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# Metals and oxidative stress in patients with Parkinson's disease

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**Summary**. - Twenty-six metals and the oxidative status in 71 patients affected by Parkinson's disease and 44 healthy individuals were compared in order to identify potential biomarkers of the disease. In the patients, the following significant imbalances were found ($p \leq 0.05$): *i)* in serum, an increment of Ca, Mg, Ni, Si and V, and a decrement of Cd, Co, Fe, Li, Sn, Zn and Zr; *ii)* in blood, raised levels of Co, Li, Ni and Si and decreased of Al, Be, Ca, Cd, Fe, Mg, Mo, Sn, Zn and Zr; *iii)* increased formation of oxidant species and lowered anti-oxidant capacity ($p \leq 0.001$ for both). Barium, Bi, Cr, Cu, Hg, Mn, Pb, Sb, Sr, Tl and W did not change with the disease. The best discriminating variables between patients and controls were Cd, Co, Fe, Ni and Si in serum (91.2% of cases correctly classified), and Al, Cd, Co, Fe, Mo and Si in blood (98.2% of cases properly classified).

*Key words*: metals, oxidative status, Parkinson's disease, serum, blood.

**Riassunto** *(Elementi chimici e danno ossidativo in pazienti affetti da morbo di Parkinson).* - Ventisei metalli e danno ossidativo sono stati confrontati in 71 pazienti affetti da morbo di Parkinson e 44 individui sani per identificare potenziali marcatori biologici della malattia. Nei pazienti sono state riscontrate le seguenti alterazioni significative ($p \leq 0{,}05$): 1) nel siero, un incremento di Ca, Mg, Ni, Si e V ed una diminuzione di Cd, Co, Fe, Li, Sn, Zn e Zr; 2) nel sangue, livelli più alti di Co, Li, Ni e Si e più bassi di Al, Be, Ca, Cd, Fe, Mg, Mo, Sn, Zn e Zr; 3) aumentata formazione di specie ossidanti e ridotta capacità antiossidante ($p \leq 0{,}001$ per entrambi). Bario, Bi, Cr, Cu, Hg, Mn, Pb, Sb, Sr, Tl e W non variavano con la malattia. Le variabili che meglio discriminavano fra pazienti e controlli risultavano essere Cd, Co, Fe, Ni e Si nel siero (91.2% di casi correttamente classificati), e Al, Cd, Co, Fe, Mo e Si nel sangue (98,2% di casi esattamente classificati).

*Parole chiave*: elementi, danno ossidativo, morbo di Parkinson, siero, sangue.

## Introduction

The overall picture of Parkinson's disease (PD) has not yet been completely clarified, although its multifactorial etiology has been rather accepted. It seems that factors as age, dietary habits, environmental and occupational exposure to chemicals may contribute to the development of PD. On the contrary, the involvement of genetic factors in this pathology is less than 5 - 10% of all cases.

In this context the relevant role of metals has been presumed from neurochemical observations. Gorell *et al.* asserted that a synergic combination of various metals could increase the risk of the disease [1]. Iron is one of the most important metals involved in the pathogenesis of PD because of its ability to generate free radicals and to promote redox reactions. It accumulates in high concentration in different brain regions and forms selectively complexes with neuromelanin that may induce oxidative stress and death of dopaminergic neurons [2, 3]. Iron target seems to be the *substantia nigra,* since in this region increased levels in parkinsonian patients were found [4, 5]. As concern Mn, this element is able to exchange single electrons and to produce reactive oxygen species and toxic cathecolamines, laying the basis for

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neurodegeneration [6]. Neurological alterations in PD seem to be associated to anomalous levels of Mn in brain because of its high permeability to this element, as well as to Fe and Zn [7]. With reference to Zn, there is not evidence on its direct involvement in developing this neuropathological condition in humans. On the contrary, it has several functions in brain development and maintenance as the participation in the superoxide dismutase (SOD) and Zn-thioneine enzymes to prevent oxidative damage. It has been reported that a depletion of Zn could led to PD in people, who tend to accumulate Cu [8].

Also Cu plays a key role in cell metabolism as transition metal acting as a cofactor in many detoxifying enzymes and proteins (SOD, ceruloplasmine, metallothioneine, etc.), but a modified brain homeostasis of this element together with oxidative stress and mitochondrial alterations could lead to cell death and, thus, to the PD [9]. As regards Ca and Mg, it has been shown that neurons involved in neurodegenerative disorders have increased intracellular free $Ca^{++}$ acting as promoter of cell death, whereas in normal subjects, the above phenomenon is limited *via* the voltage-Mg dependent blockade [10]. In addition, an altered metabolism of Ca and Mg and a subsequent very high Ca/Mg ratio was associated to central nervous system (CNS) degeneration [11]. Moreover, a depletion of these metals in brain seems to be related to a high intake of Al [12]. The amount of Al in cerebral tissues is probably due to exogenous exposure. In general, this element has very low levels in brain, but increased concentrations in the neurofibrillary tangles of PD patients have been found. Although Al is not a free radical promoter, the combination of Al-salts and $Fe^{++}$ causes the iron-induced lipid peroxidation [13, 14]. As concern Si, the silicic acid is able to bind Al, so lowering its bioavailability and showing a protective effect [15]. On the other side, Si accumulates as alumino-silicate complexes in the neurofibrillary tangles of patients with neurological diseases [16]. Mercury is a well known neurotoxin, stored in human cortical motor neuron, where it is able to promote neural damaging through the formation of free radicals and the increase of intracellular Ca. An association of Hg with PD was also hypothesized [17-19]. Neurological disorders produced by the exposure to Pb are well reported and a relationship between long-term exposure to this metal and development and acceleration of PD was also suggested [20].

In addition to this, the characteristic intracellular inclusions of the PD, i.e., Lewy bodies, are produced through the aggregation of the $\alpha$-synuclein brain protein. A study evidenced that some elements as Al, Cd, Co, Cu, Fe and Mn favoured the production of the these inclusions [21]. On the contrary, Mg seemed to inhibit spontaneous and Fe-induced $\alpha$-synuclein aggregation [22]. Although the toxicity of various elements such as Be, Cr, Ni and V has been assessed, their relation with PD was not suggested.

Accordingly to that above mentioned and due to the scarcity of information in literature about the relationship between the elements in biological fluids and the disease, a study to quantify the concentration of a large number of elements, i.e., Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn and Zr in blood and serum of PD patients and controls has been undertaken. Besides, the oxidative status (SOS) as well as the total antioxidant capacity (SAC) were assessed in serum of both population groups.

## Materials and methods

### *Study population*

Seventy-one patients (53 males and 18 females) with PD diagnosed according to the London Brain Bank Criteria were examined [23]. These subjects had a mean age of 65.5 $\pm$ 9.4 yrs with a mean duration of the disease of 4.62 $\pm$ 4.50 yrs and a Hoehn and Yahr phase range of 1-3 [24]. Approximately 25% of patients were not undergoing treatment with anti-parkinsonian drugs, while the remaining patients were being treated with dopaminergic agonists, L-dopa or a combination of both. Forty-four (33 males and 11 females) controls with a mean age of 51.9 $\pm$ 4.0 yrs, who were not affected by any central neurological disorder, were also examined. All subjects were interviewed in order to obtain detailed information on family, dietary habits, lifestyle and personal medical history. Exclusion criteria applied to both groups were: liver or renal disease, cardiological and respiratory disorders, diseases causing malabsorption, intake of vitamin or mineral supplements, active infections, assumption of thyroid hormones or lithium, vegetarian dietary habits, artificial metallic body parts and assumption of psychoactive drugs except for anti-parkinsonism medications. The study was approved by the Ethics Committee of the Neuroscience Department of Tor Vergata University, Rome.

### *Pre-treatment and analysis of samples*

Details on the sampling procedures and pre-treatment as well as on the analytical methods employed for the elements quantification of body fluids were reported elsewhere [25]. Serum oxidative status and SAC were photometrically determined and expressed in U.CARR - where 1 U.CARR corresponded to 0.08 mg of peroxyl radicals for 100 ml of hydrogen peroxide - and in μmol $ml^{-1}$ of HClO, respectively.

*Statistical analysis*

Results on the PD patients and controls were described in terms of mean, standard deviation (SD) and 25th-75th percentiles, and statistically compared by the Mann-Whitney test ($p \leq 0.05$). A stepwise discriminant analysis (SDA) has been applied on the variables resulted to be significantly different between the two groups under study. A forward stepwise procedure, guided by the statistical F values, to select the variables was used. The discriminant function was validated through the leave-one-out method. The statistical package SPSS version 12.0 was utilized (SPSS, Chicago, IL, USA).

## Results and discussion

Tables 1 and 2 give a basic description of the data in serum and blood of the PD patients and healthy control subjects. In serum of PD group, Bi, Cd, Sb, Tl, W and Zr were at level below 0.1 ng $ml^{-1}$, with the lowest value for Bi. Concentrations between 0.1 and 1.0 ng $ml^{-1}$ were observed for Ba, Be, Co, Cr, Li, Mn, Mo, Ni, Pb, Sn and V, and between 1.0 and 5.0 ng $ml^{-1}$ for Al and Hg with the highest mean value for Al (3.05 ng $ml^{-1}$). Silicon and Sr were found at level of hundreds and tens of ng $ml^{-1}$, respectively. With reference to the major elements, i.e., Ca, Cu, Fe, Mg and Zn, the lowest value resulted for Zn while the highest was for Ca.

In blood, Bi, Tl and W were again under 0.1 ng $ml^{-1}$; Be, Cd, Co, Cr, Sb, Sn, V and Zr were in the range 0.1-1.0 ng $ml^{-1}$; while Ba, Hg, Li, Mn, Mo and Ni were in the interval 1.0-10 ng $ml^{-1}$ with the highest concentration for Mn (8.57 ng $ml^{-1}$). The mean concentrations in blood of Al, Pb and Sr were in the order of tens of ng $ml^{-1}$ and those of Si one order of magnitude higher. Copper and Fe, among the major elements, presented the lowest and highest values found in this matrix.

In general, most of the quartile ranges of the patients overlapped those of the controls. This is the case of Ba, Bi, Sb, Sr, Tl and W, for which no statistical difference as a function of the disease has been found in both matrices. In agreement to this result, the available literature did not report any kind of implication of these elements in PD. The levels of Cr were ca. 2-fold and 1.5-fold higher in patients than in controls in serum and blood, respectively, although not statistically different. In this last case, similar increment was found by Jiménez-Jiménez *et al.* in the same diseased population [26]. Surprisingly, elements recognized to have some adverse effects on the nervous system such as Cu, Hg, Mn and Pb did not differ in the two groups and this is partially confirmed by previous literature. In fact, Jiménez-Jiménez *et al.* and Hegde *et al.* found no alterations when the serum Mn content of PD patients was matched with control group [26, 27]. Pamphlett *et al.* found similar results for Hg and Pb in patients affected by a different neurological condition, i.e., the sporadic motor neuron disease (SMND) [28]. As regard Cu, some studies confirmed no difference between PD or SMND subjects and controls, whilst others found statistically raised Cu content in PD [27-30].

In the case of elements like Be, Li, Ni, V and Zr, for which apparently no implication in the pathogenesis of the disease has been reported, statistically significant differences between patients and healthy subjects were observed. In particular, the levels of Ni and V in serum of patients were found increased and those of Li and Zr decreased with respect to the controls. In blood, in turn, the Li and Ni levels were higher and those of Be and Zr were lower than in healthy individuals. In all cases they were at significance level of $p < 0.001$, with the exception for Li and Zr in blood and Li in serum ($p < 0.03$).

Relevant differences ($p < 0.005$) between the two population groups have been observed for elements such as Cd, Co and Sn in both matrices and for Mo in blood. In particular, Cd and Sn lowered in both matrices, Co raised in blood and decreased in serum, whilst Mo decreased in blood. As regards Cd, it is known as a neurotoxin and one case of development of parkinsonism in a welder after severe exposure to this element has been reported [31]. In contrast with the finding of this study, higher level of Cd in serum of SMND patients respect to a control population has been found [28]. For Co, no difference in serum of individuals affected by PD was found [26]. The unbalanced levels of Sn in our PD subjects should be more deeply investigated to assess the organic species of the total Sn found because only the organic part is able to produce adverse effects on brain. Blood Al seemed to correlate with PD after comparison with controls as testified by a statistically relevant decrement in patients ($p < 0.01$). Similar observation was reported in another recent study where serum Al in patients was statistically lower than in controls [27]. Calcium in PD has been found to be significantly higher in serum and lower in blood respect to controls ($p < 0.001$, in both cases). This trend confirmed the results of Hegde *et al.* in serum of patients affected by PD [27]. Significant difference ($p < 0.001$) in the levels of haematological Fe between diseased and healthy groups was observed. In particular, patients showed lower values in both matrices with respect to those of controls, with a decrement of ca. 22% and 30% in blood and serum,

**Table 1**. - Element concentrations (ng ml$^{-1}$) in serum of Parkinson's disease (PD) patients and controls

| Element | PD patients | | Controls | |
|---|---|---|---|---|
| | Mean ± SD | 25$^{th}$-75$^{th}$ | Mean ± SD | 25$^{th}$-75$^{th}$ |
| **Al** | 3.05 ± 1.64 | 1.81 - 4.04 | 2.79 ± 1.51 | 1.65 - 3.91 |
| **Ba** | 0.66 ± 0.22 | 0.56 - 0.79 | 0.69 ± 0.36 | 0.43 - 0.84 |
| **Be** | 0.30 ± 0.17 | 0.19 - 0.47 | 0.24 ± 0.14 | 0.10 - 0.35 |
| **Bi** | 0.02 ± 0.01 | 0.01 - 0.02 | 0.02 ± 0.01 | 0.01 - 0.02 |
| **Ca*(*)** | 67,259 ± 9,938 | 61,344 - 74,486 | 61,378 ± 5,635 | 58,030 - 65,648 |
| **Cd*(*)** | 0.07 ± 0.03 | 0.05 - 0.09 | 0.10 ± 0.05 | 0.07 - 0.12 |
| **Co*(*)** | 0.11 ± 0.05 | 0.08 - 0.12 | 0.19 ± 0.10 | 0.11 - 0.25 |
| **Cr** | 0.35 ± 0.07 | 0.12 - 0.32 | 0.18 ± 0.07 | 0.13 - 0.22 |
| **Cu** | 987 ± 238 | 798 - 1,116 | 910 ± 197 | 755 - 1,062 |
| **Fe*(*)** | 1,122 ± 432 | 834 - 1,479 | 1,596 ± 442 | 1,299 - 1,892 |
| **Hg** | 2.02 ± 1.67 | 0.65 - 2.91 | 1.41 ± 0.73 | 0.95 - 1.72 |
| **Li*(*)** | 0.79 ± 0.60 | 0.35 - 1.01 | 1.01 ± 0.75 | 0.60 - 1.55 |
| **Mg*(*)** | 19,012 ± 3,215 | 16,850 - 21,199 | 17,200 ± 1,781 | 16,466 - 18,550 |
| **Mn** | 0.66 ± 0.24 | 0.52 - 0.84 | 0.65 ± 0.24 | 0.48 - 0.80 |
| **Mo** | 0.89 ± 0.42 | 0.63 - 1.20 | 0.92 ± 0.49 | 0.52 - 1.24 |
| **Ni*(*)** | 0.64 ± 0.33 | 0.41 - 0.77 | 0.43 ± 0.36 | 0.17 - 0.56 |
| **Pb** | 0.62 ± 0.34 | 0.36 - 0.81 | 0.52 ± 0.22 | 0.36 - 0.61 |
| **Sb** | 0.08 ± 0.04 | 0.04 - 0.11 | 0.11 ± 0.09 | 0.05 - 0.15 |
| **Si*(*)** | 295 ± 104 | 217 - 368 | 111 ± 55 | 74.1 - 136 |
| **Sn*(*)** | 0.48 ± 0.21 | 0.33 - 0.60 | 0.78 ± 0.47 | 0.37 - 1.10 |
| **Sr** | 36.9 ± 11.5 | 28.8 - 45.1 | 38.7 ± 14.7 | 27.7 - 45.4 |
| **Tl** | 0.05 ± 0.02 | 0.03 - 0.06 | 0.05 ± 0.02 | 0.03 - 0.06 |
| **V*(*)** | 0.11 ± 0.07 | 0.06 - 0.15 | 0.06 ± 0.03 | 0.04 - 0.07 |
| **W** | 0.03 ± 0.02 | 0.02 - 0.04 | 0.03 ± 0.02 | 0.02 - 0.05 |
| **Zn*(*)** | 717 ± 125 | 633 - 798 | 813 ± 135 | 704 - 910 |
| **Zr*(*)** | 0.09 ± 0.07 | 0.04 - 0.12 | 0.12 ± 0.05 | 0.09 - 0.15 |
| **SOS*(*)** | 317 ± 54 | 296 - 345 | 273 ± 44 | 243 - 308 |
| **SAC*(*)** | 321 ± 48 | 300 - 352 | 368 ± 74 | 340 - 378 |

*(*)*: $p \leq 0.05$

respectively. Our data on Fe corroborate those found by earlier studies carried out on PD patients [27, 32]. On the contrary, Cabrera-Valdivia *et al.* found Fe slightly higher in serum of the patients while other authors observed no difference [33, 34]. In the case of Mg, values statistically higher in serum and lower in blood of PD patients in this study were found. At level of CNS, a Mg deficit in various brain regions has been found [11]. Also Si level in PD has been found markedly increased in both matrices under study, though no recent evidence in literature can support this finding. The increase of Si could be probably related to an involuntary intake of this element as drug excipient during PD treatment rather than to its role as biomarker of the disease. Finally, a significant depletion ($p < 0.001$) of Zn in serum and blood of PD patients was observed, as it was also noticed in the study of Hegde *et al.* [27]. On the other hand, two additional papers on Zn levels did not report any unbalance between PD patients and controls both in blood and serum [29, 30].

Moreover, there is evidence that oxidative damage incurred in the patients as indicated by the statistically increased SOS ($p \leq 0.001$) in PD subjects, with mean values of 317 ± 54 *vs* 273 ± 44 U.CARR of the controls. It must be taken in mind that values higher than 300 U.CARR indicated a clear oxidative damage. At the same time, the SAC was found to be lower in the patients than in the controls ($p \leq 0.001$) with levels of HClO equal to 321 ± 48 *vs* 368 ± 74 μmol ml$^{-1}$,

**Table 2**. - Element concentrations (ng ml$^{-1}$) in blood of Parkinson's disease (PD) patients and controls

| Element | PD patients | | Controls | |
|---|---|---|---|---|
| | Mean ± SD | 25$^{th}$-75$^{th}$ | Mean ± SD | 25$^{th}$-75$^{th}$ |
| **Al*(*)*** | 13.0 ± 11.2 | 4.51 - 18.7 | 18.1 ± 10.8 | 9.20 - 23.1 |
| **Ba** | 1.82 ± 1.33 | 0.71 - 2.54 | 1.32 ± 0.68 | 0.84 - 1.72 |
| **Be*(*)*** | 0.28 ± 0.19 | 0.14 - 0.39 | 0.41 ± 0.18 | 0.30 - 0.51 |
| **Bi** | 0.03 ± 0.02 | 0.02 - 0.04 | 0.03 ± 0.02 | 0.02 - 0.04 |
| **Ca*(*)*** | 48,861 ± 9,532 | 40,565 - 56,150 | 62,870 ± 10,051 | 56,947 - 66,388 |
| **Cd*(*)*** | 0.77 ± 0.32 | 0.51 - 1.01 | 1.06 ± 0.48 | 0.82 - 1.36 |
| **Co*(*)*** | 0.26 ± 0.12 | 0.17 - 0.32 | 0.12 ± 0.09 | 0.05 - 0.16 |
| **Cr** | 0.71 ± 0.59 | 0.23 - 1.18 | 0.48 ± 0.33 | 0.25 - 0.55 |
| **Cu** | 1,042 ± 453 | 623 - 1,327 | 946 ± 137 | 869 - 1,030 |
| **Fe*(*)*** | 428,610 ± 70,062 | 362,880 - 478,254 | 547,409 ± 49,050 | 524,453 - 574,841 |
| **Hg** | 5.81 ± 3.77 | 2.90 - 8.42 | 5.85 ± 3.18 | 3.58 - 8.05 |
| **Li*(*)*** | 1.09 ± 0.76 | 0.49 - 1.59 | 0.74 ± 0.44 | 0.44 - 0.94 |
| **Mg*(*)*** | 31,370 ± 4,672 | 27,767 - 34,544 | 40,235 ± 4,967 | 38,092 - 43,134 |
| **Mn** | 8.57 ± 3.90 | 5.69 - 10.9 | 7.49 ± 3.48 | 5.41 - 9.49 |
| **Mo*(*)*** | 1.67 ± 0.73 | 1.15 - 2.21 | 3.47 ± 1.35 | 2.48 - 4.34 |
| **Ni*(*)*** | 4.36 ± 3.13 | 1.40 - 6.55 | 0.97 ± 0.53 | 0.58 - 1.30 |
| **Pb** | 57.4 ± 27.7 | 35.8 - 70.0 | 50.9 ± 20.1 | 32.8 - 64.4 |
| **Sb** | 0.43 ± 0.21 | 0.27 - 0.60 | 0.41 ± 0.25 | 0.23 - 0.58 |
| **Si*(*)*** | 241 ± 166 | 119 - 338 | 154 ± 63 | 112 - 181 |
| **Sn*(*)*** | 0.98 ± 0.72 | 0.46 - 1.54 | 1.39 ± 0.49 | 1.03 - 1.69 |
| **Sr** | 27.8 ± 8.9 | 20.9 - 33.6 | 28.2 ± 12.3 | 19.9 - 35.9 |
| **Tl** | 0.09 ± 0.06 | 0.05 - 0.12 | 0.08 ± 0.04 | 0.05 - 0.10 |
| **V** | 0.11 ± 0.07 | 0.07 - 0.14 | 0.09 ± 0.05 | 0.06 - 0.13 |
| **W** | 0.09 ± 0.07 | 0.04 - 0.13 | 0.07 ± 0.03 | 0.04 - 0.08 |
| **Zn*(*)*** | 5,313 ± 977 | 4,580 - 6,092 | 6,810 ± 787 | 6,280 - 7,286 |
| **Zr*(*)*** | 0.38 ± 0.17 | 0.27 - 0.45 | 0.48 ± 0.25 | 0.31 - 0.60 |

*(*)*: $p \leq 0.05$

respectively (normal condition, values > 350 µmol ml$^{-1}$ of HClO). The combination of increased free oxidant species and of depleted natural defence mechanisms observed in PD patients leads to a situation of oxidative stress. The unbalance in neurotoxic elements such as Al and Cd in association with an altered concentration of essential elements, i.e., Ca, Fe, Mg and Zn, could promote the production of the reactive oxygen molecules and the diminishing of the antioxidant defences of the organism.

When the SDA was applied, only a small group of variables seemed to have a role in adequately discriminating between diseased and healthy individuals. The SDA, in fact, selected five variables in serum with the standardized coefficients > 0.3, namely, in the order, Si, Co, Fe, Ni and Cd. The discriminant function classified correctly 91.2% of cases (also after cross-validation). Considering Si, the doubt that its weight in the discriminating function was due to its use in drug formulas during therapy persists. In the blood, instead, six variables having the standardized coefficients > 0.3 were selected. They were, in order of relative importance as discriminatory factor, Fe, Co, Al, Mo, Si and Cd. The classification rate was extremely good, i.e., 98.2% of cases correctly classified (both by the original and cross-validated method). It should be stressed out that both well known neurotoxins, as Al and Cd, and elements essential but involved in the generation of oxidative damage, such as Co and Fe, resulted to have a crucial discriminant role in the statistical model. Moreover, the combination of Al and Fe in the statistical function seemed to confirm earlier evidences on the role of these two metals in promoting lipid peroxidation.

## Conclusions

The obtained data give a large sight of the elemental status of PD patients, as resulting from the monitoring of the peripheral fluids. Evident imbalances in trace elements content, as an effect or cause of the neuropathology, have been observed. Although these imbalances cannot be explained through a simple model, a potential role of a number of elements as biomarkers in this kind of neurodegeneration cannot be excluded. In addition, the presence of a certain degree of oxidative damage in these patients confirms the idea that oxidative injury is a key factor in neurodegenerative status. Finally, taking into account the exact interpretation of data, these findings suggest that the SDA could be correctly applied to the PD as a predictive or diagnostic statistical tool.

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