R&B Form No. 1A

DATABANK INFO NEEDED:
☐ No
☐ Yes
Specify _____

St. Luke's Medical Center RESEARCH AND BIOTECHNOLOGY

CLINICAL RESEARCH PROPOSAL

INSTRUCTIONS:

1. *The following are the requirements for the submission of proposal:*
   - *Six (6) copies of the study protocol with **version no. and date** on the lower left hand corner (See below)*
   - *Endorsement letter from Department / Institute Research Committee Head noted by the Institute/Department Head addressed to Dr. Noel D.J. Atienza, Chair-ISRC and IERC Chair, Dr. Prospero Ma. Tuaño*
   - *Copy of Technical Review Forms for Research Committee signed by Research Committee Reviewer/s*
   - *Disclosure of Conflict of Interest Form for all authors*
   - *Data collection forms if applicable*
   - *Informed Consent Form(ICF) if applicable (English and Tagalog)*
   - *Budget Proposal Form*
   - *Curriculum Vitae (CV) of the Study Team*
   - *Good Clinical Practice (GCP) certificate of the study team*
   - *Soft copy sent in email (scientificreview.qc@stlukes.com.ph)*

2. *All information should be answered completely and accurately, and should be typewritten and **signed by proponents.***

RESEARCH TITLE:

Sparing Confirmatory Testing in Primary Aldosteronism: The Combination of Renin, Aldosterone and Potassium levels

INVESTIGATORS:	**Name & Signature**	**Unit/Position**
Project Leader/s:	Albert Macaire Chua Ong Lopez	SLMC-QC Fellow
	Diana Collen Dimayuga	SLMC-BGC Fellow
	Dylan Jansen V. Taytayon	UST Fellow
	Leo E. Tiu Jr.	MMC Fellow

Co-Project Leader/s:	Oliver Allan Dampil	Consultant
	Erick S. Mendoza	Consultant
	Michael Villa	Consultant
	Andrea Marie Macabuag-Oliva	Consultant

Research Assistant/Fellow: None

Inst./Dept./Centers:

St. Luke's Medical Center-Quezon City, Section of Endocrinology, Diabetes and Metabolism

St. Luke's Medical Center-Global City, Section of Endocrinology, Diabetes and Metabolism

University of Santo Tomas Hospital, Section of Endocrinology, Diabetes and Metabolism

Makati Medical Center, Section of Endocrinology, Diabetes and Metabolism

1. COOPERATING AGENCY OR COLLABORATORS, if any: None

2. BRIEF DESCRIPTION / PROJECT SUMMARY:

Background and Objective: The diagnosis of primary aldosteronism is comprehensive which includes case-detecting testing, case confirmation followed by subtype classification. In certain instances, one may not proceed with further confirmatory testing in the setting of spontaneous hypokalemia, undetectable renin, and PAC > 15-20 ng/dL. However, this quality of evidence is very low. This study sought to evaluate the proposed "simplified confirmatory pathway" that can spare confirmatory testing for primary aldosteronism by evaluating the diagnostic performances of the various pre-specified PAC thresholds in combination with suppressed renin and spontaneous hypokalemia.

Methodology: This will be a multi-center, cohort-selected cross-sectional study with a sampling total of 157 subjects aged 18 years and above who underwent saline infusion test between January 2010 to October 2022. Diagnostic performances including the sensitivity, specificity, negative predictive value, positive predictive value of the different combinations (baseline PAC, PRA and presence of spontaneous

hypokalemia) are the outcome measures in this study. Data analysis will be performed by *SPSS 29.0.1.0* & *MedCalc 20.218*.

Abbreviations:

1. PA – primary aldosteronism
2. PAC – plasma aldosterone concentration
3. PRA – plasma renin activity
4. K^+ - serum potassium
5. ARR – aldosterone-renin ratio
6. SIT – saline infusion test

3. INTRODUCTION

3.1. SIGNIFICANCE OF THE PROJECT:

This study shall provide a critical assessment of the current body of knowledge regarding sparing confirmatory test/s for primary hyperaldosteronism, and offer supporting evidence in lieu of the clinical practice guidelines.

3.2. RATIONALE FOR DOING THE STUDY

Primary aldosteronism is one of the most common causes of secondary hypertension. It is a prevalent disease that is often unrecognized, especially if milder in severity. In a systematic review totaling 42,510 patients, prevalence estimates ranged from 3.2% to 12.7% in primary care and from 1% to 29.8% in referral centers[1]. The prevalence was identified to be higher in patients with new onset diabetes mellitus. In a study of 256 outpatients with hypertension and diabetes mellitus, 49 (19%) were diagnosed with primary aldosteronism[2].

In the diagnosis of suspected primary aldosteronism, it requires initial case-detection testing with morning blood sample in a seated patient: plasma aldosterone concentration, plasma renin activity or plasma renin concentration. After case detection, according to Endocrine Society Guidelines 2016, patients who had a positive aldosterone renin ratio (ARR) result should undergo one or more confirmatory tests to

confirm or exclude primary aldosteronism[3]. These confirmatory testing include the saline infusion test, captopril challenge test, oral sodium loading test and the fludrocortisone suppression test. Despite its many benefits, there are some shortcomings to confirmatory testing such as the cost, time requirements and inconveniences.

There are however some exceptions to the requirement for confirmatory testing. According to the Endocrine Society Guidelines 2016, in the setting of very low plasma renin levels, a plasma aldosterone concentration (PAC) of >20 ng/dL with spontaneous hypokalemia, one may not proceed with further confirmatory testing[3]. Furthermore, in a recent updated approach to the evolution of primary aldosteronism syndrome, a suppressed renin activity (PRA) of at least less than 1.0 ng/mL/h (ideally plasma renin activity < 0.6 ng/mL/h or plasma renin concentration < 5 mU/L), plasma aldosterone concentration greater than 15 ng/dL and a high pretest probability consisting of resistant hypertension and/or hypokalemia, an "overtly positive screen" is detected and no further dynamic testing is required[4]. However, these recommendations have very low quality evidence. The suitable setting for sparing confirmatory tests is still under debate and requires more body of evidence.

3.3. BACKGROUND INFORMATION AND BRIEF LITERATURE REVIEW

The impetus for bypassing the confirmatory step and directly proceed with discriminatory step is suggested based on these few studies. Based on a retrospective study of French hypertensive population which included 173 patients, baseline plasma aldosterone concentration was 3-fold higher among aldosterone producing adenoma and idiopathic aldosteronism patients than in essential hypertension patients ($p < 0.0001$). 91% of aldosterone producing adenoma patients had baseline PAC above 550pmol/l as compared to 14% only in essential hypertension subjects. Direct renin concentrations were likewise lower in aldosterone producing adenoma and idiopathic aldosteronism patients compared to essential hypertension patients ($p < 0.001$ and $p < 0.02$, respectively). 82.9 % of aldosterone producing adenoma patients are hypokalemic versus 20.2% only for essential hypertension patients[5].

In another retrospective cross-sectional study conducted in Japan involving 327 patients, all patients with PAC >30 ng/dl were diagnosed with primary aldosteronism. All 26 patients with PAC between

20 to 30 ng/dl who had spontaneous hypokalemia were diagnosed with PA[6].

Recently, a development and validation criteria for sparing confirmatory tests in diagnosing primary aldosteronism were created and compared with the 2016 Endocrine Society guideline criteria. The criteria were developed in a Chinese cohort using the captopril challenge test as confirmatory test, verified by saline infusion test, or fludrocortisone suppression test, and further validated in an Australian cohort. In the development cohort (518 PA and 266 non-PA), the combination of PAC＞20ng/dl plus PRC＜2.5 μIU/ml plus hypokalemia had much higher sensitivity than the Endorine Society guideline criteria (0.36 vs 0.11). In the validation cohort (125 PA and 81 non-PA), the sensitivity of the optimized criteria was also significantly higher (0.12 vs. 0.02)[7].

4. OBJECTIVES:

GENERAL OBJECTIVE

To evaluate the proposed "*simplified confirmatory pathway*", which will exclude the need for dynamic confirmatory testing for primary hyperaldosteronism and compare the findings with the recent Endocrine Society clinical practice guideline criteria and recommendations.

SPECIFIC OBJECTIVES, if any

1. To describe the sociodemographic, clinical and laboratory characteristics of patients who underwent saline infusion test.

2. To evaluate the diagnostic performances of the various combinations of baseline plasma aldosterone concentration (PAC), baseline plasma renin activity (PRA), and presence of spontaneous hypokalemia (K^+) in the diagnosis of primary hyperaldosteronism

3. To elucidate the subtype classification proportion of patients with positive saline infusion test with unilateral aldosteronoma, bilateral adrenal hyperplasia, or adrenocortical carcinoma on adrenal CT and/or surgical pathology.

5. METHODS:

5.1. Type of study & time period & target population

This study will be conducted as a retrospective diagnostic accuracy cohort-selected cross-sectional study[8] at multiple outpatient referral centers namely – St. Luke's Medical Center-Quezon City, St. Luke's Medical Center-Global City, Makati Medical Center and University of Santo Tomas Hospital.

Relevant clinical and laboratory data will be obtained from medical records of all eligible participants who underwent saline suppression test. The saline suppression test will serve as the reference test which is used to confirm the presence or absence of primary aldosteronism disease. As shown in the schematic diagram below, pertinent clinical and laboratory data will be retrieved from saline infusion test patients who also underwent the screening test initially. This primarily includes the following blood exams: baseline plasma renin activity (PRA), plasma aldosterone concentration (PAC), and serum potassium, respectively. The combinations of these screening laboratory values will then be investigated for its diagnostic performance, which will serve as the main index test in this study. The time interval between the index and reference test was performed without too much delay (<1 month) and not at the same time.

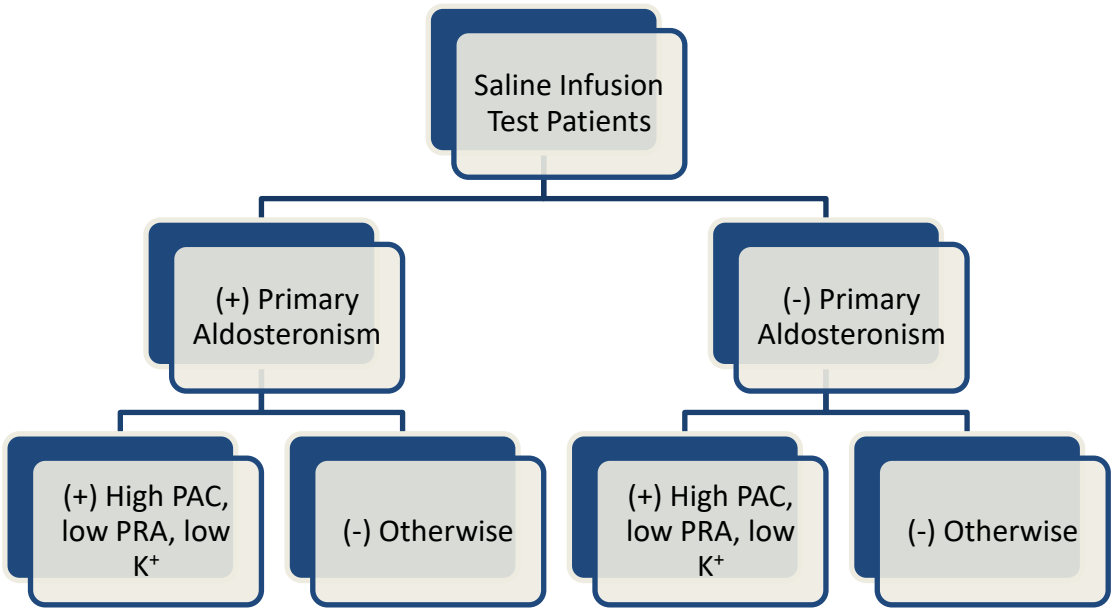

Figure 1. Schematic diagram of cross-sectional study design

5.2. Inclusion criteria and Exclusion criteria for subject selection

Patients above 18 years of age who underwent saline infusion test between January 2010 to October 2022 will be included in this study. Those who did not complete the said procedure or did not

comply with the saline infusion protocol will be excluded.

5.3. Operational definitions

a. Confirmed primary hyperaldosteronism – post saline-infusion plasma aldosterone levels of >10 ng/dL; or for indeterminate values between 5-10 ng/dL, a cutoff of 6.8 ng/dL
b. Elevated plasma aldosterone level –

   Marked aldosterone elevation PAC > 30 ng/dL

   Moderate aldosterone elevation PAC 20-30 ng/dL
c. Low plasma renin activity – PRA < 1.0 ng/ml/hr
d. Markedly suppressed plasma renin activity – PRA < 0.6 ng/ml/hr
e. Elevated aldosterone-to-renin ratio (ARR) – ARR > 30
f. Spontaneous hypokalemia – potassium < 3.5 without drug interference

5.4. DESCRIPTION OF STUDY PROCEDURE

The logbook and other registries from the respective diabetes, thyroid and endocrine centers will be used as the main source for subject selection. Lists of all the potential study participants who underwent saline infusion test will be thoroughly reviewed by looking through their electronic medical records for demographic, clinical characteristics, laboratory and CT scan findings, and other surgical and histopathology results.

The saline infusion test was conducted in accordance with a pre-specified institution-based protocol. This specific test will be primarily used to confirm primary aldosteronism. Patients remained in supine position for at least 1 hour prior to saline infusion. Samples of plasma aldosterone and serum potassium were drawn at baseline. Afterwards, 0.9% sodium chloride were infused at rate of 500 ml per hr over 4 hours for a total of 2 liters. At the end of infusion, repeat plasma aldosterone and serum potassium were extracted. A positive test result is defined as post saline-infusion plasma aldosterone levels of >10 ng/dL. For indeterminate values between 5 and 10 ng/dL, a cutoff of 6.8 ng/dL (190 pmol/L) will be used and reclassified as positive test for primary aldosteronism. The saline infusion test protocol was similar

across the involved hospital institutions in this study.

5.5. DESCRIPTION OF OUTCOME MEASURES

Primary outcome measures in this study shall include the specificity and positive predictive value of the following pre-specified cutoff combinations and will be obtained at baseline ("screening values') through electronic health records (Table 1). Secondary outcomes measures shall contain the sensitivity, negative predictive value and the global diagnostic accuracy of the stated cutoff combinations and similarly will be accessed at baseline through electronic health records (Table 1).

Table 1. Proposed index test variables

A1 – PAC > 10 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia	B1 – PAC > 10 ng/dL + PRA<1.0 ng/ml/hr
A2 – PAC > 15 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia	B2 – PAC > 15 ng/dL + PRA<1.0 ng/ml/hr
A3 – PAC > 20 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia	B3 – PAC > 20 ng/dL + PRA<1.0 ng/ml/hr
A4 – PAC > 25 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia	B4 – PAC > 25 ng/dL + PRA<1.0 ng/ml/hr
A5 – PAC > 10 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia	B5 – PAC > 10 ng/dL + PRA<0.6 ng/ml/hr
A6 – PAC > 15 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia	B6 – PAC > 15 ng/dL + PRA<0.6 ng/ml/hr
A7 – PAC > 20 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia	B7 – PAC > 20 ng/dL + PRA<0.6 ng/ml/hr
A8 – PAC > 25 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia	B8 – PAC > 25 ng/dL + PRA<0.6 ng/ml/hr

sensitivity, negative predictive value, diagnostic accuracy

5.6. SAMPLING METHOD and SAMPLE SIZE ESTIMATION

A purposive sampling method will be employed for this study to achieve the target sample size. The sample size calculation for sensitivity and specificity of a single diagnostic test with a binary outcome according to *Buderer* was performed[9]. A minimum sample size of 157 participants was computed for a confidence level of 95%, precision of 0.10, with an estimated crude prevalence rate of 26%[10] based from the prevalence study by *Song et al.*, and assuming a 100% specificity and 12% sensitivity as based on the validation cohort saline infusion test dataset of *Wang et. al.*[7]

5.7. DATA ANALYSIS

For descriptive statistics, frequencies and percentages will be presented for nominal/ordinal data, while mean ± SD (range) will be shown for continuous/scale data. Statistical significance will be determined when two-tailed p-value is <0.05. Differences between groups will be assessed by χ^2 or Fisher's exact test for dichotomous categorical variable, Mann-Whitney U test for non-normally distributed numerical variables, and Student's *t* test for normally distributed data, as appropriate. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of each combination will be reported at 95% confidence interval using the 2x2 table. Statistical analyses will be performed by *SPSS 29.0.1.0 and MedCalc 20.218.*

5.8. ETHICAL CONSIDERATIONS

The main authors in this study are responsible for ensuring that all activities meet ethical standards. The study shall abide by the Principles of Declaration of Helsinki and will be conducted along the Guidelines of the International Conference on Harmonization-Good Clinical Practice (ICH-GCP). The Clinical Protocol and all relevant documents shall be reviewed and approved by the SLMC Institutional Ethics Review Committee. The authors declare that there is no conflict of interests with study collaborators, and subjects.

Patient confidentiality shall be respected by ensuring anonymity of patient records. Each patient document is coded and will not contain any identifying information to ensure confidentiality. All study data shall be recorded, and investigators are responsible for the integrity of the data i.e accuracy, completeness, legibility, originality, timeliness, and consistency. The manner of disseminating and communicating the study results shall guarantee the protection of the confidentiality of patient's data. All study-related documents such as all versions of the protocol, ethical clearance, data collection forms, hard copies of source documents, shall be kept and stored by the Principal Investigator in strict confidentiality for at least 5 years; after which they will be shredded.

For data protection plan, the main person responsible for storage of data shall be the principal investigator. Hard copy study-related documents will be stored in a cabinet with lock and key. The key will be kept by the project leader and the cabinet which is located in a secure room, is only accessible to members of the research team.

For interhospital data dissemination plan, there will be a central shared data which will be kept anonymous, and only the principal investigator per institution will have access to it.

6. SCHEDULE OF ACTIVITIES:

Tasks	Nov 2022	Dec 2022	Jan 2023	Feb 2023	Mar 2023	Apr 2023	May 2023	June 2023
Protocol Amendment Submission to Research & Biotechnology Department								
Review by Institutional Review Board								
Data collection								
Analysis of Data								
Submission of 1st draft								
Submission of 2nd draft								
Submission of final analytical research paper								
Presentation								
Submission of revised final analytical research paper								

7. REFERENCES:

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2. Hu Y, Zhang J, Liu W, Su X. Determining the Prevalence of Primary Aldosteronism in Patients With New-Onset Type 2 Diabetes and Hypertension. *J Clin Endocrinol Metab*. 2020;105(4):dgz293. doi:10.1210/clinem/dgz293

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7. Wang K, Hu J, Yang J, et al. Development and Validation of Criteria for Sparing Confirmatory Tests in Diagnosing Primary Aldosteronism. *J Clin Endocrinol Metab*. 2020;105(7):dgaa282. doi:10.1210/clinem/dgaa282

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10. Song Y, Yang S, He W, et al. Confirmatory Tests for the Diagnosis of Primary Aldosteronism: A Prospective Diagnostic Accuracy Study. *Hypertension*. 2018;71(1):118-124. doi:10.1161/HYPERTENSIONAHA.117.10197

8. APPENDIX

8.1. INFORMED CONSENT FORM, if applicable: not applicable
8.2. DATA COLLECTION FORMS (see attached form)

8.3 Dummy Tables

Table 1. Clinical characteristics between PA vs non-PA patients

Variables	**PA**	**Non-PA**	***p*-values**
Age, mean (SD), y			
Sex at birth			
Male, No./No. (%)			
Female, No./No. (%)			
Spontaneous hypokalemia, No./No. (%)			
PAC, mean (SD), ng/dL			
PRA, mean (SD), ng/ml/h			
ARR			

Table 2. 2 x 2 Contingency Table

	PA (SIT +)	**Non-PA (SIT -)**	**Total**
Index test +			
Index test -			
Total			

Table 3. Diagnostic Performances of Laboratory Findings

	Sen	Spec	PPV	NPV	Prev	DA
A1 – PAC > 10 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia						

A2 – PAC > 15 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia						
A3 – PAC > 20 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia						
A4 – PAC > 25 ng/dL + PRA<1.0 ng/ml/hr+spont. hypokalemia						
A5 – PAC > 10 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia						
A6 – PAC > 15 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia						
A7 – PAC > 20 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia						
A8 – PAC > 25 ng/dL + PRA<0.6 ng/ml/hr+spont. hypokalemia						
Legend: Sen – Sensitivity Spec – Specificity PPV – Positive predictive value NPV – Negative predictive value Prev – Prevalence DA – Diagnostic Accuracy						

9. CODING MANUAL

Variable	Label	Measurement Level	Coding
IDS	Subject number	Scale	As is
Age	Age of patient when test was done	Scale	As is “99” = missing value
Sex	Biologic sex of patient	Nominal	“0” = Male “1” = Female
HTN	Diagnosed hypertension	Nominal	“0” = No “1” = Yes “99” = missing value
DM	Diagnosed diabetes mellitus	Nominal	“0” = No “1” = Yes “99” = missing value
CKD	Diagnosed chronic kidney disease	Nominal	“0” = Normal or G1 “1” = G2 “2” = G3a “3” = G3b “4” = G4 “5” = G5 “99” = missing value
HypoK	Spontaneous hypokalemia	Nominal	“0” = No “1” = Yes “99” = missing value

BasePAC	Baseline plasma aldosterone concentration (ng/dl)	Scale	As is "99" = missing value
PRA	Baseline plasma renin activity (ng/ml/hr)	Scale	As is "99" = missing value
PrePAC	Pre saline infusion test plasma aldosterone concentration (ng/dl)	Scale	As is "99" = missing value
PostPAC	Post saline infusion test plasma aldosterone concentration (ng/dl)	Scale	As is "99" = missing value
ARR	Aldosterone renin ratio	Scale	As is "99" = missing value
HighPAC	Elevated plasma aldosterone concentration	Nominal	"0" = No "1" = Yes
LowPRA	Low plasma renin activity	Nominal	"0" = No "1" = Yes
HighARR	Elevated aldosterone renin ratio	Nominal	"0" = No "1" = Yes
Criteria1	High plasma aldosterone concentration (PAC) + low plasma renin activity (PRA) + spontaneous hypokalemia	Nominal	"0" = No "1" = Yes
Criteria3	High aldosterone renin ratio (ARR) + spontaneous hypokalemia	Nominal	"0" = No "1" = Yes
Criteria5	High plasma aldosterone concentration (PAC) + spontaneous hypokalemia	Nominal	"0" = No "1" = Yes
Adrenal	Abdominal CT scan	Nominal	"0" = No "1" = Yes
AdreLat	Adrenal lesion laterality	Nominal	"0" = No discrete lesion "1" = Unilateral "2"= Bilateral
Surgery	Surgery done	Nominal	"0" = No "1" = Yes
HistPath	Histopathology report	Nominal	"0" = adenoma "1" = adenocarcinoma "2"= Others

10. PROPOSED BUDGETARY REQUIREMENTS

PROPOSED BUDGET FOR 2022

RESEARCH TITLE: Sparing Confirmatory Testing in Primary Aldosteronism: The Combination of Renin, Aldosterone and Potassium levels

I.	**Personnel Services:**		
	Biostatistician		P 0.00
II.	**Clinical Procedures:**		
	None		
III.	**Office Supplies** (Please specify)		
	Data Collection Forms		P 0.00
	Miscellaneous		P 0.00
		Subtotal	**P 0.00**
IV.	**Others** (Please specify)		
	None		
TOTAL AMOUNT (I + II + III + IV)			**P 0.00**