Non-HLA Abs in Solid Organ Transplantation

María Gutiérrez-Larrañaga, Marcos López-Hoyos †®, André Renaldo and David San Segundo *†

Immunology Department, Marqués de Valdecilla Hospital-IDIVAL, 39008 Santander, Spain; maria.gutierrezl@scsalud.es (M.G.-L.); marcos.lopez@scsalud.es (M.L.-H.); arenaldo137@gmail.com (A.R.)
* Correspondence: david.sansegundo@scsalud.es
† Contributed equally in the work.

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Abstract: The role of anti-HLA antibodies in solid organ rejection is well established and these antibodies are routinely monitored both in patients in the waiting list and in the post-transplant setting. More recently, the presence of other antibodies directed towards non-HLA antigens, or the so-called minor histocompatibility antigens, has drawn the attention of the transplant community; however, their possible involvement in the graft outcome remains uncertain. These antibodies have been described to possibly have a role in rejection and allograft failure. This review focuses on the most studied non-HLA antibodies and their association with different clinical outcomes considered in solid organ transplantation with the aim of clarifying their clinical implication and potential relevance for routine testing.

Keywords: non-HLA; MICA; AT1R; ETAR; perlecans; collagen; tubulin; vimentin

1. Introduction

The Major Histocompatibility Complex (MHC) was such named after initial experiments with allograft compatibility in mice. The equivalent in humans, the Human Leukocyte Antigen (HLA), has been widely studied in the field of transplantation, and the involvement of the antibodies (Abs) directed against these molecules in allograft rejection has been repeatedly demonstrated [1]. In solid organ transplantation, routine studies include the HLA typing of both donor and recipient with the aim of detecting mismatches as well as mixing the donor’s lymphocytes with the recipient’s serum to test for pre-transplant anti-HLA Abs via cytotoxicity and cytometry assays. Likewise, serum HLA Abs are monitored post-transplant and in the case of suspected rejection. However, cases of allograft rejection in kidney transplants between HLA identical siblings have been described [2]. Similarly, poor graft outcomes are reported in matched-HLA lung recipients [3]. These findings suggest that other antigen determinants might be implicated in allograft rejection. Such antigens have been called non-HLA or Minor Histocompatibility antigens. These new actors have been less studied than HLA and anti-HLA Abs. However, the interest in this area has notably increased in recent years, and testing for Abs targeting non-HLA antigens is now suggested in cases of antibody mediated rejection (ABMR) with no detectable donor-specific anti-HLA Abs (DSA) [4,5].

Many non-HLA Abs have been described that possibly have a role in rejection and allograft failure. The most well-studied non-HLA Abs are those for which determination kits are commercially available, as their detection method is more standardized, and thus the results of different studies are more comparable. This review will focus on some of the widely studied non-HLA Abs. At the beginning of the last decade, efforts were made to review and analyze the literature regarding this field [6,7]. The present work provides updated information about the clinical implication of non-HLA antibodies in different solid-organ transplantations and their relevance in routine testing.
2. Non-HLA Abs

2.1. Anti-MICA Abs

The MHC I-related (MIC) gene family involves seven members (MICA to MICG), five of which are pseudogenes and two of which are functional genes (MICA and MICB). The MHC I-related chain gene A (MICA) was first described in 1994 [8,9] as a highly polymorphic gene, with more than 80 alleles described (http://hla.alleles.org/data/refs/micarefs.html) and new variants being continuously identified [10]. It is located in chromosome 6 and is closely related to HLA-B. However, its product, a 62 kDa protein, does not bind peptides, nor does it act in antigen presentation. It is known to be the ligand of the Natural Killer group 2, member D (NKG2D) activating receptor, which is present in NK cells and CD8+ T lymphocytes [11]. MICA is expressed in several cells, such as keratinocytes, monocytes, and endothelial cells [12], and its expression has been shown to be upregulated in allograft tissues as a result of the ischemia/reperfusion injury inherent to the transplant process [13]. Its ability to activate NK cells has related MICA to allograft rejection by its direct consequence on the cellular cytotoxic response, as well as by its role in the link between innate and adaptive immunity [11].

In addition, the presence of anti-MICA Abs has been repeatedly reported in patients with allograft failure and antibody-mediated rejection (ABMR) [14–17].

Many laboratories screen for anti-MICA Abs in the recipients’ serum while the patient is on the waiting list. However, it should be pointed out that MICA is not present in resting lymphocytes, and thus Abs against these molecules would not be identifiable by classic crossmatch tests confronting the recipient’s serum with the donor’s lymphocytes [18].

In this review, we searched the recent literature addressing the effect of anti-MICA Abs on solid organ transplant outcomes.

2.1.1. Anti-MICA Abs in Renal Transplantation

MICA sensitization can occur through mechanisms similar to HLA, such as previous transplants, pregnancy, or transfusion, but patients with no such expositions can also present anti-MICA Abs, suggesting other ways of sensitization. In addition, the presence of autoreactive anti-MICA Abs has also been reported [19]. There is a large collection of publications regarding the role of anti-MICA Abs in kidney transplantation. Among those focused on Abs detected in pre-transplant samples, Sánchez-Zapardiel and colleagues published a retrospective study of 727 kidney recipients, with an incidence of pre-transplant anti-MICA Abs of 7.1%. This study showed that the presence of these Abs together with anti-HLA Abs was significantly associated with a decreased global allograft survival. It has also been shown that anti-MICA Abs increase the risk of allograft rejection measured during the third month post-transplant independently of anti-HLA [20]. In an independent cohort of 646 kidney recipients, the incidence of anti-MICA Abs was 14.6% prior to transplantation. The association between pre-transplant anti-MICA Abs and reduced allograft survival was observed to be more pronounced in patients with the anti-MICA+ anti-HLA- profile than in those with the anti-MICA+ anti-HLA+ profile, suggesting an independent role of anti-MICA Abs [21].

In contrast, other groups did not find significant associations between pre-transplant anti-MICA Abs and worse allograft outcomes. The presence of anti-MICA Abs was not significantly associated with acute rejection at 1 year post-transplant, nor with a decreased overall survival at 10 years [19]. More recently, Min et al. retrospectively studied anti-HLA, anti-AT1R (Angiotensin II Type I Receptor), and anti-MICA Abs in a cohort of 359 kidney transplant recipients; they reported 13.9% pre-transplant anti-MICA Abs. No correlation was found between anti-HLA or anti-MICA and antibody mediated rejection, microvascular inflammation or allograft survival [22].

The studies focusing on post-transplant development of anti-MICA Abs yield discrepant results. In a prospective study of 185 kidney transplant recipients (KTR), the de novo MICA Ab development rate was 16% and was associated with a higher incidence of rejection and worse graft function [17]. In another study, the development of anti-MICA was independent of graft function, but once Abs were
developed, higher MFIs were detected in those with poor function [23]. Another group reported the presence of anti-MICA Abs to be significantly associated with higher serum creatinine levels at 1 week post-transplant but not at 4 weeks in sensitized kidney transplant recipients [24]. A later prospective study of 84 KTR showed a significantly impaired allograft function in patients with anti-MICA Abs compared to patients without anti-MICA [25].

On the other hand, L.W. Zang et al. published a prospective study of 275 kidney recipients. Thirty-three patients had post-transplant anti-MICA Abs and 71 patients had anti-HLA Abs, but only the presence of anti-HLA Abs was found to be significantly associated with proteinuria after transplantation and decreased graft survival [26].

In a similar vein, Lemmy et al. published in 2012 a multi-center retrospective study with 779 kidney transplant recipients. The anti-MICA detected post-transplant were not significantly associated with 4-year death-censored graft survival [27]. Moreover, a study of 457 KTR found that 19.2% developed de novo anti-MICA Abs during the seven-year follow-up period, with no impact in graft survival [28].

Some of these studies also addressed the association of anti-MICA Abs and rejection. The multicenter study conducted by Lemmy et al. found that anti-MICA Abs were apparently associated with chronic antibody-mediated graft rejection 1 year post-kidney transplant. However, the low number of events in this outcome and the association of most of these cases with anti-HLA Abs made discerning the role of each antibody difficult [27]. The presence of anti-MICA alone was not associated with a higher acute rejection rate [24]. In a small retrospective cohort of 32 KTR with either ABMR or cellular-mediated rejection (CMR), no correlation with anti-MICA antibody development was found [29].

In a large prospective study with 442 KTR, a total of 17 had pretransplant anti-MICA donor-specific Abs (DSA) and 13 developed anti-MICA DSA post kidney transplantation. MICA mismatch was a significant factor associated with the de novo development of anti-MICA Abs. The presence of both anti-MICA and anti-HLA Abs was associated with acute cellular rejection. Nevertheless, anti-MICA DSA were significantly associated with a decreased estimated Glomerular Filtration Rate at 2 and 3 years post-transplantation [30].

Given the coexistence of anti-HLA and anti-MICA, it is difficult to assess the independent involvement of anti-MICA Abs in clinical outcomes.

2.1.2. Anti-MICA Abs in Lung Transplantation

Anti-MICA Abs have also been implicated in other examples of solid organ transplantation, though less extensively explored. Angaswamy et al. analyzed the role of post-transplant de novo anti-MICA Abs in the development of bronchiolitis obliterans syndrome (BOS) in 80 lung transplant recipients. In this study, a significantly higher proportion of patients with BOS had developed de novo anti-MICA Abs after lung transplantation. The development of antibodies either to MICA alone or to MICA and HLA together significantly correlated with the development of BOS, and the correlation was stronger when both were present, suggesting a synergistic effect. In addition, data obtained during the follow-up period showed that the presence of anti-HLA Abs in serum preceded that of anti-MICA Abs, suggesting a role of the alloimmune response in the development of anti-MICA Abs [31].

In lung transplants, some authors suggest that a possible mechanism in the pathogenesis of chronic rejection is an immune response to self-antigens which is driven by an initial alloresponse to the donor HLA [31]. However, the scarce findings in lung transplantation along with the concomitant presence of anti-HLA and anti-MICA Abs makes it difficult to draw consistent conclusions.

2.1.3. Anti-MICA Abs in Liver Transplantation

The first study in 84 liver transplant recipients found that neither the preformed or de novo development of anti-MICA Abs was associated with rejection. However, no study of anti-HLA Abs was performed [32]. Subsequently, in a large prospective study with 123 liver transplant recipients followed for 7 years, no association of anti-HLA or anti-MICA Abs with acute or late rejection or graft
survival was found, although the positivity for Abs (either anti-HLA or anti-MICA) was associated with increased late rejection episodes [33].

2.1.4. Anti-MICA Abs in Heart Transplantation

Nath et al. studied the implication of post-transplant anti-HLA DSA and anti-MICA Abs in early (<1 year) ABMR and late (>1 year) cardiac allograft vasculopathy (CAV) in 43 and 52 heart recipients, respectively. The data collected showed a significant association between anti-MICA+/anti-HLA DSA+ patients and ABMR, but no association was found in anti-MICA+/anti-HLA DSA- patients. With respect to CAV, the association was significant for both groups (anti-MICA alone or anti-MICA and anti-HLA together). Moreover, as observed in lung transplantation, anti-HLA DSA preceded the development of anti-MICA Abs in patients with ABMR [34].

Zhang et al. assessed the implication of donor-specific anti-MICA Abs by studying their presence along with the MICA genotype in 168 heart allograft recipients. Five out of 19 patients who developed ABMR had donor-specific anti-MICA Abs with or without anti-HLA DSA. From those five patients, three had only anti-MICA DSA, while two had both anti-MICA and anti-HLA DSA. The authors also found that having both HLA and MICA DSA was associated with a higher rate of transplant coronary artery disease (TCAD) at 2 years, with no differences in the 1-year graft survival. However, these results were not analyzed separately for anti-MICA Abs and anti-HLA DSA [35].

The clinical implication of MICA Abs remains controversial. In 2011, Lu Jun et al. published a systematic review on the effect of anti-MICA Abs, detected either pre- or post-transplant, on solid organ transplant outcomes. The results of the meta-analysis performed in this study showed a weak association between anti-MICA and poor transplant outcomes. However, very few studies addressed the effect of pre-transplant Abs, and most studies regarding post-transplant anti-MICA included very few patients [36].

As the data suggests, the development of anti-MICA Abs is often accompanied but preceded by that of anti-HLA Abs. This may explain the scarcity of studies involving the development of anti-MICA Abs alone, which makes it difficult to discern their role in AR, CR, ABMR, graft function, and graft survival.

2.2. Anti-Angiotensin II Type 1 Receptor (AT1R) Abs and Anti-Endothelin Receptor (ETAR) Abs

For years, endothelial cell crossmatch has been an approach to detect non-HLA Abs in transplant recipients or waiting list patients [37–40]. Indeed, many non-HLA Abs are primarily directed to antigens expressed on endothelial cells and have been named anti-Endothelial Cell Abs (AECA). However, this technique by itself cannot reveal the exact antigenic specificity of AECAs unless subsequent analyses are performed. Among other antigens expressed by endothelial cells, two G-protein-coupled receptors (angiotensin II type 1 receptor (AT1R) and endothelin type A receptor (ETAR)) have been frequently reported to have a clinical relevance in solid organ transplantation. Additionally, Abs against AT1R have also been found to be associated with immune-related diseases such as systemic sclerosis or preeclampsia [41,42]. Under physiological activation by angiotensin, AT1R activates vasoconstriction but also leads to cell proliferation through the ERK kinase pathway [43].

Unlike HLA, the AT1R gene has limited polymorphism, and Abs targeting this antigen are auto-Abs capable of binding the receptor located in both the allograft and the recipient’s own tissues. Several events could promote the production of anti-AT1R Abs in the recipient, such as ischemic injury or inflammation during transplantation, which may lead to an abnormally high expression of AT1R in endothelium, the exposure of cryptic epitopes, or neoantigen formation [44].

2.2.1. Anti-Angiotensin II Type 1 Receptor (AT1R) Abs and anti-Endothelin Receptor (ETAR) Abs in Kidney Transplantation

AT1R-specific Abs and their outcome in kidney transplantation have been widely studied in the last decade. Pre-transplant anti-AT1R Abs have been found to be significantly associated with acute
rejection. Pre-transplant AT1R Abs have been reported to be associated with acute ABMR [22,45–51] and histological characteristics typical of ABMR, such as microvascular inflammation [22], ACR [48,52–54], or mixed ABMR-ACR [49], depending on the study. Although less frequently assessed, pre-transplant anti-AT1R has been independently associated with worse graft function [48].

Graft failure has been a more controversially reported outcome. There is evidence of increased risk of graft failure in patients with anti-AT1R Abs [22,47] in both the early [48] and long-term periods [46]. However, the same group led a multi-center study comprising 940 kidney recipients and did not observe an independent association between pre-transplant anti-AT1R and acute rejection or graft failure [55]. These discrepancies might have different explanations: (1) a non-centralized histopathological diagnosis of rejection, (2) differences in the HLA-antibody detection technique, or (3) different cut-off values for considering positive anti-AT1R Abs.

Subsequent studies, on the other hand, did not find differences either in graft failure or function despite long follow-up periods [53]. Presumably, the detection of rejection with surveillance biopsies dictated early immunosuppressive interventions, which might have improved the long-term outcomes in the anti-AT1R-positive group [53]. Another study also showed no association between AT1R-specific Abs and graft loss in a long period of follow-up independently of the de novo HLA DSA [54].

The relationship between pre-transplant anti-AT1R Abs and de novo anti-HLA DSA in kidney transplant recipients has also been reported [54]. Regarding the time course of anti-AT1R Abs detection and its relation with anti-HLA DSA, a study showed that de novo, pre-, and post-transplant AT1R-specific Abs were all independently associated with decreased graft survival, with de novo Abs being the highest risk factor. Moreover, 58% of those with graft loss had anti-AT1R Abs, and the levels of these Abs increased during the post-transplant period. Another interesting finding of this study was that when anti-AT1R developed before HLA DSA, the time to graft failure was shorter than when the order of antibody appearance was reversed [47]. A synergistic effect of anti-HLA DSA and anti-AT1R on reduced allograft survival [22,47,56] and on the incidence of acute rejection at one year follow-up has also been reported [56]. The relation between anti-AT1R and anti-HLA DSA was dose-dependent, while high levels were an independent risk factor for the earlier detection of de novo anti-HLA DSA [57].

Most articles consider the clinical implication of pre-transplant anti-AT1R. A study analyzing the levels of Abs at the time of acute rejection concluded that they were significantly associated with HLA DSA and their combination was significantly associated with ABMR. Anti-AT1R positivity alone was not significantly associated with any specific type of rejection [58].

The pre-transplant anti-ETAR Abs were detected in 47.4% of 116 KTR. These patients had worse 6- and 12-month graft function with a higher frequency of arteritis and graft vasculopathy, although there were no differences between the groups in biopsy-proven acute rejection [59].

2.2.2. Anti-AT1R Abs in Heart Transplantation

In heart transplantation, the implanted mechanical circulatory support (MCS) devices, commonly used as bridges to transplantation, have been found to be a source of sensitization to AT1R. A work with 96 patients on MCS demonstrated a higher rate of anti-AT1R antibody development but was not associated with significant differences in survival at 24 months post-implantation. Moreover, in 69 heart-transplanted patients, no major difference in freedom from rejection was obtained between the pre-transplant anti-AT1R-positive and negative groups [60]. An independent group has shown similar results in terms of AT1R sensitization after MCS in 88 patients. Here, the patients with anti-AT1R Abs had a significantly lower 18-month survival post-implantation, but no differences were observed in the longer follow-up times. A total of 75 patients underwent heart transplantation afterwards and, again, no differences in the post-transplant patient survival were observed either in freedom from ABMR, ACR, or CAV [61].

Previously, a small cohort of 30 heart transplant recipients who reported the maximum levels of anti-AT1R and anti-ETAR Abs were significantly associated with ABMR, CMR, and biopsy-proven
microvasculopathy during a one-year follow-up. The maximum anti-AT1R and anti-ETAR levels corresponded to the samples taken 24 h post-transplantation, which probably reflected pre-transplant sensitization. Shortly after transplantation, the antibody levels decreased but then recovered, and the time post-transplant to reach 10U/L-titre of non-HLA Abs was found to be shorter in patients with PRA (Panel Reactive Antibody) and microvasculopathy. However, no significant association was found between the time to developing 10U/L titers and the presence of ABMR or ACR [62].

A subsequent study with 200 heart transplant recipients found a decreased rate of ABMR and/or CMR freedom when strong-binding levels of pre-transplant anti-AT1R Abs were considered together with de novo anti-HLA DSA. However, such differences were not found when anti-AT1R Abs were considered alone. In this case, survival and CAV were assessed, but no significant differences were found for anti-AT1R Abs-positive patients in the 5-year follow-up [63].

It is difficult to assess an independent role for anti-AT1R and anti-HLA Abs development in heart transplant recipients due to a lack of large multivariate analysis studies in the literature.

2.2.3. Anti-AT1R Abs in Lung Transplantation

A multi-center study with 162 lung transplant recipients tested pre- and post-transplant for anti-HLA, anti-AT1R, and anti-ETAR Abs to assess the involvement of anti-AT1R Abs in lung transplantation was conducted [64]. The authors found that patients with strong and intermediate binding pre-transplant Abs (either AT1R or ETAR) had a significantly reduced freedom from ABMR and a trend for lower freedom from CMR, respectively. The presence of these Abs was related to the development of de novo anti-HLA Abs, an association that was more marked when also considering the pre-transplant HLA sensitization status. It should be noted that only five patients developed ABMR, and all of them had de novo DSA Abs and strong-binding AT1R-specific Abs, and four out of five had strong-binding anti-ETAR Abs [64]. Thus, it was not possible to determine the size of the effect of de novo DSA, anti-AT1R, and anti-ETAR Abs independently.

2.3. Anti-Perlecan (LG3) Abs in Kidney Transplantation

Heparan sulfate proteoglycan 2, also called perlecan, is a major component of vessels’ walls. It is involved in a wide variety of functions, such as the modulation of cell growth, differentiation, and death or the lipid metabolism [65]. The LG3 or endorepellin domain, the C-terminal fragment of perlecan, can be released by caspase-dependant cleavage during apoptosis [66]. This fragment might act as a neoantigen and promote antibody production.

Although relatively uncommon, the acute vascular rejection (AVR) of renal allograft is associated with a high rate of permanent graft dysfunction and loss. This type of rejection is characterized by immune-mediated vascular injury and the increased apoptosis of endothelial cells. Cardinal et al. demonstrated that the levels of anti-LG3 Abs, both pre- and post-transplant, were significantly higher in kidney-transplanted patients with acute vascular rejection than in patients with tubulo-glomerular rejection or with normal allograft function. A multivariate analysis determined that the pre- and post-transplant anti-LG3 levels are risk factors for acute vascular rejection, independent from anti-HLA DSA [67,68]. This study also proved that anti-LG3 Abs enhanced NK infiltration, C4d deposition, and obliterative vascular remodeling in a murine model of HLA-mismatched aorta transplantation, especially when the graft was exposed to ischemic distress, which favors the exposition of LG3 for recognition by Abs.

More recently, in a study with 172 kidney transplant recipients, pretransplant anti-LG3 titers have been significantly associated with an increased risk of delayed graft function. In these patients, anti-LG3 Abs were associated with impaired graft function at 1-year follow-up independent from episodes of acute rejection [69].

Matching hypersensitized patients and potential donors is a clinical challenge in which anti-perlecan Abs might be an important piece of the puzzle. In 2018, our group published data from a cohort of 27 hypersensitized patients who had undergone kidney transplantation from donors
without forbidden HLA specificities. Fifty-two percent of these patients presented anti-LG3 Abs. Eleven patients were followed-up after transplantation and four presented early ABMR, two of which were anti-AT1R and one which was de novo HLA DSA, and all of them with positive anti-LG3 Abs pre-transplant [70].

Although the source of sensitization remains to be elucidated, the presence of anti-LG3 prior to transplantation seems to be related to worse graft function, vascular acute rejection, and ABMR. Questions still remain and large prospective studies still need to be carried out.

2.4. Anti-Collagen Abs and Anti-K-Alpha-Tubulin Abs

Autoimmunity seems to play an important role in graft rejection following lung transplantation, particularly the so-called lung-restricted self-antigens. The most studied ones include collagen type I (Col-I), collagen type V (Col-V), and k-alpha 1 tubulin (KAT), which are frequently studied together. Collagen and tubulin are both structural proteins. Collagen I is a component of most connective tissues, whereas collagen V modulates its fibrilization. KAT is an epithelial surface gap junction cytoskeletal protein.

2.4.1. Anti-K-Alpha 1 Tubulin and Anti-Collagen Abs in Lung Transplantation

Some of the studies published do not differentiate between Ab-specificities when reporting their results, but rather show the findings according to the presence of self-antigen directed Abs. A total of 142 lung transplant recipients who were negative for anti-HLA Abs had their pre-transplant serum samples tested for anti-self-antigens, including anti-KAT, anti-Col-V, and anti-Col-I. The results showed that 41 patients who had pre-transplant Abs against self-antigens also had an increased risk of primary graft disfunction (PGD). Anti-self-antigen Abs were also associated with increased levels of inflammatory mediators (IP-10, MCP-1, IL1β, IL12, and IL17) and with the development of anti-HLA Abs during the 5-year follow-up. In this work, having Abs against at least one of the studied specificities was found to be an independent risk factor for developing BOS [71]. These results were confirmed in 2013 by Tiriveedhi et al., who studied the association between Abs against KAT, Col-I, and Col-V with PGD, DSA, and BOS in 317 lung transplant recipients. First, they found that pre-transplant anti-tubulin and anti-collagen Abs were more prevalent in patients with idiopathic pulmonary fibrosis and cystic fibrosis. The authors also confirmed that the incidence of PGD and BOS was significantly higher in patients with pre-transplant anti-lung restricted Abs, and the same trend was observed regarding anti-HLA DSA development [72].

In terms of anti-KAT and anti-Col-V antibodies development after lung transplantation, a study with 108 lung recipients showed a correlation between Abs against self-antigens and DSAs. Among patients with anti-self-antigen Abs, those who received antibody-directed therapy were less likely to develop BOS. In addition, cases who had cleared anti-HLA DSA but had persisting anti-self-antigen Abs were more likely to develop BOS [73].

A retrospective study of 42 lung transplant recipients who were negative for anti-HLA, anti-KAT, and anti-Col-V Abs pre-transplant found that those who developed BOS had higher levels of both lung-restricted Abs post-transplant in their serum samples as well as in their bronchioalveolar lavage. In the same study, a prospective cohort of 103 lung transplant recipients was followed and tested for the development of anti-KAT, anti-Col-V, and anti-HLA Abs. The temporal sequence of appearance of the Abs was first anti-HLA DSA and then anti-self-antigens, suggesting that the alloimmune response to donor HLA can induce an autoimmune response to self-antigens, which contributes to graft injury. Finally, in addition to the evaluation of the humoral response, the BOS patients were also found to have a higher frequency of Col-V and KAT-specific T cells secreting IL-17 and INF-γ and less secreting IL-10 than recipients without BOS [74].

It has been proposed that the development of anti-tissue-specific Abs after lung transplantation may be produced by a two-hit mechanism. First, the apoptosis of regulatory T cells, which has been shown to occur after viral infections, and second, the exposure of the antigens, which leads to antibody
An interesting finding is that such antigens, as well as donor HLA antigens, can be found in exosomes both in the serum and bronchoalveolar lavage of lung recipients with ABMR or BOS but not in stable lung recipients [76].

2.4.2. Anti-K-Alpha 1 Tubulin and Anti-Collagen Abs in Heart Transplantation

Antibodies directed against these antigens have not been studied in depth in solid organ transplantation. A 2011 heart transplant study on 137 recipients demonstrated the involvement of anti-KAT and anti-Col-I, -II, -IV, and -V Abs on cardiac allograft antibody-mediated rejection and CAV (60 in the early period, 77 in the late post-transplant period). The authors concluded that Abs against KAT and Col-V, but not Col-I, -II, or -IV, were significantly increased in patients who developed ABMR. Moreover, patients with CAV also presented an increased frequency of Th cells specific to KAT and Col-V, secreting IL-5 and IFNY but decreased IL-10. Finally, the serial monitoring of Abs post-transplant found that anti-HLA DSA occurred first, around 2 months. This was followed by the appearance of anti-Col-V and anti-KAT Abs and subsequent ABMR at 8 months [77].

2.4.3. Anti-Collagen Abs in Kidney Transplantation

A case-control study examined the implication in renal transplantation of Abs directed against other structural antigens, such as Col-IV and fibronectin. Twenty-six patients with kidney transplant glomerulopathy (TG), 10 KTR with stable graft function, and 33 normal subjects with no kidney disease underwent assessment with pre- and post-transplant samples. The development of post-transplant Abs against Col-IV and fibronectin as well as anti-HLA DSA were associated with TG. The anti-collagen-IV and anti-fibronectin Abs found were both IgG and IgM isotypes, and the increasing titers appeared to be correlated with the development of transplant glomerulopathy [78].

2.5. Anti-Vimentin Abs

Vimentin is an intermediate filament protein of the cellular cytoskeleton. It has been demonstrated that cell activation upon injury or inflammation can lead to the rearrangement of cytosolic proteins and unmasking of cryptic epitopes, as well as to the exposure of antigens in the cell surface, allowing the production and binding of specific Abs [79]. This breakage of tolerance to autoantigens has been demonstrated to occur in patients on hemodialysis [80].

2.5.1. Anti-Vimentin Abs in Kidney Transplantation

The prevalence of these Abs in waiting list patients for kidney, liver, and thoracic transplants, as well as in KTR with failed grafts, was assessed. The patients with primary liver failure and renal graft failure had increased levels of anti-vimentin Abs [81].

During the last decade, anti-vimentin Abs have been linked with different conditions involving impaired allograft function. Interstitial fibrosis and tubular atrophy (IFTA) is the most common cause of late graft failure, and is characterized by a sum of immune-related and nonimmune-related damage responsible leading to a slow but continual worsening of renal function [82]. A retrospective case-control study screened both the pre- and post-transplant serum samples from 70 kidney recipients (40 controls and 30 biopsy-proven IFTA) for anti-vimentin and anti-HLA Abs. Although they did not find differences in the proportion of patients displaying positive anti-vimentin Abs between the control and IFTA group, they did find higher mean levels of anti-vimentin IgG Abs in the IFTA group compared to the controls, and the levels of such Abs increased significantly in the years following transplantation in the IFTA group but not in the control group. This suggests that anti-vimentin IgG Abs could play a role in patients developing IFTA. However, such differences did not apply for anti-vimentin IgM isotype Abs. Finally, this study did not find association between anti-vimentin Abs and anti-HLA Abs [83]. Similarly, another study with 24 transplant glomerulopathy patients, 24 matched and stable kidney recipients, and 22 healthy controls confirmed that a higher proportion of glomerulopathy patients developed IgG isotype anti-vimentin Abs, but the IgM Abs were similar.
in both groups, which suggests isotype switching in the immune response of the glomerulopathy group [84]. This study also has shown that the anti-vimentin Abs detected in renal transplantation are not specific to the citrullinated form of the protein, contrary to what has been reported in patients with rheumatoid arthritis [85].

López-Soler et al., in a case-control study with 97 kidney transplant recipients, showed that anti-vimentin IgG isotype Abs were higher in the IFTA group than in patients without IFTA. In addition, higher concentrations of pre-transplant anti-vimentin Abs were shown to significantly increase the risk of developing IFTA and were associated with worsening graft function at 1, 3, and 5 years post transplantation [86].

A Luminex-based assay designed to routinely detect anti-vimentin Abs was used to assess the utility of these Abs as clinical biomarkers. Seventy-one serum samples (51 patients with kidney allograft rejection and 20 age-matched controls) were tested. Using this assay, their results showed significantly higher levels of anti-vimentin Abs detected in patients with rejection compared to the control group [87].

Altogether, recent scientific evidence supports the potential involvement of IgG levels of anti-vimentin Abs in renal transplantation, especially in chronic rejection.

2.5.2. Anti-Vimentin Abs in Heart Transplantation

In a study of 50 HTx waiting list patients, the level of sensitization with anti-vimentin Abs was addressed, as well as the association with graft survival during the one-year follow-up period. The incidence of pre-transplant Abs was 34%, and their presence before transplant was not associated with either graft rejection or survival in the first year post-transplant [88].

Some evidence has also been published regarding the effect of anti-vimentin Abs in heart transplantation outcomes. Nath et al. studied the implication of post-transplant anti-vimentin and anti-myosin Abs in ABMR and CAV after heart allogenic transplantation in 148 recipients. ABMR was associated with HLA DSA and higher levels of Abs against vimentin and myosin. CAV patients were more likely to present anti-HLA DSA, higher levels of anti-vimentin and anti-myosin Abs and, in this case, increased IL-17-secreting specific Th cells and decreased IL-10-secreting specific Th cells [89].

2.6. H-Y Antibodies in Solid Organ Transplantation

H-Y antigens are proteins encoded by genes in the Y chromosome and are expressed in many different tissues. While both sex chromosomes have a high degree of homology with respect to these proteins, some regions differ between the H-X and H-Y proteins and can cause immunogenic reactions in certain settings of non sex-matched transplants, leading to T and/or B cell-mediated responses [90,91]. These minor antigens have been widely studied in hematopoietic stem cell transplantation. In this setting, antibodies directed against them have been associated with increased rates of graft versus host disease, as well as lower levels of relapse in male recipients/female donor pairs [92,93]. In solid organ transplantation, the impact of H-Y antigens has not been as extensively described. In kidney transplantation, some retrospective studies with large cohorts identified an increased risk of graft failure in female recipients of kidneys from male donors compared to the rest of the possible gender combinations [94–97]. On the other hand, a large retrospective study of simultaneous pancreas-renal transplantation found that graft survival was not influenced by different gender combinations, nor were there differences in the survival of each individual graft [98].

Another study analyzing rejection in kidney recipients from HLA-identical sibling donors did not find any correlation between HY mismatching (determined by genotyping the samples) and graft loss or rejection, possibly because of the low incidence of such events in the studied cohort [98].

Nevertheless, the published evidence on the effect of H-Y directed antibodies on renal graft outcome is scarce. A study from 2008 measured IgG antibodies against H-Y antigens RPS4Y1 and DDX3Y by ELISA and Western blotting and found a higher incidence of such antibodies in female
recipient and male donor combinations and a strong correlation between post-transplant H-Y antibodies and acute rejection episodes, which was independent of HLA DSA [99].

2.7. Conclusions

In this review, we have summarized, to the best of our knowledge, the most current evidence regarding the clinical utility of measuring the serum levels of non-HLA Abs in solid organ transplantation (Table 1). The potential mechanisms of non-HLA Abs involved in allograft dysfunction are depicted in Figure 1, whereas the characteristics of non-HLA Abs in solid organ transplantation are exposed in Table 2. Our data reveals that there is still a need for large multi-center prospective studies that address their independent role as risk markers for ABMR. Furthermore, the role they might play in stratifying the risk of graft loss in the case of concomitant HLA Abs is of great clinical relevance. As our understanding of their mechanisms improves, the use of non-HLA Abs will be continually implemented in the routine management of transplant recipients.

Table 1. Summary of the references of non-Human Leukocyte Antigen (HLA) antibody studies in solid organ transplantation.

| Specificity                | Type of Solid Organ Transplantation |
|---------------------------|------------------------------------|
|                           | Renal | Lung | Liver | Heart |
| MICA                      | [17,19–30] | [31] | [32,33] | [34–36] |
| AT1R/ETAR                 | [22,45–59] | [64] | [60–63] |
| Perlecan (LG3)            | [67–70] |       |       |
| Collagen/K-alpha-tubulin  | [73,74,78] | [71–76] |       | [77] |
| Vimentin                  | [81–84,86,87] |       |       | [88,89] |

Figure 1. Mechanisms of non-HLA antibodies in solid organ transplantation. The mechanisms depicted take place within structures that include the blood vessel (red) and its endothelial lining of cells (orange), the extracellular matrix (collagen), and inter-cellular gap junctions (black and blue). An individual cell is further depicted to illustrate antibody binding to various membrane receptors and intracellular components (e.g., tubulin). The targets are listed in the blue box and the mechanisms in the red box. The corresponding target and mechanism are defined in the white circles. Abbreviations: AT1R: Angiotensin 1 receptor. MICA: MHC class I-related chain gene A. CDC: Complement dependent cytotoxicity. HIF-1α = Hypoxia-Inducible Factor 1 alpha.
Table 2. Characteristics of non-HLA antibodies in organ transplants.

| Antibody | Solid Organ Transplant | Detection Technique | Cellular Location | Proposed Mechanism |
|----------|-------------------------|---------------------|------------------|--------------------|
|          | Renal                   |                     |                  |                    |
|          | Lung                    |                     |                  |                    |
|          | Heart                  |                     |                  |                    |
| MICA     | Pre                     | Unclear association with graft survival and ABMR | Luminex | Ligand in NKG2D receptor | Complement-dependent cytotoxicity? [100] |
|          | Post                    | Unclear association with graft survival and ABMR |          | BOS, synergistic with HLA | ABMR synergistic with HLA, CAV |
| AT1R     | Pre                     | Acute rejection      | Not associated with rejection | Commercially available ELISA | GPCR in endothelia, promoted by ischemic injury | Endothelial damage, vasoconstriction [44] |
|          | Post                    | ABMR and decreased graft survival | Possible ABMR and CMR | ABMR and CMR |
| Perlecan | Pre                     | AVR                  | -                | -                  | Home made ELISA (also available for Luminex) | Vessel wall component acts as neoantigen after caspase-dependent cleavage |
|          | Post                    | -                    | -                | -                  | - |
| Tubulin  | Pre                     | -                    | BOS, PGD         | -                  | Home made ELISA | Gap junctions, cytoskeletal protein |
|          | Post                    | -                    | ABMR driven by donor HLA, BOS | CAV, ABMR |
| Collagen | Pre                     | -                    | BOS, PGD         | -                  | Home made ELISA | Connective tissues |
|          | Post                    | TG                   | ABMR driven by donor HLA, BOS | CAV, ABMR |
| Vimentin | Pre                     | IFTA (IgG isotype)   | -                | Not associated with rejection | ELISA and/or LUMINEX | Cytoskeletal, intermediate filament |
|          | Post                    | TG (IgG isotype), rejection | - | ABMR and CAV driven by donor HLA |

Abbreviations: MICA: MHC1-related chain A gene. NKG2D: Natural Killer Group 2 D. HLA: Human Leukocyte Antigen. ABMR: Antibody-Mediated Rejection. AVR: Acute Vascular Rejection. CMR: Cell-Mediated Rejection. AT1R: Angiotensin II Type 1 Receptor. GPCR: G-protein coupled receptor. BOS: Bronchiolitis Obliterans Syndrome. PGD: Primary Graft Dysfunction. CAV: Cardiac Allograft Vasculopathy. TG: Transplant Glomerulopathy. IFTA: Interstitial Fibrosis and Tubular Atrophy. ELISA: Enzyme-Linked ImmunoSorbent Assay. HIF-1α = Hypoxia-Inducible Factor 1 alpha.
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Abbreviations

| Abbreviation | Description                      |
|--------------|----------------------------------|
| ABMR         | Antibody Mediated Rejection      |
| BOS          | Bronchiolitis obliterans syndrome |
| CAV          | coronary allograft vasculopathy   |
| DSA          | Donor Specific Antibody          |
| HLA          | Human Leukocyte Antigens         |
| IFTA         | Interstitial fibrosis and tubular atrophy |
| IRI          | Ischemia/reperfusion injury       |
| MCS          | Mechanical circulatory support devices |
| MHC          | Major Histocompatibility Complex  |
| MICA         | MHC class I-related chain gene A  |
| PGD          | Primary graft disfunction         |
| PRA          | Panel Reactive Antibody          |
| TCAD         | Accelerated transplant coronary artery disease |
| TCMR         | T Cell Mediated Rejection         |
| TG           | Transplant glomerulopathy         |

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