Cardiocyte Nuclear Chromatin Density Correlates With Transplanted Heart Left Ventricular Mass

J. Nożyński, M. Zakliczyński, E. Zembala-Nożyńska, D. Konecka-Mrówká, R. Przybylski, B. Nikiel, Joanna Mlynarczyk-Liszka, D. Lange, A. Mrówka, J. Przybylski, M. Maruszewski, and M. Zembala

ABSTRACT

Introduction. Cardiocyte hypertrophy is accompanied by polyploidy, seen as a decrease in chromatin density in the enlarged nucleus. Repeated biopsies of a transplanted heart offer the possibility of a dynamic evaluation of these phenomena. The aim of this work was an evaluation of cardiocyte nuclear chromatin density in transplanted hearts during long-term follow-up.

Materials and Methods. The material encompassed myocardial biopsy specimens taken during the first week, first month, and then on an annual basis up to 10 years after surgery. Only biopsy specimens with no rejection were considered (grade "0" International Society for Heart and Lung Transplantation [ISHLT] 122 biopsy specimens). The control group consisted of 7 donor heart specimens. We evaluated the optical density—mean gray level—of cardiomyocyte nuclear chromatin. We determined correlations of this index with the nuclear area, and with left ventricle ultrasound measurements, using correlation analysis.

Results. The chromatin mean gray level decreased with time, correlating positively with interventricular septum thickness, left ventricle posterior wall diameter, and left ventricular mass. Analysis of individual periods showed a significant positive correlation of the mean grey level with the cardiocyte nuclear surface in year 3, 4, and 9 after transplantation, thereby suggesting the occurrence of polyploidy at those times. The significant negative correlation of these values (1 week and 1 year) indicated normalization of early cardiocyte hypertrophy.

Conclusions. With the passage of time chromatin condenses, leading to pyknosis. The activity of cardiocyte chromatin correlated with left ventricular hypertrophy. Compensatory cardiomyocyte polyploidy is a periodical phenomenon.

THE DISPERSION and staining intensity of nuclear chromatin are commonly recognized indices of physiological and pathological changes within cells, encompassing the phases of rest, stimulation, hypertrophy, and mitotic division. Our earlier morphometric examination implementing the mean gray level of nuclear chromatin showed significant chromatin despiralization, lightening and dispersing during grade 3A (International Society for Heart and Lung Transplantation [ISHLT]) acute rejection of a transplanted heart.[1] One of the compensatory phenomena observed in the myocardium was cardiocyte hypertrophy characterized by nuclear enlargement, a phenomenon that was particularly noticeable in the early posttransplantation period.[2–4] Cardiomyocyte hypertrophy is most clearly expressed through nuclear enlargement; however, it is also connected with multiplication of DNA quantity—polyploidy.[5] This phenomenon presents as a decrease in chromatin density with an enlarged nucleus.[6] Polyploidy is likely to be the result of endomitosis, ie, duplication of genetic material

From the Department of Cardiac Surgery & Transplantation (J.N., M.Z., D.K.-M., R.P., A.M., J.P., M.M., M.Z.), Silesian Center for Heart Disease, Zabrze, and Department of Tumor Pathology (E.Z.-N., B.N., J.M.-L., D.L.), Comprehensive Cancer Center M. Sklodowska-Curie Institute Branch, Gliwice, Poland.

Address reprint requests to M. Zakliczyński, Silesian Center for Heart Disease, Szpitalna 2, 41-800 Zabrze, Poland. E-mail: zaklimed@onet.pl

0041-1345/09/$–see front matter
doi:10.1016/j.transproceed.2008.10.067

without division,[7] occurring in response to myocardial damage or overload, similar to cardiomyocyte multinuclearity.[8] The aim of our study was to evaluate the density of cardiocyte nuclear chromatin in a transplanted heart upon long-term follow-up seeking to determine whether chromatin thickness correlated with the size of cellular nucleus and with the thicknesses of the heart walls, and whether its microscopic activity was an indicator of hypertrophy.

MATERIAL AND METHODS

The histological material originated from the archives of our histopathology laboratory from 1995–2004. The patients received Cyclosporine, Azathioprine, and Encorton. Antihypertensive therapy was necessary in all enrolled patients from the first month after transplantation, consisting of an angiotensin-converting enzyme inhibitor in all subjects and a calcium channel blocker in 10 patients. Diagnostic biopsy specimens were obtained during weeks 1, 2, 3, and 4 after transplantation; the next 2 biopsies were performed at 2-week intervals, and then monthly until 6 months after transplantation. Subsequent biopsies were performed either due to clinical indications or annually. Myocardial biopsy specimens fixed in 6% buffered formalin were routinely embedded in paraffin. The nuclei were stained with Harris' hematoxylin for 5 minutes, then rinsed for 5 minutes (saturated solution of lithium carbonate); the cytoplasm was counterstained with 0.5% phloxin. The evaluation of acute rejection was performed according to the ISHLT protocol.[9] We only analyzed cases displaying no features of rejection (ISHLT "0"). The study encompassed the following groups: (a) controls, donor heart fragments disqualified due to disproportionate size (n = 7); (b) biopsies on the day 7 from transplantation (n = 15); (c) biopsies on day 30 from transplantation (n = 15); (d) biopsies 1 year from transplantation (n = 15); (e) biopsies 2 years from transplantation (n = 13); (f) biopsies 3 years from transplantation (n = 10); (g) biopsies 4 years from transplantation (n = 9); (h) biopsies 5 years from transplantation (n = 8); (i) biopsies 6 years from transplantation (n = 8); (j) biopsies 7 years from transplantation (n = 7); (k) biopsies 8 years from transplantation (n = 8); (l) biopsies 9 years from transplantation (n = 7); and (m) biopsies 10 years from transplantation (n = 7).

All 15 donors died of acute cerebral injuries not related to hypertension or vascular abnormalities (14 from cerebral trauma due to traffic accident, 1 from suicidal gunshot).

The image analysis was performed with the implementation of Quantimet 500+ Color Option (Leica, Cambridge, Mass, United States). Measurements were done exclusively on clearly visible longitudinal sections of muscle fibers in which cardiocyte nuclei showed clear outlines. The outlines of cardiocyte nuclei marked with a cursor on an analyzer display screen using a microscopic optical zoom (1000×) were measured for nuclear chromatin mean gray level and nuclear area.

Prior to taking a biopsy specimen in each patient an ultrasound examination was performed with a Sonos 2000 using a probe frequency of 2.5/2 MHz. We recorded diastolic thickness of the interventricular septum, left ventricular posterior wall, and left ventricular mass. The statistical analysis was performed with Statistica 99 software (StatSoft Inc., Tulsa, Okla, USA), including descriptive statistics and Spearman correlation test.

RESULTS

The measurement results are shown in Table 1, whereas the regression analysis is shown in Table 2 The mean gray level of the cardiomyocyte nuclei in the control group was 133, 575 ± 56, 757. In the first week the values were clearly higher than in the control group; between week 1 and the month 1 as well as between year 1 and 2 after transplantation, there was a significant decrease in this value. It should be noteworthy that first biopsy did not show any abnormalities or pathologies, such as cardiocyte hypertrophy or ischemic lesions, whereas the 1-month biopsy specimen showed enlarged cardiocytic nuclei with chromatin activation, despite the absence of hemodynamic compromise. Afterwards, a decreasing tendency was observed with a transitional increasing tendency in year 8. This value showed a strong negative correlation with time from surgery, suggesting gradual condensation—spiralization, ie, deactivation of chromatin, eventually leading to pyknosis. The cardiocyte nuclear surface area revealed a mean value of 54.096 ± 22.629 μm^2, showing an enlargement in the first

Table 1. Cardiomyocytic Chromatin Mean Gray Level, Nuclear Area, and Transplanted Heart Ultrasound Measurements in 10-Year Biopsy Observation: Descriptive Statistics (mean ± SD)

Values Groups	No. of Nuclear Measurements	Chromatin Mean Gray Level (gray level deg)	Nuclear Area (μm^2)	No. of Ultrasound Measurements	IVS Dilatative Thickness (cm)	LV Posterior Wall Dilatative Thickness (cm)	LV Mass (g)
Control	2250	133.58 ± 56.76	54.09 ± 22.62	—	—	—	—
1st wk after HTx	340	171.71 ± 29.20	46.23 ± 26.44	31	1.25 ± 0.08	1.26 ± 0.13	253.51 ± 27.46
1st mo after HTx	380	135.49 ± 35.95	80.93 ± 49.76	31	1.05 ± 0.16	1.14 ± 0.14	251.58 ± 65.63
1st y after HTx	341	142.99 ± 21.41	50.22 ± 30.28	33	1.07 ± 0.19	1.13 ± 0.16	250.82 ± 59.83
2nd y after HTx	425	120.82 ± 11.38	43.40 ± 20.11	28	0.98 ± 0.10	1.03 ± 0.16	243.42 ± 38.19
3rd y after HTx	280	117.67 ± 23.09	62.07 ± 40.61	23	0.91 ± 0.14	0.98 ± 0.26	207.33 ± 43.89
4th y after HTx	270	118.62 ± 14.82	62.72 ± 7.84	20	1.05 ± 0.11	1.12 ± 0.13	201.87 ± 41.46
5th y after HTx	270	115.22 ± 10.09	63.87 ± 28.09	22	1.14 ± 0.09	1.24 ± 0.11	235.56 ± 52.81
6th y after HTx	290	119.09 ± 10.01	59.88 ± 14.21	16	1.16 ± 0.08	1.26 ± 0.08	224.66 ± 44.14
7th y after HTx	322	123.53 ± 14.41	58.17 ± 14.84	16	1.18 ± 0.06	1.26 ± 0.07	212.83 ± 19.67
8th y after HTx	260	135.10 ± 22.74	62.25 ± 19.58	18	1.25 ± 0.07	1.25 ± 0.07	217.10 ± 14.29
9th y after HTx	240	130.07 ± 19.45	67.13 ± 25.71	15	1.25 ± 0.06	1.22 ± 0.04	222.65 ± 15.08
10th y after HTx	220	122.65 ± 13.10	69.73 ± 37.54	15	1.21 ± 0.07	1.20 ± 0.03	220.51 ± 18.10

Abbreviations: deg, degree; IVS, interventricular septum; LV, left ventricle wall thickness; HTx, heart transplantation.