

Babelyuk Valeriy Y., Gozhenko Anatoliy I., Dubkova Galyna I., Babelyuk Nazariy V., Zukow Walery, Kindzer Bohdan M., Kovbasnyuk Marta M., Popovych Igor L. Causal relationships between the parameters of gas discharge visualization and phagocytosis. *Journal of Education, Health and Sport*. 2021;11(6):268-276. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2021.11.06.029> <https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2021.11.06.029> <https://zenodo.org/record/5498345>

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

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The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 21.05.2021. Revised: 03.06.2021. Accepted: 30.06.2021.

CAUSAL RELATIONSHIPS BETWEEN THE PARAMETERS OF GAS DISCHARGE VISUALIZATION AND PHAGOCYTOSIS

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Abstract

Background. Previously we have been shown that between parameters of GDV and principal neuroendocrine factors of adaptation exist strong canonical correlation. In the next study, we detected very strong ($R=0,994$) integral canonical correlation between the parameters of GDV and Immunity. This study, conducted in the same contingent, will analyze the relationships between GDV parameters, on the one hand, and Phagocytosis parameters, on the other. **Material and Methods.** We observed twice ten women and ten men aged 33-76 years without clinical diagnose. In the morning in basal conditions at first registered kirlianogram by the method of GDV by the device “GDV Chamber” (“Biotechprogress”, SPb, RF). Then we estimated the parameters of Phagocytic function of neutrophils. Results processed by method of canonical analysis, using the software package “Statistica 5.5”. **Results.** According to the value of the canonical correlation coefficient R with GDV parameters, the registered Phagocytosis parameters are arranged in the following order: activity (0,616), bactericidal capacity (0,493), completeness (0,489) and intensity (0,484) of Phagocytosis of *E. coli*; completeness (0,482), bactericidal capacity (0,448), activity (0,364) and intensity (0,338) of Phagocytosis of *Staph. aureus*. Coefficient of canonical correlation between parameters of GDV, on the one hand, and Phagocytosis, on the other hand, makes 0,847. **Conclusion.** The above data, taken together with the previous ones, state that between parameters of Neuroendocrine-Immune complex and GDV exist strong canonical correlation suggesting suitability of the latter method.

Key words: Gas Discharge Visualization, Phagocytosis, Relationships.

INTRODUCTION

In 1996 KG Korotkov created a new scientific approach, based on the digital videotechnics, modern electronics and computer processing quantitative data, called as method gas discharge visualization (GDV bioelectrography). Parallel uses the terms kirlianography and electrophotonics [8-10]. Since ambiguous attitude to the method [17], we launched a study on its verification. We have been shown that between parameters of GDV and principal neuroendocrine factors of adaptation exist strong canonical correlation suggesting suitability this method for estimation at least adaptation and its changes [1]. In the next study [3], we analyzed the relationships between GDV and immunity parameters. According to the value of the canonical correlation coefficient R with GDV parameters, the immunity parameters are arranged in the following order: IgA (0,716), $CD8^+CD3^+$ Tc-lymphocytes (0,646), IgG (0,645), IgM (0,622), “active” T-lymphocytes (0,572), $CD4^+CD3^+$ Th-lymphocytes (0,566), CIC (0,491), 0-lymphocytes (0,457), $CD16^+$ NK-lymphocytes (0,396), $CD22^+$ B-lymphocytes (0,439). The integral canonical correlation between the parameters of GDV and Immunity was very strong ($R=0,994$) [3]. This study, conducted in the same contingent, will analyze the relationships between GDV parameters, on the one hand, and phagocytosis parameters, on the other.

MATERIAL AND METHODS

The object of observation were 20 volunteers: ten women and ten men aged 33-76 years without clinical diagnose but with dysfunction of neuro-endocrine-immune complex and dysmetabolism.

In the morning we registered kirlianogram by the method of GDV by the device of “GDV Chamber” (“Biotechprogress”, SPb, RF). The first base parameter of GDV is **Area** of gas discharge image (GDI) in Right, Frontal and Left projections registered both with and without polyethylene **filter**. The second base parameter is a **coefficient of Shape** (ratio of square of length of external contour of GDI toward his area), which characterizes the measure of serration/fractality of external contour. The third base parameter of GDI is **Entropy**, id est measure of chaos. Program estimates also **Energy** and **Asymmetry** of virtual **Chakras** [8-10].

In portion of the venous blood we estimated parameters of phagocytic function of neutrophils as described by SD Douglas and PG Quie [6] with moderately modification by MM Kovbasnyuk [11,23]. Here is the author's description of the method. 5 drops of blood immediately after collection, made in glass centrifuge tubes with 2 ml of 4% solution of sodium citrate. Blood samples were stored in a refrigerator at a temperature of 4°C . Further samples were centrifuged (5000 rev/min for 5 min). The supernatant was removed with the help of the Pasteur's pipette. We used a fraction of leukocytes with traces of erythrocytes. The objects of phagocytosis served daily cultures of *Staphylococcus aureus* (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and *Escherichia coli* (O55 K59) as typical representative of Gram-negative Bacteria. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC “Truskavets'kurort”. To prepare the suspension microbes did wipes with relevant shoals sterile saline, immersed tubes in boiling water for 3 seconds, cooled to room temperature. Integrity microbes controlled with the aid of a microscope. To do this, drop the suspension of microbes applied to skimmed substantive piece of glass, fixed in alcohol lamp flame. Ready preparations stained by Papenheim, microscoped during immersion, lense h90, eyepiece x10. The test samples were prepared as follows. In Vidal's plastic tubes made in the following order of 0,05 mL of heparin, 0,05 mL of sterile saline, 0,1 mL suspension of leukocytes, 0,05 mL suspension of microbial bodies. Samples shaken and placed in thermostat at 37°C for 30 min, shaking them with every 10

mins. Then, to stop phagocytosis, the sample was cooled under running water for 10 min. In further samples are centrifuged (5000 rev/min, for 5 min), the supernatant removed with the help of the Pasteur's pipette. From the suspension of leukocytes (with traces of red blood cells) prepared strokes, dried in air at room temperature and stained by Papenheim. Microscoped during immersion lens h90, x10 eyepiece.

Take into account the following parameters of phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytosis Index, **PhI**), intensity (number of microbes absorbed one phagocytes - Microbial Count **MC** or Right's Index) and completeness (percentage of dead microbes - Killing Index **KI**). Microbial number and index their digestion is determined for each phagocyte and fixed in phagocytic frame.

The integrated evaluation of phagocytic function of neutrophils is made by the number of microbes that are able to neutralize neutrophils contained in 1 liter of blood, named as Bactericidal Capacity (BCC) and calculated by formula [21]:

$$BCC (10^9 \text{Bac/L}) = \text{Leukocytes} (10^9/\text{L}) \cdot \text{Neutrophils} (\%) \cdot \text{PhI} (\%) \cdot \text{MC} (\text{Bac/Phag}) \cdot \text{KI} (\%) / 10^6$$

Every day four people were tested. A week later, all the tests were repeated.

Results processed by methods of correlation and canonical analyses, using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Following the previously accepted algorithm, for each parameter of phagocytosis we built regressive models by stepwise exclusion to reach the maximum value of Adjusted R².

KG Korotkov [8-10] put forward the concept that each Chakra is associated with a part of the finger. This approach is embodied in the "GDV Chakras" program, which allows us to quantify the state of virtual Chakras.

The activity of phagocytosis by neutrophils of E. coli as a typical representative of gram-negative microbes is most closely related to GDV (Table 1). It is significant that the top position is occupied by the energy just of the **fourth** Chakra.

Table 1. Regression Summary for Dependent Variable: Phagocytosis Index vs E. coli
R=0,616; R²=0,380; Adjusted R²=0,244; F_(7,3)=2,80; p=0,022; SE: 0,47%

		Beta	St. Err. of Beta	B	St. Err. of B	t ₍₃₂₎	p-level
	r		Intercept	99,50	,206	484	10 ⁻⁶
Ch4E f	0,45	,473	,261	1,153	,636	1,81	,079
Ch7E f	0,45	,774	,396	2,228	1,139	1,96	,059
Ch6E	0,39	,244	,195	,451	,361	1,25	,220
Ch1E f	0,38	,291	,305	,689	,722	,95	,347
Ch2E f	0,36	-,535	,394	-1,475	1,088	-1,36	,185
Ch3E f	0,31	-,362	,323	-,761	,679	-1,12	,270
Ch4E	0,27	-,322	,243	-,707	,534	-1,32	,195

Abbreviations of GDV parameters: A – Area (pixels), Sym – Symmetry (%), S – Shape coefficient, R – Right projection, F – Frontal projection, L – Left projection, f – with filter, ChE - Chakra Energy [E=(R+L)/2], ChA – Chakra Asymmetry (A=R-L).

According to Ayurvedic medicine, Chakras are power centers, related to the endocrine glands and neural plexus as well as to some organs. CR Chase [4] and LG Puchko [24] provides a table according to which the **fourth** Chakra is associated with **thymus** as well as cardiac and celiac plexus, vagus nerve, heart and circulation. It is also expected that among the Chakras that activate this parameter of phagocytosis, the **third** Chakra was found, which is associated with **spleen** as well as [endocrine] pancreas, celiac plexus ganglion, liver, gall bladder, stomach, duodenum and pancreas.

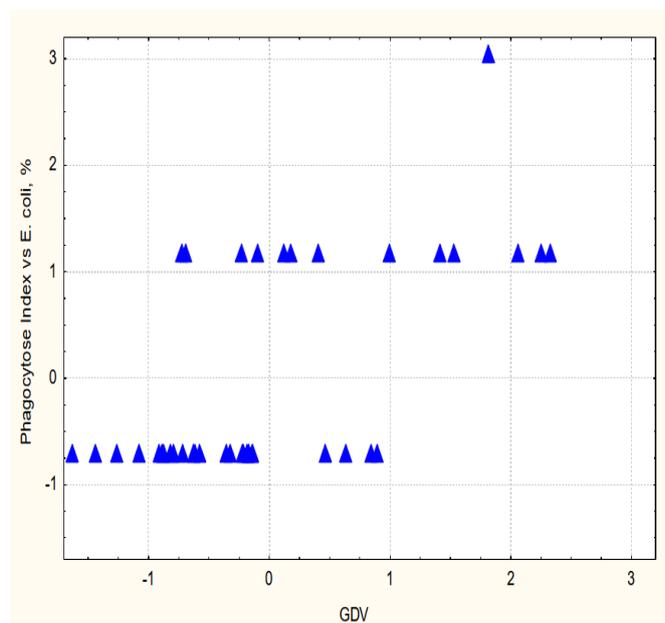
Despite the **seventh** Chakra does not directly affect the immune system, its location among the first two is quite natural, because it is associated with the pineal gland as well as the right and upper brain [4,24]. The pineal gland, in turn, is closely linked to immunity through melatonin [14,15,25]. According to KJ Tracey's [30] conception of immunological homunculus the CNSs structures that are projected onto certain EEG loci are responsible for certain immune functions. In developing this concept, we have previously shown that certain EEG loci are responsible for the regulation of phagocytosis [18].

The **sixth** Chakra is associated with pituitary and pineal glands, left and lower brain, thalamus, hypothalamus, superior cervical ganglion [4,24]. Therefore, its presence in this constellation is also fully consistent with the concept of neuro-immunomodulation [11,12,16,23,27,29-32].

The **first** Chakra is associated with adrenals and pelvic nerve plexus as well as spine, kidneys, bladder and large intestine; **second** Chakra with testes/ovaries and inferior mesenteric ganglion as well as ileum and organs of reproduction [4,24]. Immunomodulatory action of hormones of adrenals and testes/ovaries is well documented [7,22,33].

KG Korotkov [8-10] believes that GDI, taken off without filter, characterizes the **functional** changes of organism, while taken with a filter characterizes **organic** changes. With regard to the fourth chakra, we confirm this by finding a lower correlation coefficient.

Taken together, the six Chakras positively determine the activity of phagocytosis by neutrophils the most represented bacterium in the human microbiota by 38% (Fig. 1).



$R=0,616$; $R^2=0,380$; $\chi^2_{(7)}=16,5$; $p=0,021$; Λ Prime= $0,620$

Fig. 1. Scatterplot of canonical correlation between GDV parameters (X-line) and the Phagocytosis Index vs E. coli (Y-line)

The intensity of phagocytosis of E. coli is determined by the **fourth** Chakra also positively, but only by 14,7% (Table 2).

Table 2. Regression Summary for Dependent Variable: Microbial Count of E. coli
 $R=0,484$; $R^2=0,234$; Adjusted $R^2=0,147$; $F_{(4,3)}=2,68$; $p=0,048$; SE: 6,4 Bac/Phag

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(35)}$	p-level
	r		Intercept	2,338	28,861	,08	,936
Entropy F f	0,30	,164	,182	8,108	8,989	,90	,373
Entropy F	0,28	,199	,182	8,669	7,953	1,09	,283
Ch4A f	0,28	,263	,150	6,880	3,935	1,75	,089
Ch4E f	0,27	,207	,150	6,482	4,686	1,38	,175

Instead, the completion of phagocytosis of E. coli is determined by 17,6% negatively (Table 3).

Table 3. Regression Summary for Dependent Variable: Killing Index vs E. coli
 $R=0,489$; $R^2=0,241$; Adjusted $R^2=0,176$; $F_{(3,4)}=3,78$; $p=0,019$; SE: 5,9%

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(36)}$	p-level
	r		Intercept	39,71	1,27	31,2	10^{-6}
Ch7E	-0,39	-,954	,444	-25,72	11,98	-2,15	,039
Ch3E f	-0,37	-,220	,185	-5,57	4,68	-1,19	,242
Ch2E	-0,29	,744	,434	15,25	8,90	1,71	,095

The fifth Chakra was found to be involved in the downregulation of Bactericidal Capacity vs E. coli (Table 4).

Table 4. Regression Summary for Dependent Variable: Bactericidal Capacity vs E. coli

$R=0,494$; $R^2=0,244$; Adjusted $R^2=0,203$; $F_{(2,4)}=5,96$; $p=0,006$; SE: $17 \cdot 10^9$ Bac/L

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(37)}$	p-level
	r		Intercept	158,2	23,4	6,76	10^{-6}
Area L	-0,37	-,401	,144	-,0049	,0017	-2,80	,008
Ch5A	-0,29	-,324	,144	-29,98	13,28	-2,26	,030

The fifth Chakra is associated with thyroid and parathyroid glands, vagus nerve and inferior cervical ganglion as well as lungs, bronchus, larynx, pharynx and large intestine [4,24]. This is consistent with data on the effect of triiodothyronine, calcitonin, parathyroid hormone and vagus on phagocytosis [7,11,22,23].

In the Tables 5-8 show regression models for GDV parameters and phagocytosis of Staph. aureus as a typical representative of gram-positive microbes. As you can see, the number of GDV parameters included in the model is much smaller, the connections are weaker and also have the opposite direction.

Table 5. Regression Summary for Dependent Variable: Killing Index vs St. aureus
 $R=0,482$; $R^2=0,232$; Adjusted $R^2=0,191$; $F_{(2,4)}=5,60$; $p=0,008$; SE: 4,5%

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(37)}$	p-level
	r		Intercept	45,1	,771	58,5	10^{-6}
Ch7A f	0,44	,416	,145	9,743	3,388	2,88	,007
Ch7E	0,25	,205	,145	4,260	3,007	1,42	,165

Table 6. Regression Summary for Dependent Variable: Microbial Count of Staph. aureus

R=0,338; R²=0,114; Adjusted R²=0,091; F_(1,4)=4,91; p=0,033; SE: 6,6 Bac/Phag

		Beta	St. Err. of Beta	B	St. Err. of B	t ₍₃₈₎	p-level
	r		Intercept	60,8	1,22	49,7	10 ⁻⁶
Ch6E	-0,34	-,338	,153	-7,95	3,59	-2,22	,033

Table 7. Regression Summary for Dependent Variable: Phagocytosis Index vs Staph. aureus

R=0,365; R²=0,133; Adjusted R²=0,086; F_(2,4)=2,84; p=0,071; SE: 0,70%

		Beta	St. Err. of Beta	B	St. Err. of B	t ₍₃₇₎	p-level
	r		Intercept	99,3	,115	862	10 ⁻⁶
Ch2E	-0,28	-,278	,153	-,644	,355	-1,81	,078
Ch2A	-0,24	-,233	,153	-,539	,354	-1,52	,137

Table 8. Regression Summary for Dependent Variable: Bactericidal Capacity vs Staph. aureus

R=0,448; R²=0,201; Adjusted R²=0,134; F_(3,4)=3,02; p=0,042; SE: 16•10⁹ Bac/L

		Beta	St. Err. of Beta	B	St. Err. of B	t ₍₃₆₎	p-level
	r		Intercept	57,0	27,0	2,11	,042
Ch5A	-0,27	-,337	,151	-27,38	12,30	-2,22	,032
Ch3A f	-0,23	-,246	,154	-17,81	11,12	-1,60	,118
Shape R f	0,23	,218	,152	2,74	1,92	1,43	,162

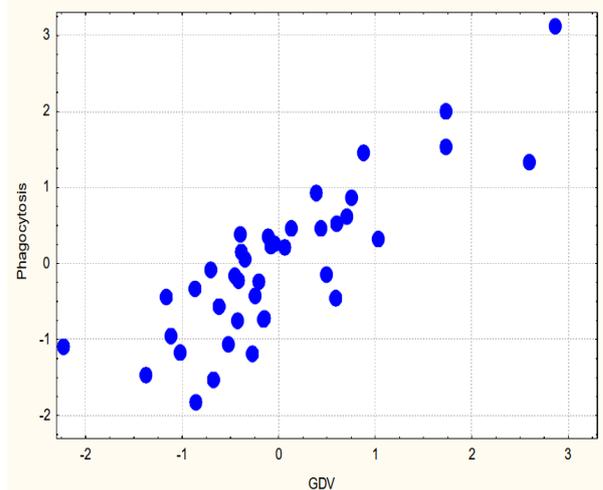
This is more clearly illustrated by the factor structure of canonical roots (Table 9). In particular, the Completion of phagocytosis and Bactericidal Capacity versus Escherichia coli **downregulated** by GDV parameters, instead the same parameters for Staphylococcus aureus **upregulated**. And vice versa, Intensity and Activity of phagocytosis **upregulated** for Escherichia coli but **downregulated** for Staphylococcus aureus.

Table 9. Factor Structure of GDV and Phagocytic Canonical Roots

Right set	R
Ch6E	,682
Ch3E f	,627
Ch7E	,563
Ch4E	,550
Ch1E f	,525
Area L	,500
Ch2E	,377
Ch7E f	,368
Entropy F f	,360
Ch2E f	,331
Ch5A	,301
Ch7A f	,175
Shape R f	-,386
Left set	R
KI E. coli	-,734
BCC E. coli	-,513
MC St. aur.	-,310
PhI St. aur.	-,179
BCC St. aur.	-,212
MC E. coli	,384
PhI E. coli	,240
KI St. aur.	,114

This state of affairs is consistent with the known data that the regulation of phagocytosis of gram-negative and gram-positive microbes is carried out by various neuro-endocrine mechanisms [11,23,28], which, as shown in this study, are associated with the Chakras.

In general, GDV parameters determine the parameters of phagocytosis by neutrophils of gram-negative and gram-positive microbes by 71,7% (Fig. 2).



$R=0,847$; $R^2=0,717$; $\chi^2_{(104)}=130$; $p=0,044$; $\Lambda \text{ Prime}=0,0097$

Fig. 2. Scatterplot of canonical correlation between parameters of the GDV (X-line) and the Phagocytosis (Y-line)

CONCLUSION

The above data, taken together with the previous ones [1-3], state that between parameters of Neuroendocrine-Immune complex [7,20,22,33] and GDV exist strong canonical correlation suggesting suitability of the latter method.

ACKNOWLEDGMENT

We express sincere gratitude to administration JSC “Truskavets’kurort” for help in carrying out immune analyzes. Special thanks to the volunteers.

ACCORDANCE TO ETHICS STANDARDS

Tests in volunteers are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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