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ORIGINAL ARTICLE

Comparison of relative telomere length measured in aortic tissue and leukocytes in patients with end stage heart failure

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Abstract

OBJECTIVES: Telomeres are repetitive non-coding DNA sequences on the ends of eukaryotic chromosomes. Relative leukocyte telomere length (LrTL) is considered to reflect biological ageing and fitness. Therefore, we examined whether LrTL would reflect rTL in aortic tissue (ArTL) and whether it could be used as a marker of biological heart age.
DESIGN: We analysed telomere length in aortic and leukocyte samples from 73 heart recipients (63 males, 10 females; age 52.2±11.7 years). Relative telomere length was measured using a quantitative PCR-based method.
RESULTS: Neither LrTL nor ArTL correlated significantly with the age of heart recipients. Mean ArTL was slightly shorter than LrTL (p=0.06) and there was a slight but significant inverse correlation between LrTL and ArTL (p=0.019).
CONCLUSIONS: The age of patients with end stage heart failure was not associated with leukocyte or aortic telomere length. An inverse correlation between LrTL and ArTL suggests that LrTL is unlikely to be an important predictor of biological ageing in these patients.

Abbreviations:

ArTL	- relative telomere length in the aorta
CAD	- coronary artery disease
DCM	- dilated cardiomyopathy
DNA	- deoxyribonucleic acid
LrTL	- relative telomere length in leukocytes
qPCR	- quantitative polymerase chain reaction
SCG	- single-copy gene
TL	- telomere length

INTRODUCTION

Heart transplantation is an effective treatment for severe end-stage heart diseases, both in patients suffering from cardiomyopathies and coronary artery disease. Thousands of successful transplantations are performed worldwide every year, with patients, in the main, displaying favourable prognoses. Median survival expectation for patients with transplanted hearts is more than ten years (Toyoda *et al.* 2013). Despite this huge success, there are still many both short-term (primary graft failure, infections or graft rejection) and long-term (neoplasma or cardiac allograft vasculopathy) complications which lead to organ failure and the subsequent death of the patient (Mangini *et al.* 2015). So far it has proved impossible to precisely or timely predict the risks of developing these complications in heart transplant patients.

One possibility that has been discussed with respect to risk prediction is to estimate the biological age of the patient using telomere length analysis (Chkhotua *et al.* 2002).

Telomeres are repetitive (the hexanucleotide tandem repeat TTAGGG, which is replicated more than 2000 times in humans) non-coding DNA sequences on the ends of chromosomes in eukaryotic cells. Telomeres maintain genome stability during cell replication and play an important role in human ageing and age-related diseases (Blackburn *et al.* 2015). Biological ageing is characterised by a reduction in telomere length and may provide an explanation for highly variable symptoms of (not only) heart failure in transplanted patients. It is accepted that short telomeres lead to a decrease in functional cells, which contributes to overall tissue and organ dysfunction (Wong *et al.* 2010). Shorter telomere length has been previously described in association with many health complications, e.g. coronary artery disease, family history of myocardial infarction, hypertension, diabetes mellitus, obesity and smoking, although the mechanisms of these associations are not fully understood (Benetos *et al.* 2001; 2004; Panayiotou *et al.* 2010; Révész *et al.* 2014; Valdes *et al.* 2005; Willeit *et al.* 2014). Telomere length seems to be a reasonable variable in helping to estimate the risks associated with heart transplantation.

However, telomere length (TL) varies between tissues and there is very limited knowledge about the correlations of telomere length between different tissues. One study of a small number of patients suggested that there could be a relation between leukocyte TL and TL in aortic tissue in patients with asymptomatic abdominal aortic aneurysms (Wilson *et al.* 2008).

The aim of this study was to test in a reasonable number of subjects whether there would be a strong correlation between leukocyte telomere length and telomere length in the aorta, and whether leukocyte telomere length would reflect telomere length in this tissue for use as a proxy of biological heart age.

MATERIALS AND METHODS

Subjects

Out of the 99 patients undergoing ortotopic heart transplantation at the Institute for Clinical and Experimental Medicine in Prague between January 2013 and December 2014, a total of seventy-three adult patients (63 males and 10 females) were included in the study. Aetiology of heart failure was coronary artery disease (N=34), dilated cardiomyopathy (N=29), congenital heart defect (N=5), myocarditis (N=2), Marfan syndrome (N=1), arrhythmogenic right ventricular cardiomyopathy (N=1) and finally non compacted cardiomyopathy (N=1).

Aorta samples of recipients were taken during heart transplantation. Blood samples were collected six months after transplantation. The protocol of this study was carried out according to the principles of the Declaration of Helsinki. All examined individuals were of Caucasian ethnicity and all signed informed consent forms, which, together with the protocol of the study, were approved by the institute's ethics committee.

DNA isolation

DNA was isolated using the modified salting out method (Miller *et al.* 1988) from i/ blood leukocytes of patients undergoing heart transplantation and ii/ from the tissue of explanted aortas obtained from identical patients. A maximum 100 mg of tissue was used for cell lysis using Proteinase K (Fermentas). The quantity and purity of isolated DNA was examined using standard spectrophotometry on the Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). DNA integrity was tested on 1.0% agarose gel (Bio Rad Power Pac 300) after visualisation using ethidium bromide.

Measurement of telomere length

Relative telomere length was analysed as described previously (Cawthon, 2002, Salpea *et al.* 2008) with slight modifications (Dlouha *et al.* 2014 and 2016). Briefly, analysis was performed on the Rotor-Gene 3000 (Corbett Research Ltd) using a quantitative polymerase chain reaction (qPCR)-based method. All samples were analysed in triplicate. Oligonucleotide sequences were as follows – telomere analysis 5′ GGT TTT TGA GGG TGA GGG TGA GGG TGA GGG TGA GGG T and 5′ TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA; single copy gene 5′ CAG CAA GTG GGA AGG TGT AAT CC and 5′ CCC ATT CTA TCA TCA ACG GGT ACA A. Relative telomere length was calculated as the ratio of telomere repeats to a single-copy gene (SCG) (T/S ratio). The acidic ribosomal phosphoprotein PO (36B4) gene was selected as the SCG. All qPCRs were performed in triplicate on a Rotor-Gene 3000 (Corbett Research Ltd). In order to examine the measurement stability of telomere length by qPCR analysis, both intra-assay (1.9–6.9%) and inter-assay reproducibility were evaluated (3.4–14.8%)

(Dlouha *et al.* 2012). Moreover, inter-plate calibration was performed to quality control each run.

Statistical analysis

Analyses were performed with GraphPad Prism 5 statistical software. Normal distribution of TL data was examined using the Shapiro-Wilk W test. Telomere length values were logarithmically normalised efore the analysis to obtain the normal distributions of data. Comparison between the two groups was performed using the Student's t-test. A linear regression model was used to evaluate the association between TL and age. P values less than 0.05 were considered to be significant.

RESULTS

The basic characteristics of the study group are described in Table 1. Patients with CAD were older than those with DCM ($p<0.01$) and CAD was more frequent in males ($p<0.01$).

Relative telomere length was analysed successfully in all included patients and their aortas. Mean relative telomere length was 0.94±0.15 in leukocytes and 0.88±0.16 in aortas, but the difference did not reach statistical significance ($p=0.06$). Neither LrTL nor ArTL was associated with the age of the patients (Figure 1). Similar results were obtained when males and females were analysed separately.

LrTL and ArTL were not different between patients with different aetiologies of heart failure (Table 2).

Unexpectedly, we detected an inverse correlation between LrTL and ArTL in patients who underwent ortotopic heart transplantation ($p<0.02$, for more details see Figure 2).

DISCUSSION

This is the first study to date to focus on relative telomere length in tissues of patients with end stage heart failure/transplanted hearts. We have detected inverse association between the LrTL and ArTL. The opposite relationship between LrTL and rTL in the aortic tissue of patients with asymptomatic abdominal aortic aneurysms has been described previously (Balistreri *et al.*

Tab. 1. Basic characteristics of the patients included in the study.

N	73
Males/females (N)	63/10
Age (years)	52.0 ± 12.1
Aetiology of heart failure	
Dilated cardiomyopathy (N)	29
Coronary artery disease (N)	34
Others (N)	10
LVAD before heart transplantation	6
LrTL	0.94 ± 0.15
ArTL	0.88 ± 0.16

Data are expressed as a mean ± SD

Tab. 2. Comparison of subgroups according aetiology of heart failure.

	Ischemic	Non Ischemic	*p*-value
N	34	39	ns
Age	56.7±7.3	48.2±13.4	0.01
Males/Females	33/1	30/9	0.01
LrTL	0.93±0.14	0.94±0.15	ns
ArTL	0.88±0.16	0.88±0.16	ns

Data are expressed as a mean ± SD

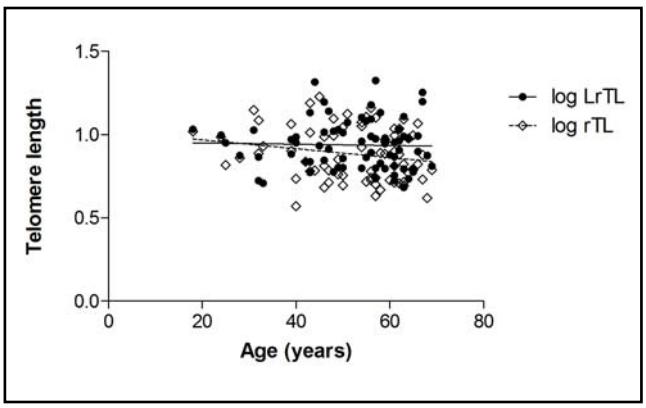

Fig. 1. Correlation between relative telomere length and age in DNA from A) peripheral blood leukocytes and from B) explanted aortas.

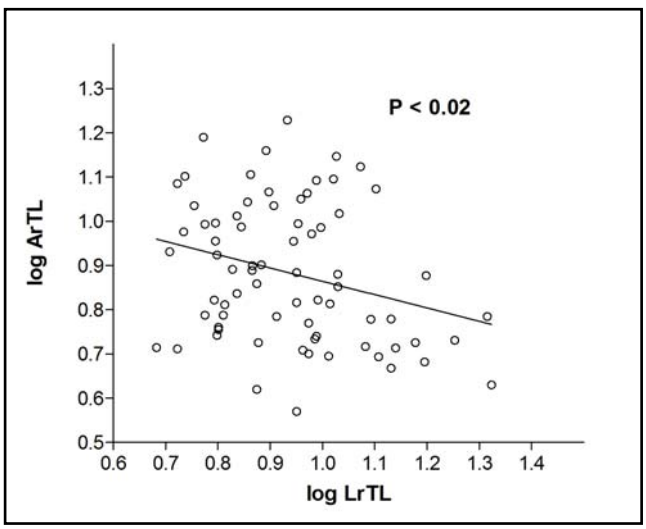

Fig. 2. Correlation between relative telomere lengths measured in DNA from peripheral blood leukocytes (LrTL) and explanted aortas (ArTL). Non parametric Spearman´s correlation $p=0.02$; Linear regression $p=0.017$.

2014; Wislon *et al.* 2008), but the question still remains whether the discrepancies in results may be due to the different methods and sample sizes used.

The role of telomere length analysis in transplantology and general medicine as a possible predictor of many pathologies has been the subject of much recent discussion. LrTL has been intensively analysed in association with a wide list of behavioural, demographic, and health variables (Sanders & Newman 2013). The results, however, are conflicting in some aspects and do not offer simple conclusions. This may be caused not only by different definitions applied to groups under study, but also by various technical difficulties. For example, methods used are often comparative and do not achieve the level of precision typical for clinical tests. Importantly, LrTL is suggested in most, but not in all, (Bojesen 2013; Njajou *et al.* 2009; Mather *et al.* 2011; Strandberg *et al.* 2011) studies as a predictor of total mortality, which indicates the general importance of telomere length for maintaining favourable health status. Finally, the inverse correlation between mean leukocyte telomere length and age has been conclusively proved (Müezzinler *et al.* 2013) and analysis of relative telomere length in leukocytes has been shown to be a promising tool for estimating biological age and cellular ageing (Zhang *et al.* 2014). Nevertheless, from the studies performed thus far, it is not clear whether shorter telomeres are a cause of disease/shorter life expectancy or whether they are only a marker of early death.

As the predictive issue of relative telomere length is of extreme interest at present, there are some issues that make this type of prediction difficult. Most importantly, studies focusing on the association between telomere length and health, disease and/or age are generally performed on more or less "normal" populations or patients (Bojesen 2013; Sanders & Newman 2013).

In our study we focused on the analysis of TL in patients with end stage heart failure/heart transplantation. Patients after heart transplantation are subjects that have undergone extensive pre-selection. Firstly, they suffer from severe heart failure (mostly cardiomyopathies or severe coronary artery disease), which can significantly influence general cellular fitness and possibly telomere length; since it is understood that many stressors shorten telomere length (reviewed by Sanders & Newman 2013). Secondly, patients after heart transplantation represent a further subset of patients with severe heart failure. For different reasons, many of these patients will not survive leading up to transplantation, or deteriorate within the first month after transplantation. These two major points may explain why we observed an unexpected inverse correlation between ArTL and LrTL, which goes against general expectation (Zhang *et al.* 2014). Finally it was suggested that immunosuppressive treatment could influence telomere length. *In vitro* experiments shown that cyclosporine A and tacrolimus have pronounced prosenescence effect and led to shortening of telomere length in human peripheral blood mononuclear cells (Welzl *et al.* 2014).

Importantly, in almost all studies TL in leukocytes is analysed (Sanders & Newman 2013; Bojesen 2013; Müezzinler *et al.* 2013), as it is the most readily available tissue sample. However, there are doubts about the relevant correlations between TL in leukocytes and TL in different tissues. In fact there are different life-spans for different cells from different tissues, which can strongly influence the potential (if any) correlation between leukocyte TL and organ TL.

Some previous studies have either focused on analysing telomere length within different tissues, including a relatively low number of examined subjects (Buttler *et al.* 1998; Dlouha *et al.* 2014), or have analysed a narrow spectrum of tissues (Lakowa *et al.* 2015), or both (Friedrich *et al.* 2000). Thus, our knowledge of how LrTL reflects the situation within the body's tissues is sparse and equivocal.

Studies of telomere length in patients after heart transplantation are still at an incipient stage. Our study suggests that there could be a weak correlation between leukocyte telomere length and aortic telomere length. Further studies are needed in order to evaluate whether telomere length is a useful marker for routine clinical use.

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