Inferring Disease Mechanisms from Epidemiological Data in Chronic Kidney Disease: Calcium and Phosphorus Metabolism

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Key Words
Chronic kidney disease · Epidemiological data · Calcium/phosphorus metabolism · Hyperparathyroidism

Abstract
Background/Aims: By applying numerical filtering to epidemiological data of 2,512 chronic kidney disease patients, we aimed to identify some of the underlying mechanisms of the calcium/phosphorus metabolism perturbations. Methods: The measured variables, serum calcitriol, calcidiol, total calcium ([Ca]s) and phosphorus ([P]s) and the urinary excretions of calcium and phosphorus, were paired in the same patients with the glomerular filtration rate (GFR) or the serum concentrations of parathormone ([PTH]s) (used as independent variables) numerically filtered with a moving average and partitioned into 15–25 frequency classes. All variables exhibited unimodal frequency distributions. Results: There was a steep fall of [PTH]s, [P]s, and urinary excretion fractions of Ca and P up to a value of GFR in the range of 25–45 ml/min/1.73 m². The increase in the phosphorus urinary excretion preceded the steep increase in [PTH]s. Except [Ca]s, all factors exhibited their physiological correlation with [PTH]s when GFR was above 90 ml/min/1.73 m² and reverted to a feedback correlation below 80 ml/min/1.73 m². Conclusion: The perturbation of mineral metabolism in chronic kidney disease results in the maintenance of a normal range of [Ca]s and [P]s acting as the controlled factors at the cost of large variations of [PTH]s, and calcium and phosphate urinary excretions behaving as controlling factors.

Introduction

Epidemiological data are currently the basis of the clinical staging in the management of chronic kidney disease (CKD)-mineral bone disorder [1–3]. The question we ask in this paper is whether its use can be extended to extract information about the underlying functional relations between the variables affected in that disease. Our ability to pursue this purpose relies on the present possibility to measure a number of biological quantities such as [Ca]s, [P]s, [PTH]s, [calcidiol], and [calcitriol], in serum and calcium and phosphorus in urine, as was done in two recent papers [5, 6]. In such patients, kidney dysfunction is most likely the primary cause of the disturbance of the calcium/phosphorus metabolism, and the usual practice is to use glomerular filtration rate (GFR) to quantify that dysfunction.
A dominant feature of the metabolic disturbances of CKD is a secondary hyperparathyroidism attributed to a fall in circulating calcitriol and calcium and an increase in circulating phosphorus [7]. This interpretation implies that in these patients the correlations between the values of variables such as \([\text{PTH}]_s\), \([\text{calcitriol}]_s\), \([\text{Ca}]_s\), \([\text{P}]_s\), etc. reflect their physiological functional links. It is widely accepted that calcitriol, synthesized in the kidney from calcidiol, inhibits its own synthesis [8] and the production of parathyroid hormone [9, 10], inhibits cell multiplication and growth of the parathyroid gland [11, 12], stimulates intestinal absorption of calcium [13] and phosphorus [14, 15] and promotes fixation of calcium in the bone [16]. It is also known that PTH promotes the synthesis of calcitriol [17, 18], the mobilization of calcium from the bone [18] and the absorption of calcium in the kidney and inhibits the absorption of phosphorus by the kidney tubule [19]. Finally, it is also widely accepted that the serum concentration of free calcium inhibits [20] while \([\text{P}]_s\), promotes [21, 22] the release of PTH. These are some of the more important functional relations of calcium/phosphorus metabolism.

In this study, we will assume that the state of a patient with CKD after a period of several months of stable GFR is in a quasi-steady state situation. However, each patient is in a different stage of his/her disease, so that in a population of patients with CKD there will be a large spread of values of the relevant variables which must reflect their functional relationships embedded in a noise caused by a large variety of other interfering factors such as age, sex, diet, etc.

The aim of this paper is to find whether by applying simple numerical filtering to the epidemiological data of a group of patients suffering from CKD we can identify some of the underlying mechanisms responsible for the perturbations of calcium/phosphorus metabolism.

**Methods**

This study was undertaken in the Nephrology Units of three hospitals in the Lisbon area (Santa Cruz Hospital (Carnaxide), São Bernardo Hospital (Setúbal) and Fernando da Fonseca Hospital (Amadora-Sintra)) over the 2003–2007 interval.

Data consisted of demographic records (age, sex, race, height, weight), clinical evaluations (CKD etiology and medications: diuretics, vitamin D and calcium supplements or other phosphorus binders) and laboratory measurements (GFR – measured by the creatinine clearance, serum concentrations of intact parathormone, calcidiol, calcitriol, total calcium, phosphorus and timed urinary excretions of calcium and phosphorus).

Each patient was represented by a single set of values (demographic, clinical evaluation and laboratory samples) collected on the same morning. The urine collections were started on the previous morning.

The use of the archived and collected data was in accordance with a study protocol approved by the review boards of each of these institutions.

**Laboratory Methods**

Serum and urinary creatinine, calcium and phosphorus were measured by standard autoanalyzer techniques. Samples were collected after overnight fasting. Creatinine clearance was calculated in a 24-hour urine sample. The patients were instructed to pass urine into the toilet on rising and then collect all urine subsequently passed over the next 24 h, including the urine passed on rising the following day. The values of the creatinine directly computed were normalized to 1.73 m² of the body surface area computed with the Mosteller formula: BSA (m²) = ([height (cm) × weight (kg)]/3,600)½.

Creatinine clearance obtained from 24-hour urine collections was used as a measure of GFR instead of the MDRD because being statistical in nature this formula cannot be used to compute glomerular loads. The relationship between the values obtained with the two methods can be seen in figure 1.

Calcium (\(\text{JUCa}\)) and phosphorus (\(\text{JUP}\)) excretions were also measured in the 24-hour urine sample (mg/24 h). They are expressed as fractions (nondimensional) of the corresponding filtered loads (\(\text{JUCa-R}\) and \(\text{JUP-R}\)), i.e. the ratio of calcium or phosphorus clearance to creatinine clearance which were independent of urinary volume. Since values for ionized (free) calcium were available for a very small number of patients, total calcium was used in all calculations. A further justification for this practice is to enable comparisons with the vast majority of published data. An estimate for the calcium excretion as a fraction of the filtered load is to assume that ionized calcium is approximately half of the total calcium. Serum intact PTH concentrations were assayed by
a chemiluminescent immunometric assay with two systems: Immulite 2000 Intact PTH from Diagnostic Products Corporation, Los Angeles, Calif., USA and by Elecsys Systems and Modular Analytics E170 from Roche Diagnostic, GmbH, Mannheim, Germany. Serum calcitriol and calcidiol were measured by radioimmunoassay either by Biosource Europe, SA, Nivelles, Belgium or by Immunodiagnostic Systems, Ltd., United Kingdom.

**Data Analysis**

Information obtained from the records of CKD patients were pooled and analyzed in relation to the following biochemical data: serum concentrations of creatinine, iPTH, calcitriol, calcidiol, calcium and phosphorus and the 24-hour urinary output of creatinine, calcium and phosphorus. Except for serum measurements of calcidiol and calcitriol and timed urinary excretions of calcium and phosphorus, all laboratory data obtained were part of routine CKD-mineral bone disorder evaluation.

Part of the data analyzed was retrospective, hence retrieved from available records. This explains why we could not obtain values for all the variables in every patient and the apparent discrepancy of the population sizes used in the different plots.

Histograms of the frequency distributions of the variables were computed and fitted with log-normal or normal curves according to whether they exhibited or not a clear skewness [23]. Although the choice of these distribution functions was arbitrary, they allowed good fits to the data, in particular for those variables represented by a larger number of samples. In addition, they provided a more meaningful parameter representation of the data than would do conventional arithmetic averages and standard deviations. Binning was performed following Hald [23].

To establish correlations between the selected variables, they were paired (i.e. GFR/[calcitriol], GFR/[Ca], etc.) and retained for further analysis only when there were values for both in the same patient. For each pair of variables, one was chosen as the independent (driving) variable, sorted in the ascending order and then smoothed out with a sliding average of 3–5 consecutive values [24] (see flow diagram below).

![Flow diagram](flow_diagram.png)

Values of i[PTH], above 800 pg/ml and the corresponding values of the other variables were rejected, since they were very few, widely spaced, did not seem to follow the trends below that limit and would have biased the calculations. The smoothed two-column set obtained was then partitioned into 15–25 frequency classes with the help of SPSS (15.0) software. For each class, averages and their standard errors were computed as well as the average and standard errors of the corresponding frequency classes of the paired variable. The original two columns of individual data points were thus replaced by two columns of average values and their corresponding standard errors which were then used in all the plots. Curve fitting was performed with the help of Origin 6.1 and NLREG software (www.nlreg.com), allowing the identification of inflexion points in the curves.

**Matrix with:**

1 patient/row

1 variable/column

Select 2 columns:

GFR+[P], GFR+[Ca], etc.

Eliminate unpaired values and sort average moving frequency classes

Group in 15–25 classes

Compute means and SEM of classes and plot

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>3.9</td>
</tr>
<tr>
<td>iPTH, pg/ml</td>
<td>8.2</td>
</tr>
<tr>
<td>Serum calcium, mg/dl</td>
<td>9.5</td>
</tr>
<tr>
<td>Serum phosphorus, mg/dl</td>
<td>3.7</td>
</tr>
<tr>
<td>Urinary calcium, mg/dl</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Results**

**Subject Population**

We analyzed data from 2,512 patients followed regularly as outpatients in the Nephrology Units of three hospitals in the Lisbon area mentioned above. Selected patients had to have a stable renal function that was determined by a change of less than 0.5 mg/dl of creatinine in the last 6 months.

Figure 2 displays the patient selection. From a pool of 2,512 patients, 1,586 had a stable renal function according to the criterion described above. Laboratory data was evaluated as described in the figure and then selected ac-

[Table 1. Characteristics of the patient population]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66 ± 16</td>
</tr>
<tr>
<td>Males, %</td>
<td>51.0</td>
</tr>
<tr>
<td>Race, white/black/other, %</td>
<td>97.2/2.7/0.1</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>CKD etiology, %</td>
<td></td>
</tr>
<tr>
<td>Hypertension/ischemic nephropathy</td>
<td>26</td>
</tr>
<tr>
<td>Diabetes</td>
<td>22</td>
</tr>
<tr>
<td>Tubulointerstitial diseases</td>
<td>16</td>
</tr>
<tr>
<td>Renal lithiasis</td>
<td>3.3</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>11.2</td>
</tr>
<tr>
<td>Polycystic kidney diseases</td>
<td>4.7</td>
</tr>
<tr>
<td>Others</td>
<td>7.6</td>
</tr>
<tr>
<td>Unknown</td>
<td>9.6</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>61.5</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>9.5</td>
</tr>
<tr>
<td>Calcium supplements</td>
<td>4.2</td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td>1.7</td>
</tr>
<tr>
<td>Serum total calcium, mg/dl</td>
<td>9.1 ± 0.67</td>
</tr>
<tr>
<td>Serum phosphate, mg/dl</td>
<td>3.78 ± 0.87</td>
</tr>
<tr>
<td>Urinary calcium, fraction of load</td>
<td>0.014 ± 0.014</td>
</tr>
<tr>
<td>Urinary phosphate, fraction of load</td>
<td>0.335 ± 0.174</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min/1.73 m²</td>
<td>49.7 ± 37.8</td>
</tr>
<tr>
<td>CKD staging (K/DOQI), %</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>13.2</td>
</tr>
<tr>
<td>Stage II</td>
<td>14.5</td>
</tr>
<tr>
<td>Stage III</td>
<td>36.4</td>
</tr>
<tr>
<td>Stage IV</td>
<td>25.8</td>
</tr>
<tr>
<td>Stage V</td>
<td>10.1</td>
</tr>
<tr>
<td>25(OH)D, ng/ml</td>
<td>26.7 ± 17.4</td>
</tr>
<tr>
<td>1,25(OH)2D, pg/ml</td>
<td>33.8 ± 15.4</td>
</tr>
</tbody>
</table>

Averages and standard deviations were calculated algebraically and are distinct from the mean and corresponding standard errors reported in figure 3 which were obtained from the fitted distribution curves. Figures in the last column indicate number of patients.
Fig. 2. Patient pool, inclusion and laboratory data evaluation.

Fig. 3. Histograms of variables studied. Lines correspond to the fitting of the normal or log-normal distribution functions according to whether there was or not a clear skewness.
Calcium and Phosphorus Metabolism in CKD

According to the flow diagram presented above. The patients in whom the urinary excretions (JUCa and JUP) were computed were selected from the group in which the GFR was measured.

Table 1 describes the general characteristics of the patient population included in the analysis. Patients were between 16 and 94 years old. The majority was of white race and 51% were males. The etiologies of CKD were predominantly nephrosclerosis, diabetes and interstitial tubular diseases. The 9.5% of the patients that were under vitamin D therapy were excluded from [calcitriol]s and [calcidiol]s measurements. Transplanted patients or patients on renal replacement therapy were excluded. Preliminary analysis showed that the fractional excretions of calcium and phosphorus were not affected by treatment with diuretics.

Parametric Description of the Patient Population

Figure 3 reports frequency distributions of the biochemical and functional variables used in this analysis. Binning was chosen following Hald [23]. The data plotted in figure 3a, b, c, d, e, f, h ([Ca]s, [P]s and [calcitriol]s in serum and phosphorus absolute urinary output) are fitted with unimodal log-normal curves. Data plotted in figure 3c, d, f, h ([Ca]s, [P]s and [calcitriol]s, in serum and phosphorus absolute urinary output) are fitted with unimodal normal curves. The numerical insets in each curve report the mean (μ) and its standard error (σ) estimated from the curve fit.
Correlations between the Variables

In a single population of patients with CKD and with perturbations of the calcium/phosphorus balances, the relations between the average values of the biochemical data ([Ca], [P], i[PTH], etc.) should be determined by their interrelations in each patient.

Figure 4 shows that the curves relating GFR to the other variables follow two basic patterns. In figure 4a, d, e, they are decreasing functions of i[PTH], [P], and JUCa-R with a clear point of inflexion. By fitting straight lines to the points before and after this inflexion, we determined interceptions at values (see insets) of GFR of 32.7 (fig. 4a), 25.1 (fig. 4d) and 28 (fig. 4e) ml/min/1.73 m². In figure 4b, c serum calcitriol and calcium concentrations are increasing functions of GFR without any points of inflexion up to around 100 ml/min/1.73 m² and decrease afterwards, but there is large spread of the values. JUP-R is a smoothly decreasing function of GFR. The straight lines fitted asymptotically to the extremes of the curve intercept at a GFR of 44.5 ml/min/1.73 m². The behavior of the system seems to change dramatically below GFR in the range of 25–45 ml/min/1.73 m². The inset in figure 4c displays the relative range of the variables studied defined as the ratio between the range (highest minus lowest value) and their corresponding values at normal (120 ml/min/1.73 m²) GFR. It shows that the relative range of variation of [Ca] is much smaller than the corresponding values for [PTH], JUCa-R and JUP-R. [P] and [calcitriol] exhibit smaller variations than these but much larger than [Ca].

The urinary excretions of both calcium (fig. 4e) and phosphorus (fig. 4f) expressed as a fraction of their filtered loads increase dramatically as GFR falls. Another way of looking at the absolute urinary excretions of calcium and phosphorus is to compare them with the serum concentrations of these two ions measured in the same patients. Figure 5 demonstrates that while [Ca] and JUCa are both nondecreasing functions of GFR, [P] falls as JUP rises with GFR.

Figure 6a shows [calcitriol], dependence on i[PTH]. The initial 3 values correspond to GFRs >80 ml/min/1.73 m², where a positive correlation between calcitriol and i[PTH] is to be expected (fig. 4). For i[PTH] values between 80 and 200 pg/ml, there is a negative correlation between [calcitriol] and i[PTH], while at higher values the serum concentration of calcitriol becomes insensitive or is an increasing function of i[PTH]. [Ca] is also a decreasing function of i[PTH] over the whole range of values represented for this hormone, while [P] is an increasing function of i[PTH]. In these 3 cases, PTH secretion is probably driven by [calcitriol], [Ca] and [P]. The fractional urinary excretions of both calcium and phosphorus rise as i[PTH] increases.

In figure 7, we report plots of i[PTH] versus [calcitriol], [calcidiol], [Ca] and [P]. Except for [Ca], all the variables have highly significant statistical correlations of opposite sign for high and low GFR values. In the case of [Ca], the correlation is negative at low values of GFR and not statistically significant at high values.

Figure 8 displays the behavior of [calcidiol]. Figure 8a shows that below GFR smaller than 50 ml/min/1.73 m², [calcidiol] is an increasing function of GFR. In figure 8b we can see that [calcitriol] is an increasing function of [calcidiol] up to a concentration 40 ng/ml of this hormone. Finally, figure 8c suggests that [calcidiol] is a decreasing function of i[PTH].
Discussion

The values of the eight variables studied had unimodal distributions and could be fitted by normal or log-normal functions. Since all the curves are unimodal, we assumed that we are dealing with a single population of values for each variable despite the fact that the data used came from three different institutions.

Since the data used consist of sets of variables measured in the same subject, we assume that the variations in their values are connected by their physiological relationships. In CKD, the metabolic perturbations should arise directly or indirectly from the kidney dysfunction, hence the choice of GFR as the driving variable. Preliminary analyses had shown that any other driving variable would generate much noisier plots. The measurement of the concentrations of calcium and phosphorus in the serum and its outputs in the urine are the observable indicators of the balances of these ions and their regulation. The choice of PTH and calcitriol as the main controlling hormones and calcidiol evaluation as a measure of body stores of vitamin D is in agreement with the accumulated clinical experience [25, 26].

The biochemical pattern of CKD is a continuum, but with a clear change as GFR falls around 25–45 ml/min/1.73 m² when there is a change of slope in relation to [P]ₙ and [iPTH]ₙ and to the urinary JUCa-R and JUP-R as functions of GFR.

From figure 3, we can see that the curves of figure 4 provide a good representation of the correlations for the central values of the represented variables, since the frequency distributions of these variables are bell-shaped.
Very low and almost normal values of GFR are poorly represented, hence less amenable to an analytical description.

The inset of figure 4c indicates that $[\text{Ca}]_s$ is regulated over the whole range of GFR and that $i[\text{PTH}]_s$ and the urinary excretions of calcium and phosphorus are the main regulators.

There is a smooth rise in the excretion fraction of phosphorus as GFR falls. This increase is an early indicator of the disturbance of mineral metabolism, with a change of slope occurring at around a GFR of 45 ml/min/1.73 m$^2$, preceding the steep rise in $i[\text{PTH}]_s$. This may reflect a stimulation in the production of phosphatonin, including FGF-23 [27] which occurs before $i[\text{PTH}]_s$ [28]. Although JUP-R increases as GFR decreases, the absolute urinary output of phosphorus decreases and serum phosphorus concentration increases, with a steep change occurring at 25 ml/min/1.73 m$^2$ (fig. 4d).

Fig. 7. Correlations between $[\text{calcitriol}]_s$, $[\text{calcidiol}]_s$, $[\text{Ca}]_s$, $[\text{P}]_s$, and $i[\text{PTH}]_s$ at low (a; <80 ml/min/1.73 m$^2$) and normal (b; >90 ml/min/1.73 m$^2$) GFRs. Plots of the original data filtered with a moving average of 3 points (see ‘Methods’). Lines are linear regression and bounds defined by 1 standard deviation. Figures at the top of each panel are the correlation coefficient and its SD, number of observations and error of the second kind (23) in the estimation of R.
Figure 5 suggests that the absolute excretion of Ca (JUCa) reflects mainly the filtered load, while the urinary excretion of phosphorus (JUP) is probably determined by the tubular reabsorption of this ion.

Although [iPTH]s is an important regulator, extremely high values (not documented) do not follow the trend observed at lower values. This suggests that they correspond to an abnormal secretion and agrees with the occurrence of hypertrophic or adenomatous hyperparathyroid glands in CKD [29].

The three variables ([calcitriol]s, [Ca]s and [P]s) involved in the control of the PTH release are part of closed feedback loops. This means that depending on the underlying functional situation, the correlation between the [iPTH]s and any of the three variables may be positive or negative. Since we assume that metabolically the patients are in a steady-state-like situation, [iPTH]s, [calcitriol]s and [calcidiol]s can be presumed to be proportional to their rates of production.

In primary hyperparathyroidism, one should expect a positive correlation between [iPTH]s and [calcitriol]s, while in the secondary hyperparathyroidism of CKD the correlation should be negative. Potentially, the analysis of this correlation might indicate which of the three variables is the earliest to be involved in the development of secondary hyperparathyroidism of CKD.

Since [PTH]s seems to be an important regulator, we made plots using it as the driving variable (fig. 6). It shows that [calcitriol]s dependence on [iPTH]s is normal when GFR is above 80 ml/min/1.73 m², as expected from PTH effect on the renal synthesis of calcitriol. For values of [iPTH]s in the range of 80–200 pg/ml, the negative correlation between [iPTH]s and [calcitriol]s reflects the inhibitory action of this hormone on the parathyroid gland. The positive correlation between the two hormones above 200 pg/ml may indicate the release of the parathyroid secretory cells from calcitriol to be expected from hypertrophic or adenomatous glands which corresponds to very low GFR (around 10 ml/min/1.73 m²), hence to very late stages of the disease. The plots of [Ca]s (fig. 6b) and [P]s (fig. 6c) versus [iPTH]s are what one would expect if the negative feedback of the two loops dominates:

[PTH]s + [Ca]s and [PTH]s + [P]s

At present, we can say that the urinary excretions of both calcium and phosphorus (expressed as fractions of the filtered loads) are increasing functions of GFR (fig. 6d, e), a behavior that cannot be attributed to variations in [iPTH]s, since this hormone has opposite effects on the tubular absorption of calcium and phosphorus. The most
likely mechanism is the effect of the reduction of tubular transporting mass on the excretion of the two ions which is not a regulatory mechanism.

The sequence of decreasing values of GFR in this work represents an idealized time series, lower values corresponding in general (albeit not always or linearly) to longer evolutions. Comparisons between the curves for [calcitriol]s, [Ca]s, and [P]s (fig. 4b–d) might suggest that [calcitriol]s is the factor firstly involved in the abnormal regulation of calcium/phosphorus metabolism in CKD. An analysis of values measured at very low and at almost normal GFR might indicate otherwise. Figure 7 is an attempt to detect which of the controlling factors ([calcitriol]s, [calcidiol]s, [Ca]s or [P]s) acts earliest in the development of CKD. With the exception of [Ca]s, all the factors exhibit their physiological correlation with i[PTH]s when GFR is above 90 ml/min/1.73 m² and revert to a feedback correlation below 80 ml/min/1.73 m². While the correlation between [Ca]s and i[PTH]s below 80 ml/min/1.73 m² follows the trend of the other variables (dominance of the feedback branch), above 90 ml/min/1.73 m² the scatter of the [Ca]s data does not allow to extract a significant correlation. A proper assessment of this mechanism implies an analysis of a larger sample of patients in the early stages of CKD.

The observations of the behavior of [calcidiol]s, reported in figure 8 cannot be interpreted at present in terms of known mechanisms. Its relation to GFR might indicate a degree of hypovitaminosis D in advanced CKD [30]. There is a positive correlation between GFR and [calcidiol]s below a GFR of 50 ml/min. This could be explained if not just [calcitriol], but also [calcidiol], production was affected in renal failure. The negative correlation between [calcidiol]s and [PTH]s might indicate an action on the parathyroid glands similar to that of [calcitriol], or through the local production of calcitriol [31] or a substrate limitation of the production of calcitriol [32].

**Conclusion**

This work was undertaken in order to obtain data that could be fitted with a previously published mathematical model of P/Ca metabolism [33]. The strategy consisted of collecting on the same day and from the same subject sets of analyzed variables combined with a moving average filtering. This procedure enabled us to extract functional relationships between the variables which would otherwise be hidden in noise. To our knowledge, this is a new approach. The shortcomings of the method resulted from the fact that the data gathering was largely retrospective. However, it revealed a number of functional relationships between the different variables. We are not aware of reports of similar studies.

**References**

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