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TELOMERE SHORTENING: A NEW PROGNOSTIC FACTOR FOR CARDIOVASCULAR DISEASE POST-RADIATION EXPOSURE

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Telomere length has been proposed as a marker of mitotic cell age and as a general index of human organism aging. Telomere shortening in peripheral blood lymphocytes has been linked to cardiovascular-related morbidity and mortality. The authors investigated the potential correlation of conventional risk factors, radiation dose and telomere shortening with the development of coronary artery disease (CAD) following radiation therapy in a large cohort of Hodgkin lymphoma (HL) patients. Multivariate analysis demonstrated that hypertension and telomere length were the only independent risk factors. This is the first study in a large cohort of patients that demonstrates significant telomere shortening in patients treated by radiation therapy who developed cardiovascular disease. Telomere length appears to be an independent prognostic factor that could help determine patients at high risk of developing CAD after exposure in order to implement early detection and prevention.

INTRODUCTION

Recent epidemiological studies have documented vascular and cardiovascular effects following exposure to radiation at doses much lower than those normally associated with cardiac injury when the irradiation is localised to the mediastinal field[1, 2]. According to the International Commission on Radiological Protection[3], excess cardiovascular risk has been proven with a threshold acute dose of ~0.5 Gy for both cardiovascular and cerebrovascular diseases[4]. In addition, several cardiovascular risk factors that could potentially account for most of the risk for coronary heart disease in a population[5] were proposed. At the individual level, there is wide variation in both the occurrence of coronary heart disease and the age of manifestation, even in individuals with the same classical risk factors. The reasons for this wide inter-individual variation in susceptibility are poorly understood. The hypothesis has emerged that inter-individual variation for the risk of coronary heart disease might result from variations in the rate of biological ageing[6, 7].

Telomeres are found at the very ends of chromosomal DNA and are involved in the maintenance of genome stability[6, 8]. Mean telomere length is considered to be a marker for biological age with shorter telomeres indicative of greater biological age[9]. Telomere shortening in peripheral blood lymphocytes has been linked to cardiovascular-related morbidity and mortality in the general population[10, 11]. However, a link between telomere shortening and the vascular effects of ionising radiation is still lacking[12].

The aim of the present study was to investigate conventional risk factors as well as radiation dose and telomere shortening in a large cohort of HL patients who developed coronary artery disease (CAD) post-treatment as biological age could affect susceptibility to coronary heart disease.

Hodgkin lymphoma is a malignant haematologic disease for which current treatment provides a very high cure rate. Because of this successes, the first amongst the best examples in oncology, and because this disease affects young patients, it is the object of intense interest amongst oncologists and radiobiologists, insofar as lessons learned from the study of this disease and its treatment can serve as a basis for the therapeutics and the follow-up of other cancers. Nevertheless, the cohort study of Hodgkin's disease patients shows that the survival of these patients who are essentially cured is much lower than that which would be expected[13]. The risk of these former patients of dying from a complication of treatment,

given in the distant past, is greater than the risk of dying from a recurrence of the disease itself. The extent and nature of these morbidities and late mortalities has been the subject of numerous and detailed studies. For these reasons, the authors decided to investigate conventional and non-conventional prognostic factors, such as telomere length, for the occurrence of CADs in HL patients.

The authors demonstrate, in this study, that following multivariate analysis, hypertension and telomere length were the only significant prognostic factors for the occurrence of secondary cardiovascular diseases.

STUDY DESIGN

Patients

A prospective cohort of 179 HL patients treated and followed up at the Gustave Roussy Institute from 2007 to 2012 without a prior history of CAD was entered into the study (Table 1). Treatment was standard: all patients underwent mediastinal radiation therapy, and chemotherapy was given to 173 of 179 HL patients. All radiation treatment charts or computerised records were reviewed, and the radiation doses were estimated according to isodose curves encompassing the area of the coronary artery origins (CAOs)[14].

Blood samples were obtained before Coronary CT angiography (CCTA). The study was approved by the local ethics committee (approval no. 97-56). All subjects signed an informed consent form.

Table 1. Clinical characteristics, modalities of treatment and CCTA findings of HL patients.

Characteristics	Patients ($N = 179$)
Age at treatment (median and range)	29 (9–75)
Age at CCTA (median and range)	42 (19–79)
Male/female ratio	82/97 (0.85)
Stage	
Early stage	151 (84 %)
Advanced stage	28 (16 %)
Treatment	
Chemotherapy	173 (97 %)
Radiation therapy	179 (100 %)
Total radiation dose (Gy)	36 (35,4–36,8)
Radiation dose to the origin of coronary arteries (Gy)	33.4 (32,4–34,6)
Patients with abnormal CCTA	46 (26 %)
Treatments	
Surgery	10 (22 %)
Angioplasty with stent placement	8
Bypass grafting	2
Medical treatment	24 (52 %)
Follow-up alone	10 (22 %)
Outcome	
Alive	43
Deceased	3

Coronary artery status

Coronary CT angiography was performed on patients using a dual-source CT scanner (Somatom Definition Flash, Siemens AG, Forchheim, Germany). The mean radiation dose delivered during the CCTA procedure was 4.5 mSv with a standard deviation of ± 2. All imaging data were reviewed by two experienced radiologists

Telomere quantification

Blood lymphocytes were cultured for 48 h, and metaphase preparations were performed using standard procedures[15]. Slides were spread and stored at $-20°C$ until use. Telomeres were stained using the Q-FISH technique with a Cy-3-labelled PNA probe specific for TTAGGG for telomere sequences (from Panagene, Daejeon, South Korea). Quantification image acquisition and analysis were performed using Metacyte software (version 3.9.1, MetaSystems, Newton, MA, USA).

Statistical analysis

All the parameters including radiation dose and telomere length were first tested for their association with CCTA abnormalities by univariate analysis. Cox Proportional Hazard regression analyses assessed the association of all parameters and CCTA abnormalities. Multivariate analysis included all the parameters whose p-values were <0.10 in the univariate analysis (all tests were two-sided; p-values of <0.05 were considered significant).

RESULTS AND DISCUSSION

The incidence of CCTA abnormalities is given in Table 1. In the total cohort of 178 patients, 3 patients died due to myocardial infarction and 46 patients had abnormalities of their CCTA. Univariate analysis of risk factors was performed and showed the presence of significant conventional risk factors for cardiovascular disease such as hypertension ($p < 2 \times 10^{-5}$), hypercholesterolaemia ($p = 0.001$), age at treatment ($p < 0.04$) and at CCTA ($p = 0.004$). The radiation dose to CAO was also a significant risk factor ($p = 0.015$). A less conventional risk factor, telomere length, was highly significant ($p = 0.006$). Multivariate analysis of conventional risk factors demonstrates that radiation dose to CAO is the most significant factor ($p = 0.005$). Age at treatment ($p < 0.02$), hypertension ($p = 0.006$) and hypercholesterolaemia ($p = 0.01$) were independent prognostic factors. The second multivariate analysis was performed with all risk factors including telomere length. Only hypertension ($p = 0.007$) and telomere length ($p = 0.03$) were found to be significant prognostic factors.

The multivariate analysis of conventional risk factors corroborated the significance of well-known

prognostic factors. The details for the conventional risk factors are given in Girinsky *et al.*[16] Figure 1 shows the mean and the range of telomere lengths of circulating lymphocytes from HL patients with CCTA abnormalities compared with patients with no evident disease. The mean age and the number of patients are represented. There was a significant association between telomere length and age in HL patients.

In this study, the authors compared only cardiovascular disease versus no cardiovascular disease. Some patients exhibit drastic telomere shortening (<5 kb). The principle finding of this investigation is the prognostic factor of telomere length for cardiovascular disease in HL patients, post-treatment. This study is the first to demonstrate the major role of telomeres in the occurrence of cardiovascular disease in a population exposed to ionising radiation for medical purposes. It is very well documented that individuals with short telomeres of their peripheral blood lymphocytes carry a higher risk for dying of cardiovascular disease in the general population[6]. Cardiovascular complications are the second most frequent fatal post-radiation therapy complication, and epidemiological studies have linked this to exposure to ionising radiation. However, the link between telomere shortening and the higher risk of cardiovascular disease post-exposure was lack.

These findings demonstrate, using multivariate analysis, on the one hand, that the dose to CAO plays a major role in the incidence of CCTA abnormalities, whereas on the other hand, the implication of telomere shortening in the occurrence of these abnormalities.

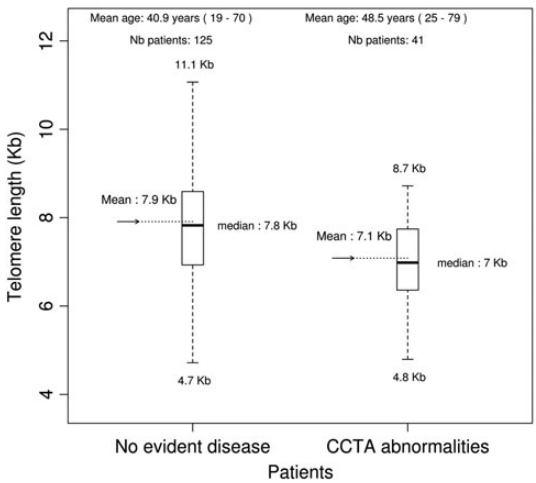

Figure 1. Telomere length in peripheral blood lymphocytes of HL patients with CCTA abnormalities compared with those with no evident cardiovascular disease. Higher heterogeneity in the mean telomere length was observed in patients with no evident cardiovascular disease, which can be explained by the short follow-up of some patients as well as the possible occurrence of another complication such as secondary cancer.

The link between telomeres and the DNA damage response has been strengthened by several studies, both at the cellular and organismal levels, demonstrating that telomere shortening is a determinant of radiation sensitivity[15, 17–19]. Interestingly, in a previous study, the authors have demonstrated that a subset of newly diagnosed, untreated patients with HL demonstrated pre-treatment telomere shortening associated with increased *in vitro* radiation sensitivity and higher frequency of chromosomal aberrations[15]. These findings suggest that HL patients exhibiting short telomeres prior to treatment who received mediastinal radiation might be at higher risk of developing CCTA abnormalities. This plausible hypothesis that telomere shortening may represent an important mediator between radiation exposure and vascular damage could be used to define new radiation protection strategies and supports the quantification of telomere as a prognostic factor of cardiovascular risk of population exposed to ionising radiation.

Two possible hypotheses, oxidative stress and viral infections, could be investigated to elucidate the mechanisms of this telomere shortening. Hodgkin lymphoma patients offer a specific profile from the double point of view of oxidative stress (immunosuppression) and viral infection (role of EBV)[20]. These hypotheses can be used in the monitoring of telomere length in populations before they are exposed to genotoxic agents in order to define new radiation protection strategies on an individual basis (Figure 2).

This study is the first to link telomere length in peripheral blood lymphocytes to cardiovascular disease in a population medically exposed to ionising radiation and provides additional evidence that telomere

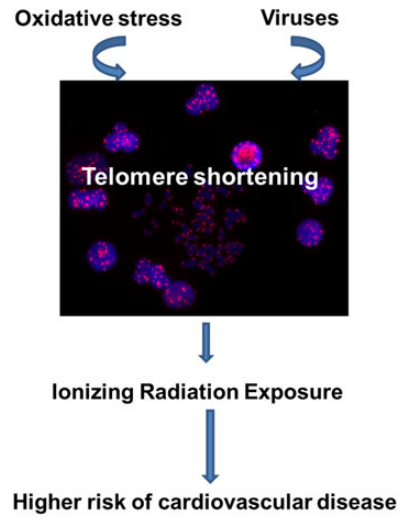

Figure 2. Schematic representation of telomere shortening and higher risk of cardiovascular disease hypothesis in HL patients.

length may be a proxy for underlying inter-individual sensitivity.

These findings need to be confirmed in larger exposed cohorts (accidentally exposed populations, professionally exposed personnel, breast cancer patients, etc.). The introduction of telomere length as a prognostic factor for cardiovascular diseases will require the development of a reliable, easy and automated method to quantify telomere length.

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