PREVENTIVE DISTINCTION OF PATIENTS WITH PRIMARY OR SECONDARY HYPERTENSION BY DISCRIMINANT ANALYSIS OF CHRONOBIOLOGIC PARAMETERS ESTIMATED ON 24-HOUR BLOOD PRESSURE PATTERNS

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This investigation deals with a statistical probatory that patients with primary (PH) or secondary (SH) hypertension may be correctly diagnosed by a discriminant analysis of the chronobiologic characteristics computed on the 24-hour blood pressure (BP) patterns. The methodology concerning non-invasive 24-h BP monitoring, chronobiologic analysis and the discrimination process is detailed. Substantial dissimilarities were found in the statistical distribution for systolic and diastolic BP rhythmometric parameters (mesor, amplitude and acrophase) by a retrospective assessment of two groups, consisting of 54 patients with PH and 16 patients with SH. The group-related distribution for rhythmometric parameters was found to be significantly different to generate a statistically significant intergroup discriminatory boundary. The discriminant analysis correctly diagnosed patients with PH and SH in a percentage of about 91% and 63%, respectively. The high incidence of success is convincing that the combination of 24-h BP monitoring/chronobiologic analysis/discrimination process can be a practical tool for confidently selecting patients with a presumable PH or SH.

NORMOTENSION and hypertension are presently diagnosed by repeated measurements of blood pressure (BP) taken on different occasions with a methodology which is called “casual”.1,2 Factually, the casual BP measurements are used as “spatial” information (one value per single subject) to binarily distinguish deviant and non-deviant states.

With the development of automated non-invasive techniques of clinical monitoring, it has become clear that the BP measurements may have a more extensive meaning under an informative point of view. The 24-h monitoring explores BP values in their dynamicity, with a methodology which can be termed “historical.” Basically, the historical series are made by time points which are relationally linked in their spatial continuity. This implies that the BP values include a temporal form of information to be exploited for exploring deviant and non-

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Key words:
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Hypertension
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### TABLE 1 CLINICAL DATA

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age* (years)</th>
<th>Sex (M/F)</th>
<th>&quot;Casual&quot; blood pressure (mmHg)**</th>
<th>Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Systolic</td>
<td>Duration</td>
</tr>
<tr>
<td>A. PH</td>
<td>56</td>
<td>17–55</td>
<td>29/27</td>
<td>190 ± 14</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>B. SH</td>
<td>16</td>
<td>17–55</td>
<td>13/3</td>
<td>198 ± 16</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>RPD</td>
<td>4</td>
<td>30–54</td>
<td>4/0</td>
<td>185 ± 8</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>RVD</td>
<td>2</td>
<td>28–40</td>
<td>2/0</td>
<td>180 ± 12</td>
<td>1–2</td>
</tr>
<tr>
<td>PHA</td>
<td>2</td>
<td>35–38</td>
<td>1/1</td>
<td>175 ± 3</td>
<td>2–4</td>
</tr>
<tr>
<td>CS</td>
<td>5</td>
<td>32–36</td>
<td>4/1</td>
<td>185 ± 6</td>
<td>2–3</td>
</tr>
<tr>
<td>11BHD</td>
<td>2</td>
<td>17–27</td>
<td>2/0</td>
<td>170 ± 2</td>
<td>0–0***</td>
</tr>
<tr>
<td>PHEO</td>
<td>1</td>
<td>20–35</td>
<td>0/1</td>
<td>200 ± 2</td>
<td>1–2</td>
</tr>
</tbody>
</table>

*Given as range; **Calculated on several measurements; ***These two subjects were treated from neonatal age; PH = Primary Hypertension; SH = Secondary Hypertension; RPD = Renoparenchymal Disease; RVD = Renovascular Disease; PHA = Primary Hyperaldosteronism; CS = Cushing’s Syndrome; 11BHD = 11Beta-Hydroxylase Deficiency; PHEO = Pheochromocytoma

deviant states.

In view of these informative capacities, the question is whether or not the 24-h BP patterns are helpful in distinguishing patients with primary and secondary hypertension. If clinical differentiation between the two groups of cases with hypertension could be achieved, this would be of considerable value. Hypertensive subjects can be cost-effectively selected, and the cases with secondary hypertension can be investigated further for the etiology of their disease.

A possible support to a distinct diagnosis is suggested by biostatistical, physiological and clinical considerations. Biostatistically, it has been demonstrated that a historical series incorporates two components, i.e., the structured sequence that generates the systematic changes, and the erratic component that creates a masking effect via its stochastic (casual, aleatory, occasional, spurious) elements. Physiologically, it has been clarified that the systematic component in the 24-h BP patterns is sustained by a period sequence of values that are arranged to disclose a circadian rhythm. Clinically, it has been demonstrated that the properties of the BP circadian rhythm are considerably uniform in patients with primary hypertension.

The “rationale” underlying the construction of using BP monitoring in the differential diagnosis is simple. In conformity with the periodic structure, the basic idea is to explore whether or not the rhythmometric properties of the 24-h BP patterns can be used for a complete separation between primary and secondary hypertension. In line with this idea, the 24-h BP measurements which were non-invasively monitored in patients with or without etiologically-known hypertension, were first chronobiologically analysed to derive rhythmometric properties. Subsequently, the rhythmometric parameters were processed to explore whether these patients could be correctly reassigned to a disease A (primary hypertension) or B (secondary hypertension) by a multivariate discriminant analysis.

### MATERIALS AND METHODS

1. **Subjects and protocol**

   The original analysis was retrospective and was confined to 54 patients with primary hypertension, and 16 patients with secondary hypertension, categorized as I or II WHO class (Table I).

   The diagnosis was made in accordance with an extensive clinical and laboratory check-up, including pyelography, renal and suprarenal echography.

   During the 24-h BP monitoring, patients were permitted to ambulate, initiating their day-time routine at 07:00. They were invited to interrupt any physical activity at 23:00, lying in bed during the nocturnal span with the lights off. Ordinary diurnal activity was recommended in order to avoid the addition of aleatory values to the 24-h BP patterns. Patients were requested to follow a meal schedule with breakfast at

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Cosinor, the periodic regression analysis.\textsuperscript{13,14} This method fits 24-h cosine function to raw data using the least squares method in order to find the best fitting sinusoidal wave that is the systematic component of a periodic time series (Fig. 1).

Cosinor procedure quantifies the rhythmometric properties by calculating the coefficients that constitute the 24-h cosine function:

\[ Y_t = M + A \cos \left( \frac{2\pi}{24} t + \phi \right). \]

Three parameters are computed, i.e., mesor, acronym for Midline Estimating Statistic of Rhythm, M, (rhythm-adjusted mean), amplitude, A, (one-half of the total extent in the sinusoidal fluctuation), and acrophase, \( \phi \), (timing of crest in waveform profile related to local midnight). More detailed information on the cosinor procedure can be found in the literature.\textsuperscript{13–15}

The cosinor-derived rhythmometric estimates (M, A, \( \phi \)) related to SBP and DBP in group A (primary hypertension) and B (secondary hypertension) were considered the variables for a stepwise multivariate discriminant analysis.\textsuperscript{12} Basically, this method is aimed to verify whether or not a number of variables or parameters in individuals “a priori” classified as members of two distinct groups (A and B) are able to properly separate the two populations with a discriminant boundary (Lo) so that a newer member can be assigned with a confident prediction to the correct population of origin. The discriminant analysis provides the “a posteriori” allocation to the disease group and, thus, the classification of a patient on the basis of the “likelihood ratio”,

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08.30, lunch at 12.30 and dinner at 18.30. Mineral water, coffee, caffeinated and alcoholic drinks were not permitted. No smoking was allowed. The above-mentioned training was initiated at least seven days before the BP monitoring was started.

The 24-h BP monitoring was made by means of a non-invasive automated device (ICR, Space Labs) programmed to take the BP recordings (microphonic method) at 30-min intervals from an inflatable cuff attached to the upper non-dominant arm. The BP measurements were stored on a solid state memory mass. At the end of the session, the recorded BP was removed by loading it into the volatile memory of a XT-IBM computer.

2. Statistical analysis

Time-qualified series for systolic (S) and diastolic (D) BP were analysed by means of
fA/fB, where f values are the probability densities of the frequency distribution for discriminated values in the two populations. The pertaining frequency curves are constructed by introducing the means (LA and LB) and their standard deviations into the formula for normal continuous "Gaussian" distribution function (Fig. 2).

The appropriate boundary is that for which the fA/fB ratio is constant so that the boundary minimizes the amounts of misclassification. The error of misclassification can be statistically established. The right separation is given by that boundary which defines the locus of dissection at a point of the frequency curve for which the variance analysis (ANOVA) of discriminated values in the two populations is statistically significant. The discriminant analysis allocates the subjects to the pertaining group considering how X observation conforms to a given group. The member is plotted by computing the standardized distance, i.e., the number of standard deviations from the mean of the group. The plot on a linear dimension of all observations with their standardized distances generates a surface divided by the discriminatory boundary in two areas (Fig. 3).

These concepts are, however, presented in greater detail in a previous publication by our research group.12

RESULTS

The results obtained by cosinor analysis are
Fig. 5. Gaussian curves of discriminated values computed by multivariate discriminant analysis on rhythmic parameters, mesor, amplitude and acrophase for systolic and diastolic blood pressure in primary and secondary hypertension.

Fig. 6. "A posteriori" assignation of patients to primary or secondary hypertension by multivariate discriminant analysis applied to rhythmic parameters of systolic and diastolic blood pressure.

shown in Fig. 4 as a trivariate plot. Data refer to the rhythmic parameters (mesor, amplitude and acrophase) that were calculated in each patient by processing the 24-h BP patterns. The graphical display was preferred to a tabulation sheet, as the tridimensional plot allows us to preview the agglomeration or dispersion in each group. Each point, thus, represents a single patient. Operatively, the discriminant analysis will decide whether or not the two distributions originate a true geometrical separation or a zone of overlap. Data dislocation is, thus, of considerable importance.

Even though the number of patients in any diagnostic category is unpaired, it is clear that the two distributions show a different compactness. The configuration of data-points pertaining to primary hypertension is less dispersed in width, height and depth. Therefore, one must convey that the parameters (mesor, amplitude and acrophase) are less uniform in patients with secondary hypertension. By the visual assessment, however, it is difficult to derive whether a concrete separation of groups is possible. The indication to a multivariate discriminant analysis was posed.

Figure 5 illustrates the frequency curves of discriminated values that the linear discriminant function was able to construct by analysing mesors, amplitudes and acrophases for SBP and DBP in groups A (primary hypertension) and B (secondary hypertension).

Despite a certain degree of overlapping, the two curves were separated by the discriminatory boundary in a portion of their tail. According to ANOVA, the two distributions of discriminated values were found to be significantly different (p < 0.01). The error of misclassifi-
TABLE II RESULTS BY BACKWARD STEPWISE DISCRIMINANT ANALYSIS APPLIED TO COSINOR-DERIVED RHYTHMOMETRIC PARAMETERS ESTIMATED ON THE 24-H PATTERNS OF SYSTOLIC AND DIASTOLIC BLOOD PRESSURE IN TWO GROUPS OF PATIENTS WITH PRIMARY (A) AND SECONDARY (B) HYPERTENSION

<table>
<thead>
<tr>
<th>Rhythmometric parameters compared in group A and B</th>
<th>ANOVA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesor + Amplitude + Acrophase</td>
<td>3.120</td>
<td>6, 63</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Mesor + Amplitude</td>
<td>3.410</td>
<td>4, 65</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Mesor + Acrophase</td>
<td>2.407</td>
<td>4, 65</td>
<td>ns</td>
</tr>
<tr>
<td>Amplitude + Acrophase</td>
<td>4.011</td>
<td>4, 65</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

ANOVA = Analysis of Variance; DF = Degrees of Freedom; ns = not significant

cation may be, thus, statistically accepted.

As the rhythmometric parameters are susceptible to discriminant analysis, the diagrammatic separation of patients was possible on a geometric ground by plotting their standardized distance on a surface divided by the discriminatory boundary. Fig. 6 displays the “one dimension” allocation of patients as they were “a posteriori” assigned by discriminant analysis to group A (primary hypertension) or B (secondary hypertension).

Taking the dislocation into consideration, it can be argued that the linear discriminant analysis would have misdiagnosed 5 patients with primary hypertension and 6 patients with secondary hypertension. The practical value of discriminant analysis in distinguishing patients with primary or secondary hypertension can be, thus, estimated in percentage. While primary hypertension may be correctly diagnosed in about 91% of cases, the correct diagnosis of secondary hypertension may be posed in about 63% of patients.

Interestingly, the discriminant analysis was processed by removing one variable at a time in order to verify, via a backward stepwise, which parameter was preponderant for the separation of patients with primary or secondary hypertension. Table II lists the results in terms of F values with their statistical significance. According to P values, the greater weight for statistical differentiation appears to be the pair amplitude-acrophase. As the remotion of amplitude results in an F value that is not significant, the amplitude has to be, however, considered the rhythmometric parameter that is more discriminant inside the pair amplitude-acrophase.

DISCUSSION

It must be stressed that this study deals with a retrospective assessment and that the validity of statistical results depends not only on the exactness of the diagnosis before the analysis but also on the number of cases under scrutiny. While it is of fundamental importance that the lowest number of cases in each group is at least equal to the number of variables additioned by 1, theoretically, there is no limitation to the highest dimension of variables (or parameters) and members which can be processed by the discriminant analysis. According to statistical theory, the likelihood ratio is a form of analysis that tends to increase in reliability when dealing with series that increase in density. One can, thus, assume that the linear discriminant analysis will forecast the results more correctly as the number of members increases.

Due to this optimization, the positive outcomes obtained on the present number of patients, confidently suggest that newer hypertensive members will be assigned to the correct diagnosis with increasing safety as they are added to the procedure. This implies that the likelihood ratio will be more accurately predictive as the number of patients inflates.

In view of this consideration, our research group has subsequently operated on other cases. Factually, the values of mesor, amplitude and acrophase, computed by cosinor analysis on 24-h SBP and DBP patterns, were appended to the pertaining variable as the last entry of the column in a randomly selected group. The attribution to primary or secondary hypertension was, thus, posed via the statistical discrimination before the clinical diagnosis. The diagnostic

check-up that was subsequently made gave encouraging results, confirming that the discriminant analysis was successfully applied in more than 90% of the newly entered patients. Furthermore, confirmation was received that the likelihood ratio is a form of analysis that can effectively increase its probability. The statistical process correctly diagnosed three patients with primary hypertension who were previously misclassified.

In our opinion, the success of the combination: 24-h BP monitoring/chronobiologic analysis/ discriminant analysis in the differential diagnosis of primary and secondary hypertension, raises further interesting, but as yet, unresolved questions.

The most intriguing point is the explanation of the phenomenon. How is it possible that the increase in BP can generate two different conditions of hypertension that are recognizable by the 24-h BP patterns? Should we realize that hypertension is a disorder of the BP time structure that substantiates the occurrence of different chronopathological types?

In our view, the interpretative key is in the conceptual acceptance that any phenomenon in nature has to be explained by taking the general rules into consideration. For bioperiodic events, their phenomenological expression has to be, thus, interpreted by taking into consideration the general rules that govern the biological rhythmicity.

In line with this concept, one can interpret the compactness of the distribution in mesor, amplitude and acrophase, as the expression of a temporally-oscillating phenomenon that is constitutively well-structured in its systematic component to give uniform characteristics at the chronobiologic resolution. Exactly the contrary is the interpretation for a bioperiodic event whose identifiable expression is a chaotic distribution of its rhythymetric properties. One can argue that its systematic sequence is intimately affected and/or strongly influenced by the erratic component.

The application of these principles may be invoked to interpret the success of the discriminant analysis in the differential distinction of primary and secondary hypertension by means of 24-h BP monitoring. As the incomparability for rhythymetric properties contributes to separation, it is a legitimate explanation that the BP elevation in primary and secondary hypertension recognizes mechanisms formally dis-

similar in their constitutive essence. The disorder in the 24-h BP patterns suggests a pathophysiology of the systematic time structure for primary hypertension and that of the stochastic time structure for secondary hypertension. The disorder is expressed by a more pronounced variability for the amplitude of the BP circadian oscillation in patients with secondary hypertension.

The practical meaning of this is that considerable promise for future scientific work is the possibility of chronobiologically exploring the pathophysiology of hypertension. The area of medical diagnosis could expand the technique of discriminant analysis applied to the rhythymetric properties of the 24-h BP patterns for the diagnostic prediction of patients with primary and secondary hypertension.

As arterial hypertension is a social disease that is sustained in more than 80% of cases by primary hypertension, it is economically appealing to reduce the diagnostic costs by making a confident preliminary prediction before laboratory tests. Today the 24-h BP monitoring may be certainly regarded as a clinical tool that is less expensive than a battery of laboratory tests, and more innocuous than any radioisotopic and contrastographic diagnostic examination.

REFERENCES
in the Clinical Management of Hypertension, ed by
GERMANO' G, Pozzi Edizioni, Rome, 1985, p55
9. DRAYER JIM, WEBER MA, DE YOUNG YL,
   WILE FA: Circadian blood pressure patterns in
   ambulatory hypertensive patients. Effects of age.
10. HALBERG F, DRAYER JIM, CORNELISSEN G,
    WEBER MA: Cardiovascular reference data base
    for recognizing circadian mesor- and amplitude-
    hypertension in apparently health men. *Chrono-
   obiologia* 11: 275, 1984
11. HALBERG F, AHLGREN A, HAUS E: Circadian
    systolic and diastolic hyperbaric indices of high
    school and college students. *Chronobiologia* 11:
    299, 1984
12. CUGINI P, LEONE G, STRAZZERA P,
    KAWASAKI T: Stepwise multivariate discriminant
    analysis (SMDA) for paired and unpaired bio-
    medical data using microcomputers. *Comput Biol
13. HALBERG F, TONG YL, JOHNSON EA:
    Circadian system phase: an aspect of temporal
    morphology- procedures and illustrative examples.
    In The Cellular Aspects of Biorhythms, ed by von
    p20
14. HALBERG F, JOHNSON EA, NELSON W,
    RUNGE W, SOTHERN RB: Autorhythmometry:
    procedures for physiologic self-measurements and
    their analysis. *Physiol Teacher* 1: 1, 1972
15. BINGHAM C, ARBOGAST B, CORNELISSEN G,
    LEE JK, HALBERG F: Inferential statistical
    methods for estimating and comparing cosinor
    parameters. *Chronobiologia* 9: 397, 1982