Stem cell-based therapy has recently emerged as an innovative approach for the treatment of ischemic left ventricle (LV) dysfunction. Nonhuman animal studies have indicated that treatment with bone marrow stem cells (BMSCs) after ST-segment elevation myocardial infarction (STEMI) might regenerate myocardium by inducing myogenesis or vasculogenesis and improve LV function. The results from a recent meta-analysis indicated a modest, yet significant, increase in the LV ejection fraction (LVEF) after BMSC injection 7 days after STEMI. Standard echocardiography and speckle tracking analysis was performed at baseline and 6 months after STEMI. No differences were found in the baseline echocardiographic parameters of LV systolic and diastolic dysfunction—the LV ejection fraction was 35 ± 6% in the BMSC group, similar to that in the control group (33 ± 7%, p = 0.42). After 6 months, the absolute change in the LV ejection fraction was significantly greater in the BMSC group than in the control group (10 ± 9% versus 5 ± 8%, p = 0.04). Significant improvement was seen in 2-dimensional systolic strain in all segments (12 ± 4 vs 14 ± 4; p = 0.0009) and in the infarcted area (5 ± 2 vs 6 ± 2; p = 0.0038) only in the BMSC group. Of the diastolic function parameters, we observed improvement in the early filling propagation velocity (30 ± 8 cm/s vs 37 ± 13 cm/s; p = 0.0008), early diastolic velocity – E’ (4.5 ± 1.5 vs 5.0 ± 1.3, p = 0.02), and the E/E’ ratio (17 ± 7 vs 14 ± 5; p = 0.03) in the BMSC group. In conclusion, intracoronary injection of unselected BMSCs in patients with STEMI improved both LV systolic and diastolic function at 6 months of follow-up.

Methods

A total of 60 patients with a first anterior wall STEMI (18 women and 42 men, age 56 ± 9 years, range 34 to 72) and a baseline LVEF <40%, were included in the present study. All the patients were successfully treated with primary PCI within 12 hours after the onset of symptoms. The STEMI diagnosis was determined from the findings of typical chest pain, the presence of ST elevations on a standard 12-lead electrocardiogram, and a significant increase in the serum markers of myocardial infarction. The symptom-to-balloon time did not differ between the treatment (7 ± 2 hours) and control (8 ± 3 hours, p = NS) groups. The exclusion criteria were previous myocardial infarction, significant stenosis in a nonculprit coronary vessel, clinical and hemodynamic instability, current infection, neoplasm, and other severe coexisting conditions that could influence a patient’s compliance to the protocol or a patient’s 1-year prognosis. The patients were randomly assigned to the treatment group follow-up period in patients undergoing BMSC therapy after their first anterior wall STEMI treated with primary percutaneous coronary intervention (PCI).
(BMSC group) or the control group in a 2:1 ratio (40:20). No placebo procedure (sham bone marrow aspiration or injection) was performed in the control group. The patients' pharmacologic treatment during hospitalization and follow-up period was administered in accordance with current guidelines and was not influenced by the assignment to either of the study groups. The local ethical committee approved the study, which was registered at the Polish Ministry of Science and Higher Education as Grant 2 P05B 178 28. All patients received oral and written information about the study and provided written informed consent before inclusion in the study.

Echocardiography was performed in all patients at baseline (day 3 after primary PCI) and after 6 months of follow-up with standard parasternal and apical views using a VIVID 7 Dimension system (GE Vingmed Ultrasound AS, Horten, Norway). The echocardiographic data were analyzed by an investigator unaware of the treatment assignment.

The LVEF was calculated using Simpson’s biplane method. Regional wall motion abnormalities were assessed qualitatively using the 16-segment LV model. Wall motion was scored in each of the 16 segments as normal (score of 1), hypokinetic (score of 2), akinetic (score of 3), or dyskinetic (score of 4). Then, the LV wall motion score index was calculated by dividing the sum of scores for all segments by 16.

The segments demonstrating a wall motion abnormality during the baseline study were considered to belong to the infarct area.

Assessment using speckle tracking imaging required greater temporal resolution of the acquired gray-scale cine loops (50 to 75 frames/s). The myocardial systolic and diastolic velocities and 2-dimensional longitudinal systolic strain in the apical, medium, and basal segments of the left ventricle were measured off-line using a dedicated workstation equipped with an EchoPAC personal computer (GE Vingmed Ultrasound 2001-2006, version 6.1.0). Two-dimensional strain analysis involved manual tracing of a LV endocardial contour, acceptance of the appropriate quality level of the region of interest, manual contour corrections as needed, and, finally, automatic calculations of local systolic and diastolic velocities and systolic strain.

The maximal velocities of E- and late diastolic velocity (A)-waves of transmitral flow were recorded using pulsed-sample Doppler, and the E/A ratio was calculated.

The measurement of the early filling propagation velocity was performed in the apical 4-chamber view using color Doppler echocardiography in M-mode. Then, adjustment of the Doppler window and Nyquist velocity to 2/3 of the blood flow peak velocity was done to display the average velocity of the mitral early wave from the mitral annulus to...
4 cm toward the apex of the left ventricle. The early filling propagation velocity of the early wave was measured as the slope of the line parallel to the recorded border between the blue and red colors. The M-mode color and pulsed Doppler signals were recorded at a horizontal sweep of 100 mm/s.

The peak velocity of early diastolic longitudinal motion of the lateral and septal corners of the mitral annulus (E) was assessed using speckle-tracking imaging, as described above.

The ratio of transmitral flow velocity to annular velocity (E/E') was calculated.

A total of 100 ml of bone marrow was aspirated from the iliac crest using local anesthesia 3 to 11 days after STEMI. The cell preparation and administration protocol has previously been described in detail.7–9 In brief, bone marrow aspirates were diluted with 20 ml of 0.9% NaCl, filtrated, and mononuclear cells (CD34+ and CD133+) were isolated by density gradient centrifugation (Ficoll-Paque Plus, 15°C, 20 minutes). The mononuclear cells were then washed 2 times with 0.9% NaCl, filtered, and subjected to quality and quantity control. Within 2 hours after the bone marrow harvest, a volume of 20 ml of mononuclear cell suspension was infused into the infarct-related artery distally to the inflated over-the-wire balloon catheter (Ninja, Cordis, Miami, Florida). The balloon was inflated with low pressure to completely block blood flow for 2 minutes, and 5 ml of the BMSC suspension was infused distally to the occluding balloon through the central port of the balloon catheter. This maneuver was repeated 4 times, interrupted by 2 minutes of reflow by deflating the balloon to minimize ischemia. After completion of the intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency. The mean radiation exposure was 975 ± 154 μGy/m².

Continuous variables that approximated a normal distribution (as assessed by the Kolmogorov-Smirnov test) and categorical variables are expressed as the mean ± SD and percentages, respectively. A paired samples t test was used to detect differences between the baseline and follow-up observations within the study groups, and an unpaired t test was used to compare continuous variables between the study groups. The variance ratio F test was used to compare the variances of analyzed samples, and, whenever the variances of the samples were significantly different, a t test correction for unequal variances (Welch test) was applied. The level of statistical significance was set at p < 0.05.

Statistical analysis was performed using MedCalc software, version 9.6.4.0 (Mariakerke, Belgium).

Results

The study and control groups were uniform with respect to the baseline demographic and clinical characteristics and drug therapy given.

During the 6 months of follow-up, 2 cardiac-related deaths occurred in the BMSC group (1 fatal STEMI and 1...
sudden cardiac death) and 2 in the control group (2 sudden cardiac deaths). Therefore, the results of the follow-up echocardiographic studies were available for 38 patients from the BMSC group and 18 patients from the control group. These 56 patients were subject of additional analysis—their characteristics are summarized in Table 1.

The procedure of bone marrow aspiration, cell preparation, and intracoronary transplantation was conducted 7 ± 2 days (range 3 to 11) after the STEMI. In 24 patients (60%), it was 6 to 8 days; in 7, it was 3 to 5 days; and in 9, it was 9 to 11 days. The procedural success rate was 100%. Details of the characterization of the cells administrated are listed in Table 2.

In the study groups, the baseline echocardiographic parameters of systolic and diastolic dysfunction before treatment assignment and BMSC transfer were fairly uniform (Table 3) and represented significant LV dysfunction. The baseline diastolic dysfunction indexes are presented in Table 3; no significant differences were found between the BMSC and control groups.

Selected parameters of LV systolic and diastolic function measured after 6 months are presented in Table 4.
The analysis of systolic function using the speckle tracking method showed a significant improvement in systolic myocardial velocities of the basal segments of LV in the BMSC group but not in the control group (Figure 1). Also, significant improvement was seen during follow-up in the mean average 2-dimensional systolic strain after BMSC transfer; however, the improvement in the control group was nonsignificant (Figure 2).

During the follow-up period, no significant treatment-related differences in the E/A ratio between the 2 groups were noted, with a trend toward impaired relaxation in the BMSC group but pseudonormalization in the control group. However, the early filling propagation velocity, increased significantly with a corresponding significant decrease in the control group (Figure 3). Consistently, we observed an improvement in the early diastolic velocity (E') of the basal segments of the septum and lateral wall in the BMSC group only. Furthermore, the E/E' ratio significantly decreased in the BMSC group, but in the control group, it remained pathologically elevated (Figure 4).

**Discussion**

Our results have demonstrated that in patients with a large first anterior STEMI intracoronary injection of autologous mononuclear BMSCs 3 to 11 days after successful primary PCI significantly improves both LV systolic and diastolic function at 6 months. To our knowledge, this is the first study showing such a benefit using speckle tracking echocardiography in patients after BMSC therapy.

We found significant differences in the standard systolic function parameters (e.g., the absolute change in LVEF was 10 ± 9% in the BMSC group and 5 ± 8% in the control group, p < 0.05). The improvement in LVEF in the BMSC group was greater than that reported in a meta-analysis by Lipinski et al.12 (3% increase, p < 0.001), probably because of the more severe baseline LV systolic dysfunction in our group (mean LVEF 35 ± 6%). Post hoc analyses from the REinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial10 suggested that BMSC therapy might be more beneficial to patients with more severely depressed LVEF. Furthermore, in the most recent meta-analysis by Martin-Rendon et al.,11 the subgroup analysis revealed a statistically significant difference in LVEF in favor of BMSC when the cells were infused within 7 days after STEMI. The investigators explained that fact by the increase in cytokines such as vascular endothelial growth factor, hepatocyte growth factor, and granulocyte colony-stimulating factor in plasma during the first week after STEMI. In the REPAIR-AMI trial, BMSC infusion was more effective when administrated 6 days after reperfusion.10 In our group, the timing of stem cell delivery (3 vs 11 days, mean 7) had no significant effect on the magnitude of improvement in LV function; however, the subgroups were to small to draw definite conclusions (most patients treated at 6 to 8 days).

In our study, we assessed the LV function using the new echocardiographic method—2-dimensional speckle tracking myocardial velocity and strain analysis. This imaging mode allows the analysis and quantification of myocardial acoustic marker motion throughout the heart cycle,12 and its accuracy for local contractility quantification has been confirmed.13-15 However, until now, this method has not been used in the setting of BMSC therapy. In our study, we found a significant increase in the systolic myocardial velocities and systolic strain, averaged from all LV segments and from the infarct area (defined as the region with wall motion abnormalities on the basal echocardiogram), which was the main component of improvement in global LV function. Our results are in concordance with a recent published study by Herbots et al.16 In the present study, regional myocardial deformation was measured using Doppler-derived strain rate imaging. At 4 months of follow-up, the end-systolic strain in the infarcted segments had improved significantly more in the BMSC group than in the control group.

Schaefer et al.17 published an analysis of standard diastolic function parameters in patients enrolled in the Bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial. Diastolic function was determined by measuring the transmitral flow velocities (E/A ratio), diastolic myocardial velocities (E'/A' ratio), isovolumic relaxation time, and deceleration time. An overall effect of the BMSC transfer was seen on the E/A and E'/A' ratios. In contrast, they found no effect of BMSC transfer on the deceleration time, isovolumic relaxation time, or E/Ea ratio. Similar to the results in our study, during the 6-month period, E/A ratio showed a trend toward evolving to impaired relaxation in BMSC-treated patients and a pseudonormalization pattern in the control group.

In the present study, we performed a more detailed analysis of diastolic dysfunction. One of the indexes of abnormal relaxation is the early inflow propagation velocity, which slows when relaxation is impaired and, in contrast to the mitral E wave, remains reduced when the left atrium pressure increases.18,19 In our study, we found significant improvement in the early filling propagation velocity in the BMSC group in contrast to an additional significant decrease in the control group during follow-up. We analyzed the ratio of early mitral valve flow velocity to early diastolic tissue velocity (E/E' ratio). It has been established that a E/E' ratio >15 indicated elevated LV filling pressures and a ratio <8 indicated normal LV filling pressures.20,21 In the present study, the baseline E/E' in the BMSC and control groups was elevated to >15. After the 6 months, the E/E' ratio had significantly decreased in the BMSC group to less than the cutoff, but in the control group it had remained elevated. To summarize, irrespective of the assessment method, we consistently showed that diastolic function improved after BMSC therapy in patients with large anterior STEMI. This is a clinically important finding, taking into account that the risk of death is increased if direct or indirect signs of increased LV filling pressures are present.22 It is well known that after myocardial infarction, cell necrosis, residual ischemia, microvascular dysfunction, and regional wall motion abnormalities influence the diastolic function. The improvement in diastolic function is probably not a direct effect of the BMSC therapy itself, because enhanced systolic function could result in an increase in restoring forces, leading to an increased rate of early diastolic relaxation.

The relative benefits of specific cell types remain unsettled as documented by a recently presented Myocardial
Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) trial. Lipinski et al. did not find a statistically significant association between the number of injected cells, the interval to PCI, and the interval to symptom onset. They found a trend toward a statistically significant association between the injected volume and LVEF (p = 0.066), suggesting the possible presence of a dose–response relation. It has been shown in the newest meta-analysis, however, that LV function improvement occurred only when the BMSC dose was >10^7 cells. This is consisted with our data—the mean number of transplanted cells was 1.44 ± 0.49 × 10^5, including 0.44 ± 0.95 × 10^5 CD133+ cells and 3.06 ± 2.18 × 10^5 CD34+ cells.

The number of the patients included in our study were insufficient for definite conclusions regarding the clinical end points and the safety of mononuclear BMSC intracoronary transfer. Therefore, although we realize that the data regarding the effect of intracoronary injection of autologous mononuclear BMSCs on the clinical end points would be of great interest, we focused our present analysis on the procedure’s influence on the parameters of myocardial systolic and diastolic function.

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5. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Chamber Quantification Writing Group; American Society of Echocardiography’s Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group. Developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440–1463.

