1　**Cardiac Fluid Dynamics Anticipates Heart Adaptation.**

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14 **ABSTRACT**

15 Hemodynamic forces represent an epigenetic factor during heart development and are supposed to

16 influence the pathology of the grown heart. Cardiac blood motion is characterized by a vortical

17 dynamics, and it is common belief that the cardiac vortex has a role in disease progressions or

18 regression. Here we provide a preliminary demonstration about the relevance of maladaptive intra-

19 cardiac vortex dynamics in the geometrical adaptation of the dysfunctional heart. We employed an *in*

20 *vivo* model of patients who present a stable normal heart function in virtue of the cardiac

21 resynchronization therapy (CRT, bi-ventricular pace-maker) and who are expected to develop left

22 ventricle remodeling, if pace-maker was switched off. Intra-ventricular fluid dynamics is analyzed by

23 echocardiography (Echo-PIV). Under normal conditions, the flow presents a longitudinal alignment of

24 the intraventricular hemodynamic forces. When pacing is temporarily switched off, flow forces develop

25 a misalignment hammering onto lateral walls, despite no other electro-mechanical change is noticed.

26 Hemodynamic forces result to be the first event that evokes a physiological activity anticipating cardiac

27 changes and could help in the prediction of longer term heart adaptations.

INTRODUCTION

Mechanical forces have an active biological role during morphogenesis. They stimulate cellular growth and multiplication at the microscopic level which eventually reflect on the macroscopic shaping of an organ as a whole (Farge, 2011; Freund et al., 2012). The importance of hemodynamic forces for heart morphogenesis was first demonstrated in the zebrafish, in which it was experimentally shown that intracardiac stresses imparted by the blood flow lead the proper heart development (Hove et al., 2003). Biological fluid forces are known to play a central role in the growth of the embryonic heart and vasculature (Reckova et al., 2003; Santhanakrishnan and Miller, 2011) so that the phenotype of congenital heart abnormalities was suggested to depend from the characteristics of blood flow forces acting on the developing tissues (Gruber and Epstein, 2004). Along the same line, it is suggested that hemodynamic forces should participate to pathological developments and therapeutic outcomes in the grown heart, although evidences are still lacking (Pasipoularides, 2012; Pedrizzetti et al., 2014).

The clinical syndrome of heart failure (HF) is the principal social threatening cardiac progressive dysfunction. It presents either as a primary pathology or because of a majority of primary diseases. The salient feature of HF is the development of left ventricle (LV) remodeling: a geometric modification and dilatation of the ventricular chamber that progressively reduce its muscular pumping ability. Despite modern treatments, hospitalization and death rate remain high, with nearly 50% people diagnosed with HF dying within 5 years (Levy et al., 2002).

The physiological causes that lead to LV remodeling are mainly ascribed to an increase of stresses on the myocardial fibres (around a scar area, because of higher systemic pressure etc.), which stimulate the growth and multiplication of cells and give rise to an increase of muscular thickness and then dilatation. Current models of cardiac remodeling, however, are not consistently predictive and remain rather primitive (Opie et al., 2006; Sengupta and Narula, 2008); although a variety of pathophysiologic

mechanisms have been suggested, there is paucity of methods capable of effectively forecasting the future risk of cardiac remodeling (Wu et al., 2008; Nijveldt et al., 2009). All existing models, in particular, do not account of the presence of hemodynamic forces that can trigger the sequence of events leading to progressive LV remodeling and eventually to HF (Pasipoularides, 2012; Pedrizzetti et al., 2014).

The distinguishing feature of cardiac blood flow is the presence of vortices. The sinuous flow paths around the vortex in the human heart were elegantly described by magnetic resonance visualization (Kilner et al., 2000). It was suggested that the asymmetric vortical arrangement was the flow functional counterpart of the looped heart structure that enhances the conservation of momentum from the entry jet to the ejected flow. It ensures an energetic balance of the longitudinal function during the filling-emptying mechanism with left ventricle asymmetry and vortex formation (Pedrizzetti and Domenichini, 2005). In recent years, numerous results about vortex dynamics in the human LV were produced using different techniques, from numerical simulations to magnetic resonance, to echocardiography (Markl et al., 2011; Sengupta et al., 2012). All these studies evidenced the presence of an intimate relationship between cardiac function and quality of intra-ventricular fluid dynamics.

A firm evidence of the relevance of LV fluid dynamics to the development and progression of a cardiac pathology, however, is still lacking. This is partly imputable to the difficulty of building comprehensive mathematical or experimental models capable of accounting of the complex transduction mechanism, where the large scale flow forces are sensed at the microscopic level, turn into cellular multiplication, and lead to alterations at the organ level (Pasipoularides, 2012). Here, we present an initial evidence that the quality of cardiac fluid mechanics could be a participating factor of heart adaptation mechanisms, namely adverse or reverse remodeling. These results may provide an interpretative ground for future clinical studies.

METHODS

We consider an *in vivo* model made of formerly HF patients with dilated LV that were subjected to cardiac resynchronization therapy (CRT, implant of bi-ventricular pace-maker) and that returned (after at least six months of therapy) to a stable condition with a LV of normal dimension and functional parameters. These subjects represent a special prototype model with a stably normal cardiac function with the support of the CRT. The same subjects, whether CRT is switched off, are expected to turn into an unstable state undergoing heart adaption and, within a few weeks, falling back into LV remodeling. The realization of both stable and unstable states on a same subject, at few seconds of distance, permits a deterministic one-to-one comparison.

These subjects were selected from a population of 30 (age 58±11years old) who underwent CRT device implant according to the in use guidelines for a non-ischemic and non-valvular dilated cardiomyopathy. Exclusion criteria were atrial fibrillation, severe renal insufficiency, acute coronary syndrome, cardiac insufficiency of advanced grade (NYHA IV), severe either pulmonary hypertension or obstructive pulmonary disease, uncontrolled systemic hypertension. At follow-up, all patients were in sinus rhythm with spontaneous atrio-ventricular conduction. In this population we identified a sub-group of 6 patients who presented a high response to the therapy (super-responders). This sub-group was characterized by a pre-CRT dilated LV with large volumes (end-systolic volume>160 ml, end-diastolic volume>200 ml) and reduced ejection fraction (EF<30%). The high response to the therapy, was defined by a reduction of more than 40% in both LV volumes and an EF above 40%. In the same population, as counter-examples, we also identified 2 subjects presenting the opposite outcome and did not get any benefit from the CRT (non-responders) whose LV volumes were not significantly reduced (<10%) after six months of therapy. The selection of extreme sub-groups (super-responders and non-responders) was driven by the objective of developing a deterministic biomechanical interpretation at an individual level and avoiding the statistical analysis typical for clinical results that are not the scope

98   of the present study. All subjects underwent echocardiographic examination. Cardiac mechanical

99   contraction was evaluated by the global longitudinal strain (GLS) while its synchronicity was evaluated

100 by the standard deviation of time to peak of transversal strain (SD-TTS) (Knappe et al., 2011) assessed

101 in bi-plane recordings (2- and 4-chambers apical views). Intra-cardiac fluid dynamics was measured

102 using an echographic adaptation of the optical particle image velocimetry, widely validated in clinical

103 applications (Echo-PIV) (Sengupta et al., 2012), on a longitudinal plane containing both the inlet and

104 outlet valves (3-chambers view). Echo-PIV permits a good temporal resolution but presents some

105 limitations in the quality of spatial distribution (noise) and in the detection of high velocities

106 (Kheradvar et al., 2010). For these reasons velocity information was here employed in averaged and

107 normalized terms only, that are less affected by local and instantaneous inaccuracies.

108 The dynamic interchange between flow and tissue was summarized by the rate of fluid momentum

$$109 \qquad \mathbf{m}(\mathbf{x},t) = \rho\left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v}\cdot\nabla\mathbf{v}\right); \qquad\qquad (1)$$

110 where ρ=1050 Kg/m^3 is the blood density and $\mathbf{v}(\mathbf{x},t)$ is the 2D velocity vector field. The field $\mathbf{m}(\mathbf{x}.t)$

111 corresponds, by Navier-Stokes balance, to the sum of the pressure gradient and the viscous terms,

112 where the latter is mostly negligible with the exception of the region next to the walls.

113 A measure of the global hemodynamic force (per unit depth) exerted by the fluid on the surrounding

114 tissue is obtained after spatial integration of (1)

$$115 \qquad \mathbf{M}(t) = \iint_{LV} \mathbf{m}\,dA. \qquad\qquad (2)$$

116 taken over the image area contained inside the LV chamber. Directional distribution of hemodynamic

117 forces during the entire heart cycle is summarized in terms of an intensity-weighted polar histogram

118 (like that used for wind description). For this, the circumference is divided in 12 sectors, centered in

119     $\theta_i=(2i-1)\pi/12$, $i=1..12$, and the force moduli during all the time instants in the heartbeat are summed up

120   when the angle falls in a corresponding sector. The resulting values are normalized to unit sum to

121   provide an intensity-weighted angular frequency distribution.

RESULTS AND DISCUSSION

123   The intraventricular fluid dynamics under stable conditions (pace-maker ON), estimated from Echo-

124   PIV (one example is shown in Figure 1), agrees with what was previously described in literature: a

125   circulatory pattern forming during the LV filling (diastole) that accompanies blood from the inlet

126   toward the outflow where it converges like in a funnel during the ejection (systole) (Kilner et al., 2000;

127   Pedrizzetti and Domenichini, 2014; Markl et al., 2011; Sengupta et al., 2012). When the pace-maker

128   therapy is discontinued (pace-maker OFF), the LV mechanical function should manifest early signs of

129   mechanical dysfunction driving toward the spiral of events leading to remodeling and HF. However,

130   the overall fluid dynamics does not evidence qualitative alterations. Some minor differences are shown,

131   for example, in Figure 1 where the entering jet is slightly displaced toward the side wall, or the

132   converging motion during ejection presents sharper bends. Thesesmall changes, however, globally

133   reflect into large deviations of intraventricular momentum away from the normal, longitudinal base-

134   apex alignment.

135   The directional deviation on intraventricular forces becomes evident in Figure 2, where the polar

136   histogram of $\mathbf{M}(t)$ during the entire heartbeat is reported for 4 subjects. In the stable configuration

137   (pace-maker ON, left column in Figure 2) the momentum is well aligned along the base-apex LV axis,

138   in compliance with the dynamics of the filling-emptying process. Few seconds after the pacing is

139   switched off (right column), the LV enters into a physiologically unstable state whose dynamics

140   anticipates heart adaptation. In this condition, flow loses its natural alignment, intraventricular forces

141 develops transversal components, despite cardiac contractility and synchrony parameters (GLS, SD-

142 TTS) do not evidence noticeable (or measurable) changes.

143 As a counter-example we performed the same analysis on the non-responders subjects. Differently

144 from before, as summarized in Figure 3, in those subjects the flow was neither aligned when the peace-

145 maker was active nor when it was switched off. Their state was unstable (or meta-stable, given the

146 extreme deformation) and the therapy was not able to create longitudinal hemodynamic forces.

147 These observations suggest that a modification of the natural fluid dynamics pattern is the first

148 recognizable mechanical phenomenon associated with an unstable condition that anticipates LV

149 remodeling. Flow changes presumably are due to minor modifications in the synchrony of tissue

150 motion, like local and short-lasting accelerations, that are difficult to detect directly but that reflect on

151 the overall dynamic balance of the incompressible fluid contained in the LV chamber.

152 Hemodynamics forces, by themselves, are not able to provoke large stresses that may deform a tissue

153 by fatigue. However, during morphogenesis, endothelial cells are able to sense vorticity and loading

154 conditions via shear changes (mechano-sensing), transforming any abnormal condition into adaptive

155 responses (mechano-transduction) (Pedrizzetti et al., 2014). The presence of forces acting on

156 inappropriate regions at inappropriate timings presumably activates, through a plethora of intracellular

157 signaling pathways, a physiological adaption mechanism that under prolonged over-stimulation leads to

158 the development of LV adaptation.

CONCLUSION

These results provide initial evidence that the natural longitudinal alignment of hemodynamic forces is a necessary condition for the presence of a physiologically LV stable state and to avoid heart adaptation. By logical equivalence, the lack of flow alignment is a sufficient condition for physiologically instability inducing heart adaptation.

Hemodynamic forces are known to participate to heart morphogenesis during the development of the embryonic heart. This study suggests that they also participate to physiological adaptations in the grown heart. In a more general perspective, large scale flow phenomena influence, through the mediation of sensing and transduction at the cellular level, the long term shaping of the cardiac organ as a whole.

Epigenetic mechanisms are a concurring factor in heart pathological adaptation and they are mediated by mechanical forces. The deeper understanding of how physical phenomena are associated to physiological outcomes could open a new comprehension about expression of phenotypes not revealed by (and not written in) the genetic structure only.

Conflict of Interest: none.

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231 **FIGURES**

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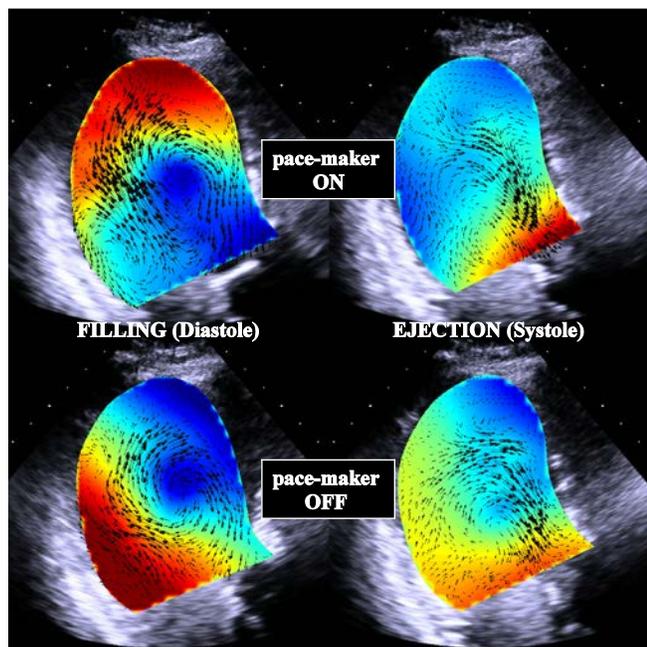
Figure 1. Example of flow changes following deactivation of the pace-maker in a subject who responded to the pacing therapy, during diastolic filling (left) and systolic ejection (right). The colormap represents the intraventricular pressure with a scale from red (higher pressure, relative to the mean value) to blue (lower).

237
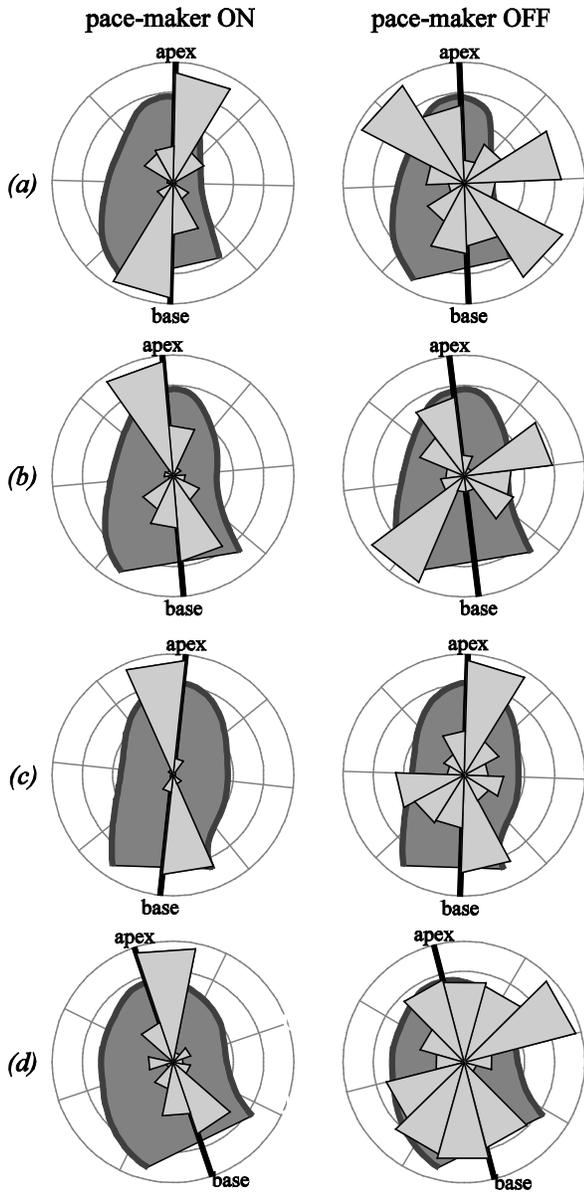
Figure 2. Polar histogram of intra-cardiac momentum distribution during the heartbeat in 4 subjects who well responded to the pacing therapy (super-responders), *(a)* to *(d)*, while the pacemaker is normally active and after a temporary deactivation.
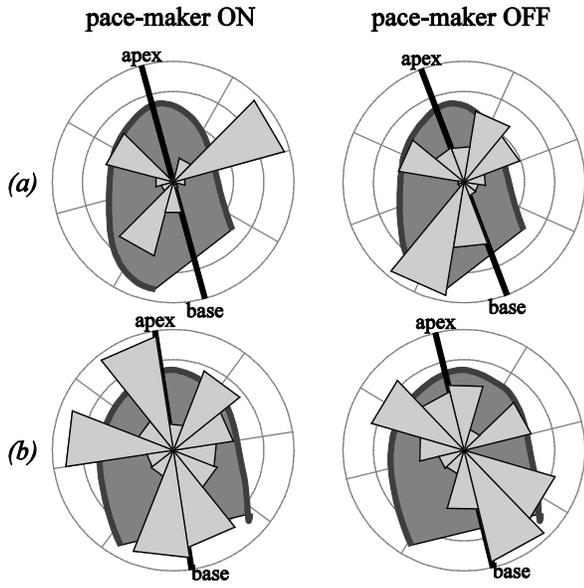
Figure 3. Polar histogram of intra-cardiac momentum distribution during the heartbeat in 2 subjects who did not benefit from the pacing therapy (non-responders), *(a)* to *(b)*, while the pacemaker is normally active and after a temporary deactivation.