**SUPPLEMENTAL DATA**

**Development and validation of a prediction score to avoid confirmatory testing in patients with suspected primary aldosteronism.**

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**Table S1. Patient Characteristics of Study Cohort: Univariate Regression Analysis**

|  |  |  |
| --- | --- | --- |
| **Variable (ref. PA confirmed)** | **OR (CI 95%)** | ***P-*value** |
| Age at diagnosis (years) | 0.99 (0.98-1.01) | 0.202 |
| Female sex, n (%) | 0.34 (0.24-0.46) | **<0.001** |
| Duration of HTN (months) | 1.01 (1.00-1.01) | 0.089 |
| Systolic BP (mmHg) | 1.01 (1.01-1.02) | **0.003** |
| Diastolic BP (mmHg) | 1.01 (1.00-1.03) | 0.052 |
| Antihypertensive medication (DDD) | 1.40 (1.27-1.54) | **<0.001** |
| BMI (Kg/sqm) | 1.02 (0.99-1.06) | 0.204 |
| PRA at screening (ng/mL/h) | 0.28 (0.14-0.57) | **<0.001** |
| Aldosterone at screening (ng/dL) | 1.06 (1.04-1.08) | **<0.001** |
| Lowest Potassium (mEq/L) | 0.13 (0.09-0.19) | **<0.001** |
| eGFR (mL/min) | 1.00 (0.99-1.01) | 0.666 |
| Diabetes, n (%) | 1.60 (0.82-3.12) | 0.166 |
| Organ damage, n (%) | 3.13 (2.28-4.29) | **<0.001** |
| CV events, n (%) | 2.16 (1.27-3.67) | **0.004** |

Odds ratio (OR) and the 95% confidence interval (CI) were evaluated by univariate logistic regression analysis for each variable. An OR greater than 1 indicates an increased likelihood of confirmed PA, and an OR less than 1 a decreased likelihood. HTN, Hypertension; BP, Blood Pressure; DDD, Defined Daily Dose (average maintenance dose per day for a drug used for its main indication in adults); PRA, Plasma Renin Activity; eGFR, estimated Glomerular Filtration Rate; CV, Cardiovascular. Organ damage is defined as presence of left ventricular hypertrophy at echocardiography and/or microalbuminuria.

**Table S2. Patient Characteristics of Study Cohort: Multivariate Regression Analysis**

|  |  |  |
| --- | --- | --- |
| **Variable (ref. PA confirmed)** | **OR (CI 95%)** | ***P-*value** |
| Female sex, n (%) | 0.42 (0.28-0.62) | **<0.001** |
| Systolic BP (mmHg) | 1.00 (0.99-1.01) | 0.566 |
| Antihypertensive medication (DDD) | 1.21 (1.07-1.36) | **0.002** |
| PRA at screening (ng/mL/h) | 0.07 (0.03-0.19) | **<0.001** |
| Aldosterone at screening (ng/dL) | 1.08 (1.06-1.11) | **<0.001** |
| Lowest Potassium (mEq/L) | 0.15 (0.09-0.23) | **<0.001** |
| Organ damage, n (%) | 2.64 (1.74-4.01) | **<0.001** |
| CV events, n (%) | 1.40 (0.72-2.72) | 0.315 |

Odds ratio (OR) and the 95% confidence interval (CI) were evaluated by multivariate logistic regression analysis for variables associated to a confirmed PA diagnosis in the univariate model. An OR greater than 1 indicates an increased likelihood of confirmed PA, and an OR less than 1 a decreased likelihood. BP, Blood Pressure; DDD, Defined Daily Dose (average maintenance dose per day for a drug used for its main indication in adults); PRA, Plasma Renin Activity; CV, Cardiovascular. Organ damage is defined as presence of left ventricular hypertrophy at echocardiography and/or microalbuminuria.

**Table S3. Characteristics of Training versus Internal Validation cohort**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Combined Cohort**  **(n=696)** | **Training Cohort**  **(n=522)** | **Validation Cohort**  **(n=174)** | ***P-*value** |
| Confirmed PA, n (%) | 421 (60.5) | 322 (61.7) | 99 (56.9) | 0.263 |
| Subtyping, UPA (%) | 133 (19.1) | 98 (18.8) | 35 (20.1) | 0.313 |
| Age at diagnosis (years) | 50 ± 9.9 | 50 ± 10.2 | 50 ± 9.3 | 0.770 |
| Female sex, n (%) | 318 (45.7) | 239 (45.8) | 79 (45.4) | 0.930 |
| Duration of HTN (months) | 64 [21; 131] | 59 [21; 128] | 75 [23; 134] | 0.300 |
| Systolic BP (mmHg) | 155 ± 20.3 | 155 ± 20.4 | 155 ± 20.2 | 0.889 |
| Diastolic BP (mmHg) | 95 ± 11.0 | 95 ± 11.1 | 94 ± 10.8 | 0.531 |
| Antihypertensive medication (DDD) | 2.15 [1.00; 4.00] | 2.00 [1.00; 3.69] | 2.33 [1.00; 4.00] | 0.765 |
| BMI (Kg/sqm) | 25.7 ± 4.28 | 25.9 ± 4.23 | 25.4 ± 4.45 | 0.224 |
| PRA at screening (ng/mL/h) | 0.30 [0.15; 0.40] | 0.22 [0.15; 0.40] | 0.30 [0.20; 0.45] | 0.086 |
| Aldosterone at screening (ng/dL) | 25.6 [18.7; 35.5] | 25.8 [18.8; 35.5] | 24.3 [18.5; 35.1] | 0.791 |
| Lowest Potassium (mEq/L) | 3.8 ± 0.62 | 3.8 ± 0.62 | 3.8 ± 0.61 | 0.414 |
| eGFR (mL/min) | 91 ± 17.0 | 91 ± 17.2 | 91 ± 16.6 | 0.914 |
| Diabetes, n (%) | 44 (6.3) | 33 (6.3) | 11 (6.3) | 1.000 |
| Organ damage, n (%) | 404 (58.0) | 298 (57.1) | 106 (60.9) | 0.375 |
| CV events, n (%) | 81 (11.6) | 62 (11.9) | 19 (10.9) | 0.733 |

Characteristics of patients included in the developmental cohort: patients from the combined cohort (n=696) were randomly assigned to training (n=522), or validation cohort (n=174). HTN, Hypertension; BP, Blood Pressure; DDD, Defined Daily Dose (average maintenance dose per day for a drug used for its main indication in adults); PRA, Plasma Renin Activity; eGFR, estimated Glomerular Filtration Rate; CV, Cardiovascular. Organ damage is defined as presence of left ventricular hypertrophy at echocardiography and/or microalbuminuria. Normally and non-normally distributed variables were reported as mean ± standard deviation or median [interquartile range], respectively. Categorical variables were reported as absolute number (n) and proportion (%).

**Table S4. Characteristics of Developmental versus Validation cohort**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Developmental Cohort**  **(n=696)** | **External Validation Cohort**  **(n=328)** | ***P-*value** |
| Confirmed PA, n (%) | 421 (60.5) | 173 (52.7) | **0.019** |
| Subtyping, UPA (%) | 133 (19.1) | 89 (27.1) | 0.299 |
| Age at diagnosis (years) | 50 ± 9.9 | 50 ± 13.5 | 0.467 |
| Female sex, n (%) | 318 (45.7) | 192 (58.5) | **<0.001** |
| Duration of HTN (months) | 64 [21; 131] | 48 [11; 138] | **0.006** |
| Systolic BP (mmHg) | 155 ± 20.3 | 150 ± 19.6 | **<0.001** |
| Diastolic BP (mmHg) | 95 ± 11.0 | 93 ± 12.4 | 0.136 |
| Antihypertensive medication (DDD) | 2.15 [1.00; 4.00] | 1.00 [0.00; 2.50] | **<0.001** |
| BMI (Kg/sqm) | 25.7 ± 4.28 | 27.0 ± 5.09 | **<0.001** |
| PRA at screening (ng/mL/h) | 0.30 [0.15; 0.40] | N.A. | N.A. |
| DRC at screening  (mU/L) | N.A. | 2.7 [2.0; 5.6] | N.A. |
| Aldosterone at screening (ng/dL) | 25.6 [18.7; 35.5] | 12.8 [8.2; 20.0] | **<0.001** |
| Lowest Potassium (mEq/L) | 3.8 ± 0.62 | 3.5 ± 0.51 | **<0.001** |
| eGFR (mL/min) | 91 ± 17.0 | 87 ± 19.9 | **0.001** |
| Diabetes, n (%) | 44 (6.3) | 36 (11.0) | **0.010** |
| Organ damage, n (%) | 404 (58.0) | 129 (39.3) | **<0.001** |
| CV events, n (%) | 81 (11.6) | 39 (11.9) | 0.907 |

Characteristics of patients included in the analysis: patients from the developmental cohort from Torino (n=696) were compared to patients from the external validation cohort from Munich (n=328). HTN, Hypertension; BP, Blood Pressure; DDD, Defined Daily Dose (average maintenance dose per day for a drug used for its main indication in adults); PRA, Plasma Renin Activity; eGFR, estimated Glomerular Filtration Rate; CV, Cardiovascular. Organ damage is defined as presence of left ventricular hypertrophy at echocardiography and/or microalbuminuria. Normally and non-normally distributed variables were reported as mean ± standard deviation or median [interquartile range], respectively. Categorical variables were reported as absolute number (n) and proportion (%).

**Table S5. Diagnostic performance of machine learning based models**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PACT Score Accuracy** | | | **Predicted Diagnosis** | | | **Performance** | | |
| **LDA Model** | **Training cohort** (N = 522) | PA confirmed | | PA excluded | Accuracy (%) | | 79.7 |
| PA confirmed | 272 | | 50 | Sensitivity (%) | | 84.5 |
| PA excluded | 56 | | 144 | Specificity (%) | | 72.0 |
| **Validation cohort** (N = 174) | PA confirmed | | PA excluded | Accuracy (%) | | 77.6 |
| PA confirmed | 82 | | 17 | Sensitivity (%) | | 82.8 |
| PA excluded | 22 | | 53 | Specificity (%) | | 70.7 |
| **Combined cohort** (N = 696) | PA confirmed | | PA excluded | Accuracy (%) | | 79.2 |
| PA confirmed | 354 | | 67 | Sensitivity (%) | | 84.1 |
| PA excluded | 78 | | 197 | Specificity (%) | | 71.6 |
| **RF Model** | **Training cohort** (N = 522) | PA confirmed | | PA excluded | Accuracy (%) | | 82.8 |
| PA confirmed | 286 | | 36 | Sensitivity (%) | | 88.8 |
| PA excluded | 54 | | 146 | Specificity (%) | | 73.0 |
| **Validation cohort** (N = 174) | PA confirmed | | PA excluded | Accuracy (%) | | 79.9 |
| PA confirmed | 84 | | 15 | Sensitivity (%) | | 84.8 |
| PA excluded | 20 | | 55 | Specificity (%) | | 73.3 |
| **Combined cohort** (N = 696) | PA confirmed | | PA excluded | Accuracy (%) | | 82.0 |
| PA confirmed | 370 | | 51 | Sensitivity (%) | | 87.9 |
| PA excluded | 74 | | 201 | Specificity (%) | | 73.1 |
| **Linear SVM** | **Training cohort** (N = 522) | PA confirmed | | PA excluded | Accuracy (%) | | 80.7 |
| PA confirmed | 272 | | 50 | Sensitivity (%) | | 84.5 |
| PA excluded | 51 | | 149 | Specificity (%) | | 74.5 |
| **Validation cohort** (N = 174) | PA confirmed | | PA excluded | Accuracy (%) | | 78.2 |
| PA confirmed | 82 | | 17 | Sensitivity (%) | | 82.8 |
| PA excluded | 21 | | 54 | Specificity (%) | | 72.0 |
| **Combined cohort** (N = 696) | PA confirmed | | PA excluded | Accuracy (%) | | 80.0 |
| PA confirmed | 354 | | 67 | Sensitivity (%) | | 84.1 |
| PA excluded | 72 | | 203 | Specificity (%) | | 73.8 |
| **Gaussian SVM** | **Training cohort** (N = 522) | PA confirmed | | PA excluded | Accuracy (%) | | 83.9 |
| PA confirmed | 284 | | 38 | Sensitivity (%) | | 88.2 |
| PA excluded | 46 | | 154 | Specificity (%) | | 77.0 |
| **Validation cohort** (N = 174) | PA confirmed | | PA excluded | Accuracy (%) | | 74.7 |
| PA confirmed | 81 | | 18 | Sensitivity (%) | | 81.8 |
| PA excluded | 26 | | 49 | Specificity (%) | | 65.3 |
| **Combined cohort** (N = 696) | PA confirmed | | PA excluded | Accuracy (%) | | 81.6 |
| PA confirmed | 365 | | 56 | Sensitivity (%) | | 86.7 |
| PA excluded | 72 | | 203 | Specificity (%) | | 73.8 |

The table shows real and predicted diagnosis (PA confirmed vs. excluded), accuracy, sensitivity, specificity for the training cohort (n=522), the validation cohort (n=174), and the combined cohort from Torino (n=696). Diagnostic performance is shown for LDA (linear discriminant analysis), RF (random forest), linear and gaussian SVM (support vector machine) models.

**Table S6. Score development and validation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PACT Score Accuracy** | | **Predicted Diagnosis** | | **Performance** | |
| **Real Diagnosis (Cut-off ≥5)** | **Training cohort** (N = 522) | PA confirmed | PA excluded | Accuracy (%) | 70.7 |
| PA confirmed | 322 | 0 | Sensitivity (%) | 100.0 |
| PA excluded | 153 | 47 | Specificity (%) | 23.5 |
| **Validation cohort** (N = 174) | PA confirmed | PA excluded | Accuracy (%) | 66.1 |
| PA confirmed | 99 | 0 | Sensitivity (%) | 100.0 |
| PA excluded | 59 | 16 | Specificity (%) | 21.3 |
| **Combined cohort** (N = 696) | PA confirmed | PA excluded | Accuracy (%) | 69.5 |
| PA confirmed | 421 | 0 | Sensitivity (%) | 100.0 |
| PA excluded | 212 | 63 | Specificity (%) | 22.9 |
| **Real Diagnosis (Cut-off ≥8)** | **Training cohort** (N = 522) | PA confirmed | PA excluded | Accuracy (%) | 84.1 |
| PA confirmed | 297 | 25 | Sensitivity (%) | 92.2 |
| PA excluded | 58 | 142 | Specificity (%) | 71.0 |
| **Validation cohort** (N = 174) | PA confirmed | PA excluded | Accuracy (%) | 83.9 |
| PA confirmed | 91 | 8 | Sensitivity (%) | 91.9 |
| PA excluded | 20 | 55 | Specificity (%) | 73.3 |
| **Combined cohort** (N = 696) | PA confirmed | PA excluded | Accuracy (%) | 84.1 |
| PA confirmed | 388 | 33 | Sensitivity (%) | 92.2 |
| PA excluded | 78 | 197 | Specificity (%) | 71.6 |
| **Real Diagnosis (Cut-off ≥13)** | **Training cohort** (N = 522) | PA confirmed | PA excluded | Accuracy (%) | 53.6 |
| PA confirmed | 80 | 242 | Sensitivity (%) | 24.8 |
| PA excluded | 0 | 200 | Specificity (%) | 100.0 |
| **Validation cohort** (N = 174) | PA confirmed | PA excluded | Accuracy (%) | 55.2 |
| PA confirmed | 21 | 78 | Sensitivity (%) | 21.2 |
| PA excluded | 0 | 75 | Specificity (%) | 100.0 |
| **Combined cohort** (N = 696) | PA confirmed | PA excluded | Accuracy (%) | 54.0 |
| PA confirmed | 101 | 320 | Sensitivity (%) | 24.0 |
| PA excluded | 0 | 275 | Specificity (%) | 100.0 |

The table shows real and predicted diagnosis (PA confirmed vs. excluded), accuracy, sensitivity, specificity for the training cohort (n=522), the validation cohort (n=174), and the combined cohort from Torino (n=696). Diagnostic performance is shown for the PACT (Primary Aldosteronism Confirmatory Testing) score. A cut-off of equal or greater than 5 identifies patients with a confirmed diagnosis of PA with the maximum sensitivity; a cut-off of equal or greater than 8 identifies patients with a confirmed diagnosis of PA with the higher accuracy; a cut-off of equal or greater than 13 identifies patients with a confirmed diagnosis of PA with the maximum specificity.

**Table S7. Distribution of PA patients according to the score**

| **Score Points** | **Total**  (n) | **PA excluded** | | **PA confirmed** | |
| --- | --- | --- | --- | --- | --- |
| (n) | (%) | (n) | (%) |
| 0.0-2.0 | 14 | 14 | 100,0 | 0 | 0,0 |
| 2.1-4.0 | 49 | 49 | 100,0 | 0 | 0,0 |
| 4.1-6.0 | 138 | 106 | 76,8 | 32 | 23,2 |
| 6.1-8.0 | 145 | 63 | 43,4 | 82 | 56,6 |
| 8.1-10.0 | 137 | 25 | 18,2 | 112 | 81,8 |
| 10.1-12.0 | 112 | 18 | 16,1 | 94 | 83,9 |
| 12.1-14.0 | 87 | 0 | 0,0 | 87 | 100,0 |
| 14.1-16.0 | 14 | 0 | 0,0 | 14 | 100,0 |
| Total | 696 | 275 | N.A. | 421 | N.A. |

Number (n) and proportion (%) of patients stratified for diagnosis (PA excluded *vs.* confirmed) is shown according to the score in the developmental cohort of Torino (n=696). N.A., Not Applicable.

**Figure S1. Diagnostic modelling**

**Immagine che contiene testo

Descrizione generata automaticamente**

Machine learning based models to discriminate patients with a confirmed diagnosis of PA (n=421) from patients with PA excluded (n=275). The models included the 6 variables with the highest prediction power. Confusion matrix shows real and predicted diagnosis, accuracy, sensitivity, and specificity for each model in the developmental cohort (n=696). Data on training and validation of the models are reported in Table S5. (**A**) Canonical plot representing diagnostic performance of LDA; each patient is indicated by a point and diagnosis are reported by colour (confirmed PA, black; PA excluded, grey). The axes (canonical component 1 and 2) are calculated by weighted linear combination of the 6 variables included in the model to maximize the separation between groups. The crosses indicate the means of (canonical 1; canonical 2) for patients with UPA or BPA, the ellipse included patients with a linear combination coefficient that falls within the mean ± SD. (**B**) The first classification tree of the forest is shown for the prediction of PA confirmed *vs.*PA excluded. (**C**, **D**) Graphs showing the performance of SVM models (Support Vector Machine, Linear and Gaussian). Axes report the two best support vector classifiers: aldosterone at screening on x-axis and lowest recorded potassium levels on y-axis. Each patient is indicated by a point and diagnosis are reported by colour (confirmed PA, dark grey; PA excluded, grey). Model prediction areas are indicated by colours, as appropriated.

**Figure S2. Flow chart for the management of PA patients**

Immagine che contiene screenshot

Descrizione generata automaticamente

Flow chart for the management of patients with a positive screening test (Developmental Cohort + External Validation Cohort; n=1,024). (**A**) PA patient management using the PACT score; the number of patients is indicated in bold; cut-offs are indicated in grey. Misclassified patients are reported in red. (**B**) Confusion matrix representing real and predicted subtype diagnosis, sensitivity, specificity, positive and negative predictive value (PPV; NPV). AVS, Adrenal Venous Sampling; PA, Primary Aldosteronism; LREH, Low Renin Essential Hypertensive patients (PA positive screening test with a negative confirmatory test); PACT, Primary Aldosteronism Confirmatory Testing Score.