Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection

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VELDHUIS, JOHANNES D., AND MICHAEL L. JOHNSON. Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. Am. J. Physiol. 250 (Endocrinol. Metab. 13): E486–E493, 1986.—Endocrine signaling provides one critical means of physiological communication within an organism. Many endocrine signals exhibit an episodic or pulsatile configuration. In an effort to provide a versatile and statistically based algorithm for investigating the regulation of endocrine pulse signals, we have formulated a computerized algorithm in which a pulse is defined as a statistically significant increase in a “cluster” of hormone values followed by a statistically significant decrease in a second cluster of values. The increase or decrease is judged in relation to the actual experimental error expressed by the replicates in the presumptive nadir and peak data clusters. The program permits the operator to specify the cluster sizes of test peaks and pre- and postpeak nadirs. This method is largely insensitive to unstable base-line hormone concentrations and is not adversely affected by varying pulse amplitudes, widths, or configurations within the endocrine series. In addition, the simple statistical basis for this algorithm renders it minimally dependent on explicit or a priori assumptions about rates of hormone secretion or disappearance. The program has been validated for false-positive errors against a wide range of intraseries coefficients of variation (4–52%). We have illustrated its performance for profiles of luteinizing hormone, follicle-stimulating hormone, growth hormone, prolactin, adrenocorticotropin hormone, and cortisol and compared these episodic patterns with those of stable serum constituents (total serum protein and calcium), which do not exhibit pulsatile fluctuation.

These methods have advanced our appreciation of pulsatile hormonal phenomena but are susceptible to one or more of the following limitations: 1) inconsistent pulse frequency estimates in the presence of fluctuating baseline hormone concentrations; 2) a requirement for relatively uniform pulse amplitudes within a given series under analysis; 3) use of a single point to define a significant increase as a peak; 4) the lack of explicit definition of a significant decrease (downslope) of the pulse; 5) the dependence on estimates of assay precision that are external to the actual experimental series; 6) substantial susceptibility to intra-assay variation; and 7) the absence of a clear or simple statistical basis for the analysis (1–4, 6–8). In addition, the lack of availability of a single robust method of pulse analysis has resulted in the application of numerous individual methodologies, with a consequently wide range of endocrine pulse parameters reported (1–8).

In an effort to address some of these limitations in pulse analysis, we have developed a simple, adaptable, and statistically based computerized pulse-detection algorithm, which we present here.

METHODS

The cluster analysis program was designed to search for significant increases and significant decreases within the data series. A significant increase was judged in relation to a specified nadir width, by using a moving nadir that began with the onset of the experimental series. The values in the test nadir cluster were compared against values in a possible peak, which was defined as a second set of consecutive samples of specified number immediately after the test nadir. Comparisons of nadir and peak clusters were made by pooled t testing (10) using the actual experimental replicates present in the test nadir and peak. For example, for an operator-specified nadir test width of two and a peak test width of three, the initial comparison by pooled t testing was between replicates in nadir samples 1 and 2 and peak samples 3, 4, and 5. The comparison used a pooled t statistic value selected by the operator (as discussed below). The search for significant increases was continued by shifting the test nadir cluster by one sample and...
testing against a corresponding possible peak shifted by one value (e.g., the second test would compare nadir samples 2 and 3 with peak samples 4, 5, and 6). This search was continued throughout the entire experimental series, so that all individually significant increases were marked.

After marking all the significant increases, the series was rescanned in consecutive order to search for significant decreases. An operator-specified test peak width was compared with an immediately following postpeak nadir (the width of the postpeak nadir was the same as that specified for the prepeak nadir). The entire data set was then analyzed serially as above to search for all significant decreases.

The occurrence of a peak is defined as a significant increase followed by a significant decrease. This formulation of a peak requires that a region of significant increase be associated with nadirs on both sides. A nadir is defined as a decrease followed by an increase, with all else representing a peak.

In performing the analysis, the operator specifies individual test cluster sizes for the nadir and peak (i.e., number of points to be used in testing nadirs against peaks), a minimum and maximum intraseries coefficient of variation, a t statistic to identify a significant increase, and a t statistic to define a significant decrease.

After the location and widths of individual peaks are identified, the total number of peaks is counted and the mean interval between peaks calculated in minutes (taken as the time lapse between the maximum values within the consecutive peaks). In addition, the following parameters are determined: mean peak width in minutes, maximum peak height (highest absolute value attained within the peak), mean peak height as a percent increase above prepeak nadir, incremental peak amplitude (expressed as the algebraic difference between the maximal peak height and the prepeak nadir value), and area under the peak (expressed as the product of the mean peak value minus the average pre- and postpeak nadirs times the peak width). In addition, significant interpeak intervals (valleys) are identified as regions embracing nadirs without intervening upstrokes. Mean valley and pre- and postpeak nadir values are calculated. The results of these analyses are printed in summary form. In addition, the program provides a graphical output in which significant increases and decreases comprising peaks within the data series are schematized.1

The detection of peaks at the very outset or end of a data series may be susceptible to "edge effects." An edge effect would ensue if only part of the peak is contained in the available data series. Such partial peaks cannot be accurately characterized with respect to width, amplitude, or location, because both upstroke and downstroke excursions are required to delineate peak shape fully. In the present program, we have attempted to minimize bias that could be introduced by edge effects at the end of the series. Thus we do not use the last "peak" in the series when calculating the statistical summary of peak characteristics, unless the last peak is followed by a fully demarcated nadir. We define the latter as a region bounded by two significant increases. This requirement reflects the following rationale: when an apparent nadir after the terminal peak is not demarcated by a subsequent significant increase, one cannot ascertain whether the full width of the peak has been observed. Accordingly, mean peak characteristics would be calculated most appropriately from internal peaks or those flanked by complete nadirs. To assist the investigator in his decision whether to include the last peak, our plotting routine depicts the last significant increase or decrease in the data series. This is actually tallied as a peak only if it is followed by a fully demarcated nadir.

The performance of the pulse-detection method was evaluated in relation to false-positive rates over a wide range of intraseries coefficients of variation, by using various measures of immunoassay variation.

The distribution of measurement error of 565 luteinizing hormone (LH) values (derived from pools of male serum, with each value assayed by radioimmunoassay in triplicate) was ascertained earlier (7). Similarly, standard deviations, or Z scores, were also determined on 829 immunoactive growth hormone values derived from pools of normal male serum. X-Square testing was used to determine whether the Z score distribution observed for the growth hormone values differed significantly from a Gaussian (normal) distribution (7, 10). To assess false-positive error rates, cluster analysis was applied to detect false-positive pulses in a series of 2,700 simulated data values each represented in triplicate, in which measurement error was made to vary in a Gaussian distribution at an intraseries coefficient of variation of 8%. Cluster sizes were defined as test nadir and test peak widths used to detect significant increases or decreases by pooled t statistics. These cluster sizes ranged from 1 by 1 (comparing a single point comprising triplicates as nadir with a single point comprising triplicates as possible peak) to 10 by 10, as indicated. The t statistics were varied from 0.82 to 6.96. False-positive rate was defined as number of pulses/100 points detected by corresponding t statistic values and cluster sizes. For a t statistic of ±4.3, the false-positive rate declined to ±0.04%.

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FIG. 2. Effect of cluster size on false-positive rate in relation to selected t statistics. For each of the indicated t statistics, test sizes of nadirs and peaks were made to vary from 1 to 10. Corresponding false-positive rates were determined as defined in legend of Fig. 1. Each error estimate reflects results from 2,700 points comprising Gaussian distributed noise that conformed to an intraseries coefficient of variation of 8%.

TABLE 1. False-positive rates in relation to intraseries variances for particular distributions of assay error

<table>
<thead>
<tr>
<th>Intraseries Coefficients of Variation, %</th>
<th>Distributions</th>
<th>LH RIA (565)</th>
<th>GH RIA (829)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2.7*</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>8</td>
<td>3.3</td>
<td>3.7</td>
<td>3.2</td>
</tr>
<tr>
<td>12</td>
<td>3.5</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td>18</td>
<td>3.9</td>
<td>3.9</td>
<td>3.0</td>
</tr>
<tr>
<td>26</td>
<td>3.8</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>38</td>
<td>3.9</td>
<td>4.3</td>
<td>3.2</td>
</tr>
<tr>
<td>52</td>
<td>4.2</td>
<td>3.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Data were analyzed by the cluster program using a t statistic of 1.89 and a minimum nadir and peak width of 2 points. Values in parentheses give the no. of standard deviates obtained empirically to characterize the distribution of measurement error in that particular assay (7). LH, luteinizing hormone; RIA, radioimmunoassay; GH, growth hormone. *False-positive rates/100 points.

system, as described earlier (7). The coefficients of variation within the replicates of each series were constrained to vary between 4 and 52%. To evaluate false-positive errors, we examined the number of pulses detected by the cluster program using the simulated series, which comprised 2,700 members each, with each member represented in triplicate. Any pulses detected in such series were considered false-positive errors.

RESULTS AND DISCUSSION

The performance of the cluster program was initially
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FIG. 5. A: lack of influence of base-line drift on endocrine pulse detection by cluster analysis. Endogenous luteinizing (LH) pulsations were analyzed by cluster method using a $t$ statistic of 2.89, a cluster size of 2 by 2, and a constant coefficient of variation of 5.5% determined in this subject's assay. Parent LH series (top) was corrupted by incremental adjustment of base line to create a constantly upsloping base line (center) or a "U-shaped" base line (bottom). Tracing schematized at top of each panel indicates significant increases and decreases in data series identified by cluster analysis method. B: lack of influence of varying pulse amplitude on cluster analysis. Serum ACTH levels were measured in blood collected at 20-min intervals for 24 h (top). One ACTH value was increased to 118.5 pg/ml (bottom) to test influence of nonuniform peak amplitudes on pulse detection by cluster method ($2 \times 2$ cluster size, $t$ statistic of 2.92, and constant coefficient of variation of 7.0%). Schematized tracings at top of each panel indicate actual significant increases and decreases in data identified by cluster program under these circumstances. C: influence of various presumed intra-assay coefficients of variation on apparent ACTH pulses detected by cluster analysis. Serial ACTH values were measured by immunoassay in blood samples withdrawn at 20-min intervals for 24 h in 1 normal man. Intra-assay coefficient of variation (CV) was presumed to be 7% (top), 3.5% (center), or 2% (bottom) for cluster analysis employing a fixed $t$ value of 2.92 and test nadir and peak widths of 2 points. Schematized tracing at top of each panel denotes significant upstrokes and downstrokes specified by cluster program.

tested on Gaussian distributed measurement error, in which 2,700 simulated data values were made to vary about a mean of 10 with an intrareplicate coefficient of variation of 8% (which approximates that in many immunoassay systems). Each simulated value was generated in triplicate. Cluster analysis was then undertaken for test nadir and peak widths (cluster sizes) of $1 \times 1, 2 \times 2, 3 \times 3, 4 \times 4, 5 \times 5, 6 \times 6,$ and $10 \times 10$. For each cluster size, the value of the operator-specified $t$ statistic was made to vary as 0.89, 1.89, 2.92, 4.30, and 6.96. These values correspond nominally to $P$ values of 0.25, 0.10, 0.05, 0.025, and 0.01 for 2 df (10). As shown in Fig. 1, as the value of the $t$ statistic increased, there was a remarkable decrease in false-positive error rates, and this rate of decrease was dependent on the cluster size. For $t$ statistics of 4.3 and 6.96 and nadir and peak cluster sizes of $\geq 2$, there were fewer than 4 false-positive pulses detected per 10,000 samples (or 0.04%).

The relationship between cluster size and false-positive rate for varying $t$ statistics was studied further, as shown in Figure 2. For any given $t$ statistic, the false-positive rate declined progressively as the size of the test nadir and peak increased. The rate of decline of false-positive errors was most prominent as the cluster size was increased from one to six and somewhat less striking for larger cluster sizes. Asymmetric clusters gave intermediate results between their two symmetric flanking cluster sizes (e.g., a $1 \times 2$ cluster gave a false-positive rate intermediate between $1 \times 1$ and $2 \times 2$) for any given $t$ statistic (not shown). Increasing the test cluster size also decreased the false-positive rate in relation to increasing $t$ values. Thus, based on Fig. 2, the investigator can select test cluster sizes for the putative nadir and peak and identify an appropriate $t$ statistic that will maintain a desired minimum false-positive rate associated with Gaussian-distributed measurement error.

To test whether the cluster program exhibited similar false-positive rates for Gaussian and non-Gaussian distributions of measurement error, false-positive errors were assessed for standard deviates conforming to a normal distribution and to the distributions characteristic of the LH (not significantly different from Gaussian) or the growth hormone assay (the latter departs significantly from normality because of skew introduced by undetectable hormone levels). As shown in Table 1, false-positive rates were similar among these three distributions and were remarkably constant over a wide range of intraseries coefficients of variation; viz. those
FIG. 6. Illustrative profiles of episodic hormone release analyzed by cluster method. A cluster size of 2 by 2 and a $t$ statistic of 2.92 were used as parameters for analysis of episodic hormonal and nonhormonal data. A: serum ACTH and cortisol fluctuations over time in blood sampled at 20-min intervals for 24 h in 1 man (estimated constant coefficient of variation of 7% for both hormones). B: serum prolactin in 1 man sampled at 4-min intervals for 8 h (estimated constant coefficient of variation of 7%). C: serum luteinizing hormone (LH) in a normal man sampled at 20-min intervals for 24 h (actual data triplicates as ±SE). D: serum follicle-stimulating hormone (FSH) in a postmenopausal woman sampled at 20-min intervals for 24 h (constant estimated assay SD of 2.0 mIU/ml). E: serum growth hormone concentrations measured in blood collected at 20-min intervals for 24 h in a normal man (actual data triplicates as means ± SE). F: total serum protein. G: total serum calcium measured in blood collected at 5-min intervals for 24 h in a normal man (cluster parameters as in A). Schematized tracing at top of each panel depicts significant upstrokes and downstrokes detected by cluster analysis. *Straight line* indicates that no significant peaks were detected in serum protein and calcium profiles.
To test the influence of unstable base lines on pulse detection, the cluster method was applied to endogenous LH pulsations observed in a young woman, in whose series the base line was deliberately made to vary substantially. Figure 5A illustrates the LH pulses detected in the original data series as well as in two corrupted series. One series was distorted algebraically by adding incremental values of 0, 0.25, 0.5 . . . 17.75 mIU/ml to the authentic series, which resulted in a progressively increasing base line over the 24 h. Alternatively, the series was corrupted by adding 0, 0.25, 0.5 . . . 8.75 mIU/ml beginning at the midpoint of the parent series and continuing 1) in reverse order to the beginning of the series and 2) in forward order to the end of the parent series. Despite such corrupted base lines, the cluster analysis method detected 11 complete peaks in each data series and identified the two "half-peaks" at each end of the series. In contrast, the Pulsar method (3), using the same external coefficient of variation of 5.5% (and a smoothing window of 6 h), detected 12 peaks in the parent series, 2 in the ascending base-line series, and 7 in the U-shaped base-line series.

This stability of the cluster analysis method over a wide range of intraseries coefficients of variation is illustrated in Fig. 3 for a t statistic of 1.89 and of 2.92. These t statistics yielded ~4 and 1% false-positive rates, respectively, independently of the 13-fold range of intraseries coefficients of variation tested. In contrast, the original single-point threshold method of Santen and Bardin (4), which uses a fixed 20% threshold to detect a significant increase (or pulse) in the data series, resulted in a major (>30 fold) augmentation of the false-positive rate as the intraseries coefficient of variation increased. These results presumably reflect the inability of a fixed threshold and single-point method to account adequately for false-positive errors associated with intrinsic variations in the experimental series.

Although available pulse detection programs, including the present cluster analysis method, can be tested for their false-positive rate in endocrine pulse analysis, there is no independently validated means to determine exact rates of false negatives (type II statistical errors; Ref. 6). When methodology is developed to independently confirm the identity of individual "true" physiological pulses in any given experimental series, available pulse detection methods will need to be compared in relation to their false-negative behavior.

The cluster analysis program as described above utilized internal standard deviates calculated from the actual assay replicates within the experimental series. We also evaluated the performance of the program when a constant coefficient of variation (or constant SD) was used instead (Fig. 4). The constant coefficients of variation (or constant SD) were derived from the actual Gaussian series being tested, which were made to vary from 4 to 36%. The use of a constant coefficient of variation or a constant SD pertinent to the test series yielded stable and very similar false-positive rates. These rates were consistently lower than those detected when the internal standard deviations of the actual replicates were employed. This difference reflects the distinct origins of the estimates for the internal standard deviates compared with the constant SD or constant coefficient of variation. The latter two terms were estimated from the mean values in the series, whereas the internal SD were calculated from the actual triplicates. Accordingly, these two estimates would differ by the square root of 3, or approximately a factor of 1.73. This is the degree of difference observed in Fig. 4.

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Where actual replicates in the experimental series are not available, i.e., only mean values are given, the investigator may use a best estimate of the coefficient of variation or SD of that assay. In this circumstance, the number of degrees of freedom for the estimate must be provided, e.g., as n - 1 for n replicates used per sample in the assay.
TABLE 2. Estimates of physiological LH pulse frequency and amplitude in normal men: comparison of methods

<table>
<thead>
<tr>
<th>Sampling Frequency</th>
<th>Methods of Pulse Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cluster</td>
</tr>
<tr>
<td>A: LH pulse frequency</td>
<td></td>
</tr>
<tr>
<td>Sampling every 4 min</td>
<td>10.2±2.0</td>
</tr>
<tr>
<td>Sampling every 12 min</td>
<td>3.6±0.51</td>
</tr>
<tr>
<td>Sampling every 20 min</td>
<td>2.2±0.20</td>
</tr>
<tr>
<td>Sampling every 32 min</td>
<td>2.0±0.0</td>
</tr>
<tr>
<td>B: LH pulse amplitudes</td>
<td></td>
</tr>
<tr>
<td>Sampling every 4 min</td>
<td>3.5±0.94</td>
</tr>
<tr>
<td>Sampling every 12 min</td>
<td>4.4±1.5</td>
</tr>
<tr>
<td>Sampling every 20 min</td>
<td>4.7±1.4</td>
</tr>
<tr>
<td>Sampling every 32 min</td>
<td>3.4±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5 men) for no. of luteinizing hormone (LH) pulses/8 h. Blood was withdrawn at 4-min intervals for 8 h in 5 normal men (5). The parent 4-min LH series were used to construct corresponding 12-, 20-, and 32-min sampling series. Amplitude in mIU/ml, except for modified Santen and Bardin method (% increases), as increment above preceding nadir. A median intraseries coefficient of variation (8%; 2-point nadir and peak test widths, and a t statistic of 2.92 for upstroke and downstroke were used as cluster parameters; modified Santen and Bardin (4) method requiring a threshold of 3-fold the mean intra-assay coefficient of variation is illustrated in Fig. 5C. Here, serial adrenocorticotropic hormone measurements. The Cycle Detector (1) found only one peak in the data series containing the prominent spurious pulse, whereas the cluster method identified the same seven peaks in both series.

The sensitivity of the cluster analysis program to variations in presumed intra-assay coefficients of variation is illustrated in Fig. 5C. Here, serial adrenocorticotropic hormone measurements were analyzed using various estimated constant coefficients of variation for the adrenocorticotropic assay. Notably, the number of individual peaks detected predictably increased as the presumptive precision of the assay improved (reflected in a decreased coefficient of variation).

The performance of the pulse detection method was then examined on various experimental series, as further illustrated in Fig. 6. The cluster parameters were set to a test nadir width of 2 points, a test peak width of 2 points, a t statistic of 2.92 (approximating a 1% false-positive rate for these immunoassay data), and either a constant assay-specific coefficient of variation or internal SD calculated from the actual experimental replicates in the data series. We have illustrated the identification of individual peaks for adrenocorticotropic hormone and cortisol (Fig. 6A), prolactin (Fig. 6B), LH (Fig. 6C), follicle-stimulating hormone (Fig. 6D), and growth hormone (Fig. 6E). These different profiles exemplify widely varying peak widths, peak amplitudes, and peak configurations. For comparison, the performance of the cluster analysis program was also tested on serial serum protein and calcium concentrations (Figs. 6F and 6G) measured in one normal man in blood withdrawn at 5-min intervals for 24 h. As anticipated, the stable serum constituents failed to exhibit any significant "pulsatile" fluctuations over the interval of observation when the same program parameters used in endocrine pulse detection were applied.

To appraise the performance of the cluster method at various pulse frequencies in relation to that of the Pulsar program (3), Cycle Detector program (1), original Santen and Bardin program (4), and the modified version of the Santen and Bardin threshold algorithm (7), we used the cluster method to analyze LH series collected in 5 men at 4-min intervals for 8 h, as were extensively compared earlier (6). As summarized in Table 2, the cluster analysis method delineated a significantly higher estimated LH pulse frequency at a 4-min sampling rate compared with a 12-, 20-, or 32-min sampling rate (P < 0.05 by analysis of variance). Moreover, the LH pulse frequency estimates provided by cluster analysis for 4-min sampling were intermediate (10.2 ± 2.0 pulses/8 h) between those of the revised threshold method and/or the Pulsar and Cycle Detector algorithms. Results from the cluster method also delineated a significantly higher estimated LH pulse frequency at a 4-min sampling rate compared with a 12-, 20-, or 32-min sampling rate (P < 0.05 by analysis of variance). Moreover, the LH pulse frequency estimates provided by cluster analysis for 4-min sampling were intermediate (10.2 ± 2.0 pulses/8 h) between those of the revised threshold method (16.9 ± 2.5 pulses/8 h) and the Pulsar algorithm (5.0 ± 0.75 pulses/8 h).

The present observations suggest that the cluster program should provide the following advantages in pulse analysis: 1) consistent pulse detection despite drifting base-line hormone concentrations; 2) lack of requirement for uniform pulse amplitudes within the series under analysis; 3) an ability to use multiple-point criteria to delineate significant nadirs or peaks; 4) a statistically explicit definition of significant increases and decreases in the data series and hence accurate demarcations of peak width; 5) sensitivity to assay precision within the actual experimental data series; and 6) substantial freedom from variable false-positive errors associated with a wide range of intra-assay coefficients of variation. Thus, we suggest that cluster analysis may be applicable to investigations of a wide variety of endocrine pulse signals.

The serum ACTH and cortisol profiles were generously provided by Dr. Barry Sherman (University of Iowa) and the serum prolactin/growth hormone and LH and FSH profiles by Drs. Michael O. Thorner and Alan D. Rogol.

This work was supported in part by National Institute of Child Health and Human Development Research Career Development Award 1 K04 HD00634, University of Virginia Computer Services Grant, and
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National Institutes of Health Biomedical Research Support Award 5S07RR05431 (to J. D. Veldhuis); National Institutes of Health Grants AM-22125, AM-30302, and GM-8928 (to M. L. Johnson); US Public Health Service General Clinical Research Grant RR-847; and Diabetes Research and Training Grant 5PC0 AM-22125-06.

Copies of this program are available to qualified investigators upon request.

Received 27 September 1985; accepted in final form 12 November 1985.

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