Title: Long-term Mortality and Cardiovascular Events in Patients with Unilateral Primary Aldosteronism after Targeted Treatments

Methods

Standard TAIPAI protocol and Aldosteronism Consensus in Taiwan

Patients were enrolled from the following hospitals:

There were 2 tertiary medical centers, 3 affiliated hospitals and 2 regional hospitals in various cities of Taiwan joining this investigator group. Patients with other secondary hypertension, including renovascular hypertension, Cushing’s syndrome, hyperthyroidism, and pheochromocytoma were excluded from this study registry. All anti-hypertensive medications were discontinued for at least 21 days before screening tests. Doxazosin and/or diltiazem were administered to control markedly high blood pressure when required.

This study included two medical centers (National Taiwan University Hospital (NTUH), Taipei, Taiwan; Taipei University Hospital, Taipei, Taiwan) and five regional hospitals (Cardinal Tien Hospital, New Taipei City, Taiwan; Taipei Tzu Chi Hospital, New Taipei City, Taiwan; Yun- Lin Branch of NTUH, Douliou City, Taiwan; Hsin-Chu Branch of NTUH, Hsin-Chu City, Taiwan; Zhongxing Branch of Taipei City Hospital, Taipei, Taiwan).

Our standard protocol to identify primary aldosteronism (PA) and functional lateralization:
The diagnosis of primary aldosteronism was established in hypertensive patients on the basis of the following criteria:

**EH patients were screened negative for secondary hypertension during the study period.**

We attempted to diagnosis of secondary hypertension by clinical clues, physical examination and biochemical assessment, i.e. measurement of serum potassium levels, 24-h urinary sodium and potassium excretion, urinary and/or plasma free
normetanephrines, metanephrines, 24-h urine free cortisol, 1-mg overnight dexamethasone suppression test and active renin concentration or plasma renin activity, but should also entail instrumental tests, e.g., apnea/hypopnea index. Imaging is recommended when the screening is positive, e.g., computed tomography (CT) or magnetic resonance angiography, and renal catheter-based contrast angiography.

**Confirmation**

Fulfillment of the following three conditions confirmed a diagnosis of PA:

1. Autonomous excess aldosterone production evidenced with an aldosterone-renin ratio (ARR) > 35;
2. A TAIPAI score larger than 60%;
3. Post-saline loading PAC > 16 ng/dL or PAC/PRA > 35 (ng/dL)/(ng/mL/h) shown in a post-capotopril/losartan test.

(Abbreviations: PAC, plasma aldosterone concentration; PRA, plasma renin activity).

The probability of PA (TAIPAI score) was equal to:

\[ P = 1 + e^{-\beta} \]

where \( \beta = (\text{PAC \hspace{0.1cm} [ng/dL]} \times [0.063]) + (\text{PRA \hspace{0.1cm} [ng/mL/h]} \times [-0.205]) + ([\text{ARR} \times 0.001] \times [0.067]) + (\text{Male} \times [-0.738] + \text{SK} \times [-1.512]) + (\text{eGFR} \times [0.017]) + ([\text{propensity score}] \times [-0.539] + [1.851])

**Lateralization**

Unilateral APA (aldosterone producing adenoma, uPA) was identified on the basis on the following four criteria:

1. Confirmed PA;
2. An adrenal adenoma or hyperplasia evidenced with a CT or MRI scan;
3. Lateralization of aldosterone secretion with adrenal vein sampling (AVS) or during dexamethasone suppression NP-59 SPECT/CT;
4. APA is further confirmed after adrenalectomy:
   - Pathologically proven a CYP11B2 adenoma or aldosterone-producing cell clusters at immunohistochemistry after adrenalectomy, and subsequent emergence of biochemical correction.

**Selectivity and lateralization indices of AVS**

The selectivity index (SI) is defined as the ratio of the sampled cortisol concentration of each adrenal vein to that of the peripheral vein without the stimulation of cosynotropin. The lateralization index (LI) is defined as the ratio of the aldosterone/cortisol concentration on the dominant side to that on the contralateral side. Successful AVS is defined as an SI value ≥2.0 bilaterally. After confirming successful bilateral AVS, lateralization of the PA was determined by an LI value ≥2.0.
**Ethical considerations**
Ethical approval (201801049RIND, NCT00917345) was obtained from the institutional review board of the National Taiwan University Hospital. Written informed consent for clinical data collection and research use was obtained from participants at study enrollment. All methods were carried out in accordance with approved guidelines.

**Validation the clinical outcome of TAIPAI with Taiwan National Health Insurance Research Database**
Our study was further validated the outcome of interests, medication and baseline characteristics with a longitudinal database created by the National Health Research Institutes (NHRI) through extracting original Taiwan National Health Insurance (TNHI) data (23.12 million insured population in 2015).
Our study used a longitudinal database through Applied Health Research Data Integration Service from Taiwan’s National Health Insurance Administration (NHIA). The longitudinal database contains comprehensive healthcare information, including but not limited to general demographic information, acute inpatient hospitals, outpatient primary care and subspecialty office visits, outpatient pharmacies, diagnoses, prescriptions, long-term care facilities and medical events. To detect possible fraud in the NHI, the NHIA has been routinely auditing data and records submitted by healthcare institutions and providers. The NHIA is the only insurance carrier of covered healthcare in Taiwan. To avoid rejection of claim reimbursement from the NHIA, physicians in Taiwan usually follow clinical guidelines/policy suggested by the consensus. The indication and guideline for hypertensive management in Taiwan have proposed and revised by the Taiwan society of Hypertension. Briefly, a diagnostic algorithm was proposed, emphasizing the ESH/ESH joint hypertension guidelines suggestion to loosen BP targets to < 140/90 mmHg for all patients.

**Outcomes of interest**

**Outcome evaluation**

All-cause mortality was the primary outcome. Our secondary outcomes included *de-novo* (incident) MACE, atrial fibrillation (Af) and congestive heart failure (CHF) after the index date of PA confirmatory diagnosis. MACE was the incidence of
new-onset coronary events including non-fatal myocardial infarction (MI), coronary artery bypass graft (CABG), stroke and coronary angiography.

The data of this study were from a prospectively designed and collected TAIPAI cohort. We had a discipline in data-collection with the whole study team and well-trained research nurses/assistants. We also have double-checked our data with Taiwan National Health Insurance Research Database (NHIRD) to assure the completeness of the data entry. Therefore, we sincerely decreased the ascertainment bias including patients PA and EH patients.

For corroborating long-term events, we further validated TAIPAI records with Taiwan National Health Insurance Research Database (NHIRD). The Taiwan NHI is a nationwide insurance program that covers ambulatory visits, hospital admissions, prescriptions, interventional procedures and disease profiles for over 99% of the whole population in Taiwan (23.12 million in 2009). The records of outcomes of interest had high accuracy as validated by previous research because they were tied to the NHI reimbursement system with regular auditing. Therefore, we further confirm that there was little bias in the records of clinical data collection and outcome follow-up. The outcomes were determined blindly and had little bias. 15,17-19 The ICD-9 code of MI at hospitalization has high accuracy, as validated by previous research. 18,20 The records of CABG and angiography are also very reliable because they were constructed on the basis of NHI procedure codes that were tied to the NHI reimbursement system with regular auditing. 18 The diagnosis of stroke was an outcome of interest with validation from both radiographic image reports and ICD-9 diagnosis codes in this study and has been well attested. 21,22

The diagnosis of CHF is compounded by the typical reliance on the first listed diagnosis, which was well vindicated using the ICD-9 code from a population-based surveillance program. 23 To ensure the accuracy of the Af identification, the diagnosis of Af needed to be confirmed by doctor(s) and recorded in the diagnosis list of the medical record more than twice in ambulatory visits, or recorded in the discharge diagnosis list more than once in in-patient setting. 24 The diagnostic accuracy of Af based on the ICD-9-CM codes has been previously validated. 25

They were instructed to measure BP while in a sitting position twice daily for 1 week before visiting, and the number of antihypertensive agents each patient was using was recorded.

Assessment of clinical and biochemical outcomes after unilateral adrenalectomy according to the PASO criteria. 26
**Perspectives**

Our comparison of the effect of adrenalectomy versus MRA on long-term risks of mortality and cardiovascular events in uPA patients demonstrated that adrenalectomy could ameliorate all-cause mortality, compared with that of EH controls, and such effect is independent of post-operative hypertension-remission. However, post-adrenalectomy not-cured hypertension or MRA therapy could still be associated with higher risks of MACE than that of EH controls in uPA patients. We further matched the uPA patients in regard to their targeted treatments and suggested an obvious beneficial effect of adrenalectomy over MRA therapy on long-term risks of mortality, MACE, and CHF. Our study generated the first in-depth analysis on the more favorable effects of adrenalectomy over MRA treatment on the survival and cardiovascular benefits among uPA patients in the long run.

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Membership of the Taiwan Primary Aldosteronism Investigation (TAIPAI) Study Group:

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Table 1. The characteristics of enrollees between PA patients underwent adrenalectomy or MRA.

**Abbreviations:** ACEI/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; Af, atrial fibrillation; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CHF, congestive heart failure; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; HTN, hypertension; K, potassium; MACE, major cardiovascular event; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; Primary Aldosteronism Surgical Outcome (PASO) criteria; sBP, systemic blood pressure; s/p, post-adrenalectomy.
**Table 2.** Risk of mortality in clinical APA patients sub-grouped by complete cure after adrenalectomy and EH by Cox proportional hazard model.

**Abbreviations:** ACEi/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; EH, essential hypertension; K, potassium; MACE, major cardiovascular event; APA, aldosterone producing adenoma; sBP, systemic blood pressure,
Table 3. Risk of subsequent MACE in clinical APA patients sub-grouped by complete cure after adrenalectomy and EH by Cox proportional hazard model, taking mortality as a competing factor.

**Abbreviations:** ACEi/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; K, potassium; MACE, major cardiovascular event; APA, aldosterone producing adenoma; sBP, systemic blood pressure,
**Table 4.** Risk of subsequent CHF in clinical APA patients sub-grouped by complete cure after adrenalectomy and EH by Cox proportional hazard model, taking mortality as a competing factor.

**Abbreviations:** ACEi/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CHF, congestive heart failure; CKD, chronic kidney disease; DBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; K, potassium; MACE, major cardiovascular event; APA, aldosterone producing adenoma; SBP, systemic blood pressure,
Table 5. Risk of subsequent Af in clinical APA patients sub-grouped by non-cure after adrenalectomy and EH by Cox proportional hazard model, taking mortality as a competing factor.

Abbreviations: ACEi/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; Af, atrial fibrillation; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CHF, congestive heart failure; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; K, potassium; MACE, major cardiovascular event; APA, aldosterone producing adenoma; sBP, systemic blood pressure,
Table S6. Factors identified as predictors of adrenalectomy or underwent MRA in clinical APA patients with the logistic regression model as the inverse probability of treatment weighted (IPTW) matching.
ACEI/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CHF, congestive heart failure; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; K, potassium; MACE, major cardiovascular event; PA, primary aldosteronism; sBP, systemic blood pressure,

Goodness-of-Fit Assessment: VIF of all factors: all <3.0; adjust generalized R square=0.81; area under the ROC curve=0.72
**Table S7.** Incidence and Risks for mortality, between Clinical APA patients underwent adrenalectomy or MRA treatment after IPTW adjusted and taking adrenalectomy as a time varying risk.

**Abbreviations:** CHF, congestive heart failure, IPTW, inverse probability of treatment weighting; K, potassium, MRA, mineralocorticoid receptor antagonist.

**Table S8.** Incidence and Risks for MACE, between clinical APA patients underwent adrenalectomy or MRA treatment after IPTW adjusted and taking adrenalectomy as a time varying risk.

**Abbreviations:** CHF, congestive heart failure; MACE, major cardiovascular event; IPTW, inverse probability of treatment weighting; MRA, mineralocorticoid receptor antagonist.

**Table S9.** Incidence and Risks for CHF between clinical APA patients underwent adrenalectomy or MRA treatment after IPTW adjusted and taking adrenalectomy as a time varying risk.

**Abbreviations:** CHF, congestive heart failure, IPTW, inverse probability of treatment weighting; K, potassium; MRA, mineralocorticoid receptor antagonist.

**Table S10.** Incidence and Risks for Af, between clinical APA patients underwent adrenalectomy or MRA treatment after IPTW adjusted and taking adrenalectomy as a time varying risk.

**Abbreviations:** Af, arterial fibrillation, IPTW, inverse probability of treatment weighting; K, potassium, MRA, mineralocorticoid receptor antagonist.
Table s11.  Long-term outcomes of interest grouped by biochemical outcomes.
(s/p biochemical remission vs EH)

**Abbreviations:** Af, atrial fibrillation; CHF, congestive heart failure; CI, confidence interval; EH, essential hypertension; HTN, hypertension; MACE, major cardiovascular event.
Table s12. Comparison of patients treated with MRA in whom renin remained suppressed vs not.

**Abbreviations:** ACEI/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; Af, atrial fibrillation; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CHF, congestive heart failure; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; HTN, hypertension; K, potassium; MRA, mineralocorticoid receptor antagonist; PA, sBP, systemic blood pressure; s/p, post-adrenalectomy.
**Figure S1.** Standardized mean difference (SMD) between the covariates regarding adrenalectomy and MRA use after propensity score matching.
Dash line, the SMD of +/-0.1

**Abbreviations:** ACEI/ARB, angiotensin converting enzyme inhibitors and angiotensin-receptor blockers; ARR, aldosterone to renin ratio; CAD, coronary artery disease; CHF, congestive heart failure; CKD, chronic kidney disease; dBP, diastolic blood pressure; DM, diabetic mellitus; eGFR, estimated Glomerular filtration rate; K, potassium; MACE, major cardiovascular event; SMD, Standardized Mean Difference ;APA, aldosterone producing adenoma; sBP, systemic blood pressure,

**Figure S2.**
GAM plot for the probability of all -cause mortality or major cardiovascular events (MACE) for post operative aldosterone, post MRA treatment renin in clinical APA patients. The model incorporates the subject-specific (longitudinal) random effects, expressed as the logarithm of the odd (logit). The probability of outcome events was constructed with biochemistry level and was centered to have an average of zero over the range of the data as constructed with the GAM. (A) Plasma aldosterone concentration higher than 27ng/dL after adrenalectomy and (B) Plasma renin activity less than 0.6 ng/mL/hr after MRA treatment were independent risk for all -cause mortality or MACE .The curve was centered to have an average of zero over the range of the data. The dashed lines indicated approximated point-wise 95% CIs.

(A)

(B)

**Figure S3.** Central Illustration of Long-term Mortality and Cardiovascular events in Patients with Aldosterone Producing Adenomas after Target Treatments†

**Abbreviations,** Af, atrial fibrillation; CHF, congestive heart failure; CI, confidence interval; CtL, control; EH, essential hypertension; HR, hazard ratio; HTN, hypertension; MACE, major cardiovascular event; MRA, mineralocorticoid receptor antagonist; OP, operation.

† Complete clinical success (hypertension-remission) was defined as normal BP
without usage of anti-hypertensives, those with residual hypertension were HTN Not cured according to the PASO consensus criteria. 26 (supplementary methods)

Reference

8. Wu VC, Yang SY, Lin JW, Cheng BW, Kuo CC, Tsai CT, Chu TS, Huang KH, Wang SM,


