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Telomere Length Assessment for Prediction of Organ Transplantation Outcome. Future or Failure: A Review of the Literature

Authors' Contribution:
Study Design A
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Statistical Analysis C
Data Interpretation D
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Literature Search F
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Telomeres are located at each end of eukaryotic chromosomes. Their functional role is genomic stability maintenance. The protective role of telomeres depends on various factors, including number of nucleotides repeats, telomere-binding proteins, and telomerase activity. Organ transplantation is the preferred replacement therapy in the case of chronic kidney disease and the only possibility of sustaining recipients' life in the case of advanced liver failure. While the prevalence of acute rejection is constantly decreasing, prevention of transplanted organ long-term function loss is still challenging. It has been demonstrated that post-transplant stressors accelerate aging of the allografts manifested through telomere shortening.

The aim of this paper was to evaluate the importance of telomere length assessment for prediction of organ transplantation outcome. Literature review included the 10 most important studies regarding linkage between allograft function and telomere erosion, including 2 of our own reports.

Telomere length assessment is useful to predict organ transplantation outcome. The importance of telomere length as a prediction marker depends on the analyzed material. To obtain reliable results, both graft cells (donor material) and lymphocytes (recipient material) should be examined. In the case of kidney transplantation, assessment of telomere length in the early post-transplant period allows prediction of the long-term function of the transplanted organ. To increase the accuracy of transplantation outcome prediction, telomere length assessment should be combined with evaluation of other aging biomarkers, like CDKN2A (p16). Large-scale clinical studies regarding telomere length measurement, including genome wide association analysis introducing relevant genetic factors, are needed for the future.

MeSH Keywords: **Aging • Allografts • Cell Aging • Organ Transplantation • Telomere Homeostasis**

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Background

Telomeres are noncoding structures located at each end of eukaryotic chromosomes. Their functional role is genomic stability maintenance [1]. The protective role of telomeres depends on various factors, including number of nucleotides repeats, telomere-binding proteins, and telomerase activity [2]. Disturbances in telomere erosion have serious consequences. Among them premature aging and cancer predisposition, associated with telomerase insufficiency, are the most common [3]. Critical reduction of telomere length may contribute to the proliferative potential limitation through inability to attach proteins [1]. A normal human cell responds to telomere dysfunction by undergoing apoptosis or arresting the cell cycle. While apoptosis – programmed cell death – is the cause of aging, alteration of the cycle and cell immortality are the hallmarks of cancer development [4]. It is known that factors like age, sex, or stress influence telomere length [5-8]. However, not least important are the genetic factors, especially because several polymorphisms significantly affect telomere erosion. Genetic *loci* within *hTERT*, *TERC*, and *BICD1* genes, as well as two *loci* within chromosome 18, were studied thoroughly [9–13].

Organ transplantation is the preferred replacement therapy in the case of chronic kidney disease and the only possibility of sustaining recipients' life in the case of advanced heart or liver failure [14–16]. While the prevalence of acute rejection is constantly decreasing, prevention of transplanted organ long-term function loss is still challenging [17]. Moreover, it was demonstrated that post-transplant stressors accelerate aging of the allografts manifested through telomere shortening, resulting in organ function impairment [18]. These observations were based on earlier reports regarding both biological aging and chronic rejection of transplanted kidney [19–21]. It seems obvious that long-term allograft dysfunction is linked with telomere erosion; thus, attempts to use telomere length assessment for prediction of organ function have been made [22]. Nevertheless, these studies are scarce and need systematization.

The aim of this paper was to evaluate the importance of telomere length assessment for prediction of organ transplantation outcome. Literature review included the 10 most important studies regarding linkage between allograft function and telomere erosion, including 2 of our own reports. To help readers, the most important features of the described studies are presented in Table 1.

Telomere Length Assessment in Kidney Allografts

The problem of replicative senescence *in vivo* encouraged Ferlicot et al. to assess telomere length together with a specific marker, senescence-associated beta-galactosidase (SA- β -Gal), in

human kidney allograft undergoing chronic allograft nephropathy (CAN) [19,21]. Currently, this term has been replaced with chronic allograft dysfunction (CAD), which is a much broader definition of long-term transplanted kidney function impairment. The diagnosis of CAD is based on functional and morphological (biopsy confirmed) deterioration of renal allograft at least 3-6 months after transplantation, whereas diagnosis of CAN was based on tissue examination [23–25]. Ferlicot et al. studied 67 cases of CAN and 13 controls. They measured telomere length in cells expressing or not expressing SA- β -Gal as a marker associated with CAN and found that telomere length was significantly lower in SA- β -Gal(+) cells ($p < 0.01$). Not entirely directly, these results showed that long-term dysfunction of transplanted kidney is inextricably linked with telomere erosion. The second, most important observation was that the age of the donor was correlated with the occurrence of SA- β -Gal(+) cells and appeared to be the major determinant factor in replicative senescence [19]. Ferlicot et al. opened the discussion on the clinical importance of transplanted organ aging and its implications. Thus, the next question was: what other factors influence the allograft cells' senescence? The answer to this question showed that although chronological donor age is the most potent predictor of long-term kidney transplantation outcome, the individual differences and post-transplant stressors might also affect the allograft aging process [26]. Koppelstaetter et al. sought a specific biomarker that would be of high predictive value for kidney transplantation outcome [26]. They analyzed telomere length in 54 zero-hour biopsy samples and its association with various clinical parameters, including graft function. The potential benefits of such an approach in kidney transplantation are clinically important. Indeed, it was shown that telomere length is a significant negative factor (the shorter the telomeres, the greater the function deterioration) associated with transplanted kidney function expressed as a 1-year creatinine concentration measurement ($p = 0.036$). However, another aging marker – CDKN2A (p16) – together with donor age turned out to be the best predictors of the transplantation outcome ($p < 0.001$ and $p = 0.001$, respectively) [26]. The main difference between telomere erosion and CDKN2A as aging biomarkers is their correlation with chronological age. While telomere length is negatively associated with donor age, levels of CDKN2A correlate positively. A quite recent study by Gingell-Littlejohn et al. once again proved that telomere length, the “most celebrated” biomarker, might not be as useful as CDKN2A for prediction of kidney transplantation outcome [27]. However, despite the demonstrated strength of CDKN2A as a pre-transplant predictor of post-transplant serum creatinine 6 and 12 months after transplantation ($p = 0.02$ and $p = 0.012$, respectively), telomere length was also significantly associated with 6- and 12-month estimated glomerular filtration rate (eGFR) ($p = 0.038$ and $p = 0.041$, respectively) [27].

Table 1. Characteristic of chosen studies regarding association between telomere length and organ transplantation outcome.

First author and year	No of ref.	Type of organ	Study sample	No of study sample	p-value for correlation between TL and relevant clinical features			
					DGF	AR	CAN/CAD	Cr/eGFR
Ferlicot, 2003	[19]	Kidney	Human, kidney cells	67	–	–	<0.01 [#]	–
Koppelstaetter, 2008	[26]	Kidney	Human, kidney cells	54	–	–	–	0.036 ^{**}
Trzonkowski, 2010	[29]	Kidney	Human, CD4 ⁺ and CD8 ⁺ cells	36	–	0.0002	–	–
Gelson, 2010	[30]	Liver	Human, lymphocytes	97	–	–	–	–
Gingell-Littlejohn, 2013	[27]	Kidney	Human, kidney cells	43	–	–	–	0.038 [*] and 0.041 ^{**}
Uziel, 2013	[31]	Liver	Human, lymphocytes	62	–	0.005	–	–
Aini, 2014	[33]	Liver	Human, hepatocytes	20	–	–	–	–
Oetting, 2014	[28]	Kidney	Human, lymphocytes	1805	–	NS	NS	–
Domański, 2015	[34]	Kidney	Human, kidney cells	119	<0.05	0.047	0.038	0.01 ^{**} and 0.006 ^{***}
Kłoda, 2015	[25]	Kidney	Human, kidney cells	119	–	–	–	NS ^{****}

TL – telomere length; Cr – creatinine; eGFR – estimated glomerular filtration rate; NS – not statistically significant. [#] p-value reflecting the correlation between TL and SA-β-Gal used as the marker of CAN; ^{*} p-value for 6-month measurement; ^{**} p-value for 12-month measurement; ^{***} p-value for 18-month measurement; ^{****} p-value for 24-month measurement.

Oetting et al. went with their assumptions even further in a very comprehensive and large report [28]. They hoped to establish a marker that would allow them to determine the period of allograft functionality. Their study enrolled 1805 recipients and 1038 living kidney donors. Peripheral blood white cells were collected for telomere length analysis from all participants. The authors found no associations between acute rejection (AR) episodes or chronic graft dysfunction and mean telomere length. Significant correlations were merely regarding donors' and recipients' age. It should be noted, however, that Oetting et al. did not use allograft cells in their study, but lymphocytes only [28]. The origin of the biological material used in the analysis is extremely important, because telomere length seems to be a double-edged sword. Although donor age is negatively correlated with transplantation outcome, the age of the recipient might be an ally in increasing allografts' acceptance rate. Trzonkowski et al. recruited 36 kidney recipients and divided them according to age and history of AR [29]. The telomere length was evaluated in CD4⁺ and CD8⁺ T-cell subsets. Elderly (≥60 years of age) individuals without AR were characterized as having shorter telomeres in comparison to elderly recipients with AR (p=0.0002). What's interesting is that

these differences were not found in the younger group (<60 years of age). The main conclusion of that study was that a decrease in recipients' T-cell telomere length resulted in lower response to kidney allograft [29].

Telomere Length Assessment in Liver Allografts

Apart from reports on cellular senescence in kidney transplantation, similar studies also were conducted among liver transplant recipients. Although delayed graft function (DGF) is characteristic for kidney transplantation, the pathogenesis of AR and CAD is common for all solid organ transplants. Gelson et al. enrolled 97 individuals with liver allografts and observed that lymphocytes from liver transplant recipients expressed more phenotypic markers of maturity (shorter telomeres) than did lymphocytes from controls [30]. Increasing age and previous cytomegalovirus infection were also associated with a decrease in telomere length. Unfortunately, these authors did not evaluate the possible linkage between AR episodes and long-term organ function [30]. The idea of not only the graft's, but also

the recipient's, accelerated aging was a novel one. Therefore, other authors did continue investigating this concept. In 2013 Uziel et al. published a very interesting study on 62 liver transplant recipients and 59 healthy control subjects [31]. They assessed telomere length in peripheral blood lymphocytes and assumed that not only the allografts, but also their recipients, may exhibit premature aging. Indeed, mean telomere length was significantly shorter among the transplant group, but this group was also older. However, the authors proved that increased telomere erosion among the recipients resulted rather from immunological background. In confirmation, they noted a direct correlation between AR episodes and shortened telomeres ($p=0.005$). Moreover, there was no significant association between telomere length and underlying liver disease or presence of the metabolic syndrome [31].

Aini et al. analyzed telomere length not in lymphocytes, but in liver allograft cells [32]. They recruited 17 pediatric patients with good organ tolerance and a long median of post-transplant observation (10.4 years). The measurement of telomeres was based on their signal intensity assessment. It was demonstrated that mean telomere signal intensity in liver biopsy specimens was significantly lower than predicted according to age and that its decline was correlated with development of idiopathic post-transplantation hepatitis (paper not presented in Table 1) [32]. Two years later, the same authors published a second report on 20 pediatric patients, but this time they measured the telomere length with real-time polymerase chain reaction (PCR). They obtained similar results to those described earlier. However, this time they emphasized that allografts age more rapidly than in the normal population, even in the state of tolerance [33]. This conclusion is of great practical importance, since it clearly shows that a lot of effort should be put into stopping the progressive aging of transplanted organ.

Authors' Own Research on Kidney Transplantation

Based on the aforementioned studies and because of the growing need for increasing the vitality of transplanted organs, we analyzed kidney allografts' telomere length in 119 Caucasian recipients. Biopsy specimens were collected in zero hour and at 3, 6, 12, 18, 24, 36, 48, and 60 months after transplantation. We found significant differences in telomere length between patients with DGF and without DGF ($p<0.05$) and assumed that they resulted from ischemia-reperfusion injury, because they were present only in the early period after transplantation. Moreover, significantly shorter telomeres in biopsy specimens collected ≥ 18 months after transplantation were associated with AR episodes ($p=0.047$). Although the majority of AR

episodes occur during the first 12 post-transplant months, the destructive effect of the host's immune response is long-term. Consequently, we observed accelerated telomere erosion in recipients with CAD ($p=0.038$). Strong associations between long-term creatinine concentrations (12, 12–18, and 18 months) and telomere length in early collected biopsy specimens were also present ($p=0.01$, $p=0.009$, and $p=0.006$, respectively). These results convinced us that assessment of the graft cells' telomere length in the early period after transplantation predicts the long-term function of kidney transplant. Moreover, post-transplant stressors like DGF or AR contribute to increased telomere erosion and, thus, accelerated graft aging [34]. To complete these conclusions, we have evaluated polymorphisms influencing the telomere length. Analysis of grafts' rs2735940 *hTERT*, rs2630578 *BICD1*, and rs7235755/rs2162440 chromosome 18 polymorphisms in the same group of kidney allograft recipients ($n=119$) gave additional information. The TT genotype of the rs2735940 *hTERT* gene polymorphism was significantly associated with shorter telomere length but only in biopsy specimens taken 0 and 0–6 months after transplantation ($p=0.036$ and $p=0.047$, respectively). Such observations were partly contrary to those of Zhang et al. [10]. However, they described the *hTERT* gene promoter haplotypes, whereas we focused on one polymorphism. In contrast to the evidence presented by previous publications of other authors, we found no differences in telomere length regarding rs2630578 *BICD1* gene polymorphism genotypes. In the case of the rs7235755 chromosome 18 polymorphism AA genotype, significant correlations were found, but they differed depending on the time of material collection. Nevertheless, we showed that genetic factors also influence telomere length and must be considered in further studies [25].

Conclusions

Telomere length assessment is useful to predict organ transplantation outcome. The importance of telomere length as a prediction marker depends on the analyzed material. To obtain reliable results, both graft cells (donor material) and lymphocytes (recipient material) should be examined. In the case of kidney transplantation, assessment of telomere length in the early post-transplant period allows prediction of long-term function of the transplanted organ. To increase the accuracy of transplantation outcome prediction, telomere length assessment should be combined with evaluation of other aging biomarkers, like CDKN2A (p16). Large-scale clinical studies regarding telomere length measurement, including genome wide association analysis introducing relevant genetic factors, are needed for the future.

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