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Impact of immunosuppressive therapy on arterial stiffness in kidney transplantation: are all treatments the same?

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Abstract

Arterial stiffness is a biologic process related to ageing and its relationship with cardiovascular risk is well established. Several methods are currently available for non-invasive measurement of arterial stiffness that provide valuable information to further assess patients' vascular status in real time. In kidney transplantation recipients, several factors could accelerate the stiffness process, such as the use of calcineurin inhibitors (CNIs), the presence of chronic kidney disease and other classical cardiovascular factors, which would explain, at least in part, the high cardiovascular mortality and morbidity. Despite the importance of arterial stiffness as a biomarker of cardiovascular risk, and unlike other cardiovascular risk factors (e.g. left ventricular hypertrophy), only a few clinical trials or retrospective studies of kidney recipients have evaluated its impact. In this review we describe the clinical impact of arterial stiffness as a prognostic marker of cardiovascular disease and the effects of different immunosuppressive regimens on its progression, focusing on the potential benefits of CNI-sparing protocols and supporting the rationale for individualization of immunosuppression in patients with lower arterial elasticity. Among the immunosuppressive drugs, a belatacept-based regimen seems to offer better vascular protection compared with CNIs, although further studies are needed to confirm the preliminary positive results.

Key words: arterial stiffness, augmentation index, immunosuppression, kidney transplantation, pulse wave velocity

Introduction

High cardiovascular mortality and morbidity in kidney transplant patients remains a great concern. Although the 1-year survival rates post-transplantation are high, cardiovascular risk in these patients is higher than in healthy subjects [1, 2].

Cardiovascular disease is the leading cause of death and the second cause of graft loss in kidney recipients [3]. However, classic cardiovascular risk factors are not reliable predictors of cardiovascular events in this population; in fact, the risk score calculators used for the general population usually underestimate the cardiovascular risk in kidney recipients [4]. Immunosuppressive therapy and other unconventional risk factors (such as arterial stiffness) could explain the failure in predicting cardiovascular events in this population. Among immunosuppressive drugs, steroids and calcineurin inhibitors (CNIs) have a negative impact on the cardiovascular system

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[5, 6], as they are potent vasoconstrictors that directly lead to vascular fibrosis [7]. Moreover, treatment regimens based on the use of CNIs and corticosteroids increase blood pressure (through salt retention and/or hyperactivation of the renin–angiotensin system) and low-density lipoprotein cholesterol levels, thus indirectly affecting the vasculature [8, 9].

Prednisone withdrawal and CNI-sparing therapy protocols are tempting strategies to reduce the cardiovascular burden in kidney transplant recipients, but these immunosuppressive regimens might increase the risk of rejection, thereby limiting their potential clinical benefit [10]. Instead, an individualized immunosuppressive protocol could improve outcomes in selected patients with high cardiovascular and low immunological risk. Biomarkers would be useful towards such personalized medicine, but unfortunately they are still lacking. Consequently, transplant clinicians are looking for tools to predict and prevent cardiovascular events.

In contrast to this discouraging scenario, some recent studies have addressed the importance of arterial stiffness parameters as powerful predictive variables of cardiovascular events in kidney transplant recipients [11, 12].

Arterial stiffness is a biologic process related to ageing [13, 14] and blood pressure [15], but also with inflammation [16, 17], arterial calcification [18] and stage of chronic kidney disease (CKD) [19]. In kidney transplant recipients, several studies have also associated arterial elasticity with donor age [20], donor vascular stiffness (in the case of living donors) [21], new-onset diabetes post-transplantation [22], cold ischaemia time [23], renal graft function [glomerular filtration rate (GFR)] [24], hypomagnesaemia [25] and resistance training [26]. In addition, CNI therapy is known to contribute to vascular stiffness acceleration [27].

Arterial stiffness in hypertensive patients has been studied in previous clinical trials [28, 29] that showed certain classes of blood pressure–lowering drugs appear to decrease stiffness more effectively than others, although this might be related to their better control of blood pressure [30]. In kidney transplant studies, except for a few minor studies that analysed the effect of several immunosuppressive protocols on arterial stiffness, only two recent randomized clinical trials have introduced arterial stiffness as a secondary endpoint [31, 32].

Here we provide a descriptive review of the literature focusing on the usefulness of pulse wave velocity (PWV) as a predictor of cardiovascular events and on how immunosuppressive therapy could modify arterial stiffness in kidney transplant recipients.

Arterial stiffness evaluation in kidney transplantation

The three layers of the arterial wall contribute, each to a different extent, to its elastic property, which can be measured at both macro- and microscale levels [33]. With ageing and/or due to concurrent diseases (e.g. arteriosclerosis), the balance between elastin fibres and collagens tends to be disrupted in favour of the latter. This process involves several players, including matrix metalloproteinases [34–36], which degrade elastin fibres and the connections between them, calcium deposition in the tunica media and collagen glycosylation by advanced glycation end products (AGEs) [37]. Also, endothelial and vascular smooth muscle cells can affect the elastic property of the arterial wall, although the mechanisms are not well understood [33]. All the aforementioned factors cause an acceleration of arterial stiffness progression.

The gold standard for measuring arterial stiffness is intra-aortic arterial pressure measurement, an invasive method requiring arterial catheterization [38]. Nowadays, there also exist several non-invasive methods for arterial stiffness assessment [39, 40], with PWV calculation being the most widely used. Since the aorta has elastic properties, after each systole, pressure is transmitted through the aortic wall and branches, generating a forward wave with a propagation speed (PWV) that depends on the wall elasticity, being faster in patients with greater stiffness [41]. When the wave reaches the impedance points on the arterial tree, it generates a reflected, backward wave that, in presence of increased stiffness, reaches the aorta during the systole of the same cardiac cycle, thus causing an augmentation of the central aortic pressure (AP) that can be quantified as the augmentation index (Aix), defined as the percentage of the central pulse pressure attributed to the reflected pulse wave [42].

Figure 1 shows how structural changes in the aorta, small arteries and arterioles modify the PWV, Aix and pulse pressure, the three parameters commonly evaluated in studies of arterial stiffness.

A detailed description of the definition of arterial stiffness and of the validity of all methods currently available for the evaluation and measurement of aortic stiffness has been recently and exhaustively reviewed by Adenwalla *et al.* [43].

Increased arterial stiffness in kidney transplant recipients is a powerful predictor of cardiovascular events

Results from PWV measurements should be evaluated based on the patient's age. Although the 2007 European Society of Hypertension/European Society of Cardiology hypertension guidelines recommend a fixed threshold value of 12 m/s to detect patients with high cardiovascular risk [44]. More recently, a consensus document has set this value at 10 m/s [45]. However, irrespective of the cut-off value, cardiovascular risk is increased even at a lower threshold [44].

In a Dutch study including a kidney transplant cohort of 330 patients, the PWV was found to be predictive of cardiovascular events and survival, irrespective of the patient's age. Interestingly, patients with a PWV of $\geq$7.5 m/s showed worse survival rates than those with a PWV $<$7.5 m/s [46].

In 2011, in a prospective study of a cohort of 512 kidney transplant recipients, PWV, together with central AP and Aix were measured at the time of kidney transplantation. After a mean follow-up of 5 years, PWV and AP were included in a model based on clinical variables and laboratory data to predict cardiovascular events. Adding PWV and AP data led to a net reclassification improvement for cardiovascular events of 15.9%. Moreover, patients with a PWV of $\geq$8.1 m/s had worse cardiovascular survival compared with patients with a PWV $<$8.1 m/s [11].

Lastly, a recent study from a Norwegian group including 1022 kidney transplant recipients showed that below a cut-off value of 12 m/s, each increment in PWV of 1 m/s starting from 8 m/s was associated with a 36% increase in mortality risk [12].

The aforementioned studies demonstrate the powerful predictive value of PWV for cardiovascular events and mortality (see Table 1), irrespective of age and other clinical or laboratory variables, thus confirming data obtained from other studies involving different patient populations [47].

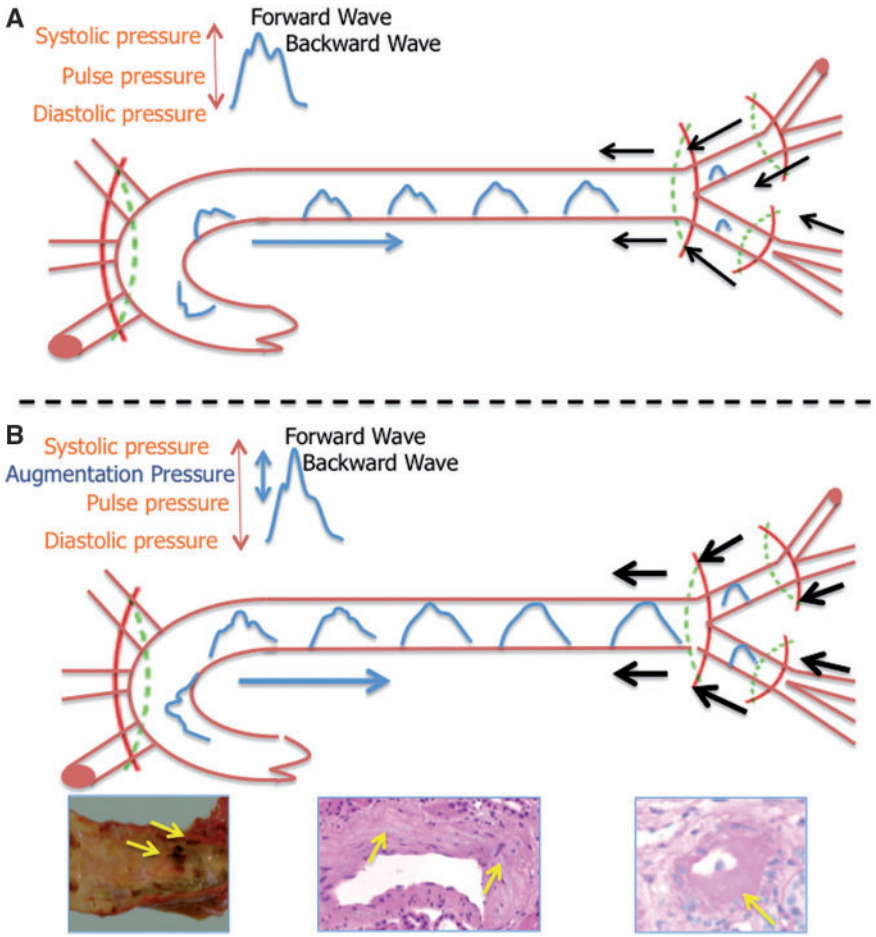

Fig. 1. Scheme of wave propagation according the PWV model. (**A**) Healthy subject/healthy artery: PWV is slow at the aortic level and fast at small (muscular) arteries. Each wave represents the sum of a unique forward wave (blue arrow) and multiple backward waves (black arrows). Backward waves are generated from reflection points located in the circulatory system: bifurcations (green dashes) and small arteries and arterioles (not shown in the figure). Due to the PWV gradient (i.e. the length and resistance at reflection points), backward waves reach, with a delay, the systolic peak of the forward wave, so there is no significant augmentation pressure (or Aix). (**B**) Patients with arterial stiffness: aortic media calcification and atherosclerotic plaque (red arrow - pulse presure) tend to increase the PWV at the aortic level. Moreover, changes in muscular tone and structure in small arteries and/or arteriolar hyalinosis (yellow arrows) increase resistance, amplifying the magnitude of reflected waves. As a consequence, more prominent backward waves reach the forward wave near the systolic peak, thus generating a notable increase in central AP, expressed as augmentation pressure.

Table 1. Main studies on the predictive power of arterial stiffness for cardiovascular endpoints (mortality and event)

Source	Number of patients	Follow-up (mean years)	Independent predictive power for CV death: PWV	Independent predictive power for CV death: Aix or AP
Mitchel *et al.* 2010 [46]	330	3.8	YES	NA
Verbeke *et al.* 2011 [11]	512	5	YES	YES
Dahle *et al.* 2015 [12]	1040	4.2	YES	NA

Aix, augmentation index; AP, aortic pressure; CV, cardiovascular; PWV, pulse wave velocity. All studies used a Sphigmocor device for calculation of PWV and Aix or AP.

Arterial stiffness and immunosuppression

As previously described, CNI toxicity on arteries is well known, at least at the microcirculatory level. Two types of toxicity for cyclosporine have been described: acute and chronic toxicity [48]. Acute toxicity is a functional alteration due to an imbalance between vasoconstrictors and vasodilators, leading to a decrease in renal blood flow and to an increase in vascular resistance, particularly at the arteriolar level. Since arteriolar network resistance is the last barrier against pulsatile pressure and represents the gate of the backward wave [49], this vasoconstriction at the arteriolar level is probably the cause of augmentation of certain stiffness parameters (Aix and AP) in patients treated with cyclosporine. Several factors seem to play a role in this acute toxicity: hyperactivation of the renin–angiotensin system [50], upregulation of endothelin receptors [51], endothelial cell injury [52], alteration in L-arginine nitric oxide production and hyperactivation of the sympathetic system [53]. Calcium antagonists and angiotensin receptor blockers (ARBs) or angiotensin-converting enzyme inhibitors (ACEis) are known to mitigate the acute toxicity, which makes

Table 2. Main studies on the effect of IMS on arterial stiffness in kidney transplant recipients

Source	Design	N	IMS	Results	Limitation
Zoungas *et al.* 2004 [63]	Longitudinal	36	24 CYC 12 TAC	PWV: no difference Aix: TAC ↓↓↓/CYC ↓	Small
Ferro *et al.* 2002 [65]	Transversal	250	146 CYC 62 TAC	PWV: NA Aix: TAC ↓ versus CYC	Design
Strzóecki *et al.* 2007 [66]	Transversal	152	76 CYC 76 TAC	PWV: TAC ↓ versus CYC Aix: NA	Design
Seckinger *et al.* 2008 [67]	Conversion CYC to EVR	27	10 CYC 17 EVR	PWV: CYC ↑; EVR ↓ Aix: NA	Small Short follow-up
Joannidès *et al.* 2011 [68]	Conversion CYC to SRL	44	21 CYC 23 SRL	PWV: CYC ↑; SRL ↓ Aix: CYC ↑; SRL ↓	Small Selection criteria
Gungor *et al.* 2011 [69]	Transversal	81	47 CNI 34 imTOR	PWV: no difference Aix: no difference	Small Mixed CNI/imTOR
Seibert *et al.* 2014 [70]	Transversal	46	23 BLC 23 CYC	PWV: no difference AP BLC ↓ versus CYC	Small Selection
Melilli *et al.* 2015 [71]	Transversal	40	20 BLC 20 CNI	PWV <8.1: BLC 60%, CNI 40% Aix: NA	Small CNI mixed
Cruzado *et al.* 2016 [72]	Conversion TAC to EVR	60	32 TAC 28 EVR	PWV: no difference	Normal PWV Small
Holdaas *et al.* 2017 [31]	Conversion CNI to EVR	164	95 CNI 69 EVR	PWV: no difference Aix: NA	Normal PWV CNI mixed

Aix, augmentation index; AP, augmentation pressure; BLC, belatacept; CYC, cyclosporine; EVR, everolimus; IMS, immunosuppression; PWV, pulse wave velocity; SRL, sirolimus; TAC, tacrolimus.

it difficult to understand how all these factors contribute to acute vascular toxicity [54, 55].

Chronic toxicity is characterized by a structural change in vessels, particularly small arteries and arterioles [56]. Hyalinosis lesions on the arteriolar wall, a hallmark of CNI toxicity, are present in protocol biopsies at 10 years in the majority of patients receiving tacrolimus or cyclosporine [57]. Treatment using the mammalian target of rapamycin (mTOR) inhibitors (imTORs) sirolimus and everolimus has been shown to attenuate allograft vasculopathy in heart transplant recipients [58–60].

Whether immunosuppressive drugs can modify PWV, directly or indirectly influencing vasculature, remains largely speculative and difficult to demonstrate for the following reasons [61]. First, transplantation per se ameliorates arterial stiffness [22, 62, 63], probably as a result of recovery in renal function. Renal function is, in fact, closely related to PWV, as shown by Ford *et al.* [19]. Moreover, transplantation allows better control of blood pressure, thus decreasing PWV, although Seibert *et al.* [64] showed that high PWV was related to cardiovascular events after kidney transplantation, regardless of peripheral blood pressure. Finally, the majority of studies published so far are small-scale, retrospective and case–control (see Table 2).

Despite these limitations, there is some evidence of a protective effect of CNI-sparing protocols on the progression of arterial stiffness in kidney transplant recipients. Table 3 summarizes the impact of different immunosuppressive drugs on PWV, Aix and blood pressure.

CNIs and stiffness

Initial data on CNI effects on large arterial functions have been conflicting. In a prospective study, Zoungas *et al.* [63] compared PWV before and after kidney transplantation in 36 patients. At 12 months post-transplantation, PWV improved in all patients, irrespective of cyclosporine or tacrolimus use, although Aix reduction was greater in patients treated with tacrolimus ($-8.0 \pm 16.5\%$ versus $-27.4 \pm 18.2\%$; $P = 0.01$).

Table 3. Main effects of different immunosuppressive drugs on PWV, Aix and blood pressure

Drugs	Systemic blood pressure	PWV	Aix or AP
Cyclosporine	+++	++	++/+
Tacrolimus	+/++	–/+	+
imTOR (everolimus or sirolimus)	–	–	–/+
BLC	–	–	–
Mycophenolate mofetil	–	?	?
Steroid	+	?	?

?, stand for No Data.

In a small study, Covic *et al.* [73] showed that cyclosporine acutely decreased the Aix. However, the study lacked a control group and the decrease in Aix after cyclosporine uptake was related to a decrease in the timing of the reflected wave, which could lead to an increased PWV in the long term.

Interestingly, in the same period, a cross-sectional study (including 250 stable kidney transplant recipients) showed that cyclosporine increased Aix and blood pressure considerably more than tacrolimus [65]. In 2007, Strózecki *et al.* [66] compared the PWV in 76 patients taking cyclosporine with 76 patients taking tacrolimus. The two study groups were matched for main clinical characteristics (age, blood pressure, time on haemodialysis, diabetes). The cyclosporine group had higher PWV values compared with the tacrolimus group (9.33 ± 2.10 versus 8.54 ± 1.35, respectively; $P < 0.01$). In another study by the same group, stepwise multiple regression analysis showed that age, male sex, mean arterial pressure (MAP), cyclosporine (versus tacrolimus) and fasting glucose concentration were independently associated with increased PWV [74]. The effect of cyclosporine on stiffness is probably due to an increase in vascular tone or to impaired nitric- oxide

vasodilation, although a study from Silverborn *et al.* [75] did not confirm this hypothesis. In their proof-of-concept study, 18 lung transplant recipients (all treated with cyclosporine) were compared with patients waiting for lung transplantation and healthy controls. Arterial resistance, non-endothelial-dependent relaxation and arterial stiffness (by echo tracking) were analysed. Lung recipients had significantly less elastic arteries than healthy controls or patients on the transplant waiting list, even though no difference in blood pressure or endothelial response to nitric oxide was seen.

Since cyclosporine was found to be related to higher PWV, conversion to tacrolimus could be an option to improve arterial stiffness. This hypothesis was tested in a small study where stable kidney recipients taking cyclosporine (>10 years) were converted to tacrolimus. PWV (by echo tracking) and ambulatory blood pressure monitoring (ABPM) were performed at baseline and repeated at 3 months post-conversion. No difference was observed in blood pressure or PWV, probably due to the short time span from conversion [76].

Despite the limitations in their study design, all the aforementioned studies suggest a possible negative impact of CNIs, and especially cyclosporine, on PWV.

imTORs and arterial stiffness

The imTORs everolimus and sirolimus are used in immunosuppressive regimens in kidney transplantation. Yet since their first use, they have not shown superior efficacy in terms of renal survival or prevention of rejection compared with tacrolimus [77, 78]. Beyond their immunosuppressive property, imTORs exert certain pleiotropic effects on atherogenesis [79, 80] and fibrosis [81], so at least in theory, kidney transplant recipients may benefit from the use of imTORs in terms of arterial elasticity.

In a randomized clinical trial, 17 of 27 patients were switched from cyclosporine to everolimus 6 months after kidney transplantation. PWV remained stable in the everolimus group (9.50 ± 1.92 versus 9.13 ± 1.62 m/s, ΔPWV −0.37 ± 1.14 m/s), whereas it was increased in the cyclosporine group (9.93 ± 1.94 versus 10.8 ± 2.24 m/s, ΔPWV + 0.89 ± 1.47 m/s) [67].

In a substudy of the CONCEPT trial [68], 23 of 44 patients were converted from cyclosporine to sirolimus 12 weeks after kidney transplantation. PWV and Aix were evaluated at weeks 12, 26 and 52. Patients in the sirolimus group experienced a decrease in PWV, whereas those in the cyclosporine group had an increase in PWV, with a significant difference at week 52. Both groups experienced an increase in Aix, which was more marked in the cyclosporine group. According to the authors, the progressive decrease in PWV in the sirolimus group was a cause, rather than a consequence, of the better blood pressure control.

Despite these encouraging results, a cross-sectional study by Gungor *et al.* [69] showed no benefit in terms of PWV or Aix in a group of patients treated with an imTOR (for at least 6 months, with either sirolimus or everolimus) compared with treatment with CNIs (cyclosporine or tacrolimus). In a linear regression analysis, only conventional risk factors (age, blood pressure, cholesterol level and proteinuria) were predictive of arterial stiffness.

More recently, a randomized clinical trial on the effect of late conversion from CNIs (tacrolimus) to an imTOR (everolimus) showed a small benefit related to regression of left ventricular hypertrophy in both groups. As secondary outcomes, changes in blood pressure (measured by ABPM) and PWV were evaluated before and after conversion. The median time from transplantation was 1.7 years for the tacrolimus group (25 patients) and 1.3 years for the everolimus group (31 patients). At 24 months from randomization, both groups had very well-controlled blood pressure, although the dipper status was preserved in more patients on everolimus (30% of tacrolimus-treated patients were non-dippers versus 22% of patients on everolimus). PWV values at baseline and 12 and 24 months were in the normal range, with no significant differences between the two study groups [72].

Another ancillary study from a recent trial [31] evaluated PWV and blood pressure by ABPM. PWV data were obtained for 277, 223 and 184 patients at randomization and months 12 and 24, respectively. Patients converted to everolimus had a slight decrease in PWV (month 12: −0.24 m/s; month 24: −0.03 m/s), whereas patients on cyclosporine experienced a progressive increase in PWV (month 12: 0.11 m/s; month 24: 0.16 m/s). Although the difference was not significant, one can argue that baseline values were in the normal range (mean 7.8 m/s for the everolimus group and 7.6 m/s for the cyclosporine group). Follow-up at 24 months confirmed the predictive value of PWV, since the incidence of cardiovascular events in the entire cohort was low (2.8% in the everolimus group and 4.8% in the cyclosporine group). In such low-risk populations, a greater number of patients is necessary in order to show any benefit in a cardiovascular endpoint (or PWV) from any therapeutic intervention (such as conversion to an imTOR). Moreover, since such small variations (0.4–0.5 m/s) usually occur over a long time span, follow-up at 24 months was probably too early a time point to detect any significant change in PWV [82, 83].

Since patients with high PWV at baseline are susceptible to a steeper increase in PWV [13], we cannot exclude the possibility that conversion to imTORs is beneficial for these patients.

Co-stimulatory blockade and arterial stiffness

The biologic immunosuppressant belatacept (BLC) is a fusion protein comprising the common fragment Fc of human immunoglobulin G (IgG) and CTL4, which, upon binding to CD80 and CD86 receptors on antigen-presenting cells (APCs), inhibits co-stimulatory signals essential for T-lymphocyte activation. In the two non-inferiority clinical trials BENEFIT and BENEFIT-EXT [84, 85], BLC was demonstrated to have an anti-rejection efficacy similar to cyclosporine. At the 3-year follow-up, patients treated with BLC showed better renal function, less renal fibrosis in protocol biopsies and a better cardiovascular profile [86]. In particular, at 12 months, systolic and diastolic blood pressures were lower in patients treated with BLC than in those treated with cyclosporine, as shown in the BENEFIT and BENEFIT-EXT trials, even though both treatment groups had the same baseline level of blood pressure. Moreover, in the BENEFIT trial, both BLC regimens [more intensive (MI) and less intensive (LI)] were associated with a 30% reduction in the odds of requiring a higher number of antihypertensive medications at month 12 ($P = 0.02$, BLC-LI versus cyclosporine A) [87].

Data analysis from these trials at different time points (12, 36 and 84 months) also showed that patients treated with BLC had a better GFR compared with patients treated with cyclosporine [87]. A long-term analysis from the BENEFIT trial at 7 years showed a 43% reduction in mortality risk or risk of graft loss with both the BLC-MI and BLC-LI regimens compared with the cyclosporine regimen [88]. Since GFR is a powerful predictor of cardiovascular events and mortality in kidney transplant recipients [89, 90], these results were not unexpected.

In addition to better survival rates related to GFR, data from other studies suggest improved control of arterial stiffness in BLC-based regimens. In an experimental model of hypertension induced by angiotensin or deoxycorticosterone acetate (DOCA)–salt, Vihn *et al.* [91] administered treatment infusion based on CTLA4-Ig (a drug with effects mimicking genetic CD80/CD86 deficiency) in mice, thus preventing hypertension acceleration. A possible influence of the immune system on hypertension is not new, since the contribution of T cells in DOCA–salt-induced hypertension in thymectomized mice was shown >25 years ago [92]. In mild hypertension, endothelial vessel damage causes the release of damage-associated molecular patterns and altered self-proteins. Hypothetically these molecules could be recognized as antigens presented by dendritic cells and thus trigger the immune system, activating T cells and stimulating cytokine production and inflammation [93], the latter being closely associated with increased vascular stiffness [16].

From a clinical perspective, only two studies have analysed the impact of BLC on arterial stiffness in kidney transplant recipients. In the first study, Seibert *et al.* [70], in a case–control retrospective study, compared 23 patients treated with BLC with 23 patients treated with cyclosporine. The two groups showed no significant differences with regard to gender distribution, age, body mass index, time on dialysis prior to transplantation and time since transplantation. After a mean follow-up of 88 months (all patients included had a minimum time from transplantation of 20 months and were first-kidney recipients), augmentation pressure was significantly better in the BLC group [augmentation pressure 12.7 mmHg (range 8.3–16) versus 7.3 (2.3–11.7); $P = 0.048$], despite no differences in systolic and diastolic blood pressures (both peripheral and central). PWV mean values were identical in both groups (8.8 m/s).

In the second study [71], our group compared 20 patients treated with BLC with 20 patients on CNIs (16 on tacrolimus and 4 on cyclosporine). The control CNI group was matched for all the main variables affecting PWV. There were no differences in median PWV between the two groups: 7.9 ± 3.4 m/s (range 4.1–12) in the CNI group and 7.4 ± 4 m/s (range 5.2–15.5) in the BLC group ($P = 0.4$). Due to the large discrepancy in age in our population study, we chose a value of 8.1 m/s of femoral-carotid PWV as the cut-off value for high arterial stiffness, which was shown to correlate with an increased cardiovascular mortality risk in a recent retrospective study performed in a transplant population [11]. In that study, 50% of patients in the CNI group had a PWV >8.1 m/s versus 25% of patients in the BLC group ($P = 0.08$). Regression logistical analysis showed that age, renal resistive index at 3–6 months after transplantation and BLC [odds ratio 0.008 (95% confidence interval 0.004–0.890); $P = 0.045$] were predictive variables of PWV.

Although these two studies have some limitations (transversal, lack of a baseline record of arterial stiffness measurements), data on PWV and augmentation pressure suggest that improvement in arterial stiffness could be obtained using BLC as the main immunosuppressant.

Limitations

Although the number of publications on arterial stiffness in kidney transplantation is increasing, most studies present limitations that warrant caution in interpreting the results. As with any method of measurement performed by an operator, there is a risk of high interobserver variation. Although the techniques used to measure arterial stiffness have been validated in terms of reproducibility in healthy and CKD patients [29, 94, 95], most published studies on kidney transplantation do not report data on intra- or interobserver variability, making it difficult to assess and compare the data quality of each study. In fact, only a few studies on kidney transplantation have reported an acceptable variation coefficient index (intraclass correlation coefficient) for operator variability [21, 76, 93]. There is also an extreme paucity of data on intrapatient variations in stiffness parameters.

Confounding factors represent another limitation relevant to arterial stiffness studies, including blood pressure and the duration of kidney disease, both closely related to arterial stiffness. In kidney transplantation, studies published so far have only reported the impact of time on dialysis [22, 25, 66], whereas data on the duration of disease and/or blood pressure are scarce. Moreover, most of these studies analysed only the relationship between a single determination of blood pressure and stiffness parameters. More rigorous blood pressure determinations using ABPM and repeated arterial stiffness measurements are needed in order to confirm a possible independent effect of immunosuppression on arterial stiffness. Other confounding factors such as diabetes and disorders of mineral and bone metabolism are also related to arterial stiffness in kidney transplantation [25, 68, 96]. The use of vitamin D supplementation may produce a decrease in PWV in CKD patients [97], although no data on vitamin D repletion in recipients are available. Nonetheless, paricalcitol did not seem to exert a reducing effect on PWV after 1 year of treatment, as shown by Pihlstrøm *et al.* [32] in a randomized trial on the effect of paricalcitol on parathyroid hormone levels in kidney transplant recipients.

In conclusion, all these confounders mask the true magnitude of the impact of immunosuppressive regimens on arterial stiffness.

Open questions and ideas for future clinical studies

Measurements of PWV, Aix and AP usually take between 20 and 30 min when performed by an experienced operator, which stands as one of the obstacles to widespread use of arterial stiffness evaluation in patients on transplant waiting lists or after transplantation. Although magnetic resonance and/or certain blood pressure devices can assess stiffness parameters, thus rendering these tests less cumbersome, these parameters have not yet been proven to be predictive of cardiovascular events [43, 98, 99]. Moreover, measurement devices approved by regulatory agencies is often unaffordable in many health care systems, thus restricting their use to research purposes only.

Since baseline high PWV values predict a higher cardiovascular risk, future trials in transplantation could include basal PWV as a biomarker to discriminate those patients at very high cardiovascular risk who could benefit from a CNI-free immunosuppressive regimen (BLC-based, for example). Moreover, monitoring of PWV after transplantation at different time points could identify those patients with rapid progression of arterial stiffness who could benefit from conversion of CNIs to imTORs or who need tighter control of mineral and bone metabolism or blood pressure.

Conclusion

Kidney transplant recipients with higher values of PWV are at increased cardiovascular and mortality risk. Preliminary data from small studies indicate that CNIs, and especially cyclosporine, could increase PWV in renal transplant recipients. Although some studies suggest a possible protective effect of

imTORs on arterial stiffness, data from two randomized trials have not shown significant differences after either early or late conversion of CNIs to imTORs. The reduction in cardiovascular mortality risk shown by long-term results from BLC trials could be due to a decrease in arterial stiffness, which warrants further investigation.

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Conflict of interest statement

None declared.

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