LONG-TERM CONTROL OF ARTERIAL HYPERTENSION
AND REGRESSION OF LEFT VENTRICULAR HYPERTROPHY WITH
TREATMENT OF PRIMARY ALDOSTERONISM

Short title: Cardiac effects of hyperaldosteronism

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EXPANDED MATERIALS AND METHODS

Echocardiography

M-mode and 2-D echocardiography (Megas, Esaote Biomedica, Italy and Vivid 7 Pro, General Electric, USA) was performed in all patients with a 3.5-MHz transducer by a Cardiologist blind to the cause of hypertension and ongoing medical therapy. All measurements were performed on the average of at least three cardiac cycles according to the American Society of Echocardiography guidelines. In the PA cohort the first 34 echo studies at baseline were performed by a different Cardiologist on the cohort reported\(^1\) albeit in the same laboratory with the same overall methodology. Thus, 81% of all echocardiograms of the PA patients at baseline and 100% of the follow-up examinations were performed by the same Cardiologist (M.C.) at the same echo-laboratory, both for the Clinica Medica 4 and for the Endocrinology patients.

Two different echo machines were used over this period: first an Esaote Megas (Esaote Biomedica) and thereafter (from 2008) a Vivid 7 Pro (General Electrics). When used in parallel in a pilot study overall the two machines provided almost identical results, particularly as regards the M-mode parameters used to assess LV mass and Doppler parameters to evaluate diastolic function.

The Cardiologist who performed the echo study was instructed to remain blind to the diagnosis and to record the images for the measurements, which were thereafter performed offline without access to the medical history of the patient. The data collected were thereafter entered in a database by another investigator blind to the final diagnosis. A file with the latter was thereafter merged. In the essential hypertensive patients examined at the University of Milan all the echo were performed by the same operator (CC) using a similar procedure. A comparison of their echo parameters of these patients with those of the essential hypertensive patients recruited in Padua showed no systematic differences. Hence, the likelihood that there were observer-related biases in our analysis was minimized.

LV wall thickness and internal dimensions were measured from 2-D-guided M-mode echocardiographic tracings obtained at mid-chord level in the parasternal long axis view. The LV mass (LVM) was estimated according to Devereux et al.\(^2\) LV mass index (LVMI) was calculated by indexing LV mass to height\(^{2.7}\). Relative wall thickness (RWT) was calculated at end-diastole to estimate LV geometry, as

\[
\text{RWT}= \frac{\text{inter-ventricular septum thickness + posterior wall thickness}}{\text{LV diameter}}.
\]

The criteria for LV hypertrophy (LVH) were LVMI>50 g/m\(^{2.7}\) and >47 g/m\(^{2.7}\) for men and women, respectively. LVH was classified as concentric or eccentric using a cut off for RWT>0.45 or <0.45, respectively; an RWT ≥ 0.45 along with a normal LVMI identified LV concentric remodeling.\(^3,4\) LV end-diastolic and end-systolic volumes were calculated with the Teicholz’ correction of the cube formula.\(^5\) Ejection fraction was calculated by standard methods.\(^6\) Stroke work (SW) was estimated as systolic BP (measured after the echocardiographic study) times stroke volume and converted into gram-meters by multiplying by 0.0144.

The theoretical (predicted) value of LV mass, which provides an estimate of LV mass expected for cardiac workload, height\(^{2.7}\) (used as surrogate for genetically programmed lean body mass for that height) and gender,\(^6,7\) was calculated using an indicator variable for gender, height\(^{2.7}\), and stroke work, as a measure of cardiac workload.
Predicted LV mass (preLVM) was $= 55.37 + 6.64 \times \text{height (m}^2\text{)} + 0.64 \times \text{SW} - 18.07 \times \text{gender (where gender was coded as male = 1 and female = 2)}.\) The observed LV mass divided by the preLVM expressed as a percentage (observed LV mass/predicted LV mass*100) was categorized as inappropriate when in excess >35% from the predicted value using the 97.5th percentiles of the distribution in normotensive, taken as a normal reference adult population.\(^8\)

**Statistical Analysis**
The data are expressed as mean ± SD (or SEM, or median and range), as appropriate. Variables that were not normally distributed were analyzed after appropriate transformations. The group comparison was performed with one-way ANOVA and post-hoc Bonferroni’s test when needed. The within-patient comparison of baseline and follow-up echocardiographic and hemodynamic variables was carried out on normally distributed value with student t-test for paired data. ANCOVA using baseline systolic, diastolic or mean BP, each in separate analyses, as covariate was performed to test for differences in LVMI, either normalized for BSA or for height\(^{27}\). Moreover, as regression analysis on LVMI showed that BP values predicted LVMI, the BP-predicted values were saved and examined with ANOVA. Again the results and conclusions were identical as mentioned in the text. Statistical significance was defined as $P < 0.05$. SPSS 20.00 for Mac (SPSS Italy Inc., Bologna, Italy) was used for all analyses.
References for expanded materials and methods


### APA diagnosis by the four corners criteria.

<table>
<thead>
<tr>
<th>1) Biochemical diagnosis of PA:</th>
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<tr>
<td>a) Aldosterone ≥ 15 ng/dl, and</td>
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<tr>
<td>i) baseline aldosterone-renin ratio ≥ 26 or</td>
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<td>ii) post captopril aldosterone-renin ratio ≥ 11;</td>
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<td>2) Lateralized aldosterone secretion;</td>
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<td>3) Identification of an adrenal adenoma at imaging and/or pathology;</td>
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<td>4) Cure of the hyperaldosteronism and cure or improvement of hypertension post-adrenalectomy.</td>
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**Supplemental table S1.** APA diagnosis by the four corners criteria.
Supplemental Figures

**Supplemental Figure S1:** Flow chart of the study.
**Supplemental Figure S2: Panel A.** The graph shows the mean systolic and diastolic blood pressure at baseline and follow-up in the surgically- and medically-treated PA patients and in the primary hypertensive patients group. **Panel B.** The bar graph shows the change in the average number of anti-hypertensive drugs needed to achieve BP control at long-term follow-up in the surgically- and medically-treated PA patients and in the PH group. All changes were significant from baseline. Moreover, there was a significant difference across group by ANOVA due to the fall in the surgically-treated PA patients and the increase in the medically-treated PA and in the PH group.
Supplemental Figure S3: Left ventricular changes during primary aldosteronism and after specific treatment. LV: left ventricular; LVH: left ventricular hypertrophy; MR: mineralocorticoid.