti-HLA class II antibodies the B-Screen ELISA was used (GTI, Waukesha, USA). Serum samples positive in this first screening step were afterwards investigated using the miniaturized chip technology named DynaChip HLA antibody analysis (Invitrogen/ Dynal, Bromborough, UK) until the year 2011. This chip-based technique was the only completely automated system available for the detection and specification of anti-HLA antibodies. In its second generation design 106 positions on glass microchips were covered with HLA class I molecules and 48 positions with HLA class II molecules of different single donors, respectively. Although this assay consequently did not provide a resolution at the single antigen level the combination of the single donors' immobilized HLA class I or class II antigens, respectively, allowed the identification of the patients' antibody specificities in most cases (70-80%) especially if the immunization level/PRA-level(see below) was not too high. Unfortunately this system was discontinued for commercial reasons by the manufacturer in 2011 leading to the implementation of the Luminex technique in our laboratory which currently represents the dominating tool for anti-HLA antibody specification. Its technical aspects and drawbacks for antibody specification have in detail been reviewed elsewhere [13-15]. This technology is generally composed of a series of polystyrene microspheres, on which recombinant HLA molecules of only one phenotype (single antigen assay) or a group of a single donor's HLA class I or II molecules, respectively, (single donor/ID level) are immobilized. For their identification all beads carrying the same antigen(s) are characterized by a unique signal due to embedded fluorochromes of different intensities. Depending on its availability during the last years the one or other system was used for anti-HLA antibody specification. The approach of so-called virtual cross-matching i.e. of the identification of anti-HLA antibody specificities which are directed against donors' HLA phenotypes was generally used as plausibility check of *de facto* cross-matching results investigated as described above.

The CDC-based procedure is also suitable for antibody monitoring/ specification using so-called "cell tray analyses". In this context it is noteworthy that the general degree of anti-HLA pre-sensitization termed "panel reactive antibodies" (PRA) which is also definable with adequate DynaChip or Luminex-based assays has originally been defined as CDC-based reactivity against either a cell panel of peripheral blood lymphocytes (PBL) from various donors or a cell panel from various chronic lymphatic leukemia (CLL) patients. As a matter of course, this cell panel has to comprise all HLA phenotypes of a given patient's population and has to represent the phenotypes' frequencies of the patient's population. It is noteworthy that this statistical (percentaged) PRA-value is not to be equated with DSA. The statistical PRA-value determined for all patients on the kidney waiting list indicates the likelihood of an individual positive *de facto* crossmatch. A high value [%] additionally indicates an increased relative risk for allograft rejection mediated by anti-HLA antibodies. Thus, this statistical value easily allows the identification of those patients who have to be monitored and crossmatched very carefully as a consequence of a high anti-HLA pre-immunization status.

Results

Acceptance of a cadaveric kidney donation through the additional use of the AMS-ELISA leading to the non-consideration of implausible positive CDC-based crossmatches and antibody specifications

Our approach to circumvent a strikingly false-positive CDC-CM outcome may be shown using the example of a 43-year-old female person with renal end stage disease who was HLA-class I and -class II typed to be listed as cadaver kidney recipient. HLA class I: HLA-A1; B8 (Bw6); Cw7. HLA-class II: HLA-DR17; DR52; DQ2. After only 17 months on the waiting list she received a kidney offer of very rare and high HLA-compatibility. The offered organ was completely HLA-compatible at the level of low resolution typing not only for the antigens A-B-DR (resulting mismatch scheme 0-0-0) but also for the HLA class I Cw and the HLA class II DQ antigens. In spite of this extraordinarily high HLA-compatibility the CDC-based prior-to-grafting-crossmatch was positive at the highest level (score 8). According to the guidelines two sera were used for this procedure i) the current serum from October 2010 and ii) a second one taken one year ago in October 2009 (Table 1a). This second serum had historically been tested as positive in the CDC-based cell tray analysis although it was impossible to define distinct anti-HLA specificities. However, exhibiting a PRA of 68% the serum had been stored as positive retain sample (PRA>5%) to be available for future cross-matching. The current serum from October 2010 was as well positive in the cell tray assay (PRA=61%) but, in accord with the historical one, never in any solid phase-based assay such as screening ELISA, Luminex assay or DynaChip analysis (Table 1b). Hence, the definition of the degree of anti-HLA PRA resulted from positive outcomes only of the CDC-based cell tray analyses performed in accordance with the guidelines of Eurotransplant (ET) and the European Federation of Immunogenetics (EFI) due to which these analyses have to be performed at least for one of the four quarterly antibody screening runs.

Serum Sample	CDC-CM (#)			ELISA-CM	
	PBL	T-cell	B-cell	Class I	Class II
10/2009	6	6	8	neg.	neg.
10/2010	6/8	6	8	neg.	neg.

Table 1A: CDC- and AMS-ELISA-based pretransplant crossmatch results of a 43-year-old female recipient with her completely HLA-matched cadaveric kidney donor.

neg.: negative; pos.: positive; #: NIH score system of the standard CDC-based crossmatch as a percent of positive/dead (red colored) cells (%); 1: <10% (negative); 2: 10-20% (doubtfully positive); 4: 20-50% (weakly positive); 6: 50-80% (positive); 8: 80-100% (strongly positive)

Serum sample	Screening ELISA/*DynaChip		CDC cell tray (PBL) Class I / II
	Class I	Class II	
07/2009	neg./*neg.	neg./*neg.	n.d.
10/2009	neg./*neg.	neg./*neg.	pos. (68% PRA)
01/2010	neg.	neg.	n.d.
04/2010	neg.	neg.	n.d.
07/2010	neg.	neg.	n.d.
10/2010	neg./*neg.	neg./*neg.	pos. (61% PRA)

Table 1B: Corresponding analyses for anti-HLA antibody screening/specification.

neg.: negative; pos.: positive; PRA: Panel Reactive Antibodies (%); n.d.: not done; the CDC-based cell tray analysis was performed during the autumnal screening [i.e. once a year in accordance with the guidelines of the European Federation of Immunogenetics (EFI)] using the samples taken in October 2009 and October 2010; *: additionally performed DynaChip analyses in all cases exhibiting negative results in accordance with the screening ELISA.

It is noteworthy that the divergent results of solid phase-based assays on the one and cell tray-based antibody detection on the other hand had prompted us already after the first CDC-based cell tray screening in October 2009 to ask the patient's dialysis center for possible disruptive factors. The consequent information that the patient suffered from SLE led to our insight that any CDC-based prior-to-grafting crossmatch would in all probability be positive due to this underlying immune complex disease. In accordance with a prior agreement with the involved transplant center the obligatory pre-transplant CDC-CM was performed in parallel to the alternative AMS-crossmatch ELISA. This assay led to an unequivocally negative outcome (Table 1a) based on which the transplantation was performed without any immunological complications for the hitherto existing follow-up time of about 39 months.

After gaining experience with solid phase-based cross-matching for more than three years (2005-2008) this approach was on the whole followed no more than two times in the year 2009/2010 for the judicial reasons described and discussed below. However, ELISA-based cross-matching has increasingly turned from a methodological scientific to an absolute routine application for living kidney donations over these years. Living donations for a total of 15 recipients suffering from SLE have been performed in the meantime (i.e. since January 2011) based on plausibly negative solid phase crossmatches in spite of artificially positive CDC-based ones due to the underlying SLE disease [10,12,13]. This is legally allowed as the restrictive guidelines hold true only for cadaver kidney donations. However, only a minority of patients on the kidney waiting list have the possibility to receive an allograft from a living donation. Furthermore, this way of allo-grafting is often performed in spite of very poor HLA phenotype-matching in contrast to cadaver kidney donations where good HLA-matching leads to quite a high number of allocation points. This holds especially true for countries such as Germany where so-called cross over living donations in order to result in better HLA-matches and to circumvent adverse effects by DSA are not allowed by law. However, it was the aim of this article to demonstrate and discuss the situation of SLE patients waiting for a kidney allograft in the "regular way" of cadaver donations and as shown below the situation for this group of patients is not promising due to insufficient diagnostic approaches.

Accumulation of SLE patients on the kidney waiting list due to obligatory CDC-based cross-matching

In contrast to this highly successful exemplary approach presented above, patients are listed in Table 2 most of whom have not yet received a kidney allograft due to an amended version of the guidelines of the German Federal Medical Association from December 2010. This amendment no longer allows the alternative approach of ELISA-cross-matching but has clearly fixed the CDC-CM for cadaver kidney offers via the allocation system of Eurotransplant (Leiden, Netherlands). Thus, in Table 2 positive CDC-based crossmatch outcomes of 15 SLE-patients are contrasted with corresponding ELISA-based crossmatch outcomes and antibody specifications leading to no other conclusion that the CDC-based result is definitively false positive as a consequence of the underlying SLE disease.

Patient's ID/ pos. CDC-CM (n)	CDC-CM (Score)			ELISA-CM		Antibody Detection/ Specific. (PRA max.)
	PBL	T-cell	B-cell	Class I	Class II	
I.A. (8)	4	2/4	6/8	neg.	neg.	(PRA = 0%)
G.T. (6)	4	2/4	6	neg.	neg.	(PRA = 0%)
K.S. (4) #	4	2	6	neg.	neg.	(PRA = 22%)§
B.M. (9)	2	1	6	neg.	neg.	(PRA = 0%)

B.S. (4)	2	1/2	6	neg.	neg.	(PRA = 0%)
M.I. (3) #	2/4	1/2	6	neg.	neg.	(PRA = 0%)
R.G. (4)	2	1/2	6	neg.	neg.	(PRA = 34%)§
P.H. (2) $	2	1	2/4	neg.	neg.	(PRA = 55%)
K.L. (3) ∞	2	1/2	4	neg.	neg.	(PRA = 0%)
R.M. (6)	2/4	2	4/6	neg.	neg.	(PRA = 0%)
H.J. (2) #	1/2	1	6/8	neg.	neg.	(PRA = 0%)
H.K. (2) $	1/2	1	2/4	neg.	neg.	(PRA = 0%)
E.U. (4) +	4/6	4	8	neg.	neg.	(PRA = 0%)
M.I. (8)	4	2	6/8	neg.	neg.	(PRA = 0%)
K.S. (4) ∞	2	2	4	neg.	neg.	(PRA = 0%)

Table 2: SLE patients exhibiting various numbers of positive CDC-based crossmatch outcomes (n) during emergency duties with the score-values of the last one shown. Additional ELISA-based crossmatch results performed in parallel and corresponding solid phase-based anti-HLA antibody specification analyses (without CDC-based cell tray analyses) are presented.

#: patients were finally allografted by living kidney donations; §: no donor-specific antibodies were identifiable using the Luminex– or DynaChip specification assays in spite of general pre-immunization (PRA); $: faintly positive reaction of B-cells was ignored due to a full house kidney offer (HLA A-B-DR–mismatch: 0-0-0) and the known underlying disease. Retrospectively performed ELISA-based cross-matching was definitely negative; +: living kidney donation projected; ∞: patients were transplanted after the acute attack of the underlying SLE had passed and historical sera identified as false positive had been sorted out

Ten out of 15 patients have undergone more than four crossmatches always leading to positive outcomes to some extent characterized by high scores (≥ 6). Alternative approaches, however, using ELISA-based cross-matching in our laboratory never led to the detection of DSA in best accordance with antibody specifications not pointing onto anti-HLA antibodies in general or DSA by virtual cross-matching through the use of solid phase assays either. Since the current guidelines do not allow alternative solid phase–based cross-matching to circumvent artificially positive outcomes these patients as a matter of fact accumulate on the waiting list without exhibiting any *de facto* contra-indication for receiving a kidney allograft. Due to the knowledge of the poor chance to receive an organ by regular CDC-CM three of the patients (#-patients in Table 2) have been transplanted in the meantime by providing a living kidney donor. As mentioned above pre-transplant cross-matching using solid phase-based procedures is allowed for living donations as the restrictions hold true only for cadaver allocations (via Eurotransplant). However, the degree of HLA-matching derived from living donations is often very poor which may lead to other well-known disadvantages for the recipients. Furthermore, although the number of living kidney donations increases due to the striking lack of cadaver kidney offers, for most of the prospective recipients no living donor is available. As is also visible ($-patients in Table 2) a certain area of discretion may be used if the underlying disease as source of irritation is known and the signal is quite faint i.e. at the border to positive (score 2/4 for B-cells) in combination with the quarterly solid phase-based antibody specifications which have not at all detected anti-HLA antibodies for several runs.

However, the impossibility to ignore CDC-based signals becomes apparent if pre-immunized recipients additionally suffering from SLE are concerned. To ignore clear CDC-based signals in those cases is unacceptable as DSA may additionally exist in combination with a false positive SLE-mediated signal thus leading to highest uncertainty concerning the assay's interpretation. To draw a conclusion for those cases which is based only on the virtual crossmatch outcome is not justifiable as it is unfeasible to definitely exclude all additional antibody specificities not virtually demonstrable and possibly leading to life-threatening hyper-acute rejections. Thus, the only chance for this group of patients to receive a kidney is indeed the living donation. It is generally noteworthy in this context that nearly all (>90%) of the CDC-based antibody specifications (cell tray analyses) dictated to be performed for one of the quarterly antibody screening runs per year were as well artificially positive in accord with the data of cell tray-based antibody specifications (presented in Table 1, not shown in Table 2) again pointing on the unrivaled relevance of solid phase assays in this regard. There are two patients (∞-patients in Table 2) who were transplanted after the acute attack of the underlying SLE had passed away and historical sera identified as false positive had been sorted out to use only sera without "SLE-factors" for cross-matching. However, this situation to our knowledge represents rather the exceptional case. Apparently most of the sera from SLE patients falsify CDC-based crossmatch results for periods longer than a year. Thus, the strategy to wait for a fading of the disruptive factors is successful only in a limited number of cases. Whatever the strategy may be which is chosen to provide SLE patients with a kidney allograft, based on the data presented in Tables 1 and 2 the general conclusion must be drawn that any CDC-based approach is generally no adequate diagnostic tool for SLE patients. Thus, the approaches shown in Table 2 (#,$,∞) always represent only the second choice for the patients as an adequate diagnostic tool for cross-matching indeed exists for SLE patients. The difficulties and main obstacles to implement the diagnostically and immunologically meaningful ELISA-based cross-matching are discussed in the following section.

Discussion and Conclusions

There is generally no doubt that the screening of anti-HLA antibodies is very important both for patients of the kidney waiting list and for patients after allograft transplantations to detect highly deleterious preformed pre-transplantation antibodies or an upcoming humoral immune reaction after grafting, respectively, as both are accompanied by a high probability of graft loss. In order to fulfill this requirement the conventional CDC-based antibody specification and cross-matching procedures were developed as "prototype techniques" in the early 1960s and widely introduced in the transplant clinics in the late years of the same decade. These diagnostic approaches have over the last 40 years highly improved the quality of life for many transplant recipients as the number of hyper-acute and acute rejections has efficiently been reduced. In spite of additional major improvement in the field of immune suppression deleterious rejection episodes remain a serious problem for the transplantation of solid organs if pre-formed or upcoming DSA are not detectable by CDC-based detection/specification that is cell tray-based systems. Especially anti-HLA class II antibodies are for various reasons hardly identifiable using those systems [13] although their association with a markedly increased risk of kidney allograft rejection has recently been described [16]. Accordingly solid phase-based (i.e. ELISA or microsphere-based) techniques using immobilized recombinant single antigens or isolated groups of these antigens have alternatively and successfully been implemented by all HLA-laboratories and represent the actual basis of reliable anti-HLA antibody diagnostics [16-18] although some puzzling aspects specific for the Luminex single antigen testing of one manufacturer have still to be cleared [19-22]. Furthermore, the drawbacks of CDC-based antibody diagnostics demonstrable in many respects have in detail been described and discussed [12,13,23,24].

With our investigations we point onto the rather rarely described fact that patients suffering from SLE have a highly reduced chance to get an organ if the allocation is performed using the CDC-based standard crossmatch procedure. This auto-immune type III (immune complex) disease represents a disruptive factor which in many cases leads to positively manipulated results of CDC-based assays. Unfortunately but as expected cell tray-dependent antibody specification does not lead to valid diagnostic results either as the technical principle of this assay is as well the complement-dependent cytotoxicity assay. Thus, both CDC-based assays without any additional solid phase procedure for cross-matching and for anti-HLA antibody detection/specification generally deprive SLE patients of an allograft as these assays' positive outcomes simulate the existence of donor-specific antibodies. A hypothetical scheme of complement activation by disruptive immune complexes on the surfaces of B-cells is shown in Figure 3. Molecules of auto-reactive IgG as parts of auto-immune complexes are bound to cell surface expressed Fc gamma-receptors thereby activating the cascade of rabbit complement components added. This finally leads to a positive reaction in the CDC-based crossmatch procedure. However, other mechanisms of complement activation most probably exist as T-cells which do generally not bear Fc gamma-receptors are also lysed even though with a decreased intensity/score in comparison to B-cells. The scores of false positive CDC-crossmatches are shown in Table 2 for prospective recipients many of whom have not received a cadaveric kidney allograft due to false positive outcomes of the CDC-CM. These are ordered by PBL, T-cells and B-cells, respectively, and clearly demonstrate this phenomenon.
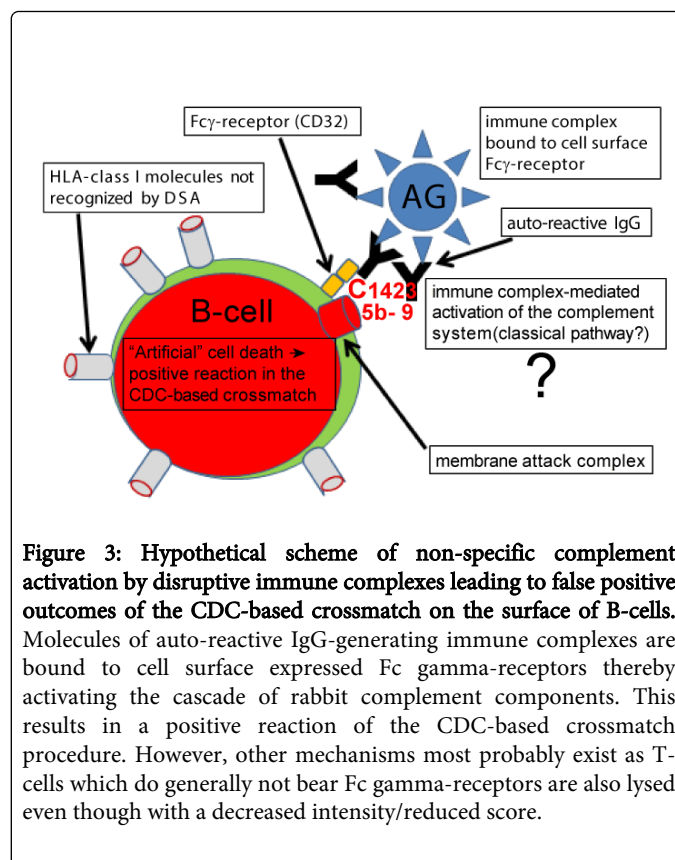

Figure 3: Hypothetical scheme of non-specific complement activation by disruptive immune complexes leading to false positive outcomes of the CDC-based crossmatch on the surface of B-cells. Molecules of auto-reactive IgG-generating immune complexes are bound to cell surface expressed Fc gamma-receptors thereby activating the cascade of rabbit complement components. This results in a positive reaction of the CDC-based crossmatch procedure. However, other mechanisms most probably exist as T-cells which do generally not bear Fc gamma-receptors are also lysed even though with a decreased intensity/reduced score.

The cases presented in this article strongly suggest the use of the AMS-ELISA as alternative solid phase-based crossmatch procedure to overcome the general problem of artificially positive CDC-CM results. Hence, the results presented here are not at all in accord with attempts to declare the CDC-based procedures as leading methods and as "gold standard" as proposed a few years ago [25]. Quite contrary to this proposal the cases presented here show the general insufficiency of CDC-based assays to lead to valid results under the accompanying immune complex disease SLE. Unfortunately the update of guidelines of the German Federal Medical Association as amended in December 2010 clearly has defined the CDC-based crossmatch assay as the only procedure allowed for cadaver kidney donations. Prior to this amendment (i.e. until December 2010) the guidelines only claimed to "exclude the existence of cytotoxic donor-specific anti-HLA antibodies" thus allowing the alternative use of ELISA-based cross-matching which clearly detects cytotoxic but additionally non-cytotoxic antibodies. However, after December 2010 any approach to perform alternative ELISA-based cross-matching as described for the 43-year-old female recipient with her completely HLA-identical cadaver kidney donor was immediately stopped by us for the last three years in order to fulfill our duties in accord with those updated guidelines. From December 2010 the determining factor was not whether these new guidelines were immunologically and diagnostically worthwhile to circumvent erroneous outcomes or not since to date they have been obligatory. Both the publication of the Eurotransplant authorities Doxiadis and co-workers [25] and the corresponding amendment of the guidelines of the German Federal Medical Association from 2010 must be regarded as anachronistic as the use of novel crossmatch assays to substitute or at least to complement the standard CDC-CM has increasingly been discussed

over the last 8-10 years due to this procedure's apparent drawbacks. More than 30 years ago, Ozturk and Terasaki already reported that autoantibodies and immune complexes such as rheumatoid factors may lead to false-positive results of CDC-crossmatches [26]. In this context, "cytotoxic antibodies" were detectable in patients with autoimmune diseases such as SLE even without previous alloimmunization. About 20 years later Sumitran-Holgersson described false-positive reactions of the CDC-CM as a consequence of autoantibodies and immune complexes as a frequent event [27]. In order to avoid CDC-CM based artefacts dithiothreitol/dithioerythritol (DTT/DTE) as reducing agents were early introduced to reduce the confounding influence of autoantibodies of the IgM-isotype. Apparently this procedure led to an improvement in the interpretability of CDC-CM results in many cases [28-30]. Accordingly these two agents are routinely used to destroy antibodies of the IgM-isotype with the aim of depleting autoantibodies. However, for more than 10 years it has been known well that autoantibodies which are detectable during autoimmune-mediated diseases such as SLE do not necessarily belong to the IgM-isotype. These autoantibodies may also represent lymphocytotoxic i.e. complement-activating IgG sub-isotypes (IgG1/IgG3) which are not destroyed through the use of DTT/DTE [27]. Furthermore, detrimental HLA-specific alloantibodies of the IgM-isotype have been detected in some studies which clearly point out the need to detect and not to destroy them [31,32]. Unfortunately, anti-HLA alloantibodies of the IgM-isotype are also dissociated/eliminated with DTT/DTE. However, they are detected with any type of crossmatch ELISA which is modified by using secondary anti-IgG/M antibodies [10,12].

In conclusion our data strengthen the urgent requirement for ELISA-based cross-matching as methodical substitution for SLE patients who are deprived of kidney allografts since the current mandatory standard CDC-based procedure is highly susceptible to auto-immune complexes leading to false positive crossmatch results. We here draw the final conclusion that exclusive CDC-based cross-matching is completely inadequate for a correct diagnosis of SLE patients with endstage renal disease in order to enable kidney allocations. Thus, we postulate that the procedure of ELISA-based cross-matching should again be legitimized by the certifying societies, the national transplantation laws and corresponding guidelines.

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