Original Articles

Early renal graft function deterioration in recipients with preformed anti-MICA antibodies: partial contribution of complement-dependent cytotoxicity

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ABSTRACT

Background. We previously reported that preformed anti-MHC class I–related chain A (MICA) antibodies increase the risk for renal graft rejection and enhance the deleterious effect of PRA+ status early after transplantation.

Methods. We studied 727 kidney recipients. Days to reach optimal serum creatinine level, estimated glomerular filtration rate (eGFR) at Month 3 and chronic kidney disease (CKD) stages were recorded. Anti-MICA specificities and C1q binding were tested by solid-phase assay. Complement-dependent cytotoxicity (CDC) and flow cytometry (FC) cross-matches with HeLa and PMA/CD28-T-blasts were performed.

Results. PRA+MICA+ recipients exhibited longer time to reach optimal serum creatinine level after transplantation (P = 0.005) and had the lowest eGFR at Month 3 (P = 0.006). PRA+MICA+ status independently increased the risk for CKDT stage 5 at Month 3 [hazard ratio (HR) 4.92, P = 0.030]. Pre-transplant anti-MICA antibodies were polyspecific and showed stronger reactions when coexisting with anti-HLA antibodies (mean standard fluorescent intensity 112 157 ± 44 426 in HLA+MICA+ sera versus 49 680 ± 33 116 in HLA−MICA+ sera, P = 0.0006). Anti-AYVE supereplet reactivity was significantly higher in HLA+MICA+ versus HLA−MICA+ patients (P < 0.001) and significantly superior than anti-CMGWS supereplet within HLA+MICA+ patients (P = 0.001). Three of 13 anti-MICA+ pre-transplant sera were positive for the C1q binding assay; one of them (serum 3) exclusively recognized AYVE supereplet with a strong reactivity against MICA*027 antigen (same as MICA*008). Anti-MICA antibodies in anti-HLA-absorbed serum 3 bound native MICA molecules in MICA*008+ HeLa and PMA/CD28-T-blasts and mediated cell death by activating complement.

Conclusion. Preformed anti-MICA antibodies may occasionally be cytotoxic by fixing and activating complement. This way they might contribute to worse early kidney graft function.

Keywords: anti-MICA antibodies, cytotoxicity, pretransplantation, renal transplant

INTRODUCTION

The role of MHC class I–related chain A (MICA) polymorphic proteins in alloresponse has been increasingly recognized. MICA products are constitutively present in endothelial cells, and its expression increases in other renal compartments after stress accompanying transplantation [1]. MICA-driven deleterious immune response against grafts may be mediated by the activation of cytotoxic cells through engagement of NKG2D receptor [2]. Additionally, anti-MICA antibodies show the capacity to trigger complement-dependent or FcγR-dependent the cytotoxicity and/or the capacity to activate endothelia and promote thrombosis [3].
Zwirner et al. [4] found anti-MICA antibodies for the first time in sera of transplanted patients after rejection. Later studies showed a relationship between pre- or post-transplant anti-MICA antibodies and kidney allograft loss [5, 6], dysfunction and acute rejection [7]. Serial long-term follow-ups [8, 9] confirmed the harmful effect of anti-MICA antibodies by reporting an association with chronic graft failure. In an analysis of 727 kidney recipients, we obtained similar results. By categorizing transplanted patients either according to pre-transplant panel reactivity antibody (PRA) and anti-MICA sensitization status or to anti-HLA and anti-MICA sensitization status, we reported decreased graft survival in PRA−MICA− patients and the highest risk for rejection at Month 3 in HLA−MICA+ patients [10]. Anti-MICA antibodies have been correlated with C4d-positive rejections [11, 12], and they have been shown to mediate complement-dependent cytotoxicity in kidney microvascular endothelial cells, HeLa or MICA-transfected cells [5, 13, 14]. However, lymphocytes do not express MICA, and cytotoxic anti-MICA antibodies cannot be detected by traditional complement-dependent cytotoxicity (CDC). This fact has made it difficult to systematically analyze the contribution of anti-MICA antibodies complement fixation capacity to graft deterioration.

Here, we further delineate the impact of preformed anti-MICA antibodies in kidney allograft function and analyse the anti-MICA specificities. We demonstrate that pre-transplant serum anti-MICA antibodies can bind native MICA molecules on cell membranes. By using both the C1q single-antigen beads assay and CDC, we show that these antibodies are able to mediate cell death by fixing and activating the complement cascade.

**MATERIALS AND METHODS**

**Patients and samples**

We retrospectively analysed 727 consecutive kidney allograft recipients transplanted between 2005 and 2011 in our hospital (cohort previously described [10]). The study was performed under informed consent and approved by the institutional review board (reference 10/014). Pre-transplantation sera were collected the day of transplantation. Allograft function was compared among patients categorized into four groups according to PRA status and flow cytometry (FC)−recorded anti-MICA antibodies: PRA−MICA+ (n = 7), PRA−MICA− (n = 610), PRA−MICA− (n = 65) and PRA−MICA+ (n = 45). Anti-MICA specificity and supereplet analysis was performed in MICA+ patients either with or without Luminex−recorded anti-HLA antibodies: HLA−MICA+ (n = 12) and HLA−MICA+ (n = 13). A serum creatinine level ≤1.20 mg/dL for males and ≤0.90 mg/dL for females was considered optimal kidney function. We measured creatinine serum levels at Month 3 and calculated eGFR using the four-variable Modification of Diet in Renal Disease (MDRD-4) equation. Chronic kidney disease (CKD) stages were based on eGFR [15].

**Anti-MICA-specific antibody and C1q binding assays**

Anti-MICA-positive samples in the screening test [10] were tested by Luminex® for individual anti-MICA specificities [Luminex single-antigen (LSA) assay, LABScreen MICA Single Antigen kit, One Lambda, Canoga Park, CA, USA]: 001, 002, 004, 007, 009, 012, 015, 017, 018, 019 or 027. C1q binding was tested in LSA-positive samples with the C1qScreen™ kit. Both LSA and C1q binding were analysed by the Luminex platform LABScan 100 Flow Cytometry and HLA Fusion™ software (One Lambda). A standard fluorescent intensity (SFI) ≥10 000 was considered positive. Some HLA−MICA+ sera were incubated with pools of peripheral blood mononuclear cells (PBMCs) to remove anti-HLA specificities.

**HLA and MICA genotyping**

Blood donor DNA samples were typed by LabType® SSO HLA-A, HLA-B, HLA-DR and MICA and analysed by Luminex (One Lambda). HeLa HLA-A, -B and -C and blood donor HLA-C were analysed by PCR-SSO using INNO-LiPA kits and LiRAS® software (Innogenetics). Figure 1 shows a flow chart of main analysis in our population.

**CD3+ T lymphocyte isolation, induction of MICA expression and FC**

CD3+ T cells sorted from healthy donors’ PBMCs (CD3 MicroBeads, MACS Miltenyi Biotec, San Diego, CA, USA) were resuspended in culture medium plus IL-2, PMA and anti-CD28 [16]. HeLa cells or PMA/CD28-T-blasts were incubated with MICA/B, isotype control and anti-HLA MoAbs (BD Biosciences, San Jose, CA, USA). For cross-matches, cells were first incubated with anti-MICA-positive sera followed by anti-human IgG-FITC (MACS Miltenyi Biotec). FC was analysed using a Navios™ Flow Cytometer (Beckman Coulter, Brea, CA, USA).

**Complement-dependent cytotoxicity**

PMA/CD28-T-blasts were incubated in Terasaki plates with recipient serum and goat anti-human IgG antisera (AHG, Sigma-Aldrich, St Louis, MO, USA), followed by rabbit complement. After staining with 5 μL of FluoroQuench™ (One Lambda), cell mortality was examined by fluorescence microscopy.

**Statistical analysis**

Frequencies were compared by Fisher’s or Pearson chi-square tests and means were analysed by Student’s t-test or one-way ANOVA. Cox regression was performed in the univariate analysis to obtain the HR for clinically relevant covariables. Variables with a P-value <0.10 were introduced in the multivariate model to assess their independent effect. P-values <0.05 was considered significant. SPSS (version 18.0; IBM, Armonk, NY, USA) and MedCalc (version 11.4.2.0; MedCalc Software, Ostend, Belgium) were used for statistical analysis.

**RESULTS**

Pre-transplant PRA+MICA+ status is associated with decreased eGFR, longer time to reach optimal serum creatinine levels and the highest risk for CKD stage 5 in the early post-transplantation period

In the analysed cohort, anti-MICA-positive and -negative patient subgroups showed no statistically significant differences.
regarding sex and age of donor and recipient, donor type, immunosuppressive treatments and original disease [10].

We previously observed that pre-transplant PRA+MICA+ sensitization significantly decreased allograft survival and was associated with more biopsy-proven rejection [10]. To further analyze how preformed anti-MICA antibodies enhanced the deleterious effect of PRA+ status on allograft function, the number of days to reach optimal serum creatinine levels and eGFR at post-transplant Month 3 were compared among PRA+MICA+, PRA+MICA−, PRA−MICA+ and PRA−MICA− patients. eGFR at Month 3 was significantly lower in recipients with both preformed anti-HLA and anti-MICA antibodies (PRA+MICA+ group) compared with the remaining groups (Figure 2A; PRA+MICA+ 38.23 ± 29.61 mL/min/1.73 m², PRA−MICA− 50.63 ± 24.55 mL/min/1.73 m², PRA+MICA− 59.02 ± 24.18 mL/min/1.73 m² and PRA−MICA+ 44.11 ± 19.26 mL/min/1.73 m²; P = 0.006). PRA+MICA+ recipients showed the longest time to reach an optimal creatinine level, followed by the PRA−MICA+ group, and global differences among groups were statistically significant (Figure 2B; PRA+MICA+ >90 days, PRA−MICA− 72.37 ± 29.44 days, PRA+MICA− 61.94 ± 35.68 days and PRA−MICA+ 79.47 ± 25.94 days; P = 0.005). Interestingly, the PRA+ groups were similar regarding the percentage of highly immunized (PRA >50%) patients and frequency of anti-HLA class I and/or class II antibodies as recorded by Luminex (no significant differences; Figure 2). However, we noticed that PRA−MICA− patients showed the highest eGFR (Figure 2A) and reached optimal creatinine levels earlier than the remaining groups (Figure 2B). This could be explained by differences in immunosuppressive treatment since more PRA+MICA− recipients (91%) received induction therapy (with monoclonal antibody or thymoglobulin) compared with the other groups (86% in PRA−MICA−, 75% in PRA+MICA− and 76% in PRA−MICA+; P = 0.051).

Finally, we analysed the relationship between PRA+MICA+ status and CKD stage 5 in the early post-transplantation period (CKD5T, very severely reduced kidney function, eGFR <15 mL/min/1.73 m² [15]), including other epidemiological and clinical variables (Table 1). Remarkably, coexistence of anti-HLA and anti-MICA antibodies emerged as an independent risk factor for CKD5T compared with PRA−MICA− (HR 4.92, P = 0.030) in the multivariate analysis. Donor age (71–85 years) was also independently associated with a higher risk for CKD5T (HR 3.34, P = 0.028).

Preformed anti-MICA antibodies are polyspecific and show stronger reactions when coexisting with anti-HLA antibodies

Among our pre-transplant anti-MICA-sensitized recipients (n = 52), 48% showed a positive anti-MICA single-antigen study (n = 25). Anti-HLA were present together with anti-MICA antibodies in 12 of these patients (HLA+MICA+) whereas 13 subjects had isolated anti-MICA antibodies (HLA−MICA−). Preformed anti-MICA antibodies were mostly polyspecific, with a mean of five different specificities per serum in both groups (Figure 3A). Antibodies against MICA*001 were the most frequent, followed by anti-MICA*018 (64 and 60% of patients, respectively). Those specificities were more prevalent in patients without HLA immunization versus double-immunized patients (69 versus 58% and 69 versus 50%, respectively). Among

![Flow diagram for the analysis of the renal transplanted (RTx) patient cohort (n = 727). First level shows the four main groups of patients classified according to PRA and MICA status. Levels 2–4 show the single-antigen (LSA MICA) and donor-specific antibody (DSA/non-DSA) analysis for MICA-positive recipients. The two final levels show C1q binding analysis (C1q MICA). In every square, positive sera for the corresponding test appear in brackets (see also Figure 3).](https://academic.oup.com/ndt/article-abstract/31/1/150/2460097/Early-renal-graft-function-deterioration-in/16921645)
HLA+MICA+ recipients, anti-MICA*017 antibodies were the most prevalent and more frequent than in the HLA−MICA+ group (75 versus 38%). Interestingly, preformed anti-MICA antibodies were stronger when coexisting with anti-HLA antibodies (Figure 3B; mean SFI 112 ± 44.26 in HLA+MICA+ versus 49 ± 33.16 in HLA−MICA+ sera, P = 0.0006).

Table 1. Univariate and multivariate analysis by Cox regression for CKD5T at Month 3 in patients categorized by PRA and anti-MICA antibodies

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CKD5T, chronic kidney disease stage 5 for transplantation; Tg, thymoglobulin; mAb, monoclonal antibodies (basiliximab); PRA, panel reactive antibody; OR, odds ratio; CI, confidence interval; DM, diabetes mellitus; GN, glomerulonephritis; NP, nephropathy.

*Univariate analysis by Cox regression for transplant number, cold ischaemia time, recipient weight, recipient sex and donor category showed P-values >0.100 and are not included in the table.
FIGURE 3: Preformed anti-MICA antibody specificities in HLA°MICA° (n = 12) or HLA°MICA+ (n = 13) kidney transplant recipients. (A) Colour code shows the strength of the reaction for each specific antibody: black squares = strong reaction (SFI >80 000); dark grey squares = intermediate reaction (SFI 40 000–80 000); light grey squares = weak reaction (SFI 10 000–40 000). (B) Comparison of preformed anti-MICA antibody SFI between HLA°MICA° and HLA°MICA+ groups. (C) Comparison of preformed anti-MICA antibody SFI for CMGWS and AYVE supereplets between HLA°MICA° and HLA°MICA+ patients. (D) Comparison of preformed anti-MICA antibody SFI between HLA°MICA° and HLA°MICA+ groups for each specificity within CMGWS and AYVE supereplets.
After transplantation, the MICA genotype was available in 17 donors. Preformed anti-MICA antibodies were donor specific in nine patients (anti-MICA DSA, sera 2, 3, 5, 6, 9, 13, 14, 16 and 17) and non-donor-specific in eight patients (anti-MICA non-DSA, sera 8, 11, 12, 15, 18, 19, 22 and 24) (Figure 3A). We did not find significant differences in either eGFR at Month 3 post-transplantation (51.09 ± 23.02 mL/min/1.73 m² in anti-MICA DSA versus 42.14 ± 19.99 mL/min/1.73 m² in anti-MICA non-DSA, P = 0.409) or days to reach optimal creatinine (79.67 ± 23.04 in DSA versus 80.13 ± 22.43 in non-DSA, P = 0.967); however, more patients with anti-MICA DSA also had anti-HLA antibodies versus patients with anti-MICA non-DSA (55 versus 38%, respectively).

Supereplet profile of preformed anti-MICA antibodies

Duquesnoy et al. [17] classified a total of 11 anti-MICA specificities into two reaction groups by developing an eplet-based version of the HLAMatchmaker algorithm: CMGWS supereplet included anti-MICA*001, *002, *007, *012, *015, *017 and *018 specificities and AYVE supereplet contained anti-MICA*004, *009, *019 and *027 specificities (this last one showing the same amino acid sequence in the extracellular domain as MICA*008) [18]. Among our MICA+ patients, 52% had anti-MICA specificities that included both CMGWS and AYVE supereplets (Figure 3A). All HLA MICA+ patients (n = 12) had specificities directed against CMGWS, but only half had specificities against both CMGWS and AYVE supereplets. Twelve of 13 HLA MICA+ patients had anti-CMGWS reactivity, and 6 of them also had anti-AYVE reactivity. Only one HLA MICA+ recipient showed exclusive immunization against AYVE (serum 25).

However, mean antibody levels were higher against AYVE supereplet, and AYVE reactivity was significantly higher in HLA MICA+ versus HLA MICA- individuals (SFI 152 773 ± 36 177 versus 37 783 ± 7866, P < 0.001) and significantly superior than reactivities against CMGWS within HLA MICA+ patients (SFI 152 773 ± 36 177 versus 88 497 ± 30 112, P = 0.001). Antibodies against CMGWS were significantly more intense in HLA MICA+ than those in HLA MICA- patients (SFI 88 497 ± 30 112 versus 56 479 ± 40 602, P = 0.034), whereas, within HLA MICA+ group, the intensity of anti-CMGWS or anti-AYVE antibodies was not significantly different (SFI 56 479 ± 40 602 versus 37 783 ± 7866, P = 0.116) (Figure 3C). Finally, every individual AYVE supereplet specificity reacted with significantly more intensity in HLA MICA+ than that in HLA MICA- patients (Figure 3D). Regarding CMGWS supereplet, only anti-MICA*002 and anti-MICA*015 antibodies were significantly more intense in double-immunized versus HLA MICA- patients (P = 0.005 and P = 0.020, respectively).

Preformed anti-MICA antibodies may bind native MICA antigens on cell surfaces

We tested by FC if anti-MICA antibodies were able to bind MICA antigens on cell surfaces in addition to binding MICA molecules captured on microbeads, which may show conformational differences versus native MICA. Two MICA*008 homozygous cells were chosen (Hela and PMA/CD28-T-cell blasts) to improve the sensitivity of the binding assay, since MICA*008-positive cells express 7- to 10-fold higher levels of MICA proteins at the cell surface [19]. Reactions due to the presence of anti-HLA antibodies were avoided by performing FC with six anti-MICA*027/008-positive pre-transplantation sera samples from HLA MICA+ recipients (anti-MICA*027 SFI: serum 13: 87 878; 14: 33 603; 16: 42 118; 17: 53 771; 22: 14 143; 25: 13 622). All these FC binding tests were negative with HeLa and PMA/CD28-T-cells.

Because we observed that anti-MICA antibodies accompanied by anti-HLA antibodies showed stronger reactions, we selected two pre-transplant sera from the HLA MICA+ group with anti-MICA antibody screening >150 000 SFI (sera 3 and 4) to test their binding capacity to MICA native proteins. The anti-MICA*027/008 reaction was strong for serum 3 (SFI 120 604) and weak for serum 4 (SFI 30 348). After absorption with lymphocyte pools, an intense decrease of most anti-HLA antibodies in both sera 3 and 4 was observed. Anti-HLA specificities corresponding to HeLa HLA-A68, -B72 and -Cw12 and to T blasts HLA-A3, -A11, -B27, -B52, -Cw7 and -Cw14 were virtually absent (Figure 4A and B). FC showed that HeLa cells constitutively express MICA antigens, in a similar amount to HLA class I expression (Figure 4C and D), whereas CD3+ T cells present HLA class I but not MICA proteins on their surface. Nevertheless, mitogen stimulation of CD3+ T cells increased membrane HLA class I and induced the surface expression of MICA (Figure 4E and F). FC-recorded binding for absorbed serum 3 was clearly positive with HeLa and slightly positive for T blasts (Figure 4G and H), but absorbed serum 4 binding was negative with both cell lines (Figure 4I and J), consistent with the higher anti-MICA*027/008 SFI in sample 3. Superior constitutive MICA antigen density on HeLa cell membranes (98% MICA-positive cells, MFI: 7.35, Figure 4D) versus induced MICA antigen in PMA/CD28-T-blasts (85% MICA-positive cells, MFI: 3.91, Figure 4F) may explain differences in binding intensity of serum 3 between both cell lines and indicates that FC with HeLa cells is probably a more sensitive assay for the detection of specific anti-MICA*027 antibodies. HLA-absorbed serum 3 contained a small amount of anti-HLA-DRB1*13 antibodies (SFI 41 142) that bind neither HeLa cells, due to the lack of HLA class II on their membranes, nor T blasts, which expressed HLA-DR1 and -DR15 after PMA/CD28 activation. Thus we conclude that preformed anti-MICA antibodies may bind native MICA proteins on cells membranes.

Preformed anti-MICA antibodies bind C1q and activate CDC

The C1q Luminex assay uses microbeads exclusively covered by individual MICA antigens. We studied 13 positive anti-MICA single-antigen pre-transplant samples, all 9 anti-MICA DSA sera (2, 3, 5, 6, 9, 13, 14, 16 and 17; Figure 3A) plus 4 samples from anti-MICA non-DSA recipients with a rejection event recorded and/or C4d+ kidney biopsy (sera 4, 8, 20 and 25; Figure 3A). Among the 13 sera, 10 were anti-MICA specific C1q negative (SFI values between 0 and 143) and 3 (23%) had C1q-fixing anti-MICA antibodies (SFI serum 3: 355 550; serum 4: 69 631; serum 17: 45 578). Interestingly, whereas anti-MICA antibodies were polyspecific in all three sera and recognized MICA antigens included in both CMGWS and AYVE
FIGURE 4: Single-antigen specificities and flow cytometry cross-match (FCXM) analysis of serum 3 and serum 4. Amount of antibodies against HLA class I antigens of HeLa cells (black arrows, HeLa cells HLA typing: A*68:02,-/B*15:03,-/Cw*12:03,-/MICA*008:01/04,-) and PMA/CD28 T-cell blasts (red arrows, T-cell blasts HLA typing: A*3,*11/B*27,*52/Cw*7,*14/DRB1*01,*15/MICA*008:01,-) in (A) serum 3 and (B) serum 4 (B), after antibody absorption treatment. FC-recorded expression of HLA class I and MICA antigens on HeLa cells (C and D, respectively) and mitogen-stimulated T cells (E and F, respectively). (G) Absorbed serum 3 binding by FCXM was clearly positive with HeLa cells and (H) slightly positive with T-cell blasts. (I) Absorbed serum 4 binding was negative for HeLa cells as well as for (J) T-cell blasts. Negative control (light grey histogram) consisted of cells incubated with a serum without antibodies and positive control (black histogram) consisted of cells incubated with a pooled serum from three hyperimmunized patients with >50% PRA.
supereplets (Figure 5A, C and E), C1q-fixing anti-MICA antibodies were exclusively directed against AYVE in serum 3, CMGWS in serum 4 and against both supereplets in serum 17 (Figure 5B, D and F). The highest C1q-positive anti-MICA reactivity corresponded to MICA*027/*008 in serum 3 (SFI 355 550). Sample 3 then exemplifies that pre-transplant anti-MICA

**FIGURE 5**: Global preformed anti-MICA single-antigen specificities and C1q binding anti-MICA specificities categorized according to CMGWS and AYVE supereplets. (A) Serum 3 had anti-CMGWS and anti-AYVE antibodies but (B) only anti-AYVE ones bind C1q. (C) Serum 4 had anti-CMGWS and anti-AYVE antibodies but (D) only anti-CMGWS ones bind C1q. (E) Serum 17 had both supereplet reactivities and (F) both anti-CMGWS and anti-AYVE bind C1q.
antibodies are able to recognize membrane MICA antigens and to fix C1q.

Finally, we analysed the absorbed serum 3 in a modified CDC-AHG assay. Whereas all PMA/CD28-T-cell blasts incubated with negative control plus complement remained alive and all cells died when incubated with positive control plus complement, ∼60–80% of cells died after incubation with serum 3 and complement (Figure 6A–C). We conclude that preformed anti-MICA antibodies in the serum of transplant recipients are able to bind native MICA antigens on cell surfaces and activate classical complement cascade leading to target-cell death.

**DISCUSSION**

We previously reported that preformed anti-MICA antibodies increase the risk for rejection and enhance the deleterious effect of PRA⁺ status in the early post-transplantation period [10]. Here we provide further evidence that preformed anti-MICA antibodies deteriorate renal graft function after transplantation by showing that the time to reach optimal serum creatinine level after transplantation is longer for patients with pre-transplant anti-HLA and anti-MICA antibodies and, 3 months post-transplant, eGFR is the lowest in these patients. PRA⁺ MICA⁺ recipients showed the highest risk for CKD5T, which frequently involves retransplantation. Nevertheless, a limitation of our work exists, since results explaining the main and synergistic effects of anti-MICA antibodies are not fully consistent and partially depend on observations from a small cohort of patients (PRA⁺MICA⁺). In our series, PRA⁺ status included both anti-HLA DSA and non-DSA. Although anti-HLA DSA have the greatest impact on allograft evolution [20, 21], a deleterious effect of anti-HLA non-DSA has also been reported [22–24]. Finally, systematic follow-up biopsies in patients with anti-MICA antibodies would help to further understand their potential role in worsening graft function.

From our initial cohort of 52 anti-MICA-sensitized patients as shown by the screening Luminex assay, 25 sera remained positive when analysed by the anti-MICA LSA. This reduction could be related to low strength of preformed anti-MICA antibodies together with bead-dependent dilutional effects (2 groups of beads bearing MICA antigens in the screening versus 11 groups in LSA) [25]. All recorded specificities corresponded to either one or both CMGWS and AYVE MICA [17, 18]. A higher frequency of preformed anti-MICA antibodies directed against anti-CMGWS than anti-AYVE supereplet was observed, and in particular against antigens MICA*001, *018 and *017. These specificities are also more prevalent in patients after kidney transplantation [18]. However, MICA*001, *018 and *017 are not the most frequent MICA antigens in Spanish or other related Caucasian populations, where MICA*008 (25% in Spanish population, included in AYVE supereplet), followed by *002, *004, *001, *009, *016 and *010, are mainly found [26–28]. Consequently the most frequent sensitizing events correspond to subjects bearing AYVE MICA antigens exposed to CMGWS alloantigens.

We observed that preformed anti-MICA antibodies are more intense when accompanied by anti-HLA antibodies, and this was especially noticed for anti-AYVE antibodies recognizing the most prevalent antigen MICA*027/*008. This suggests that the generation of anti-MICA*027/*008 antibodies corresponds mainly to an anti-allogeneic humoral immune response. These results support data from the study by Tonnerre et al. who observed that MICA*008 protein expresses 7- to 10-fold higher levels at the endothelial cell surface and is the preferential antigen leading to anti-MICA sensitization after renal transplantation in MICA-mismatched donor/recipient pairs [19].

By using the C1q Luminex assay, we show for the first time that some preformed anti-MICA antibodies may bind complement. From an analysis of 13 pre-transplant sera, 3 were C1q-positive: sera 3 and 4 corresponded to third kidney recipients and serum 17 corresponded to a female with past pregnancies. This further supports the existence of an allogeneic response against MICA antigens. This low proportion is in contrast with data from post-transplant anti-MICA antibodies that showed 8 C1q-positive out of 10 analysed sera (unpublished data). It is possible that complement-binding IgG1, IgG3 and IgM may

![Figure 6](https://academic.oup.com/ndt/article-abstract/31/1/150/2460097/Early-renal-graft-function-deterioration-in)
principally arise after transplantation, whereas pre-transplant antibodies may correspond to non-complement-binding subclasses. However, we noticed that anti-MICA antibody SFI was superior in post- versus pre-transplant samples, supporting the notion that positivity in the C1q Luminex assay is more frequent in sera with higher antibody titres [29, 30]. Interestingly, we observed that the highest C1q-positive SFI corresponded to anti-MICA*008, further supporting the immunogenicity of MICA*008 antigen and its role in the alloimmune response.

The capacity of anti-MICA antibodies to bind MICA antigens and trigger cell death in the presence of complement has been observed in the past. However, our data include two main novelties. First, we provide evidence that complement-mediated cell death is a capacity of anti-MICA antibodies existing in the serum of patients before transplantation, whereas previous authors have demonstrated the complement-mediated cytotoxicity capacity for post-transplant anti-MICA antibodies from patients rejecting kidney [5, 13] or heart [14] grafts or for anti-MICA*008 monoclonal antibodies generated in mice [13]. Second, we demonstrate cell death in CDC assays by incubating pre-transplant serum with mitogen-stimulated MICA-expressing T cells instead of the MICA-transfected or endothelial cell lines used in previous works. In our hands, contrary to data from Zou et al. [13], CDC was negative with HeLa cells, maybe because the anti-MICA antibody titre in pre-transplant serum was not high enough to overcome protection from death mediated by membrane-bound complement regulators. Interestingly, CDC-positive serum 3 corresponded to an anti-MICA DSA-positive patient with suspected acute rejection, who responded to treatment with steroids. Studies are needed to understand the significance of positive MICA cross-matches in the clinical setting.

CONCLUSION

In conclusion, we demonstrate that complement-mediated cytotoxicity is a property of some preformed anti-MICA antibodies. This capacity may play a role in deterioration of the renal allograft function early after transplantation in pre-transplant anti MICA-sensitized recipients. The relative contribution of this mechanism to renal graft dysfunction, compared with others such as NKG2D-mediated activation of NK cells by MICA-expressing renal grafts, cannot be definitively established yet. MICA-specific cross-matching and CDC tests with higher sensitivity may help to answer this question.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract format. The authors declare that there is no conflict of interest.
Plasma levels of marine n-3 polyunsaturated fatty acids and renal allograft survival

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ABSTRACT

Background. Marine n-3 polyunsaturated fatty acids (PUFAs) may exert beneficial effects on inflammation, fibrosis, endothelial function, lipid profile and blood pressure that may prevent graft loss.

Methods. In this observational cohort study in Norwegian renal transplant recipients (n = 1990), transplanted between 1999 and 2011, associations between plasma marine n-3 PUFA levels and graft loss were assessed by multivariable Cox proportional hazard regression analysis. Plasma phospholipid fatty acid composition was determined by gas chromatography and individual fatty acids recorded as weight percentage (wt%) of total fatty acids in a stable phase 10 weeks after transplantation.

Results. During a median follow-up time of 6.8 years, 569 (28.6%) renal allografts were lost, either due to patient death (n = 340, 59.8% of graft loss) or graft loss in surviving patients (n = 229, 40.2%). Plasma marine n-3 PUFA levels ranged from 1.35 to 23.87 wt%, with a median level of 7.95 wt% (interquartile range 6.20–10.03 wt%). When adjusting for established graft loss risk factors, there was a 11% reduced risk of graft loss for every 1.0 wt% increase in marine n-3 PUFA level [adjusted hazard ratio (HR) 0.89; 95% confidence interval (CI) 0.84–0.93], and a 10% reduced risk of graft loss in surviving patients (adjusted HR 0.90; 95% CI 0.84–0.97).

Conclusion. High levels of plasma marine n-3 PUFAs were associated with better renal allograft survival.

Keywords: marine n-3 fatty acids, rejection, renal graft survival

INTRODUCTION

The two major marine n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may exert beneficial effects on inflammation, fibrosis, endothelial function, lipid profile and blood pressure that may prevent graft loss. These effects are believed to be mediated by their ability to modulate the ratio of pro-inflammatory to anti-inflammatory eicosanoids. However, the role of marine n-3 PUFAs in transplant settings, particularly when used for prophylaxis or treatment of rejection, has not been extensively studied.