Alterations in the expression of genes related to contractile function and hypertrophy of the left ventricle in chronically paced patients from the right ventricular apex

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Aim

Long-term right ventricular apical (RVA) pacing may lead to left ventricular (LV) remodelling and heart failure. This study assessed changes in the expression of genes regulating LV contractile function and hypertrophy, after permanent RVA pacing and investigated whether such changes proceed or even predict LV remodelling.

Methods and results

We enrolled 52 consecutive patients (age 79.1 ± 7.7 years, 34 males) who underwent pacemaker implantation for bradycardic indications: Group A, 24 individuals with atrioventricular conduction disturbances and group B, 28 patients with sinus node disease. In group A, peripheral blood mRNA levels of gene sarcoplasmic reticulum calcium ATPase decreased at 3, 6, and 12 months' follow-up, while α-myosin heavy chain (MHC) decreased and β-MHC increased until 6 months follow-up. In this group, 25% of patients demonstrated significant LV remodelling. At 4 years, LV end-systolic diameter increased from 29.67 ± 3.39 mm at baseline to 35.38 ± 4.22 mm, LV end-diastolic diameter increased from 50 ± 4.95 to 56.71 ± 5.52 mm, and ejection fraction declined from 63.04 ± 10.22 to 52.83 ± 10.81%. Early alterations in gene expression were associated with a deterioration in LV function and geometry that became apparent months later. In group B, echocardiographic indexes and mRNA levels of the evaluated genes demonstrated no statistically significant changes.

Conclusions

Permanent RVA pacing in patients with preserved ejection fraction is associated with alterations in the expression of genes regulating LV contractile function and hypertrophy, measured in the peripheral blood. These alterations are traceable at an early stage, before echocardiographic changes are apparent and are associated with LV remodelling that becomes evident in the long term.

Keywords

Gene expression • Right ventricular apical pacing • Ventricular remodelling

Introduction

The right ventricular apex has been the most commonly used pacing site, while alternative pacing sites in the right ventricle (RV), although they may provide some advantages, have not proven to be superior regarding hard outcomes such as morbidity and mortality.3 However, soon after its introduction, it became clear that RVA pacing entails abnormal impulse conduction to the ventricles, leading to asynchronous activation and contraction. This abnormal activation pattern provokes alterations in regional mechanical function and loading, myocardial perfusion and autonomic innervation, leading to LV remodelling and heart failure (HF) in a considerable proportion of paced patients.4 Several studies have demonstrated the development of new-onset HF after RVA pacing in patients with preserved LV function and no prior history of HF or coronary artery disease (CAD).4,5 Heart failure development seems to be related with both the duration of pacing and the cumulative percentage of ventricular pacing (VP).5

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What's new?
- Permanent RVA pacing leads to changes in the expression of genes that regulate myocardial contractile function and hypertrophy, which are traceable in the peripheral blood.
- These changes are evident early after pacemaker implantation (even at 3 months’ follow-up) and proceed LV remodelling.
- These early detected alterations in the expression of genes seems to be associated with LV remodelling in the long term.
- If solid data can be established concerning factors for the early prediction of LV remodelling, then patients who might benefit from other pacing modalities, such as biventricular pacing, could be identified during a very early phase.

On the other hand, it is known that certain genes regulate excitation—contraction coupling and contractile proteins and therefore are associated with HF development.6,7

We hypothesized that RVA pacing may provoke changes in gene expression that precede a deterioration in LV function and geometry that only becomes echocardiographically or clinically evident at a much later stage. Therefore, the aim of our study was to assess early changes in the expression of selected genes related to the contractile apparatus and excitation—contraction coupling proteins, in peripheral blood cells of patients with preserved LV function, who underwent long-term RVA pacing.

Methods

Study design
This was a prospective controlled study, conducted between January 2008 and December 2013. Data concerning gene expression in the peripheral blood were assessed at enrolment (just prior to pacemaker implantation), and at 3, 6, and 12 months. Echocardiographic indexes were assessed at the time of enrolment, and at 3 months, 1 year, 2 and 4 years.

The study complied with the Declaration of Helsinki, all subjects gave written, informed consent prior to their inclusion in the study and the experimental protocol was approved by the hospital’s Ethics Committee.

Study population
We enrolled 52 consecutive patients (34 males, age 79.1 ± 7.7 years), with preserved LV ejection fraction (EF ≥ 45%) who underwent pacemaker implantation for bradycardic indications; the ventricular lead was placed in the RV apex in all patients. Participants were divided into two groups, based on the post-implant cumulative percentage of VP. Group A consisted of 24 individuals who were paced for atrioventricular conduction disturbances with a post-implant VP cumulative percentage that exceeded 90%, while group B (control group) consisted of 28 patients who received a permanent pacemaker because of sinus node dysfunction with preserved intrinsic atrioventricular conduction and whose percentage of post-implant VP was lower than 5%; the devices were appropriately programmed to minimize VP. Patients with ventricular conduction disturbances (QRS duration ≥ 120 ms) were excluded from this latter group. All patients underwent pacemaker interrogation to determine the percentage of paced ventricular systoles. In addition, we obtained 24-h Holter recordings from all patients, in order to determine whether the pacemaker-reported VP percentage was indeed referring to fully captured beats, rather than fusion or pseudofusion beats.

Echocardiography
At the time of implantation and at certain time points during follow-up, patients underwent echocardiographic examination for the evaluation of LV function and structure. Echocardiographic images were obtained with patients in the left lateral decubitus position, by two experienced echocardiographers blinded to the study protocol, using a Vivid 7 (General Electric, Horten, Norway) ultrasound device with a 1.5–3.6 MHz wide angle phased-array transducer (M4S).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (n = 24)</th>
<th>Group B (n = 28)</th>
<th>P-value</th>
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<td>Age (years)</td>
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<td>78.9 ± 8</td>
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<td>Sex (male/female)</td>
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<td>12/16</td>
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<td>0.86</td>
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<tr>
<td>LVESD (mm)</td>
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<td>EF (%)</td>
<td>63 ± 10.2</td>
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<table>
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<th>Time points</th>
<th>mRNA SERCA</th>
<th>mRNA α-MHC</th>
<th>mRNA β-MHC</th>
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<tbody>
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<td>Group B</td>
<td>P-value</td>
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<td>7.87 ± 5.51</td>
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<td>3 months</td>
<td>3.48 ± 3.46</td>
<td>6.72 ± 3.9</td>
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<tr>
<td>6 months</td>
<td>4.21 ± 2.77</td>
<td>9.83 ± 12.1</td>
<td>0.031</td>
</tr>
<tr>
<td>1 year</td>
<td>3.56 ± 2.68</td>
<td>8.82 ± 11.05</td>
<td>0.021</td>
</tr>
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</table>
All measurements were performed off-line on digitally stored images. Left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) were assessed from M-mode recordings in parasternal long-axis view. Left ventricular end-systolic and end-diastolic volumes were assessed from apical two- and four-chamber views, and LVEF was calculated using the biplane Simpson’s rule.

Expression of genes

RNA isolation and quantitative RT–PCR

Blood samples were collected into ethylenediamine-tetra-acetic acid (EDTA) collection tubes. Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll-paque plus (Stem Cell Technologies Inc., Vancouver, BC, Canada) gradient centrifugation, total RNA was isolated using the TRI-Reagent (Ambion, Life Technologies, Carlsbad, CA, USA). RNA samples for each patient’s full time course were stored at −80°C until analysis. One microgram of total RNA was reverse transcribed with oligo-(dT) using the Reverse Transcription System (Promega, Madison, USA) in 20 μL reactions. Measurements of mRNA levels were performed by quantitative real-time reverse-transcription polymerase chain reaction (RT–PCR) using the STRATAGENE Mx3000P Detection System. Polymerase chain reaction assays were performed in 2 μL of the cDNA templates using the SYBR Green PCR Master Mix (Bio-Rad, CA, USA) in 20 μL reactions. Primer sequences for sarcoplasmatic reticulum calcium ATPase (SERCA), α-myosin heavy chain (α-MHC), β-MHC, and glyceraldehyde-3-phosphatedehydrogenase (GAPDH), as well as the experimental design strategy to achieve specificity were as previously described.8 Thermal profiling for the real-time PCR was an initial denaturation step at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, annealing temperature for 60 s, 72°C for 30 s, followed by melting curve generation from 55 to 95°C. Annealing temperature was the optimal for each primer set and ranged from 55 to 60°C. All samples were run in duplicate. The standard curve method was used for absolute quantification of the amplification products and specificity was determined by performing a melting curve analysis. A uniform amplification of the products was rechecked by analysing the melting curves of the amplified products (dissociation graphs). Standard curves for the expression of each gene were generated by serial dilution of known quantities of cDNA template. The housekeeping gene GAPDH was used as an endogenous reference gene and relative quantification was performed by normalizing the signals of the different genes with the GAPDH signal. Expression levels for each gene after absolute quantification and normalization to the GAPDH signal were presented as arbitrary units.

Statistical analysis

Summary descriptive statistics are expressed as mean ± standard deviation or as frequencies and percentages, as appropriate. Continuous variables were compared between the two QRS groups using independent samples t-tests. Categorical variables were compared with using Fisher’s exact test. Repeated measures analysis of variance was employed to assess time, group, and interaction effects between the echo parameters and gene expressions. Pearson correlation coefficients were also computed to assess the association between changes in echo variables and gene expression changes. All statistical tests were carried out at the two-sided 5% level of significance. The statistical package IBM-SPSS 21 was used for all analyses. In regard to echocardiographic measurements, intra- and inter-observer variability and agreement between the measurements obtained were calculated according to Bland and Altman’s method. Lower and upper limits of agreement (95% limits of agreement of the mean bias) and coefficients of variation were calculated as the within-subject SD divided by the mean of the observations. The differences (the difference between paired measurements divided by the average of the two measurements times) were calculated for all Bland–Altman plots.
Results

Patient characteristics

Patients’ demographic and clinical characteristics, together with baseline echocardiographic parameters, are summarized in Table 1. The majority of patients were on angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers and diuretics. Patients in group A exhibited a high percentage of VP (94% ± 2%) with a mean paced QRS duration of 142 ± 12 ms, while in the control group VP was 3% ± 1% with QRS duration 85 ± 5 ms. Echocardiographic parameters and the mRNA levels of the genes measured did not exhibit statistically significant differences between the two groups at the time of pacemaker implantation. Further analysis of patients’ clinical characteristics (arterial hypertension, diabetes mellitus, hyperlipidaemia, and CAD) did not reveal any statistically significant correlation to the clinical outcome. This could probably be attributed to the small number of patients included in this study.

Alterations in the expression of genes

Repeated measures ANOVA showed significant time × group interaction effects on SERCA (P = 0.048) and α-MHC (P = 0.032). In group A, significant time effects were observed for SERCA (P < 0.001) and borderline effects for α-MHC (P = 0.06). Serca decreased sharply from the time of implantation to 3 months’ follow-up (P < 0.001), with no further changes, while α-MHC differed between 3 and 6 months’ follow-up (P = 0.015). For β-MHC, there were no group (P = 0.38) or interaction effects (P = 0.58), but a significant time effect was observed (P = 0.004). Post hoc tests showed that levels of gene expression at 12 months’ follow-up were significantly less than at the time of implantation.

No significant changes were observed in group B.

The exact values and the time course of the gene expression of these three genes in the two groups are presented in Table 2 and Figure 1, respectively.

Alterations in echocardiographic parameters/left ventricular function and geometry

No statistically significant intra- and inter-observer variation was found between the measurements obtained from the patients. Significant time × group interaction effects were also observed for LVESD, LVEDD, and EF (P < 0.001 for all). More specifically, in group A, a very significant time effect was apparent for LVESD (P < 0.001), whereas in group B no time effect was observed (P = 0.109). Post hoc Bonferroni-adjusted tests in group A showed a significant increase at each time point (P < 0.001). Similarly, a significant increase in LVEDD at each time point (P < 0.001) was evident in group A, while in group B there was a significant, but rather small difference at 1 and 2 years’ follow-up (P = 0.005) compared with baseline. On the other hand, EF showed a significant steady decline at each time point in group A (P < 0.001), while in group B no time effect was observed (P = 0.246).

The exact values and the time course of the echocardiographic indexes in the two groups are presented in Table 3 and Figure 2, respectively.

Association between gene expression alterations and left ventricular remodelling

We sought to evaluate whether alterations in the expression of genes at an early stage (from the time of implantation up to the first year) could be correlated with a deterioration in LV function and geometry that became more evident later in the long-term follow-up.

Indeed, changes in the genes at an early stage seemed to be associated with LV remodelling that took place months later. More specifically, an increase in β-MHC during the first 6 months appeared to be associated with an increase in LV dimensions and a decline in EF that became apparent almost 2 years later (Figure 3). In addition, a decrease in the expression of the gene SERCA during the first year, was correlated with an increase in LV dimensions and a decrease in EF at 2 and 4 years of follow-up (Figure 4).

To assess the extent of LV remodelling in patients in group A at 4 years’ follow-up, we defined significant adverse LV remodelling, as an increase in LVESD by ≥ 15% and a decrease in EF by ≥ 15%. Based on these criteria, 25% of the patients in group A demonstrated significant adverse remodelling of the LV. Moreover, patients with significant LV remodelling demonstrated more extensive changes of the gene β-MHC at 6 months and the SERCA gene at 1 year, compared with those that did not fulfil the criteria for significant LV remodelling; the difference between the two subgroups was statistically significant (Table 4).

Table 3 Values of the echocardiographic indexes LVEDD, LVESD, and LVEF in two groups of patients at various time points during 4 years’ follow-up

<table>
<thead>
<tr>
<th>Time points</th>
<th>LVEDD (mm)</th>
<th>LVESD (mm)</th>
<th>LVEF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
</tr>
<tr>
<td>Implantation</td>
<td>50 ± 4.95</td>
<td>49.64 ± 4.93</td>
<td>29.67 ± 3.39</td>
</tr>
<tr>
<td>3 months</td>
<td>50.54 ± 4.7</td>
<td>49.79 ± 5.04</td>
<td>30 ± 3.41</td>
</tr>
<tr>
<td>1 year</td>
<td>51.63 ± 4.49</td>
<td>50.57 ± 4.91</td>
<td>31.21 ± 3.75</td>
</tr>
<tr>
<td>2 years</td>
<td>53.79 ± 5.46</td>
<td>50.46 ± 4.94</td>
<td>33.13 ± 4.43</td>
</tr>
<tr>
<td>4 years</td>
<td>56.71 ± 5.52</td>
<td>50.36 ± 4.84</td>
<td>35.38 ± 4.22</td>
</tr>
</tbody>
</table>
**Discussion**

In this study, we demonstrated for the first time that permanent RVA pacing leads to changes in the expression of genes that regulate myocardial contractile function and hypertrophy, traceable in the peripheral blood. These changes are evident early after pacemaker implantation (even at 3 months’ follow-up) and proceed LV remodelling. Furthermore, these early detected alterations in gene expression seem to be associated with LV remodelling in the long term.

Specifically, in group A, mRNA levels of SERCA gene decreased at 6 and 12 months’ follow-up, while \( \alpha \)-MHC decreased and \( \beta \)-MHC increased until 6 months’ follow-up, as has already been described in patients who are susceptible to LV deterioration.\(^9,10\) It should be noted that, at 12 months’ follow-up mRNA levels of \( \alpha \)-MHC had risen again and mRNA levels of \( \beta \)-MHC decreased. However, the reasons for these changes at 12 months are unclear. In group A, 25% of patients demonstrated significant LV remodelling, and the changes in the gene \( \beta \)-MHC at 6 months and SERCA at 12 months appeared to be associated with LV remodelling.

In contrast, patients in group B, with a low percentage of VP, did not demonstrate statistically significant changes, either in the expression of these genes in the peripheral blood or in echocardiographic parameters.

**Myocardial gene expression and heart failure**

The pathophysiology of HF is known to involve alterations in gene expression. Adverse cardiac remodelling is characterized by the re-employment of developmental transcription factors and the activation of the ‘foetal cardiac gene program’, including an isoform switch in MHC.\(^11,12\) Other molecular changes include alterations in the expression of genes encoding excitation–contraction coupling, such as SERCA, resulting in impaired calcium cycling and therefore accounting for contractile deficiency and pathological remodelling in HF.\(^7\) In that respect, SERCA expression and functional alterations seem to contribute to major cardiac diseases that often progress to HF; thus SERCA is assumed to be a marker and a therapeutic target in heart disease.\(^13\)

Although, this altered gene profile has been postulated as an adaptive response to mechanical overload, it further deteriorates LV function and remodelling, precipitating HF.\(^14\) Conversely, an increase in SERCA levels in experimental models has been associated with an improvement in LV function.\(^15\) Even in humans, when a therapeutic decision targeting this vicious circle has been made, amelioration of LV mechanics and reversal of this abnormal gene profile is observed. Specifically, in patients with idiopathic dilated cardiomyopathy \( \beta \)-blocker therapy not only improved LV function but also led to deactivation of the ‘foetal gene program’ with an increase in SERCA gene expression and a decrease of \( \beta \)-MHC gene.\(^9\) Similarly, in end-stage HF patients, implantation of an LV assist device had the same effects on gene expression.\(^10\) Finally, in HF patients, the clinical response to cardiac resynchronization therapy (CRT) was also associated with favourable changes in genes regulating LV contractile function and hypertrophy.\(^16\)

In our study, the changes observed in gene expression represented an early indication of LV dysfunction that would gradually lead to negative LV remodelling and deterioration in LV function.
Based on current guidelines, patients with a classical indication for pacemaker implantation and preserved EF are not eligible to receive a CRT device. In the case of HF development, a late upgrade is recommended with a view to achieving remission of the HF symptoms and restoration of LV function.17

By upgrading patients with an unfavourable gene profile to biventricular pacing at an early stage, we could prevent HF from developing. In this way, we could overcome several complications of the late upgrade due to vein thrombosis or device infection due to reoperation. Of course, this should translate to early upgrades in a very limited number of patients, based on the severity of the abnormal gene profile.

**Study limitations**

The gold standard for evaluation of the expression of genes regulating contractile function and hypertrophy is myocardial biopsy. However, in our study we chose to use peripheral blood to evaluate gene expression. The reason was that myocardial biopsy is a technique that requires highly trained personnel and technology and, even if proved to be of value in predicting LV remodelling, it cannot be performed in everyday clinical practice. Therefore, there is a need for parameters that are easily accessible on an everyday basis, such as peripheral blood measurements.

Additionally, peripheral blood has been suggested as a powerful substitute material that can monitor cardiovascular disease states and can probably act as a sensor of the disease severity.18 The significant overlap in the molecular mimicry of PBMCs and cardiomyocytes that is observed in animal models, implies that transcriptional signatures of PBMCs may serve as early non-invasive and novel sentinels, predictive of impending pathologic remodeling.19 Gene expression signatures associated with HF patient outcomes have also been identified in PBMCs.20 In addition, we have reported that alterations in the expression of α-MHC, β-MHC, and SERCA in PBMCs are associated with LV diastolic dysfunction in patients with idiopathic dilated cardiomyopathy and HF.21 Therefore, measurement of mRNA in peripheral blood, though not the gold standard, is still a reliable index. A possible strong correlation between gene expression in peripheral blood and deterioration in the contractile function of the LV could be of great clinical use.

A second limitation of this study could be that for the assessment of LV function and geometry we used simple indexes such as LV end-systolic and end-diastolic diameters and volumes, rather than...
using more advanced techniques, such as 2D strain or 3D echocardiography, that are more sensitive in detecting LV remodelling earlier. However, we preferred the former indexes as they are commonly used, can be easily implemented in everyday practice, and do not require particular expertise.

Finally, it should be noted that the majority of our patients were taking ACE inhibitors, angiotensin receptors blockers, and β-blockers. This kind of medication has been proven to slow down and/or reverse LV remodelling, so we cannot predict the impact that the absence of these medications would have on our results.

**Figure 4** Scatter plots of changes in SERCA at 12 months’ follow-up and changes in LVEDD and EF at 24 (left) and 48 (right) months’ follow-up. Note with the solid circle, patients that demonstrated significant LV remodelling.

**Table 4** Comparison of changes in β-MHC gene at 6 months and SERCA gene at 1 year, between patients of group A that demonstrated significant LV remodelling and those that did not; the difference between two subgroups was statistically significant

<table>
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<th></th>
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<th>ΔSERCA 12</th>
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<tr>
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Conclusions

In patients with preserved EF after permanent RVA pacing, alterations in the expression of genes regulating LV contractile function and hypertrophy measured in the peripheral blood are traceable at an early stage, before any changes in echocardiographic parameters are evident. These alterations seem to be associated with LV deterioration, even though larger studies are needed to confirm this finding. If solid data can be established concerning factors for the early prediction of LV remodelling, then patients who might benefit from other pacing modalities, such as biventricular pacing, could be identified during a very early phase.

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References