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ŞI MEDICINĂ VETERINARĂ A BANATULUI
TIMIŞOARA**

FACULTATEA DE MEDICINĂ VETERINARĂ

LUCRĂRI ŞTIINŢIFICE

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COMPARATIVE RESEARCH REGARDING THE EVOLUTION OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS, IN UROLITHIC CONDITIONS, IN DOGS

COTOR G.[1], GÂJÂILĂ G.[1], GHIȚĂ M.[1], COTOR D.C.[2], ZAGRAI (MĂIEREAN) A.M.[2], ZAGRAI G.[1]

[1]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Bucharest-050097, Splaiul Independentei 105, Bucharest,Romania
[2]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca
E-mail: gavrilazag@yahoo.com

Summary

In our experiment we did a comparative study between the hematological profiles of dogs with urolithiasis, in correlation with the type and specificity of urinary stones. In our study we analyzed 20 dogs of different breeds and ages. Depending on the diagnosis, the cases were grouped into two categories: animals with urolithiasis formed on the basis of crystals with concretion in alkaline medium and animals with urolithiasis constituted on the basis of crystals with concretion in acidic medium. The differences between the two experimental groups in terms of hematocrit and hemoglobin were statistically insignificant. The differences between the two experimental groups, in terms of the level of leukocytes in the blood were statistically significant (P <0.05), the value of this parameter being 85.46% higher in the case of group 1, compared to group 2. The differences between the two experimental groups in terms of fibrinogen level were statistically significant (P <0.05), the value of this parameter being 60.94% higher in group 1 compared to group 2. The results obtained by us after the analysis of the urine samples collected from the animals from the two experimental groups showed that in the case of group 2 the results were negative in the case of all 8 samples. In the case of group 1, we recorded 8 cases out of 12 (66%) in which we found the presence of red blood cells and hemoglobin in the urine, suggesting the presence of lesions in the urinary tract. The results obtained by us after analyzing the urine samples collected from the animals from the two experimental groups and interpreted in order to evaluate the presence of proteins and/or leukocytes in the urine showed that in group 2 the results were negative in all 8 samples, indicating the absence of proteins and leukocytes in the urine. In the case of group 1, we registered 8 cases out of 12 (66%) in which we found the presence of leukocytes in the urine. Also, in the case of group 1, the same animals that had leukocytes in the urine, showed in the urine levels of proteins between 30 and 500 mg/dl.

Keywords: dog**,** urolithiasis, hematological and biochemical parameters

Canine urolithiasis is a pathological condition often described in veterinary clinics. Urinary lithiasis is a condition characterized by the formation of crystalloid concretions in the structures specific to the urinary tract. These concretions lead to an increase in urine pressure, upstream of the obstruction, which leads to (5, 10, 11):

-decreased glomerular ultrafiltration, due to increased hydrostatic urine pressure in the Bowman capsule;

-glomerular ultrafiltrate retrodiffusion;
-oliguria or anuria, which result in secondary local infections;
-compressions on the renal parenchyma.

The pathogenesis of urinary stones involves four factors (13, 14, 15):
-decreased urinary flow due to oliguria or obstructions;
-the presence of a crystallization nucleus, consisting of an organic matrix, released mainly after the local inflammatory processes;
-presence in urine of a sufficient concentration of lithiasogenic components (calcium ions, phosphorus, ammonia, uric acid, oxalic acid, cystine, etc.);
-ensuring a pH of urine, optimal for the precipitation of lithiasogenic salts, which can be (3):

- ~ acid, favoring the precipitation of oxalate (calcium, ammonium or magnesium), uric acid, calcium sulphate and cystine;
- ~ alkaline, favoring the precipitation of phosphates (calcium or ammonia-magnesium), ammonium urate (ammonium salt of uric acid), calcium phosphate and calcium carbonate (4).

It results that urinary lithiasis can be classified into two categories: urolithiasis formed on the basis of crystals with concretion in alkaline medium and urolithiasis constituted on the basis of crystals with concretion in acidic medium (6, 7, 8).

The aim of our approach is to make a comparative study between the hematological profiles of dogs with urolithiasis, in correlation with the type and specificity of each type of urinary stones identified above (1, 2). During our experiments we also tried to correlate hematological parameters with the evolution of urinary biochemical parameters. In parallel, we followed the evolution of all parameters considered by us during the treatment applied to sick animals (9).

The data necessary for the study were collected from the case studies of a veterinary clinic in Bucharest.

Materials and methods

In our study we analyzed 20 dogs, presented in the veterinary clinic during 6 months. These dogs were of different breeds and ages. The dogs studied were presented to the veterinary clinic because they had urinary problems. Detailed examinations of each case were performed. In parallel, blood samples and urine samples were taken for laboratory tests. These examinations were doubled by ultrasound examinations, as well as by microscopic evaluations of the urinary sediments and, finally, it was possible to establish a clear diagnosis in each situation.

Depending on the diagnosis, the cases were grouped into two categories:
-animals with urolithiasis formed on the basis of crystals with concretization in alkaline medium (n = 12);
- animals with urolithiasis formed on the basis of crystals with concretion in acidic medium (n = 8).

Blood samples were analyzed for the purpose of assessing hematological parameters, such as:
-number of leukocytes;
-hematocrit;
-hemoglobin;
-plasma fibrinogen.

Hematological parameters were determined in the same veterinary clinic. An IDEXX VetAutoread™ Hematology Analyzer was used for this purpose.

Urine samples were used to assess pH and some pathologically relevant components, such as:
-red blood cells;
-hemoglobin;
-plasma proteins;
-glucose;
-leukocytes;
-ketone bodies;
-bilirubin.

Hematological parameters were determined in the same veterinary clinic. An IDEXX VETLAB UA Analyzer was used for this purpose.

The data obtained were statistically analyzed by calculating the average. The statistical significance between the groups was assessed using the t-Student test.

Results and discussions

The results regarding the hematocrit in the case of the two categories of dogs are presented in (Table 1).

Table 1

The average hematocrit in the case of the two experimental groups

Hematocrit (%)	**Group**	
	1 Crystals concretions in the alkaline medium	**2** Crystals concretions in the acid medium
	49.16	48.62

The differences between the two experimental groups, regarding the hematocrit, were statistically insignificant ($p > 0.05$), the value of this parameter being only 1.09% higher in the case of group 1, compared to group 2.

The results obtained by us in the case of the two experimental groups fall within the limits of the reference values (36-59%), a sign that in the conditions we investigated the hematocrit was not affected. These results are in line with those

reported by Houston et al. (12), which shows that these hematological parameters are not affected in urolithiasis conditions in dogs.

The results obtained by us for the purpose of evaluating hemoglobin in the case of the two experimental groups are presented in (Table 2).

Table 2

Mean hemoglobin level in the case of the two experimental groups

Hemoglobin (g/dl)	**Group**	
	1 Crystals concretions in the alkaline medium	**2** Crystals concretions in the acid medium
	17.91	18.37

The differences between the two experimental groups in terms of hemoglobin levels in the blood were statistically insignificant ($p > 0.05$), the value of this parameter being only 2.5% higher in group 2 compared to lot 1.

The results obtained by us in the case of the two experimental groups fall within the limits of the reference values (14-20 g/dl), a sign that in the conditions investigated by us the hemoglobin level was not affected. These results confirm those stated by us above, in terms of hematocrit level, being also in agreement with those reported by Houston et al. (12).

The results obtained by us in order to evaluate the number of leukocytes in the case of the two experimental groups are presented in (Table 3).

Table 3

Average leukocyte level in the case of the two experimental groups

Leukocyte (10^3 x µL)	**Group**	
	1 Crystals concretions in the alkaline medium	**2** Crystals concretions in the acid medium
	20.03	10.8*

*= $p<0.05$

The differences between the two experimental groups, in terms of the level of leukocytes in the blood were statistically significant ($p<0.05$), the value of this parameter being 85.46% higher in the case of group 1, compared to group 2.

The results obtained by us in the case of lot 2 fall within the limits of the reference values (6-17 10^3 x µL), a sign that in the conditions investigated by us the level of leukocytes was not affected. In the case of group 1 (animals with urolithiasis formed on the basis of crystals with concretion in alkaline medium) the level of

leukocytes far exceeded the normal limits (6-17 10^3 x µL), suggesting the presence of a urinary infection.

The results obtained by us in order to evaluate fibrinogen in the case of the two experimental groups are presented in (Table 4).

Table 4

The average level of fibrinogen in the case of the two experimental groups

Fibrinogen (g/dl)	**Group**	
	1 Crystals a concretions in the lkaline medium	**2** Crystals concretions in the acid medium
	8.9	5.53*

*= p<0.05

The differences between the two experimental groups, regarding the level of fibrinogen were statistically significant (p<0.05), the value of this parameter being 60.94% higher in the case of group 1, compared to group 2.

The results obtained by us in the case of group 2 fall within the limits of the reference values (5-7 g/dl), a sign that in the conditions investigated by us the fibrinogen level was not affected. In the case of group 1 (animals with urolithiasis formed on the basis of crystals with alkaline material), the level of fibrinogen exceeded the normal limits (6-17 g/dl), suggesting the presence of a urinary infection. These results are consistent with those obtained by us in this experiment and which indicated a significantly increased level of leukocytes in dogs with urolithiasis based on crystals with alkaline material.

The results obtained by us in order to evaluate the presence of erythrocytes and/or hemoglobin in the urine, in the case of the two experimental groups, are presented in (Tables 5 and 6).

The results obtained by us after analyzing the urine samples collected from the animals from the two experimental groups and interpreted in order to evaluate the presence of red blood cells in the urine and/or hemoglobin are different depending on the group. Thus, in the case of group 2 (animals with urolithiasis formed on the basis of crystals with acidification), the results were negative in all 8 samples, indicating the absence of red blood cells and hemoglobin in the urine, results that indicate a normal status. In the case of group 1 (animals with urolithiasis formed on the basis of crystals with alkaline material), we recorded 8 cases out of 12 (66%) in which we found the presence of red blood cells and hemoglobin in the urine, suggesting the presence in the urinary tract of lesions, probably inflammatory, with potential for hematuria and hemoglobinuria.

Table 5

Evaluation of the presence of red blood cells and/or hemoglobin in the case of dogs in group 1 (animals with urolithiasis formed on the basis of crystals with concretion in alkaline medium)

Dog	Red blood cells	Hemoglobin
1	-	-
2	+	+
3	++	+
4	-	-
5	+	+
6	++	+
7	+	+
8	+++	++
9	++	++
10	++	+
11	-	-
12	-	-

Table 6

Evaluation of the presence of red blood cells and/or hemoglobin in the case of dogs in group 2 (animals with urolithiasis formed on the basis of crystals with concretions in the acid medium)

Dog	Red blood cells	Hemoglobin
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-

The results obtained by us in order to evaluate the presence of proteins and leukocytes in the urine, in the case of the two experimental groups, are presented in (Tables 7 and 8).

Table 7

Evaluation of the presence of proteins and/or leukocytes in the case of dogs from group 1 (animals with urolithiasis formed on the basis of crystals concretions in the alkaline medium)

Dog	Proteins (mg/dl)	Leukocytes
1	-	-
2	30	+
3	100	+
4	-	-
5	30	+
6	100	++
7	30	+
8	500	++
9	100	++
10	100	+
11	-	-
12	-	-

Table 8

Evaluation of the presence of proteins and/or leukocytes in the case of dogs from group 2 (animals with urolithiasis formed on the basis of crystals with concretions in the acid medium)

Dog	Proteins (mg/dl)	Leukocytes
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-

The results obtained by us after the analysis of the urine samples collected from the animals from the two experimental groups and interpreted in the sense of evaluating the presence of proteins and/or leukocytes in the urine are different depending on the group. Thus, in the case of group 2 (animals with urolithiasis formed on the basis of crystals with concretions in the acid medium), the results were negative in all 8 samples, indicating the absence of proteins and leukocytes in the urine, results that indicate a normal status. In the case of group 1 (animals with urolithiasis based on crystals with concretions in the alkaline medium), we recorded 8 cases out of 12 (66%) in which we found the presence of leukocytes in the urine, confirming the presence of urinary tract infections. Also, in the case of group 1, the same animals that had leukocytes in the urine, showed in the urine protein levels between 30 and 500 mg/dl, confirming the presence of urinary tract lesions, which were able to induce proteinuria.

The results obtained by us in order to evaluate the presence of glucose, ketone bodies and bilirubin in the urine, in the case of the two experimental groups, are presented in (Tables 9 and 10).

Table 9

Evaluation of the presence of glucose, ketone bodies and bilirubin in the case of dogs in group 1 (animals with urolithiasis formed on the basis of crystals with concretions in the alkaline medium)

Dog	Glucose (mg/dl)	Ketone bodies (mg/dl)	Bilirubin (mg/dl)
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-
11	-	-	-
12	-	-	-

Table 10

Evaluation of the presence of glucose, ketone bodies and bilirubin in the case of dogs in group 2 (animals with urolithiasis formed on the basis of crystals with concretions in the acid medium)

Dog	Glucose (mg/dl)	Ketone bodies (mg/dl)	Bilirubin (mg/dl)
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-

The results obtained by us after analyzing the urine samples collected from the animals from the two experimental groups and interpreted in order to evaluate the presence in the urine of glucose, ketone bodies and bilirubin are identical. Thus, in both groups the results were negative, indicating the absence of glucose, ketone bodies and bilirubin in the urine, results that indicate a normal status.

Conclusions

The results obtained by us highlighted the following conclusions:

- Hematological parameters related to the erythrogram were unchanged in the case of the two categories of dogs, being placed between the physiological limits;
- The number of leukocytes and fibrinogen was significantly higher in the case of dogs with urolithiasis based on crystals with alkaline material, compared to dogs in group 2 (dogs with acidic urine pH), indicating an inflammatory status;
- Urine samples collected from dogs with urolithiasis based on crystals with alkaline material were positive, in a proportion of 66%, in terms of: hemoglobin, red blood cells, proteins and leukocytes, indicating the presence of lesions inflammatory urinary tract infections;
- Urine samples collected from dogs with urolithiasis formed on the basis of crystals with acidification in an acid medium were negative, in a proportion of 100%, in terms of all parameters taken for analysis, showing the lack of inflammatory lesions in urinary tract;
- Resulted in an obvious positive correlation between the inflammatory status in the urinary tract, on the one hand, and the alkaline pH of the urine, but also the presence of urinary stones in the category of ammonium urates and ammonia-magnesium phosphates, on the other hand.

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RESEARCH ON THE EFFECT OF A HYPOALLERGENIC DIET IN DOGS WITH ATOPIC DERMATITIS

CUCERZAN A., KALMAN I., MORAR A., CZISZTER, L.T., TÎRZIU E.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, 119 Calea Aradului, Timișoara, Romania
E-mail**:** alexandra_cucerzan@yahoo.com

Summary

The research looked at the effect of a commercial hypoallergenic diet in dogs with atopic dermatitis, compared to the effect of the same diet in dogs diagnosed with food allergy. The study was conducted on twenty dogs, grouped in two experimental groups, as follows: ten dogs with skin hypersensitivity, diagnosed with atopic dermatitis and ten dogs diagnosed with a food allergy. The commercial diet tested was the Royal Canin Hydrolyzed Protein product, the effect of which was followed for a period of three months. After the end of the experimental period, the dogs returned to control, being divided into three groups, depending on the results obtained: dogs whose symptoms disappeared completely (G1), dogs whose symptoms decreased (G2) and dogs whose symptoms remained unchanged (G3). In the first group, of the dogs with atopic dermatitis, the following results were recorded: in category G1 no dogs were classified, in category G2 four dogs were classified (40%) and in category G3 six dogs (60%) were included. In the group of dogs with food allergy, group 2, we found the following results, regarding the intensity of the pruritus: ten out of ten dogs (100%) showed a significant improvement, nine dogs (90%) were included in group G2 and one dog (10%) in group G1. Diet with soy hydrolyzed protein has been good in dogs who have been diagnosed with food allergy, including dogs who have reacted positively to chicken allergens and soy being the main components of the hypoallergenic diet. In dogs with atopic dermatitis, the positive effects were restricted to the improvement of clinical signs with pruritus being prezent.

Keywords: canine, soy hydrolyzed protein, atopic dermatitis

In this study we tried to evaluate the effects of a hypoallergenic diet in dogs diagnosed with atopic dermatitis and in dogs diagnosed with food allergy. The effect of pruritus reduction and improvement of skin lesions were assessed.

The study was conducted on a group of twenty dogs, that were devided into two experimental groups, as follows: ten dogs with skin hypersensitivity, diagnosed with atopic dermatitis and ten dogs diagnosed with food allergy.

The tested diet was the Royal Canin Hydrolyzed Protein product, the effect of which was followed for a period of three months.

According to the data in the literature, diets containing hydrolyzed proteins have positive effects in dogs with food allergies, because the proteins in diets consumed by the dogs, which would normally trigger an allergic reaction, in hydrolysed form do not have this property. Hydrolysis is the chemical process through which the chemical bonds of a substance are broken by combination with water. Through this reaction they are broken down into metabolic processes, with

the help of enzymes, proteins, polysaccharides and lipids resulting in small molecules called monomers (3).

One of the factors that influence the ability of a molecule to become an allergen is its shape and size. For example, polypeptides composed of a small number of amino acids (2-4) are less immunogenic than those containing 10-12 or more amino acids. Thus, by the hydrolysis reaction, the proteins are transformed into much smaller structures, and most of them are no longer capable of activating the cascade of immunological reactions (6).

There are still many issues that need to be clarified about the allergenicity of a substance and the immunological mechanism involved. It is known that IgE is the immunoglobulin involved in triggering allergies, however there are certain substances that can trigger allergies, without the involvement of immunoglobulin E. This category includes non-IgE-mediated food allergies. It is not yet known exactly which mechanism contributes to triggering these allergic reactions. The two types of food allergies, IgE-mediated and non-IgE-mediated, can be distinguished only by the appearance of the symptomatology (much later in the case of non- IgE mediated allergy) and by the dosage of the immunoglobulin E (very low titration in the case of non- IgE mediated allergy) (2).

In Romania allergic tests are still used only in certain veterinary clinics, due to the long-term treatment and the high costs that the owners are not willing to pay, most clinicians resort to hypoallergenic diets, the diagnosis being made by exclusion from testing these diets.

The researchers, are trying to reduce the allergenic capacity of a substance by hydrolyzing the proteins with certain enzymes. Although this test has had results in food allergies in terms of aero allergies, the positive results are contradictory.

In this study we proposed to test the hypoallergenic diet on two groups of dogs with allergic dermatitis, diagnosed with the help of allergic tests.

Materials and methods

The study was conducted on twenty dogs, divided into two experimental groups, as follows: ten dogs with cutaneous hypersensitivity, diagnosed with atopic dermatitis and ten dogs diagnosed with a food allergy.

Allergy diagnosis was made with the help of the Polycheck® test and with the Sensitest® test - food allergens, tests performed at the Synevovet laboratory. The Sensitest test identifies allergen-specific-IgE and total IgE antibodies for 19 dog foods. These include: beef, pork, lamb, duck, chicken, turkey, rabbit, deer, wheat, soy, barley, rice, potato, corn, oats, milk, eggs, salmon, white fish (8).

Dogs with atopic dermatitis, which were included in this study, had the following symptoms: localized or generalized pruritus, erythema, exudation, excoriation, dryness, cracks and lichenification, scratching and self-injury, pyodermitis, dermatitis caused by *Malassezia* sp. with or without otitis.

Dogs with food allergy, included in the second group, had the following symptoms: vomiting, diarrhea, moderate or intense pruritus, alopecia, erythema, excoriation and self-injury. Dogs with ectoparasites, those suffering from other acute or chronic diseases, or who were treated with anti-inflammatory products more than three weeks after the start of the study were not included in the study.

The dogs under study generally belonged to the small breeds, as the Royal Canin Hydrolyzed Protein Small Dog product with a specification for dogs up to 10 kg was tested.

Following the anamnesis, data were obtained on the history and evolution of hypersensitivity, all patients were pruritic. The duration of clinical manifestations was at least six weeks before the clinical examination, and in some patients it exceeded two years.

The dog owners were responsible for recording the severity of the pruritus on a linear, weekly, analog scale. The scale had the following values: 0 (absent pruritus), 1 (mild, occasional pruritus), 2 (moderate, constant or intermittent pruritus, which does not disturb sleep), 3 (severe, annoying pruritus, disturbing sleep). All owners were informed about the therapeutic behavior and the nature of this study, according to the compliance to the unique diet imposed for three months with the complete exclusion of other types of foods.

The clinical signs were evaluated using the CADESI test in a simplified version (5), at the beginning of the study on day 1 and then monthly for three months. The maximum value that can be obtained by an individual is 240 points.

For the CADESI test, 15 body regions were inspected: the ears, the eye and the eyelid area, the muzzle area, the neck area, the interdigital area of the anterior and posterior limbs, the axillary area, the thoracoabdominal area and the flank area.

Four clinical signs were evaluated on a scale from 0 to 4 (erythema, primary efflorescence, secondary efflorescence and lichenification):

Erythema was evaluated according to the following classification:

- 0 - erythema absent
- 1 - erythematous macula without visible edema
- 2 - visible erythema without edema and high temperature in the neighboring tissues
- 3 - erythema with visible inflammatory edema
- 4 - erythema with inflammatory edema and efflorescence

Primary and secondary efflorescence were classified into:

- 0 - absent
- 1 - a single type of efflorescence, except for erythema: papules as primary efflorescence or scales as secondary efflorescence
- 2 - two types of efflorescence except the erythema present on the skin: papules and pustules as primary efflorescences or scales and scabs as secondary efflorescences

- 3 - three types of efflorescence except the erythema present on the skin: papules, pustules and nodules as primary efflorescences or scales, crusts and erosions as secondary efflorescences
- 4 - four types of efflorescence except the erythema present on the skin: papules, pustules, nodules and boils as primary efflorescences or scales, crusts, erosions and ulcerations as secondary efflorescences

The presence of lichenification was classified as follows:

- 0 - absent
- 1 - accentuated marks of the skin
- 2 - accentuated markings of the skin and hyperpigmentation
- 3 - accentuated skin markings, hyperpigmentation and hyperplasia
- 4 – accentuated skin markings, hyperpigmentation and hyperplasia, with visible loss of skin flexibility, coexisting ruptures and cracks

The diet designed by Royal Canin consists of: rice, hydrolyzed soy protein, chicken fat, natural flavors, vegetable oil, dried beet pulp, sodium silicoaluminate, monocalcium phosphate, calcium sulphate, salt, fish oil, fruit. oligosaccharides, potassium chloride, calcium carbonate, sodium tri-polyphosphate, taurine, vitamins [DL-alpha tocopherol acetate (source of vitamin E), niacin supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), calcium pantothenate D, biotin, pyridoxine hydrochloride (vitamin B_6), riboflavin supplement, thiamine mononitrate (vitamin B_1), acetate vitamin A, vitamin B_{12} supplement, folic acid], DL-methionine, choline chloride, mineral traces (zinc proteinat, zinc oxide, manganese proteinat, manganese oxide, copper sulfate, ferrous sulfate, sodium selenite, copper proteinate, calcium iodate), *Tagetes erecta* extract, magnesium oxide, rosemary extract, preserved with tocopherols and citric acid (7).

Sampling for allergic tests was performed by puncture of the cephalic vein and blood collection in vacutainers without anticoagulant, with or without separating gel. The samples were centrifuged at 30 minutes after harvesting, for 3-5 minutes, at 3000 rpm, then the expressed serum was transferred into Eppendorf tubes and kept in the refrigerator at 2-8°C until the Polychek® test was performed, or were sent to the Synevovet laboratory for the Sensitest test.

After obtaining the allergic tests, three dogs were required to receive corticosteroid and antihistamine drug treatment and they continued the treatment until week 3 after the start of the diet, then this treatment was discontinued. After finishing the dog's diet, they returned to control and were divided, according to the results obtained, into three categories:

- dogs whose pruritus has completely disappeared (G1);
- dogs whose pruritus has diminished (G2);
- dogs where the pruritus did not disappear at all (G3).

Results and discussions

The dogs tested belonged to the following breeds: Yorkshire Terrier (n = 1), Bichon Maltez (n = 5), Poodle (n = 4), Shih-tzu (n = 2), Shar-pei (n = 1), Pekingese (n = 2), West Highland White Terrier (n = 3), Mops (n = 2). The age of these dogs was between 2-6 years of age being 4 ± 1 years.

According to sex, of the 20 dogs that make up the two experimental groups, 60% (12/20; 95% CI 38-79) were females and 40% (8/20; 95% CI 21-61) males. Thus, in the first lot there were five females and five males, and in the second lot there were seven females and three males. The demographic data and the pruritus results obtained in group 1 are shown in Table 1.

The dogs had the following distribution acording to the daily diet: in group 1, 60% (6/10; 95% CI 31-83) of them received only dry food, from the "low cost" category, and 40% (4/10; 95;% CI 16-68) received a mixed food, consisting of commercial dry food, commercial wet food, various diets cooked by the owners and rewards, and in group 2, 40% (95% CI 16-68) from dogs received dry feed and 60% (95% CI 31-83) received a mixed ration.

Of the 10 dogs that participated in the study 80% (95% CI 49-94) reacted to *D. farinae*; 60% (95% CI 31-83) reacted to *D. pteronyssinus*; 50% (95% CI 23.6-76.3) reacted with *Lepidoglyphus destructor*; 40% (95% CI 16-68) reacted to the grass-mix; 30% (95% CI 10-60) reacted to *Tyrophagus putrescentiae*; 20% (95% CI 5 -50) reacted to *Acarus siro*, rye pollen, *Malassezia* sp., plantain / willow / poplar mixture; 10% (95% CI 1.7-40.4) in ambrosia and plantago.

Table 1

The demographic data and the pruritus results obtained in group 1

Breed	Age (years)	Sex	Allergen reactivity	Initial pruritus	Final pruritus
Yorkshire Terrier	6	F	Df, Dp, Ld, Rg, RP, Mix 3	3	3
Maltese	5	M	Df, Dp, As	4	↓3
Maltese	3.5	M	Df, Dp, Ld, Mix 2, M	3	3
Maltese	4	F	Df, Ld, Mix 3	3	3
Caniche	4	F	Df, Dp, Ld	4	↓3
Caniche	6	F	Df, Tp, Mix 3	3	3
Pekinez	3	F	As, Tp,	3	↓1
Pekinez	4	M	Df, Dp, Tp	3	↓2
Westie	5	M	RP, Mix 3, plantain	3	3
Mops	3	F	Df, Dp, Ld, Mix 2, M	2	2

Df: *D. farinae*; Dp: *D. pteronyssinus*; As: *Acarus siro*, Tp: *Tyrophagus putrescentiae*; Ld: *Lepidoglyphus destructor*; Rg: ambrosia; RP: rye pollen; Mix 2 plantain / willow / poplar mixture; Mix 3: grass-mix; M: *Malassezia* sp.

The demographic data and the results of the allergic tests for group 2 are shown in Table 2. According to the data in table 2 it can be observed that 80% (8/10; 95% CI 49-94) of the dogs reacted to the poultry meat; 70% (7/10; 95% CI 39.6-89.2) to rice and soy; 50% (5/10; 95% CI 23.6-76.3) to corn; 40% (4/10; 95% CI 16.8-68.7) to oats; 30% (3/10%; 95% CI 10.7-60.3) to barley and eggs; 20% (2/10; 95% CI 5.6-50.9) to wheat; 10% (1/10; 95% CI 1.7-40.4) to milk.

Table 2

The demographic data and the results of the allergic tests for group 2

Breed	Age (years)	Sex	Allergen reactivity
Maltese	3	F	Chicken, corn, rice, oats
Maltese	5	F	Chicken, rice, potato, egg
Shih-tzu	4	M	Chicken, pork, soy, wheat, barley, rice, oats
Shih-tzu	4	F	Chicken, rice, corn, oats
Caniche	3	F	Chicken, soy, wheat, egg
Caniche	3	F	Chicken meat, soy, milk, egg
Shar-pei	5	F	Oats, rice, corn, soy
Westie	5	M	Wheat, rice, oats, soy, barley
Westie	6	M	Chicken, oats, rice, soy, barley, corn
Mops	4	F	Chicken meat, rice, soy, corn

Thus, in the first group, three months after the beginning of the diet we recorded the following results: in category G1 (no pruritus) no dogs were included; 40% of the dogs were included in G2 category (diminished pruritus) and 60% of the dogs in G3 category (no changes). The first month in which anti-inflammatory medication was administered was not taken into account. The evolution of pruritus, following the diet with hydrolyzed protein is shown in Fig. 1.

We mention that all dogs in group 2 showed a reduction of the clinical signs, especially of pruritus, following the consumption of the diet with hydrolyzed protein of soy, the following results were observed regarding the intensity of the pruritus: ten out of ten dogs had a reduction of the pruritus intensity, 90% (95% CI 59-98) were classified in category G2 with a level 1 pruritus and 10% (95% CI 1-5) in category G1 that no longer had pruritus.

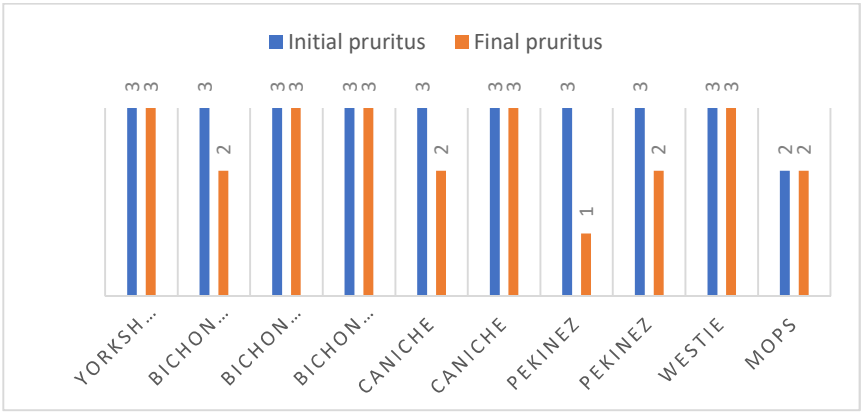

Fig. 1. The evolution of pruritus, following the diet with hydrolyzed protein in group 1

Vomiting and diarrhea diminished after the second week of use of the diet and disappeared completely by the end of the study period. The intensity of pruritus decreased, after three weeks from the beginning of the diet, reaching the stage of mild occasionally pruritus, until the third month. The intensity of pruritus three months after the beginning of the diet with soy hydrolyzed protein is shown in Fig. 2.

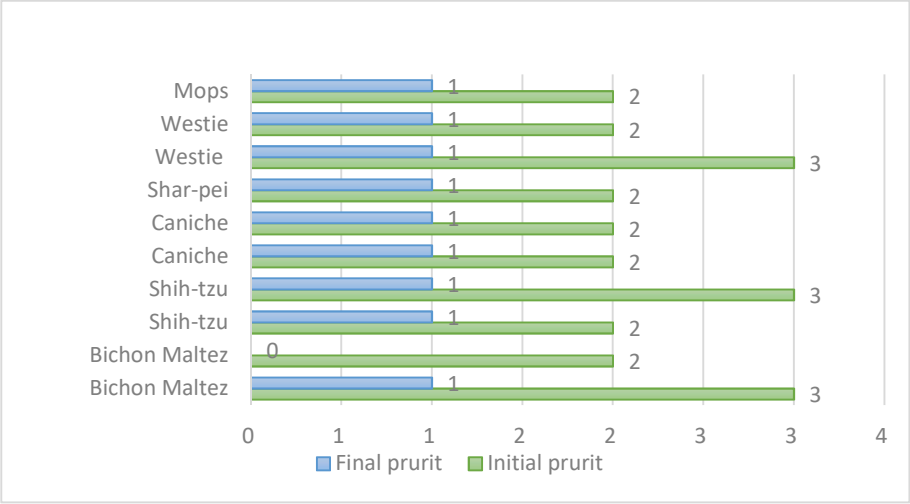

Fig. 2. The evolution of pruritus, following the diet with hydrolyzed protein in group 2

Regarding the evolution of the skin signs for group 1 and 2, they were evaluated on day 0, then in month 2 and 3. The values obtained are shown in Table 3.

Table 3

Evolution of clinical signs during the diet testing period

Day of ex.	Group	E	P1	P2	L	Total (max=2400)
Day 0	L 1	153 (40.8%)	94 (25%)	102 (27.2%)	26 (6.9%)	375
	L 2	174 (40%)	107 (24.5%)	115 (11.4%)	39 (8.96)	435
After 2 months	L 1	120 (39.2%)	73 (23.8%)	78 (25.4%)	35 (11.4%)	306
	L 2	100 (35.7%)	75 (26.7)	81 (28.9%)	24 (8.7%)	280
After 3 months	L 1	112 (41.3%)	65 (23.9%)	64 (23.6%)	30 (11%)	271
	L 2	75 (38.2%)	49 (25%)	53 (27%)	19 (9.6%)	196

E (Erytema), P1 (Primary efflorescences), P2 (Secundary efflorescences), L (Lichenification). The results are expresed as total points/group (%)

In the CADESI test on day 0 the dogs from group 1 obtained a total score of 375 points and group 2 a total score of 435 points. The highest score was recorded when evaluating the presence of erythema, group 1 recorded 153 points (40.8% of the total amount of the group), and group 2 recorded 174 points (40% of the total amount of the group).

At the clinical control of the 2nd month the reduction of the erythema, the primary efflorescence, the secondary efflorescence in both groups was observed and the intensification of the lichenification in group 1. In group 1 the score of the primary efflorescence decreased from 94 to 73 points, a reduction by 22.3%, and in group 2 the efflorescence score decreased from 107 to 75 points, a reduction by 29.9%. Erythema was reduced by 21.5% in group 1 and in group 2 by 35.6%.

At the clinical control of the 3rd month, the score of group 1 decreased from 375 to 271 points, the CADESI score decreasing by 43.7%. The score of group 2 decreased from 435 to 196 points, registering a decrease of the CADESI score of 54.9%. Erythema decreased by 26.7% compared to day 0 in group 1 and by 56.89% in group 2. Primary efflorescences decreased by 30.85% in group 1 and 54.2% in group 2, secondary efflorescence were reduced by 37.25% for group 1 and 60.78% for group 2. The lichenification was reduced by 51.28% in group 2, and in group 1, it intensified.

The Mann-Whitney test revealed a significant difference between the two groups in terms of erythema and lichenification on day 0 ($p<0.01$). The evolution of clinical signs is distinct in dogs with atopic dermatitis compared to those with

food allergy, at least in terms of erythema and lichenification in the examinated dogs (Table 4).

Table 4

Statistical analysis of the differences in group 1 and group 2 for the CADESI index

	Atopic(n=10)		Food allergy (n=10)		The difference and the level of significance
	x±Sx	SD	x±Sx	SD	
Erytema Day 0	15.3±0.63	2	17.4±0.4	1.26	2.1**
Erytema Month 2	12±0.69	2.21	10±0.61	1.49	2**
Erytema Month 3	11.2±0.87	2.78	7.5±0.32	1.08	3.7***
Primary EF. Day 0	9.4±0.22	0.69	10.7±0.61	1.94	1.3
Primary EF. Month 2	7.3±0.36	1.15	7.5±0.26	0.84	0.2
Primary EF. Month 3	6.5±0.4	1.26	4.9±0.23	0.73	1.6***
Secundary EF Day 0	10.2±0.51	1.61	11.5±0.5	1.58	1.3
Secundary EF Month 2	7.8±0.38	1.22	8.1±0.43	1.37	0.3
Secundary EF Month 3	6.4±0.54	1.71	5.3±0.15	0.48	1.1
Lichenification Day 0	2.6±0.3	0.96	3.9±0.1	0.31	1.3**
Lichenification Month 2	3.5±0.5	1.58	2.8±0.32	1.03	0.7
Lichenification Month 3	3±0.36	1.15	1.9±0.1	0.31	1.1***

Legend: EF(efflorescences), x ± Sx (mean ± standard error), SD (standard deviation), *** very significant correlation (p<0.001), ** significant correlation (p<0.05);

In week 2, a significant correlation was observed only in the evaluation of erythema in the 2 groups (p<0.01), and in week 3 there were significant correlations in the evaluation of erythema, primary efflorescence and of lichenification (p<0.01).

There was a significant correlation with the evolution of erythema, primary and secondary efflorescence in week 2 and on the first day (p<0.01) in group 1, and in group 2 a significant correlation was obtained in the evolution of erythema, primary and secondary efflorescence, and lichenification in the second and third week (p<0.05), and in the second week compared to the third week a significant correlation was observed in the evolution of erythema, primary and secondary efflorescences (p<0.01).

Thus the value p<0.01 indicates a significant correlation with regard to the reduction of the skin signs under the action of the hypoallergenic diet in both groups. There is a significant correlation p<0.05 regarding the difference of the results obtained in the two groups. In group 2, the CADESI score dropped by 54.9%.

After the end of the three months study, the dogs from group 2, which had a reduction of both the pruritus and the skin lesions, were subjected to a test of reactivity to the previous diet, for 2 weeks they were fed the food they received initially, and after a week the pruritus recurred. After the trial period, the dogs again received the hydrolysed protein diet and the pruritus diminished.

Diet with soy hydrolyzed protein has good results in dogs who have been diagnosed with food allergy, even in dogs who have reacted positively to chicken meat allergens and soy, these being the main components of the hypoallergenic diet, but in hydrolysed form they did not trigger an allergic response from the body.

Similar results were obtained by Puigdemont et al. (3) who demonstrated that in dogs sensitized to soy protein, oral administration of hydrolyzed soy protein does not cause allergic reactions.

Other researchers have also achieved very good results with the diet based on soy hydrolyzed protein in dogs and cats with enteropathies, which are manifested by vomiting and diarrhea, these clinical signs diminishing after three months of diet (1, 4).

In dogs with atopic dermatitis, the diet with hydrolyzed protein had positive effects only in dogs that had a high reactivity in mites, mainly storage mites. One of the reasons for obtaining these results could be the purchase of food in smaller packages, sealed and with a closing system after the packaging was opened, compared to the initial diet which was usually purchased in bulk form, the storage mites being more representative in this food category. The positive results on reduction of dermatological lesions make this diet suitable for atopic dogs, with the need for additional medication to control the pruritus.

Conclusions

Diet with soy hydrolyzed protein is a solution for dogs with food allergy, including soy protein allergy, in hydrolyzed form it is not capable of triggering allergic symptoms.

Hydrolyzed protein diets can also be a solution for dogs with various digestive disorders, because they are easily assimilated.

In dogs with atopic dermatitis, the diet with soy hydrolyzed protein can have positive results mostly in dogs that react mainly to storage and dust mites, a decrease of CADESI score of 43.73% has been observed, so a decrease of skin lesions, but the pruritus has not diminished.

We also recommend testing other diets that are found in packs with a controlled atmosphere and which after consumption are consumed in a relatively short time (less than 3 months) to avoid the multiplication of storage mites.

In dogs with atopic dermatitis, the effect of the diet with hydrolyzed soy protein did not have positive results în pruritus reduction, but it can be recommended as a food source due to its positive effects on skin and fur maintenance, eliminating this way feeding the animal with various commercial diets with a low nutritional content.

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EXTERNAL SPLINTING FOR TREATMENT OF PECTUS EXCAVATUM IN A PUG DOG: A CASE REPORT

IGNA C., SCHUSZLER L., DASCĂLU R., SICOE B., ZAHA C.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului, No. 119, Timisoara, Romania
E-mail: ignacornel@gmail.com

Summary

The congenital, neonatal and pediatric orthopedic diseases among which osteochondrodysplasias (cartilage and bone disorders that occur due to defective endochondral or intramembranous ossification) have often concurrent thoracic abnormalities, including pectus excavatum and pectus carrinatum, that have been previously documented in a variety of species. A recent scientific study demonstrated that the greater risk for pectus excavatum occurrence was carried by the Maltese and the English Bulldog, and pectus carrinatum by the Pug and the French Bulldog breeds. The aim of our case report was to describe, clinically and radiologically, a case of pectus excavatum in a Pug and to evaluate the surgical correction using a U-shaped external splint. The severities of thoracic deformity were evaluated by deformation indices such as fronto-sagittal index and vertebral index. A diagnosis of moderate pectus excavatum was established based on radiological measurements. Post-operative thoracic radiography showed that the concavity of the sternum was reduced and at two months after surgery, the dog was clinically normal.
Keywords: dog, pectus excavatum, external splint

Pectus excavatum (PE) is an uncommon congenital abnormality of the chest wall and it is characterized by a concave deformity of the caudal sternum. It has been reported in dogs, kittens, lambs, rabbits and calves (1, 5, 6, 14, 21, 23, 26). Burmese kittens and brachycephalic dogs are more predisposed (11, 23). This is a congenital abnormality and is considered to be a heritable disease. Pectus excavatum is a disease of unknown etiology, but observed to be linked to an autosomal recessive gene usually identified in brachycephalic dog breeds, without sex predisposition (16) or in humans, linked to an autosomal dominant gene (16, 20).

This anatomical PE abnormality is acquired during intrauterine life, and may be the result of expressions inheritable genomics, shortening of the central tendon of the diaphragm, intrauterine pressure abnormalities and congenital deficiency of the musculature in the cranial portion of the diaphragm (16, 19). In the canine species, brachiocephalic breeds have a high prevalence in the manifestation of PE, which could indicate that it is also related to a genetic component; some of these individuals present tracheal hypoplasia or other framed manifestations within brachiocephalic syndrome (nasal stenosis, soft palate hyperplasia) so it has come to think that the variations in the ventilatory gradient of

a patient could contribute to the development of the disease (16). It has been reported that this congenital abnormality can have other causes such as an alteration in intrauterine pressure, excessive growth of connective tissue of chondrocostal joints, smaller size of the central tendon of the diaphragm, thickening of the substernal ligament and a congenital malformation (dysgenesis) of the musculature in the cranial portion of the diaphragm (7). Another hypothesis states that alterations of mucopolysaccharides (16, 22) cause these changes in the canine species, especially when females received low protein diets during gestation (16, 22). Regarding the feline species, it has been described that these conditions may be related to taurine deficiency (24).

Animals with thoracic limb involvement (osteochondrodysplasias - defective endochondral or intramembranous ossification) commonly have concurrent thoracic abnormalities, including pectus excavatum, sterna concave, or dorsoventral flattening of the chest (12, 17). “Swimmer’s syndrome”, also known as “swimming puppy syndrome”, is one of the musculoskeletal disorders in puppies and cats associated with pectus excavatum (9, 10, 17, 25).

A recent scientific study (13) demonstrated that the greater risk of pectus excavatum occurrence was carried by the Maltese and the English Bulldog, whilst pectus carrinatum (PC) by the Pug and the French Bulldog breeds. The prevalence of PE in brachycephalic dog breeds was 44%, and PC was 11.3% (13).

The bibliographic introspection of the Romanian veterinary literature did not find any studies on pectus excavatum in dogs.

Materials and methods

Pectus excavatum is initially suspected after a visual examination of the ventral thorax. Diagnosis of pectus excavatum is based on clinical and radiographic examinations (7, 8, 18). The most common clinical signs were: dyspnea, compressive cardiopulmonary dysfunction, exercise intolerance, respiratory distress, cyanosis, cough, decreased appetite, vomiting, weight loss, muffled heart sound, and cardiac murmur (7, 8).

Radiographic evaluation is used to objectively grade the degree of PE by calculating the frontosagittal and vertebral indices (2, 8). The frontosagittal index (FSI) is the ratio between the thoracic width at the 10th thoracic vertebra (T_{10}) on a ventrodorsal radiograph and the distance from the ventral surface of T_{10} and the nearest point on the sternum, whereas the vertebral index (VI) is the ratio between the depth of the thorax measured at the center of the dorsal surface of T_{10} and the dorsoventral diameter of T_{10} at the same level (2, 8). An increase in the FSI (reference range for brachycephalic dogs, 1 to 1.5) and a decrease in the VI (reference range for brachycephalic dogs, 12.5 to 16.5) are usually reported in PE (8). Radiographic images may also reveal vertebral deformities, cardiomegaly, and malposition of the heart (1, 8).

The decision for surgical repair of deformities should be made on the basis

of clinical signs of disease. Frontosagittal and vertebral indices provide objective criteria for determining severity. Three types of surgical repair for PE have been described in dogs and cats: external splinting (5, 15, 19, 26, 27), internal splinting (20), and longitudinal sternebral pining combined with external splinting (3).

Results and discussions

Case description

The study was performed on a 3-month-old female Pug, with a body weight of 3.9 kg presented to the Surgery Clinic, Faculty of Veterinary Medicine, Timișoara with respiratory distress. Clinical examination revealed a marked inward deformity in the caudal sternum (Fig. 1 A, B) and thoracic radiographic examination revealed a dorsal displacement of the caudal sternebrae (Fig. 2 A, B).

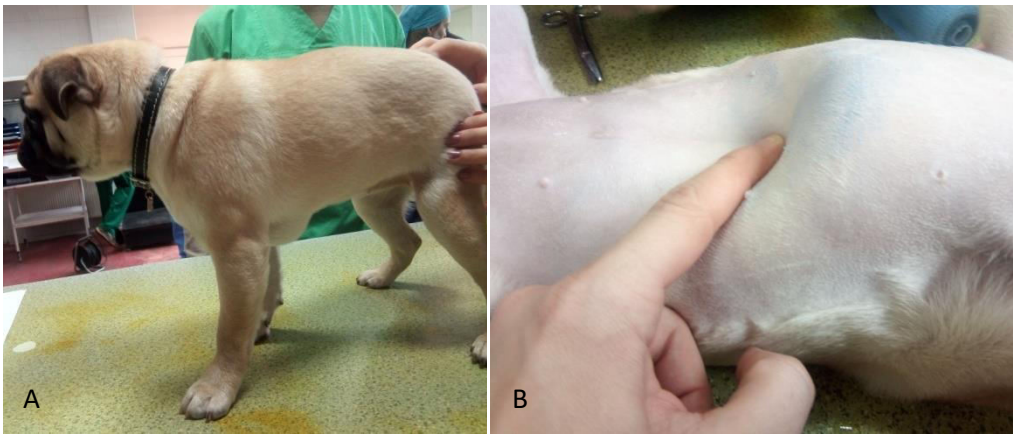

Fig. 1. Dorsal deviation of the caudal aspect of the sternum

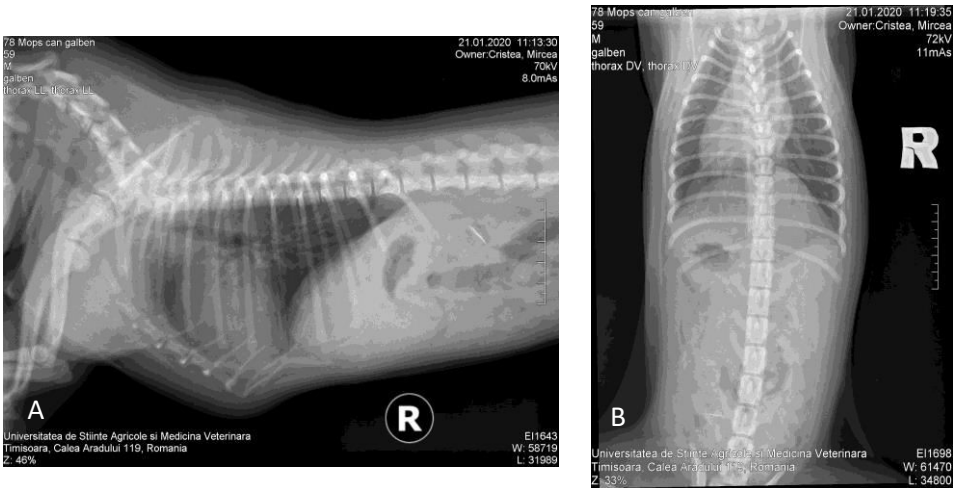

Fig. 2. Right lateral (A) and ventrodorsal (B) radiographic views of the patient

A diagnosis of PE with mild dorsal deviation of the caudal aspect of the sternum was made. The FSI determinate value was 1.21 (reference interval 1–1.5) – Fig. 3, and VI value was 17 (reference interval 12.5–16.5) – Fig. 4. The severity of PE was mild (reference interval FSI ≤ 2 and VI > 9 – after Fossum 2012 (8).

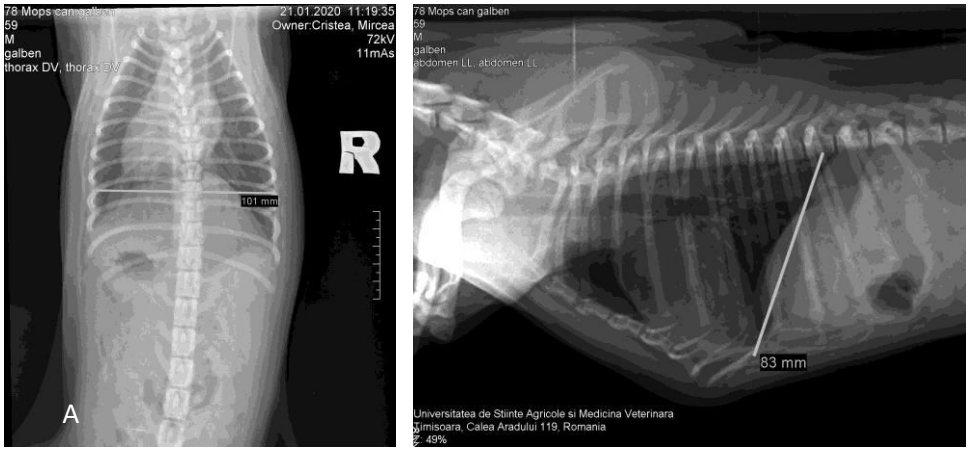

Fig. 3. The frontosagittal index (FSI) is the ratio between the thoracic width at the 10th thoracic vertebra on a ventrodorsal radiograph (A) and the distance from the ventral surface of T_{10} and the nearest point on the sternum (B)

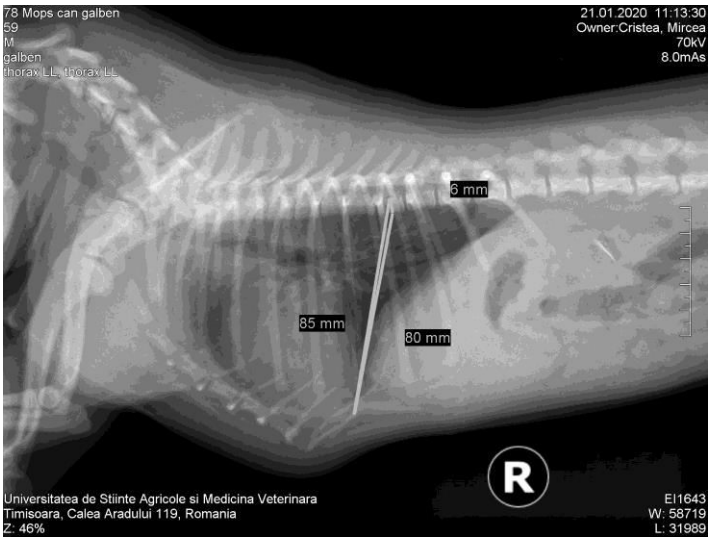

Fig. 4. The vertebral index (VI) is the ratio between the depth of the thorax measured at the center of the dorsal surface of T_{10} and the dorsoventral diameter of T_{10} at the same level

Due to its young age, the surgical method chosen was to use apply a U-shaped external splint. Prior to surgery, the U-shaped external splint was contoured to the normal shape of the dog's thorax. Preoperatively, a U-shaped, two sided splint made of fiber glass material was prepared by making holes at 1 cm intervals to include the sternum. Under general anesthesia, which consisted of premedication with diazepam (Terapia SA, Romania) and ketamine (Ketamidor, Richter Pharma AG, Austria), induction with propofol (Fresenius SE&C, Germany), and maintenance with isoflurane (Anesteran, Rompharm Company, Romania) in oxygen, the patient was positioned in dorsal recumbency and the sutures (2m, monofilament polypropylene, BioSyntex, Romania) were passed under the internal surface of the sternum starting from the caudal to the xiphoid process (Fig. 1 B).

All stay sutures were passed through the holes of the splint and then tied securely (Fig. 5 A, B).

The edges of the splint were padded. A bandage was applied lightly to cover the splint (Fig. 6 A, B).

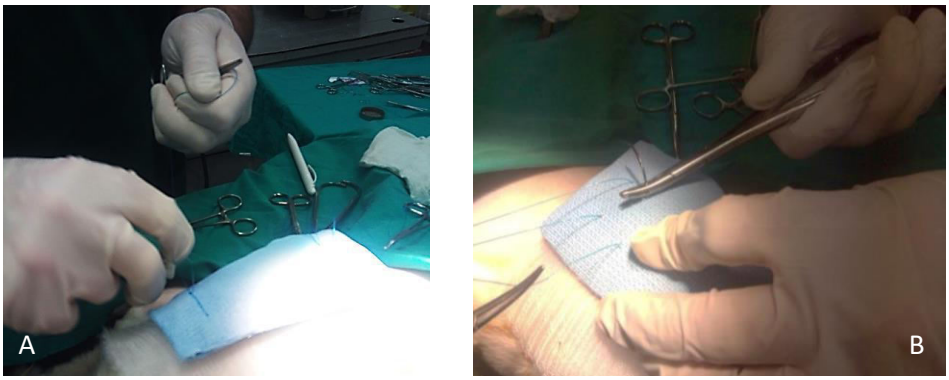

Fig. 5. Applying the U-shaped splint - intraoperative views

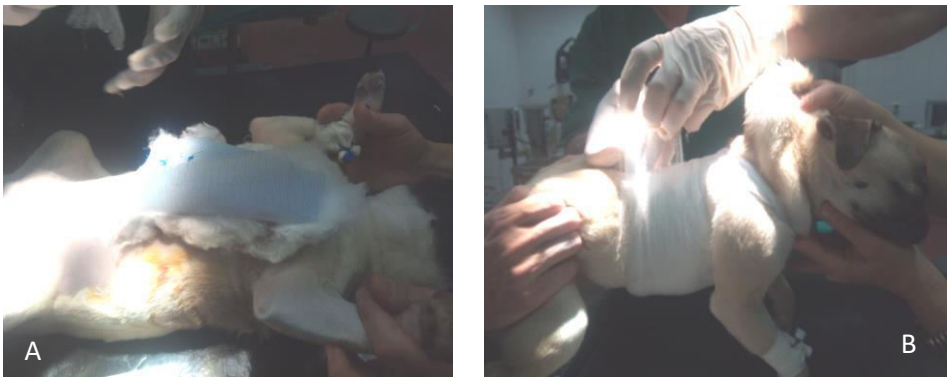

Fig. 6. The U-shaped splint - postoperative views

Immediately postoperatively, a lateral radioscopy of the thorax was performed to confirm the correct placement of the splint (Fig. 7).

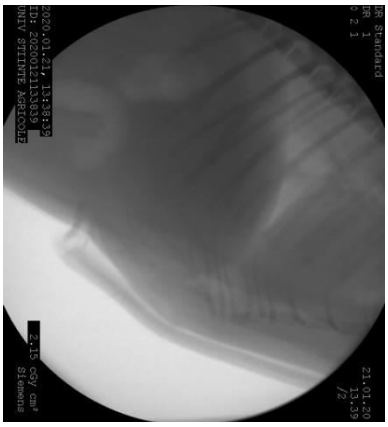

Fig. 7. Postoperative lateral radioscopic view

Meloxicam (Rompharm Company SRL, Romania) - 0.2 mg/kg subcutaneous, was given for 5 days post-operatively.

The splint was maintained for 4 weeks. Respiratory distress resolved completely after surgery. After removal of the splint, an almost normal thoracic depth was observed in physical and radiographic examinations. Postoperative thoracic radiographs revealed that the concavity of the sternum was reduced and at two months after surgery, the dog was clinically normal. Frontosagittal and vertebral indices were 1.2 and 10.6, respectively.

PE is a malformation of the sternum and costocartilages that results in dorsoventral flattening and narrowing of the thorax (1, 4, 5, 6, 14, 23, 26, 27). Clinical signs, if present, are typically attributed to chronic pulmonary and cardiovascular compression (1, 4, 5). Dogs diagnosed with PE have variable clinical signs which depend mainly on the site/type of deviation rather than the degree of deviation (11).

In 1989, Fossum (6) stated that in 8 dogs diagnosed with this defect, 7 were brachycephalic dogs. Fossum (7, 8) and Hassan (11) also indicate that PE frequently occurred in brachycephalic dogs. The exact cause of PE is unclear.

Although according to prevalence studies (13) PC frequently affects the Pug breed, our case report presents a Pug with PE.

Corrective treatment using an external splint may alleviate the impaired ventilatory performance when performed in young animals (19). In young animals, the costal cartilages and sternum are flexible, and the thorax can be reshaped by applying permanent traction to the sternum with an external splint (5). External splinting has provided good results and is cited as the treatment of choice to repair PE deformity in young dogs and cats younger than four months of age (5, 6, 15). Passing the needle as close as possible to the sternum can help avoid iatrogenic pneumothorax. Possible complications of this method are: damage to the internal

thoracic vessels, heart, or lungs during the passage of the needle around the sternum, fatal reexpansion due to pulmonary edema, and postoperative skin abrasions, suture abscesses, and dermatitis (19). However, none of these was observed in our case.

Internal splinting or longitudinal sternebral pinning combined with external splint can be considered as an alternative technique when permanent sternebral rigidity is encountered (27).

Conclusions

This report describes, according to our knowledge, the first approach and evaluation of treatment outcome in pectus excavatum in the canine patient, from Romania.

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DOES THE PROBIOTIC COMBINATION OF *BACILLUS SUBTILIS, BACILLUS LICHENIFORMIS* AND *PEDIOCOCCUS ACIDILACTICI* INFLUENCE THE BIOCHEMICAL PARAMETERS ON HEALTHY DOGS? A PILOT STUDY

MATEI M.C., BUZA V., NEAGU D., POPOVICI C.P., SZAKACS A.R., ȘTEFĂNUȚ L.C.

Faculty of Veterinary Medicine, University of Agricultural Science and Veterinary Medicine, 3-5 Calea Mănăștur Street, 400372 Cluj-Napoca, Romania
E-mail: catalina.matei@usamvcluj.ro

Summary

Probiotics are live microorganisms that have a benefic effect when administered in the right amounts. Innovative combinations are now used in veterinary medicine with data extrapolated from human medicine. The aim of the present study was to assess the influence of a probiotic product based on *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* on the biochemical parameters of 6 healthy adult dogs. The probiotic was administered once a day, together with the normal daily food of the dogs for a period of 30 days. Before enrolling the dogs in the study, a general clinical exam was performed. Moreover, at day 1 and 31/36 of the study blood samples were collected in order to evaluate the biochemical parameters. The results obtained showed a dynamic change of the biochemical parameters. However, all the values remained in the physiological parameters before and after the probiotic cure. Thus, the results obtained show that the combination between *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* prove to be safe for dogs, without any negative impact on the biochemical parameters.

Keywords: *Bacillus subtilis,* biochemical parameters, canine*, Pediococcus acidilactici,* probiotic

Probiotics are described as live microorganisms, usually bacteria or yeast that are able to confer a health benefit to the host when consumed in an adequate quantity (16, 12). Various type of products can be considered probiotics as long as they fulfill this criterion. Probiotics can be found as feed supplements, food or therapeutic drugs (1).

Importance of probiotics arise from their ability to reestablish the gastro-intestinal equilibrium. The gastrointestinal tract is one of the most complex and important organs of the body. It is representing a considerable ecosystem formed by a trillion ($10^{\wedge 12}$- $10^{\wedge 14}$) microorganisms. The term used to define this complex ecosystem is microbiota. It represents about ten times more than the total number of host cells (7).

These microorganisms are numerous and specific to each species and even each individual, about 500 different species of microorganisms in the gastrointestinal tract being counted (15).

In animals' field, probiotics have been used as dietary supplement but also as growth promoters and competitive exclusion agents especially in pigs and chickens (4). Cutting (4) reports also the use of probiotics in aquaculture (mainly shrimps) to enhance the growth and disease-resistance.

Composition of probiotics varies from one product to another. As long as the safety criteria are met, they can be used in animals and human medicine. Probiotics should be non-pathogenic, non-toxic and a normal inhabitant of the gastrointestinal tract. More than that, stability during processing, storage and delivery should be shown (6).

Bacillus spore forming bacteria have the advantage to be more stable and can be store at ambient temperature without suffering degradation. Moreover, the entire dose of probiotic ingested will reach the GIT and not be deteriorate before. This confers much more power to spore forming probiotics (4). Due to its high stability *Bacillus* is more suitable for health promoting formulation (5). Hence *Bacillus* is a free-living organism the safety concern has been raised. The importance to study their phenotype, genotype and life cycle in GIT of human and animals is primordial (5). Their ability to sporulate make them more resistant in difficult conditions, which is a controversial subject since a lot of researchers sustain that bacteria does not have to be viable in order to act like a probiotic (3, 12).

Some *Bacillus* strains appear to be involved in human diseases. In fact, *Bacillus anthracis* and *Bacillus cereus* are pathogenic. Concerning *B. cereus*, it seems to be case-by-case basis and result from opportunistic infections (4).

Others extensive studies in human and animals have been performed. The safety of several species has been recorded. *B. subtilis* var Natto, *B. indicus*, *B. coagulans, B. licheniformis* show no adverse effects (4). The usage of *Bacillus* species as probiotics started in the last about 20 years. *B. subtilis*, *B. clausii*, *B. cereus*, *B. coagulans*, *B. licheniformis*, *B. indicus* are the predominant species (4).

Since probiotics are able to influence the health status of the host enhancing the intestinal barrier function, that capacity helps in fighting pathogenic microorganisms (10). At the same time, the dogs' dietary behavior had been drastically modified due to anthropomorphizing (13), so it is important to develop an alternative treatment to keep their gastro-intestinal health.

The aim of the present study was to evaluate the influence of a probiotic product with *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* on the biochemical parameters on healthy dogs.

Materials and methods

The study was conducted at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

Study population

A total number of 6 healthy adult dogs, aged between 1year and 7 years old entered in the study (D-01, D-02, D-03, D-04, D-05, D-06). The dogs were selected based on inclusion/exclusion criteria: absence of diarrhea, vomiting, no antibiotic treatment in the last 6 months, clinically healthy, one meal/day and for exclusion- presence of diarrhea or vomiting and/or antibiotic treatment in the last 6 months. In order to ensure that the dogs enrolled in the study fulfill those criteria a full individually clinical exam was performed.

D-01, 4 years old is a castrated male, Czechoslovakian wolfdog, D-02, 3 years old castrated male, Swiss White Shepherd, D-03, 3 years old female, Border collie, D-04, 1 year old female, Shetland shepherd, d-05, 7 years old female, Shetland shepherd and D-06, 1.8 years old female, mixed breed. All dogs were current in vaccination, without internal or external treatment.

All dogs were enrolled in the study after their owners were fully informed about all the procedures and after they signed an informed consent. All procedures were conducted without producing any harm to the animals. The study design was approved by the Institutional Bioethics committee by the decision no 130/December 2018.

Study design

The study design took 31 days. On day 0 dogs were clinically examined and biological samples were collected (blood) for para-clinical examinations. Between day 1 and day 30 the probiotic product was administered to the study population together with the meal and respecting the manufacturer recommendations. On day 31/36 the clinical examination and the collection of blood samples were performed again.

The probiotic product, composed of *B. subtilis* HU58, *B. licheniformis* SL307 and *Pediococcus acidilactici* (Fidospore® provided by Microbiome Labs LLC) presented as capsules was administered to the dogs during 30 days, one capsule per day together with the regular meal.

Biochemistry investigations

The biochemistry analysis was performed on the six cases before and after the period of administration of probiotics. The objective was to see if the probiotics administrated to the dogs influence the biochemistry results. The blood was collected without anticoagulant. Serum was separated after the centrifugation and used to assess the biochemical parameters. Vet Scan Chemistry Analyzer, using Comprehensive Tests was used. The parameters assessed are represented in Table 1.

Table 1

Investigated biochemical parameters and their methods

No	Parameter	Indication	Principle of method
1.	**Alanine aminotransferase (ALT)**	Liver diseases, including viral hepatitis and cirrhosis; heart diseases.	ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. The absorbance value is directly linked with the amount of ALT in the sample
2.	**Albumin**	Liver and kidney diseases.	Dye binding technique is used. Bromocresol green (BCG) is bind to albumin.
3.	**Alkaline phosphatase**	Liver, bone, parathyroid, and intestinal diseases.	Alkaline phosphatase hydrolyses p-NPP in a metal- ion buffer and forms p-nitrophenol and phosphate. Absorbance is measured.
4.	**Amylase**	Kidney and pancreatic disease.	The substrate 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) reacts with α-amylase of the sample releasing 2-chloro-p-nitrophenol (CNP). The release of CNP creates a change in color. Absorbance is measured.
5.	**Creatinine**	Renal disease.	Endogenous creatinine is measured in the blank cuvette, which is subtracted from the combined endogenous creatinine and the creatinine formed from the enzyme reactions in the test cuvette.
6.	**Globulin**	Globulin concentration will increase with dehydration and should also increase with antigenic stimulation.	Globulin fraction is generally determined by subtracting the albumin from the total protein.
7.	**Glucose**	Diabetes, hyperglycemia, hypoglycemia, diabetes and liver disease.	Quantitative procedures using the enzymes hexokinase and glucose oxidase.
8.	**Total bilirubin**	Hepatic disorders.	Bilirubin is oxidized by bilirubin oxidase into biliverdin. Absorbance is measured.
9.	**Total protein**	Dehydration, kidney, liver disease, metabolic and nutritional disorders.	The Cu (II) ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a colored Cu-Protein complex (Biuret reaction). Absorbance is measured.
10.	**Blood Urea Nitrogen**	Liver and kidney diseases.	Urease hydrolyses urea into ammonia and carbon dioxide. Upon combining ammonia with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidizes NADH to NAD^+. Absorbance is measured.

Statistical analysis

Data were analyzed using GraphPad Software. Descriptive statistics was assessed for all the parameters. Significance was set at $P < 0.05$, which was determined using the T Test. For correlations evaluation, Pearson (Pearson r) Test Correlation was used with the expression of correlation coefficient (r) and coefficient of determination (R2).

Results and discussions

Clinical investigations

A general clinical examination is performed on all the six dogs before to start the probiotics treatment. The table (Table 2) shows the values obtained for the main physiological parameters for the six dogs.

Table 2

Clinical investigations result on day 0

Parameter	Temperature* (37,9 – 39,9° C)	Cardiac Frequency* (70-120 Beats/min)	Respiratory frequency* (18-34 Breaths/ min)	Clinically healthy	Vomiting/ Diarrhea	Antibiotic treatment**	A meal/ day
D-01	38,5	45	89	+	-	-	+
D-02	38,5	64	43	+	-	-	-
D-03	38,3	84	52	+	-	-	+
D-04	39,0	90	150	+	-	-	+
D-05	38,5	88	48	+	-	-	+
D-06	38,7	116	200	+	-	-	+

*- (Merck et al. - 10); **- In the last 6 weeks

All the dogs presented no significant modifications at general examination and were clinically healthy. The temperature for all the dogs was normal between 37.9-39.9°C. The cardiac frequency suggested a mild bradycardia for D-01 and D-02 without significant importance. For the other dogs the cardiac frequency was normal, being situated between 70-120 beats per minute. All the dogs presented a higher value for the respiratory rate between 43 breaths per minute for D-02 and 200 breaths per minute for D-06. The normal value at rest for the respiratory frequency was considered between 18 -34 breaths per minute, according to Merk (9). The fact that all the dogs had an increased respiratory rate was not considered alarming, being considered the result of the excitement, stress and/or the high ambiental temperature in the room. However, the dogs were clinically healthy, without any signs of illness, diarrhea or vomiting. Moreover, they were not under any kind of medication, especially not antibiotics.

Biochemical parameters

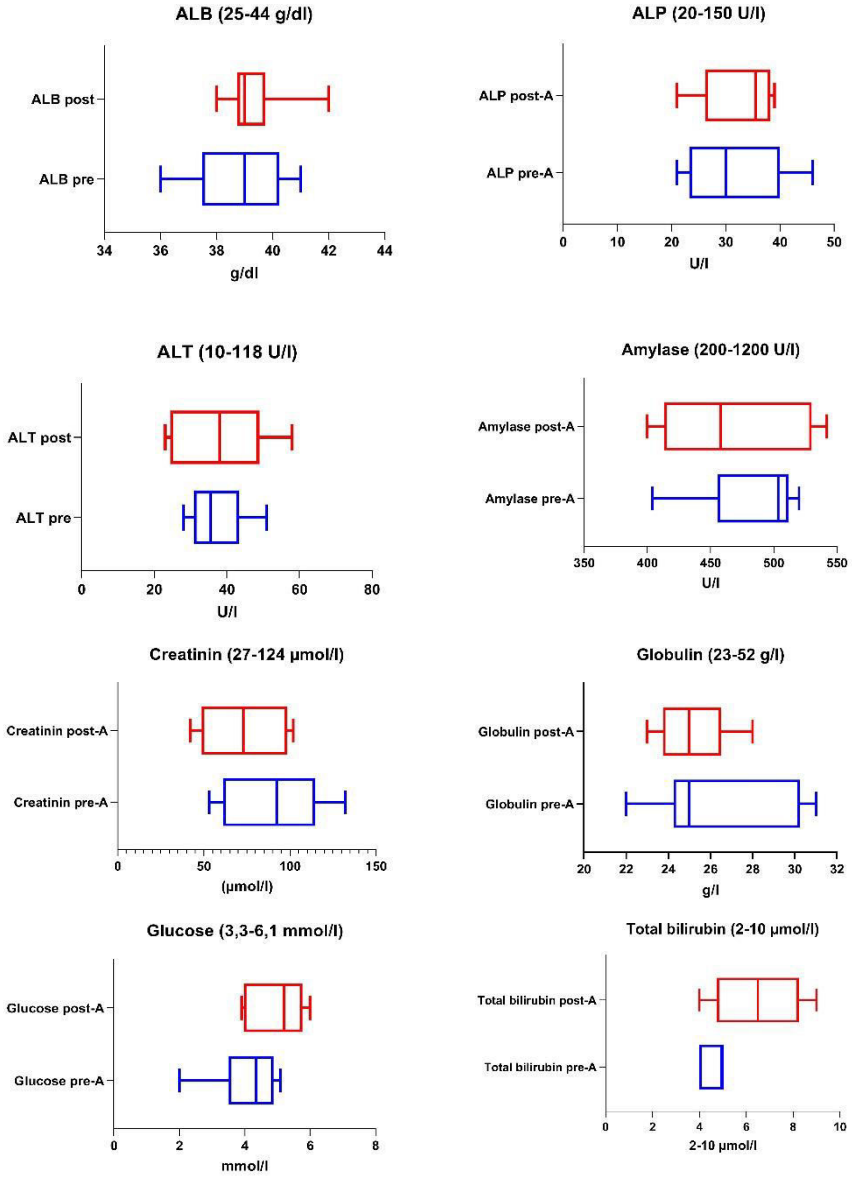
The Table 3 and Figure 1 presents the results for the biochemical parameters before and after the probiotic's treatment for all the six cases. For D-01, D-02 and D-06 blood samplings have been realized day 0 and day 31 of the study. For D-03, D0-4, D0-5 blood samplings have been realized day 0 and day 36 of the study.

Table 3

Results of the biochemical parameters before and after the probiotics treatment

	Descriptive statistic					T test	
Parameter	Min-Max	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)	p value (p<0.05)	Significance
ALT pre-A	28-51	37.17	8.183	3.341	22.020	0.7507	Ns
ALT post-A	23.0-58.0	38.00	13.100	5.348	34.470		
ALB pre-A	36.0-41.0	38.83	1.722	0.703	4.435	0.5177	Ns
ALB post-A	38.0-42.0	39.33	1.366	0.558	3.474		
ALP pre-A	21.0-46.0	31.50	9.397	3.836	29.830	0.7389	Ns
ALP post-A	21.0-39.0	32.83	7.139	2.915	21.740		
Amylase pre-A	404.0-520.0	485.50	42.960	17.540	8.849	0.5122	Ns
Amylase post-A	400.0-542.0	467.00	61.460	25.100	13.160		
Creatinine pre-A	53.0-132.0	90.50	28.920	11.810	31.950	0.0142	s*
Creatinine post-A	42.0-102.0	73.00	24.920	10.170	34.130		
Globulin pre-A	22.0-31.0	26.33	3.445	1.406	13.080	0.3933	Ns
Globulin post-A	23.0-28.0	25.17	1.835	0.749	7.291		
Glucose pre-A	2.0- 5.0	4.10	1.009	0.449	26.810	0.214	Ns
Glucose post-A	3.0-6.0	5.00	0.890	0.363	17.800		
Total bilirubin pre-A	4.0-5.0	4.667	0.5164	0.2108	11.07	0.0791	Ns
Total bilirubin post-A	4.0-9.0	6.5	1.871	0.7638	28.78		
Total protein pre-A	60.0-70.0	65.17	3.251	1.327	4.98	0.7971	Ns
Total protein post-A	63.0-68.0	64.83	2.229	0.9098	3.43		
BUN pre-A	4.0-9.0	7.2	1.513	0.6175	21.01	0.1092	Ns
BUN post-A	7.0-10.0	8.66	1.366	0.5578	15.76		

*statistically significant


ALB (25-44 g/dl)
ALB post
ALB pre
34
36
38
40
42
44
g/dl
ALP (20-150 U/l)
ALP post-A
ALP pre-A
0
10
20
30
40
50
U/l
ALT (10-118 U/l)
ALT post
ALT pre
0
20
40
60
80
U/l
Amylase (200-1200 U/l)
Amylase post-A
Amylase pre-A
350
400
450
500
550
U/l
Creatinin (27-124 µmol/l)
Creatinin post-A
Creatinin pre-A
0
50
100
150
(µmol/l)
Globulin (23-52 g/l)
Globulin post-A
Globulin pre-A
20
22
24
26
28
30
32
g/l
Glucose (3,3-6,1 mmol/l)
Glucose post-A
Glucose pre-A
0
2
4
6
8
mmol/l
Total bilirubin (2-10 µmol/l)
Total bilirubin post-A
Total bilirubin pre-A
0
2
4
6
8
10
2-10 µmol/l

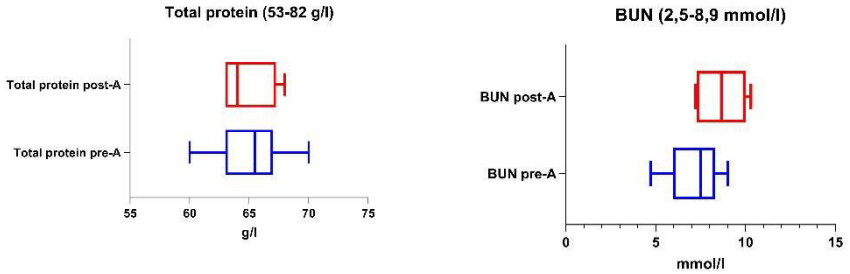


Fig. 1. Graphical representation of results of the biochemical parameters before and after the probiotics treatment

From a physiological point of view, the results show a normal variance between the results before and after the probiotic administration. However, all the parameters remain in the normal range despite the fluctuation. However, the decrease of creatinine after the treatment is constant and presented in the whole population study (Fig. 2). Moreover, from a statistical point of view, in case of creatinine a strong correlation between the pre-A and post-A data is present (Fig 3).

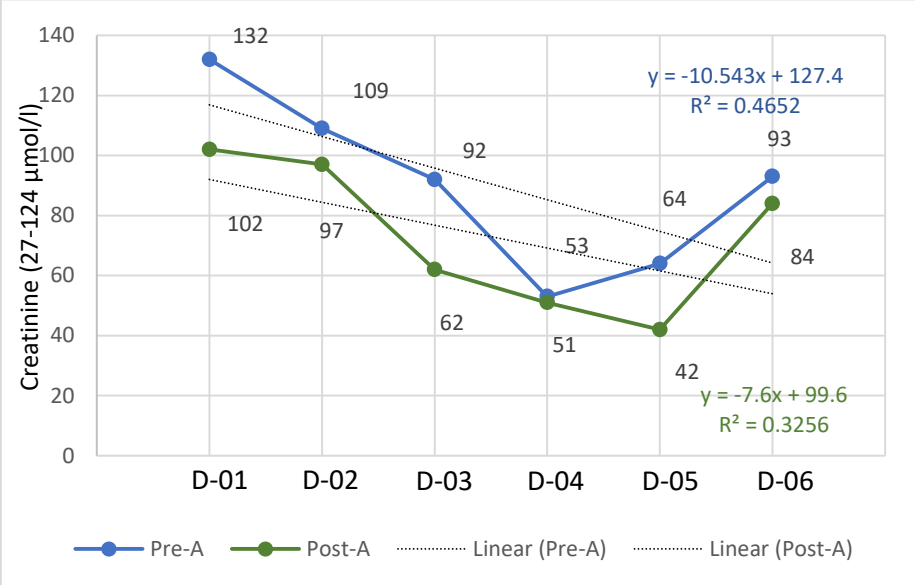


Fig. 2. Evolution of the creatinine before and after the probiotic's treatment

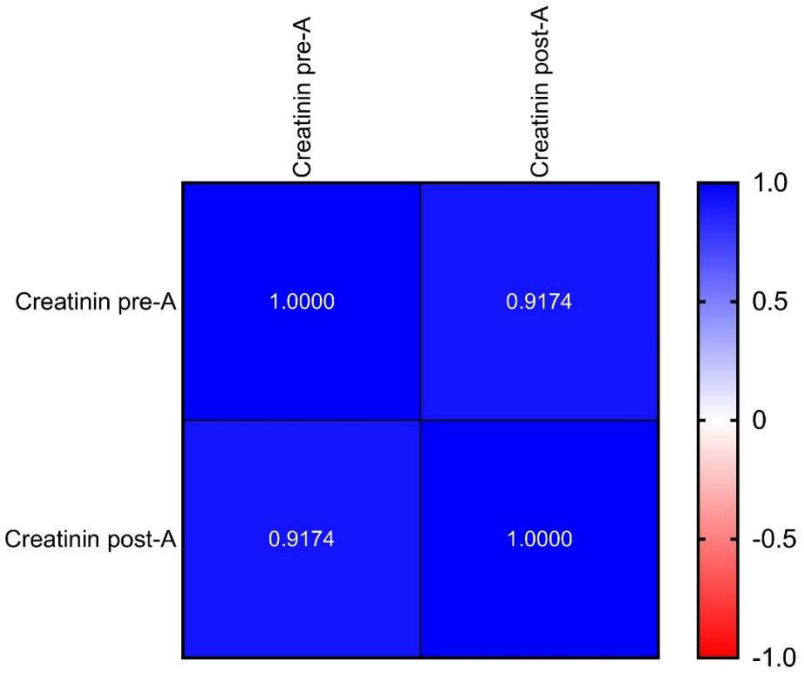


Fig. 3. Pearson correlation of creatinine levels pre and post administration

The analyzed biochemical parameters are good indicators for a normal functioning of the organs since the probiotic metabolization and elimination process took place in those organs. It can be observed the heterogeneity of those parameters, but with an evolution always in the normal range.

D-01, D-02 and D-06 showed an increase of the ALT. D-03 and D-05 present a decrease in ALT after the treatment. While for D-04 there is no variation of the ALT. Nevertheless, the values are in the normal range. The ALT is produced mainly by the hepatocytes and the results show a normal function of the liver (11).

We observe in D-01 and D-03 an increase of the ALB. While for D-02 and D-04 there is no variation of the ALB. For D-06 there is a decrease in ALB after the treatment. The values are still in the normal range and translate a good functioning of the liver (11).

The ALP results for D-01, D-04 and D-06 show a decrease of the ALP. The other dogs presented an increase in ALP. However, the differences are not significant. This enzyme is produced mainly by bile canicular membranes, bones and kidney and the normal range of values obtained confirm a good state of these organs (11).

The amylase increases for D-01, D-02 and D-03 while decreases for D-04, D-05 and D-06. The differences are not significant. The normal values show a healthy pancreas and liver (11).

For D-01, D-02 and D-06 there is a decrease of the globulin while it is increasing for D-03, D-04 and D-5 after treatment. For D-04 the value is slightly above the normal range but it is not significant. The protein is representative of the state of the liver (11).

There is no variation in glucose for D-01 before and after the treatment. For D-02 and D-06 there is a decrease of the glucose after the treatment and D-03, D-04 and D-05 present an increase in glucose. Before the probiotic treatment D-05 shows a glucose value under the normal range but it increased in the normal range after the treatment. These results are not significant and translate a good functioning of the pancreas for the dogs (11).

We observe for D-01, D-03, D-04 and D-05 an increase of the total bilirubin. No variations are shown for D-02 while for D-06 there is a decrease in total bilirubin. However, the results are not significant. It shows a good functioning of the liver and bile duct (11).

The total protein decreases for D-01, D-02 and D-06 while there is an increase for D-03, D-4 and D-05 after the treatment. However, the values are in the normal range. An increase of total protein can represent: a dehydration, inflammatory or neoplastic diseases or an infection. While a decrease of this parameter can show an over hydration, liver malfunctioning, a nephropathy, a hemorrhage or an acute tissue injury (11).

D-01, D-03, D-04 and D-5 have an increase of the BUN. D-02 and D-06 present a decrease in BUN after treatment. The values are still in the normal range and reflect a good functioning of the kidney and liver (11).

All those variations are considered normal and cannot be directly related with the probiotic administration. The same heterogeneity pattern was observed also on hematological parameters during probiotic administration on rabbits (8) and dogs (14).

Conclusions

The results obtained showed no important modification after the probiotic administration. Clinical examination results together with the biochemical data obtained before and after the treatment indicate that the combination between *Bacillus subtilis, Bacillus licheniformis* and *Pediococcus acidilactici* have not a negative impact on the general health status of the healthy dogs and do not have a remarkable influence at the level of biochemical parameters.

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EVALUATION OF THE BIOCHEMICAL CONTENT OF FRUITS ON SOME PLUM GENOTYPES

PARASCHIV M.[1,2], HOZA D.[1], NICOLA C.[2], FLOREA A.[3], BUTAC M.[2], CHIVU M.[2]

[1]University of Agronomic Sciences and Veterinary Medicine, 011464, 59 Marasti Boulevard, District 1, Bucharest, Romania
[2]Research Institute for Fruit Growing, 117450, Str. Marului, No. 402, Pitesti-Maracineni, Romania
[3]National Research and Development Institute for Biotechnology in Horticulture, Str. Bucuresti-Pitesti, No. 37, Stefanesti-Arges, Romania
E-mail: mihaela1mail@yahoo.ca

Summary

In Romania, the plum has a wide spread area (about 50% of the country's orchards), its fruits being consumed fresh or processed since long times ago. Fruits quality through its indicators (soluble dry matter and acidity) is influenced by the intake of water and fertilizers (in this case of the foliar ones) and especially the agro-biological value of the variety. Plums are a significant source of antioxidants with potential in neutralizing free radicals. The present paper presents the results of the plums quality as regard the fruits chemical indicators (dry mater content, total titrable acidity, sugar content, anthocyanins and polyphenols). The genotypes studied were: 'Agent', 'Alina', 'Andreea', 'Carpatin', 'Centenar', 'Gras ameliorat', 'Iulia', 'Roman', 'Romanța', 'Tita', 'Tuleu timpuriu', 'Tuleu gras', 'HR 7/48', 'HL 10/31', 'H 6/78 P' compared with 'Stanley' and 'Jojo', which are the most spread cultivars in commercial orchards from Europe. In this study, we observed the tendency to increase the total anthocyanins, polyphenols and total sugar content with the loss of water from the fruit.
Keywords: fruit quality, sugar content, total acidity, anthocyanins, polyphenols

Prunus domestica L. is an important fruit species in Romania, occupying an area of 75,292 ha (7). In 2017, a production of 434,390 tons (27) was registered in our country for this species. Due to the particularly favorable climate and soil conditions in our country, plum varieties have spread widely. The plum genetic breeding program in Romania aims to create varieties with resistance to diseases and pests but also with very good fruits quality (7). Plums are considered some of the most important fruits on the market due to the increased interest of consumers (16). Biochemical compounds in plums have shown beneficial effects on the body (15). Plums are a significant source of antioxidants with the potential to neutralize free radicals (12), radicals that in large quantities can cause many diseases (24). Plums have a content of high phenolic substances (between 298 and 563 mg / 100g) (5). Polyphenols in plums and other plant products have a role in protecting cells and cell organs by acting against chronic diseases, coronary heart disease and type 2 diabetes (25, 26). Phenolic substances are unevenly distributed inside the fruit, these being present in larger quantities in the fruit epicarp and in smaller quantities

in the mesocarp (4). Following the research conducted (8) on 12 plum genotypes, the distribution of phenolic substances in the skin was 4.5 times higher than in the pulp and 3.2 times higher compared to the whole fruit. Anthocyanins belong to a class of chemicals called flavonoids, which give red, purple or blue colors to fruits or vegetables. They are pigments in glycosidic form in which the hydroxyl groups of phenols are combined with reducing carbohydrates. They have anti-inflammatory, antioxidant, anticancer (17, 19), antidiabetic (13) and prevent cardiovascular diseases such as atherosclerosis (1). Anthocyanin pigments are found in the vacuolar juice of plants and their color is influenced by pH as follows: at acidic pH (pH = 3) they are red, at pH = 8.5 they are purple and at pH = 11 the color is blue (5, 11). Among anthocyanins, in plum, predominates: cyanidin 3-rutinoside, followed by peonidine 3-rutinoside, cyanidin 3-glucoside, cyanidim 3- xyloside and peonidine 3-glucoside (23). The total anthocyanin content of plums differs depending on the variety, the degree of fruit ripening and the environmental conditions (22). The objectives of the study were to determine the content of substances with antioxidant role in plums and the comparison between Romanian varieties and two varieties of foreign origin.

Materials and methods

The experimental field was placed at Research Institute for Fruit Growing Pitesti, in the Genetics and Breeding Laboratory, on a terrace of the Argeș River, on the ground flat, with a clay-brown soil type, with a loamy to loamy-clayey texture in the first 60-70 cm, and in depth the texture becomes sandy. The determined agrochemical fertility indicators (total nitrogen content, organic matter and mobile phosphorus) characterize a soil with very low fertility, and the pH is moderately acidic (2). The biological material studied in 2019 is made up of fifteen Romanian plum genotypes (‘Agent’, ‘Alina’, ‘Andreea’, ‘Carpatin’, ‘Centenar’, ‘Gras ameliorat’, ‘Iulia’, ‘Roman’, ‘Romanța’, ‘Tita’, ‘Tuleu timpuriu’, ‘Tuleu gras’, ‘HR 7/48’, ‘HL 10/31’, ‘H 6/78P’). Two cultivars (‘Stanley’ and ‘Jojo’) were used as controls. The samples were harvested at the optimal stage of maturity, between the last decade of July and the first decade of September.

Chemical analyzes and laboratory determinations consisted in determining the total content of anthocyanin pigments, total polyphenols, vitamin C, total sugar, organic acids and soluble dry matter. All biochemical determinations were performed in three repetitions.

The dosage of total anthocyanin pigments in fruits was performed by the Fuleki method (10). The method consists in the extraction of anthocyanins with appropriate extractive solutions and the measurement of the absorbance of the extract, spectrophotocolorimetric at the wavelength λ = 535 nm. The determined total anthocyanins were expressed as cyanidin-3-rutinoside mg / kg fresh fruit.

The determination of total polyphenols was performed spectrophotocolorimetrically, by the Folin-Ciocâlteu method (20) and was expressed

as mg GAE / kg fresh fruit. For the extraction of polyphenols was used as solvent methanol: water in a volume ratio of 80:20. The extracts obtained were read on a Zeiss Jena spectrophotometer.

The soluble dry matter content (% Brix) was determined using a refractometer, the organic acids (%) were measured by titration with 0,1N NaOH, ascorbic acid by the iodometric method and expressed in mg / 100g fresh fruit and total sugars (%) by the Fehling-Soxhlet method, 1965.

Results and discussions

Soluble dry matter content. In the Romanian plum genotypes studied, the soluble dry matter content varied between 11.63% and 11.69%, respectively ('Iulia', 'Carpatin') and 15.34% ('Tuleu gras'). The 'Stanley' and 'Jojo' varieties, used as a control had a high soluble dry matter content (15.35% and 15.05% respectively), similar to that of the 'Tuleu gras' variety (Fig.1).

In general, a normal distribution is symmetrical when the value of the asymmetry coefficient is zero. The average of the sample was 13.73% values of soluble dry matter content ranging between the minimum value 10.97% and the maximum value of 15.03% with an oscillation of 1.44%. The histogram of all soluble dry matter values is asymmetric on the left (higher than average values predominate), being different from the normal distribution, a sign that there are significant influences between the studied varieties in terms of soluble dry matter (Fig. 2).

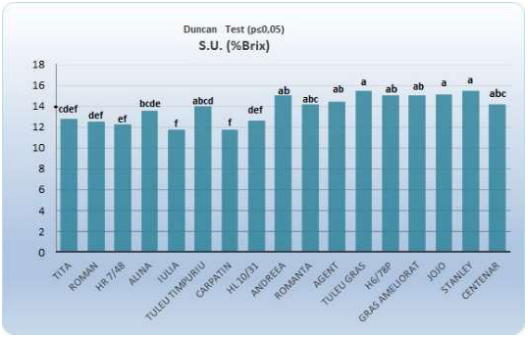

Fig. 1. Soluble dry matter content (%) of the fruits on the plums varieties

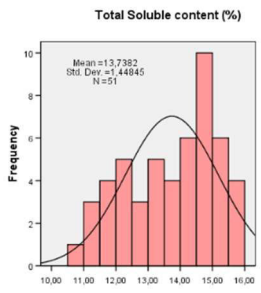

Fig. 2. Histogram of the distribution by absolute frequency classes of soluble dry matter (%), on the studied varieties

Polyphenol content. The results obtained regarding the polyphenol content varied between 1,524.07 ('Tita') or 1,526.73 ('Romanta') and 4,571.27 ('H 6/78P'),

average values registering the genotypes ‘Andreea’, ‘Alina’ and ‘H 10/31’ (Fig. 3; Fig. 4). The ‘Stanley’ and ‘Jojo’ varieties used as a control had a content of 3,835.64 and 4,145.98 mg GAE / kg of fresh fruit. Najafabad and Jamei (18) and Burzo (5) obtained similar results.

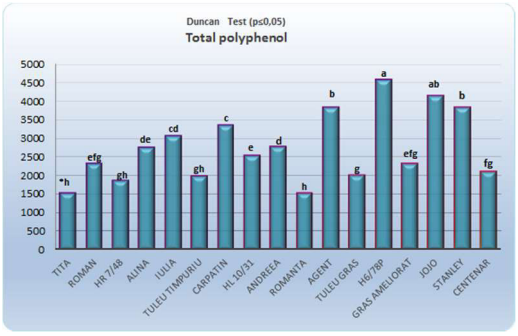

Fig. 3.Total polyphenol content (mgGAE/kg) of the fruits on the plums varieties

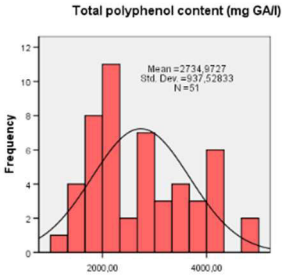

Fig. 4. Histogram of the distribution by absolute frequency classes of the fruit content in polyphenols (mg GA / l), on the studied varieties

Content of anthocyanin. An important feature of the quality of plums is the color. It depends on the content of anthocyanin pigments. In the fruits of the 15 Romanian plum genotypes studied, the content of anthocyanin pigments varied within very wide limits, from 378.89 mg / kg fresh fruit (‘Romanta’) to 43.12 mg / kg fresh fruit (‘H 6/78P’). In the ‘Stanley’ control the concentration of anthocyanin pigments was higher than of the Romanian genotypes (Fig. 5).

From Figures 4 and 6 or Table 1 it can be seen that both the histogram of all the values regarding the fruit content in total polyphenols and that of the fruit content in anthocyanins are asymmetric to the right (+0.558; +0.490), which means that the values predominate lower than average.

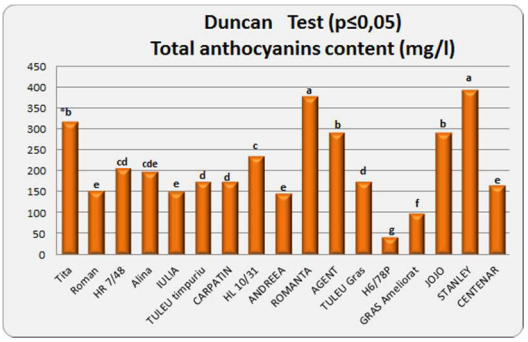

Fig. 5.Total anthocyanins content (mg/kg) of the fruits on the plums varieties

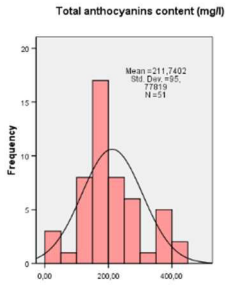

Fig. 6. Histogram of the distribution by absolute frequency classes of the fruit content of anthocyanins (mg/kg), on the studied varieties

Table 1

Indicators sample central tendency (mean, median and mode) and indicators value dispersion around the average (maximum amplitude, limits, standard deviation and asymmetric coefficient)

	Total Soluble content (%)	Total titrable acidity	Total Sugar content (%)	Vitamin C	Total anthocyanins content (mg/kg)	Total polyphenol content (mg GA/l)
Mean	13.7382	.7943	10.9110	10.0724	211.7402	2734.9727
Median	14.1100	.7800	10.5000	9.8000	181.8700	2548.2800
Mode	12.89(a)	.62	8.79(a)	8.36(a)	165.33(a)	2134.48
Std. Deviation	1.44845	.15895	1.71679	1.59359	95.77819	937.52833
Skewness	-.450	.318	-.019	-.012	.558	.490
Std. Error of Skewness	.333	.333	.333	.333	.333	.333
Kurtosis	-1.003	-.817	-1.318	-.826	-.198	-.830
Std. Error of Kurtosis	.656	.656	.656	.656	.656	.656
Range	4.91	.60	6.40	6.08	372.41	3512.92
Minimum	10.97	.55	7.74	6.68	40.92	1311.22
Maximum	15.88	1.15	14.14	12.76	413.33	4824.14

Ascorbic acid content (vitamin C). The lowest value of ascorbic acid content was also recorded in the 'H 6/78 P' genotype (7.65%) and the highest were obtained in the 'HL 10/31', 'Tita' and 'Andreea' genotypes (12.61%; 12.28% and 12.03%, respectively), higher than the varieties used as control 'Stanley' and 'Jojo' (10.08% and 10.43%) (Fig.7).

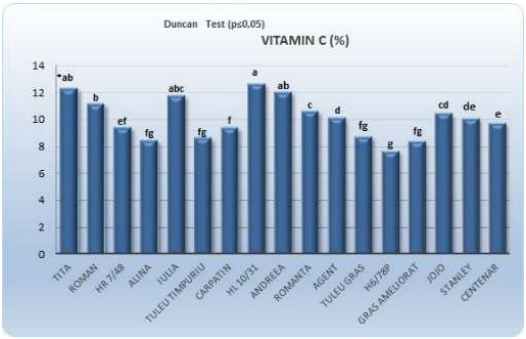

Fig. 7. Vitamin C (%) of the fruits on the plums varieties

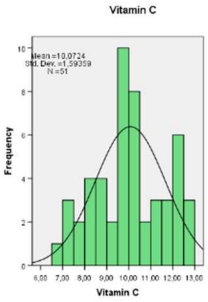

Fig. 8. Histogram of the distribution by absolute frequency classes of the fruit content in Vitamin C (%), on the studied varieties

Regarding Vitamin C, the average of the sample is 10.0724, the values being between the minimum value 6.68 and the maximum value 12.76, with an oscillation of 6.08 (Fig. 8). The histogram of all the analyzed values regarding the fruit content in vitamin C in the 17 genotypes studied is asymmetric on the left, the asymmetry coefficient being -0.012, which means that values higher than average predominate (Fig. 8), and the sample is no longer homogeneous due to the influence of the variety on the content of vitamin C*.*

Titratable acidity, (expressed as malic acid%) an important indicator of fruit quality from an organoleptic point of view in relation to sugars and tannins, recorded the lowest values (0.61%) in the varieties 'Gras ameliorat' and 'Agent' (0.62%) (Fig. 9) and maximum values for genotypes 'HL10/31' (1.04%) and 'H 6/78 P' (1.06). Average values were obtained in 'Tita' and 'Carpatin'. These were higher than in the 'Stanley' control which recorded 0.58% malic acid, a value also confirmed by Bozhkova (3). Similar data were reported by Coman et al. (7). For human consumption, a low level of acidity is appreciated (6). In the case of organic acids from fruits, the average of the values was 0.7943, with a standard deviation of 0.1595 and with an oscillation of 30.60 (Fig.10).

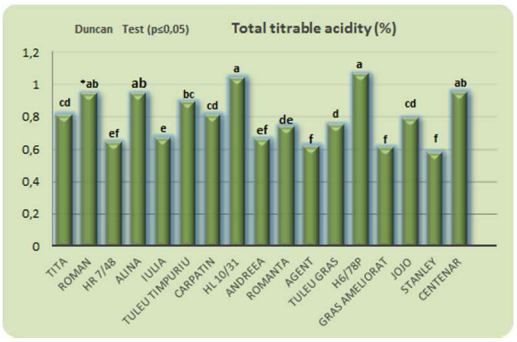

Fig. 9.Total titratable acidity (%) of the fruits on the plums varieties

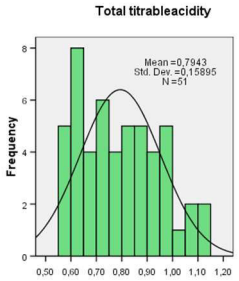

Fig. 10. Histogram of the distribution by absolute frequency classes of the total acidity of the fruit (%), on the studied varieties

The sugar content of the fruit varied between the minimum values for the 'Andreea' (8.18) and 'Romanța' varieties (8.67) and the maximum values for the 'Tuleu gras' variety (13.22). The varieties used as a control, 'Stanley' and 'Jojo', also had a high total sugar content (12.83 and 12.35%) (Fig. 11). Several factors (variety, maintenance technology, soil conditions, fruit position in the crown) can influence the value of fruit sugar content (9).

Analyzing the indicators of dispersion or genetic and experimental diversity, in terms of total sugar content of fruits, the average was 10.9110 (%), the values being between the minimum value 7.74 and the maximum value 14.14 (Fig. 12.).

The histogram of total fruit sugar (%) is bimodal, a sign that the sample is no longer homogeneous due to the influence of the different varieties studied, on the content of total sugar fruits (%) (Fig. 12).

Table 2 and the Fig. 13 show the correlations between the indicators taken in the study.

A significant negative correlation is observed between the soluble dry matter and the content of organic acids expressed as malic acid (r = -0.98), (14). The increase of the total sugar content in fruits has the effect of decreasing the content in organic acids (21). Also, the soluble dry matter correlates negatively, significantly with vitamin C (-0.306 *).

The total sugar content correlates negatively with the water contained in the fruit, through a distinctly significant correlation (r = -0.552 **). A distinctly significant negative correlation is observed between the sugar content and the vitamin C content in fruits r = -0,637. Also, a negative correlation (r = -0.152), insignificant, is observed between the total sugar content and the anthocyanin content in the fruit.

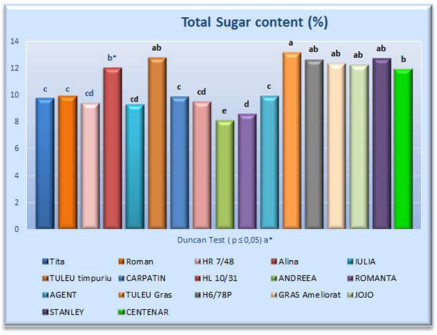

Fig. 11.Total sugar content (%) of the fruits on the plums varieties

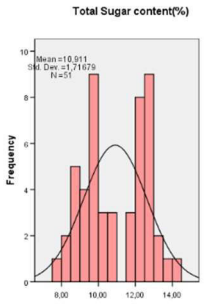

Fig. 12. Histogram of the distribution by absolute frequency classes of the fruit content in total sugar (%), onthe studied varieties

Table 2

Matrix of correlation (Pearson "r" correlation coefficients "r") of the main biochemical indicators (average for the 17 genotypes of plum studied)

Indicators		Water (%)	Total Soluble content (%)	Total titrable acidity	Total Sugar content (%)	Vitamin C	Malic acid	Total anthocyanins content (mg/l)	Total polyphenol content (mg GA/l)
Water (%)	Pearson Correlation	1	-1.000 (**)	.180	-.552 (**)	.306 (*)	.098	-.093	-.264
Total soluble content (%)	Pearson Correlation	-1.000 (**)	1	-.180	.552 (**)	-.306 (*)	-.098	.093	.264
Total titrable acidity	Pearson Correlation	.180	-.180	1	.149	-.058	.879(**)	-.340 (*)	.011
Total sugar content (%)	Pearson Correlation	-.552 (**)	.552 (**)	.149	1	-.637 (**)	.205	-.152	.260
Vitamin C	Pearson Correlation	.306 (*)	-.306 (*)	-.058	-.637 (**)	1	-.050	.344 (*)	-.163
Malic acid	Pearson Correlation	.098	-.098	.879 (**)	.205	-.050	1	-.308 (*)	.016
Total antho-cyanins content (mg/l)	Pearson Correlation	-.093	.093	-.340 (*)	-.152	.344 (*)	-.308 (*)	1	-.088
Total polyphenol content (mg GA/l)	Pearson Correlation	-.264	.264	.011	.260	-.163	.016	-.088	1

**Correlation is significant at the 0.01 level (2-tailed
* Correlation is significant at the 0.05 level (2-tailed)

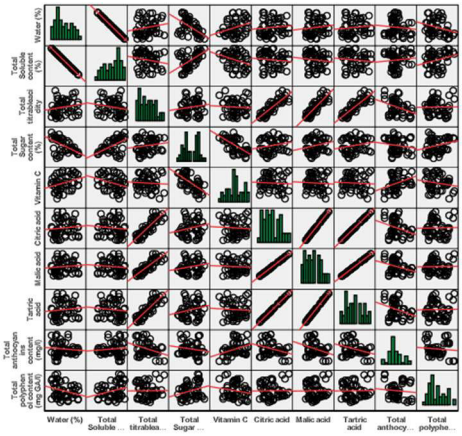

Fig. 13. Matrix of correlations between biometric and biochemical indicators studied in plum

Conclusions

The results obtained showed to the 'Tuleu gras' variety, the highest soluble dry matter content among the Romanian plum genotypes analyzed, content similar to the 'Stanley' and 'Jojo' control.

Of the 15 Romanian plum genotypes studied, only the 'H 6/78 P' genotype had a higher polyphenol content compared to the 'Stanley' and 'Jojo' varieties. The 'Agent' variety had a polyphenol content similar to control 'Stanley'.

From the point of view of the total anthocyanin content, nine of the Romanian plum genotypes studied had higher anthocyanin pigment content than the 'Stanley' control. The Romanian plum genotypes with higher anthocyanin content than the 'Jojo'' control were 'Romanța' and 'Tita'.

The Romanian plum genotypes with a higher vitamin C content than the control varieties 'Stanley' and 'Jojo' were: 'HL 10/31', 'Tita', 'Andreea', 'Iulia', 'Roman' and 'Romanța'. The 'Agent' variety had higher vitamin C content than the 'Stanley' variety but lower than the 'Jojo' variety.

The analyzed fruits had a rich content of sugars and organic acids, important components that determine their sensory quality. Of the plum genotypes studied, the highest total sugar content had 'Tuleu gras'. Results similar to the two control varieties in terms of total sugar content were also recorded by the genotypes 'Tuleu timpuriu', 'H 6/78 P', 'Gras ameliorat' and 'Centenar'. Among the plum genotypes studied, 'Stanley', 'Gras ameliorat', 'Agent' and 'Andreea' had the lowest total acidity content, expressed as malic acid.

The biochemical composition of Romanian plum genotypes is similar to the results obtained in the literature.

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RADIOGRAPHIC ASSESSMENT OF THE DISTAL RADIUS AND ULNA IN DOGS

PROTEASA A.[1], SCHUSZLER L.[2], DASCĂLU R.[2], IGNA C.[2]

[1]The Veterinary Health Center, Lytham St. Annes, United Kingdom, 4 Greenways, Lytham St. Annes, FY8 3LY
[2]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului, No. 119, Timisoara, Romania
E-mail: ignacornel@gmail.com

Summary

Objective: to report the values for four radiographic parameters of the distal radius in dogs to provide fundamental data for normal radiographic anatomy. Study design: clinical radiographic anatomical study. Animals: healthy dogs of different ages, weights and breeds (n=33 for the Hulten variance values, n=32 for the radial height and radial inclination values, n=57 for the volar tilt values). Methods: extended thoracic limb radiography was performed in two views and the images were analyzed to determine the Hulten variance, radial height, radial inclination and volar tilt. The mean values ± SD, 95% confidence intervals (95% CI) and frequencies were calculated. Kruskal-Wallis and Wilcoxon tests were implemented to determine the significance of the differences between the groups. Results: the mean Hulten variance in dogs was found to be -3.3 ± 2.4 mm. The mean radial height in dogs was found to be 4.72 ± 2.52 mm, whereas the mean radial inclination was found to be 15 ± 6.81. The radial angular inclination value was found to be depending on the patients' weight. The mean volar tilt in dogs was found to be 14.82 ± 5.42. Conclusions: the baseline values for the four radiographic parameters provide a starting point for further investigation of the canine distal radius and selection of treatment method.

Keywords: dog, Hulten variance, radial height, radial inclination, volar tilt

To quantify the amount of displacement or angulation that has occurred due to a distal radius fracture to define indications for operative treatment (using conventional or minimally invasive methods) and to evaluate the fracture reduction (particularly that due to indirect reduction), it is necessary to determine the objective radiographic parameters.

Since 1898, when Beck demonstrated that X-rays reveal any interruption of the bone outline associated with distal radius fractures (11), a number of authors have contributed to the development of radiographic measurements that were used to anatomically assess human distal radius, including the Hulten variance, radial height, radial inclination and volar tilt (1-3, 5, 7, 13-16, 19, 22, 29-32, 35, 36). These basic parameters have been used to evaluate the biomechanical function of the wrist and can be used to assess closed reduction of fractures as the use of minimally invasive techniques gain importance in veterinary medicine as in human medicine.

Currently, no study has investigated the values for these parameters in

dogs. The aim of this study was to establish the baseline values for these parameters in healthy dogs.

Materials and methods

Ulnar variance, also called Hulten variance, refers to the relative lengths of the distal articular surface of the ulna and radius and the relationship between them (19). As a specific feature, the ulnar variance can be neutral (Fig. 1) when the two articular surfaces are on the same level, negative (Fig. 2) when the ulnar articular surface is situated proximal to the radial articular surface and positive (Fig. 3) when the ulnar articular surface is distal to the radial articular surface).

Fig. 1. A neutral Hulten variance (craniocaudal view) – means that the most distal points of both the ulna (A) and radius (B) are on the same level

Fig. 2. A negative Hulten variance (craniocaudal view) – means that the most distal point of the ulna (A) is proximal to the most distal point of the radius (B)

Fig. 3. A positive Hulten variance (craniocaudal view) – means that the most distal point of the ulna (A) is distal to the most distal point of the radius (B)

As shown in Fig. 4, this parameter can be given a value. We proposed to use the same features in dogs that are considered in humans and to evaluate this parameter by assessing the positioning of the most distal points of the distal radius and ulna. The ulnar variance is measured in the craniocaudal view of the distal forearm with the shoulder and elbow joint extended.

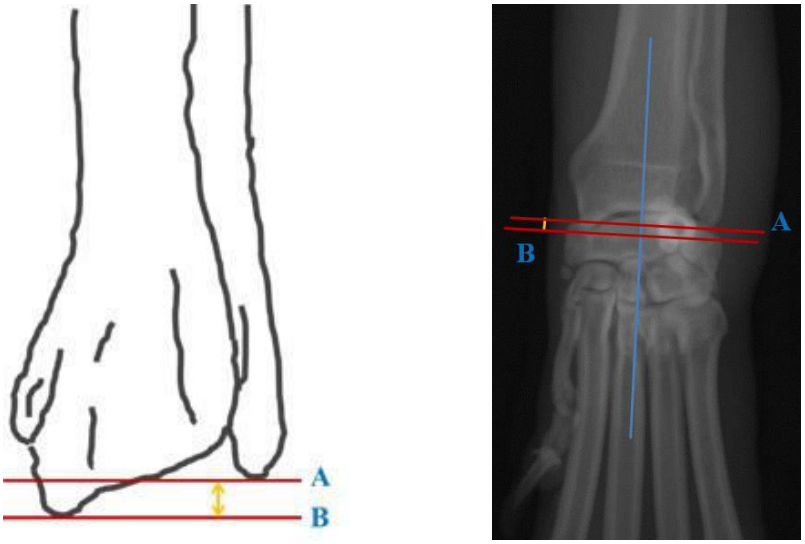

Fig. 4. The Hulten variance value is the distance between lines A and B. Line A runs perpendicular to the long axis of the distal radius crossing the most distal point of the ulna. Line B runs perpendicular to the long axis of the distal radius, crossing the most distal point of the radius, parallel to line A

The radial height which is also measured in the craniocaudal view, most often in addition to the ulnar variance, is the distance between the two perpendicular lines to the anatomic long axis of the distal radius, with one line crossing the most distal point of the styloid process of the radius and the other line following the articular surface of the distal radius (3, 15, 16, 27, 30, 36) – Fig. 5.

The radial inclination, which is measured in the same type of radiographic view, is the angle between the tangent to the distal radial articular surface and the perpendicular to the anatomic long axis of the distal radius (25, 37) - Fig. 6.

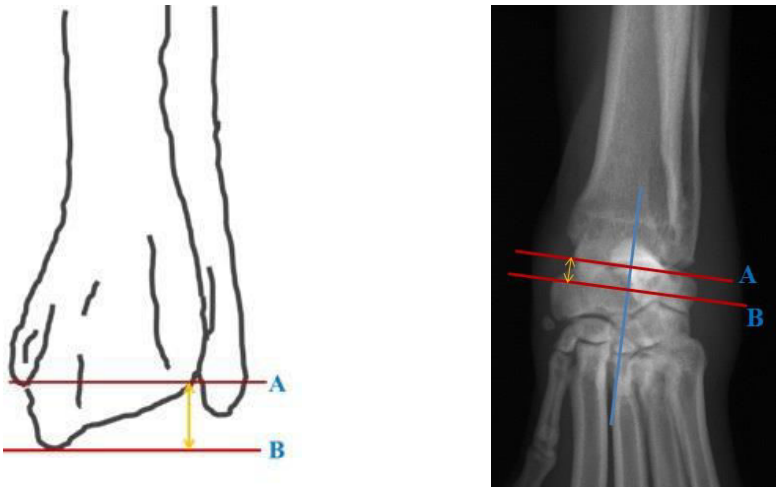

Fig. 5. Radial height (craniocaudal view) is the distance between lines A and B. Lines A and B run perpendicular to the long axis of the radius and parallel to one another, delimiting the articular surface of the distal radius

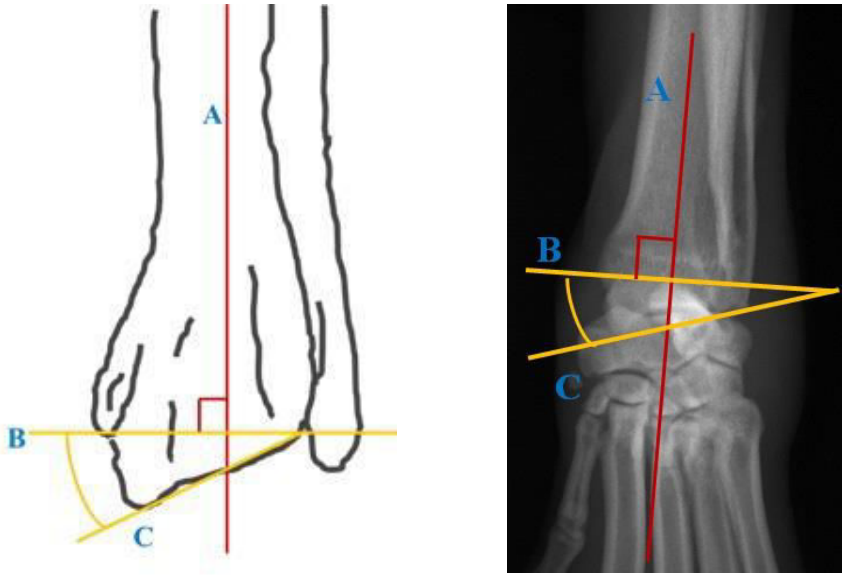

Fig. 6. Radial inclination (craniocaudal view) is the angle between lines B and C. Line A is the anatomic long axis of the distal radius and line B runs perpendicular to line A. Line C runs tangentially to the articular surface

In the mediolateral view of the forearm, the volar tilt is measured as the angle between the tangent to the articular surface and the perpendicular to the long axis of the distal radius (37) - (Fig. 7).

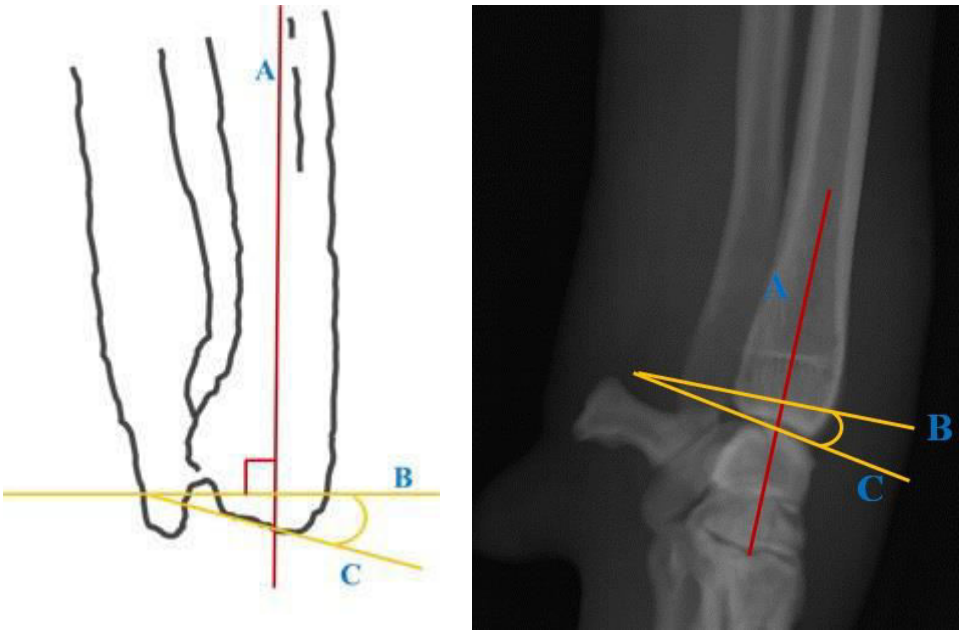

Fig. 7. The volar tilt (mediolateral view) is the angle between lines B and C. Line A is the anatomic long axis of the distal radius and line B runs perpendicular to line A. Line C runs tangentially to the articular surface

We adapted the measurement methods used in human medicine to evaluate our subjects (dogs).

Results and discussion

To determine the Hulten variance in dogs, we evaluated 33 healthy dogs using the craniocaudal view, with both the shoulder and elbow extended. Of these animals, 69.7% had a negative Hulten variance, whereas 24.2% had a negative ulnar variance and the remaining of 6.1% had a neutral positioning of the ulna relative to the radius.

The values for the Hulten variance and the mean values for the group are presented in Table 1.

To determine the radial height and radial inclination values, we evaluated 32 healthy dogs. The results are presented in Table 2.

The radial height in dogs was found to be 4.72 ± 2.51 mm and the radial inclination was found to be 15.00 ± 6.81°.

There was a significant positive correlation between the radial height and the radial inclination *r*=0.493 at p=0.004 with the correlation being significant at the p<0.010 level).

In evaluating the radial inclination values, we implemented the Kruskal-Wallis test on weight-based groups and rejected the null hypothesis at p>0.05, p=0.004. The radial inclination values depending on the weight of the dogs are shown in Table 3.

Table 1

Hulten variance values for 33 healthy dogs of different ages and weights

Hulten variance (-/+)	Indicator		Age (months)	Weight (kg)	Value (mm)
Negative	Mean Value		50.96	18.37	3.30
	SD		46.59	15.27	2.40
	SEM		9.717	3.185	0.501
	95% CI	Lower	30.81	11.76	2.27
		Upper	71.11	24.95	4.34
Positive	Mean Value		63.75	26.81	3.00
	SD		50.89	20.17	1.60
	SEM		17.995	7.131	0.567
	95% CI	Lower	21.20	9.94	1.66
		Upper	106.30	43.67	4.34

Table 2

Radial height and radial inclination values for 32 healthy dogs of different ages and weights

Indicator		Age (months)	Weight (kg)	Radial height (mm)	Radial inclination (°)
Mean Value		53,47	21.53	4.72	15.00
SD		46.58	16.22	2.51	6.81
SEM		8.235	2.868	0.445	1.204
95% CI	Lower	36.67	15.68	3.81	12.54
	Upper	70.26	27.38	5.63	17.46

Table 3

Radial inclination values for groups of healthy dogs according to their bodyweight

	Weight (kg)	Mean Value(°)	SD
n=10	Less than 10	18.50	8.46
n=7	11-20	14.14	7.10
n=8	21-30	12.25	5.28
n=7	More than 30	14.00	4.08

To determine the volar tilt value, we evaluated 57 dogs of different breeds, ages and weights. The data are shown in Table 4.

Table 4

Volar tilt values of 57 healthy dogs of different ages and weights

Indicator		Age (months)	Weight (kg)	Volar tilt (°)
Mean Value		37.08	20.17	14.82
SD		45.91	13.38	5.45
SEM		6.082	1.772	0.723
95% CI	Lower	24.90	16.62	13.38
	Upper	49.26	23.72	16.27

The mean value of the volar tilt in dog was 14.8 ± 5.45°.

Prior to determining this value, we split the group into two categories according to age (less than 12 months and greater than 12 months old) and four weights (less than 10 kg, between 10 and 20 kg, between 20 and 30 kg and greater than 30 kg) to analyze the significance of the differences between the groups.

After implementing the Wilcoxon test, the null hypothesis was accepted, meaning that there was no difference in the volar tilt value of the young (less than 12 months old) and adults (greater than 12 months old) at p=0.289. There were no differences in the volar tilt values among the four age groups according to the dogs' bodyweight at p=0.641 (determined using the Kruskal-Wallis test).

The ulnar variance is a parameter used in the context of distal radius fractures to quantify the loss of radial length. A positive ulnar variance means that the ulna extends distal to the radius and a negative ulnar variance means that the radius extends distal to the ulna. In humans, the Hulten variance is -0.6 ± 1 mm. A reduction of more than 5 mm requires operative treatment (20). Because we have so little information regarding this subject, we do not know the limit of the tolerance for ulnar variance changes.

Changes in ulnar variance lead to biomechanical disorders of the carpal joint (6, 9, 10, 12).

The greatest difficulty in evaluating this parameter has been shown to the positioning the limb for radiography and measurement (8). The smallest degree of rotation of the forearm induces changes in the ulnar variance value (12). Our results were obtained with the dog in ventral recumbency, with the shoulder and elbow joints extended.

The majority of our subjects exhibited a negative Hulten variance, whereas 24.2% of them had a positive value and 6.1% had a neutral value. These results may be due to individual variability. In an unpublished study, we also examined 12 dogs with radio-ulnar simple diaphyseal fractures. Five of these cases had a positive ulnar variance. The rest had neutral (three dogs) and negative ulnar variances (four dogs).

In humans, the value of this parameter differs not only with the position used for radiography, but also with race, age and sex of the patient (4, 8, 17, 24, 26, 34). Based on the results obtained in this study, there were no significant differences between the ulnar variance values of adult and young dogs. We did not evaluate the ulnar variance values depending on the dog breeds due to great variability of the breeds of the subjects of this study, but an evaluation of this parameter according to breeds or breed categories (e.g., chondrodystrophic or nonchondrodystrophic breeds) could be conducted in the future.

In humans, the radial inclination value is 24° (33). In general, displaced fractures of the distal radius reduce the value of this parameter and its reduction to less than 15°, is a definite indication that the fracture requires operative treatment. In veterinary medicine, there is no value of a parameter that indicates the necessity of providing an operative treatment.

Additionally, the radial height is used to assess the decrease in radial length due to a fracture. The normal value for this parameter in humans is 11.6 mm and it is dependent on the radial inclination value and the width of the bone. The radial height of dogs was found to be positively correlated with the radial inclination, with the former having a value of 4.72 ± 2.51° and the latter having a value of 15.00 ± 6.81°, respectively.

In the mediolateral view of the forearm, the volar tilt value is used to assess the angular changes in the articular surface. In humans, displaced fractures that increase the volar tilt value to greater than 10 mm tend to be highly instable and require some form of stabilization.

The study of Pennock (28) demonstrated that forearm rotation is an important factor in accurately evaluating wrist measurements and that a slight rotation of the wrist during radiographic imaging may significantly alter management decisions based on established surgical criteria.

The appropriate indirect reduction followed by the application of a method of biological osteosynthesis strategy favors rapid bone healing and massive callus formation. For healing to be adequate and the limb to be functional, the applied treatment methods, whether conservative or operative, should restore articular congruence (21, 23). Using the values of the parameters determined in this study,

we aim to develop a standard method for assessing whether an indirect reduction of a distal radius fracture can be performed and to define the indications for the necessity of providing operative treatment instead of casting/splinting.

It is important to raise awareness of the significance of radiographic examination of distal radius fractures in deciding whether to provide an operative or conservative treatment. To specify the limits of these values of parameters indicating that an operative treatment is mandatory, further investigation is needed. Even when the displacement due to the fracture is minimal and casting/splinting is the treatment of choice, a quantifiable parameter to assess when to perform an indirect reduction is required.

One drawback of this study was that the software used to determine the values did not allow further extending the places for the decimal points.

Conclusions

The mean Hulten variance in dogs was found to be -3.3 ± 2.4 mm. Most of the subjects (69.7%) exhibited a negative ulnar variance.

The mean radial height in dogs was found to be 4.72 ± 2.52 mm, whereas the radial inclination was found to be 15.00 ± 6.81°. Based on the results of this study, the radial angular inclination value depended on the weight of the subjects.

The volar tilt in dogs was found to be 14.82 ± 5.42°.

Because there is a certain amount of normal variation in the values for most of the parameters in dogs as occurs in humans, it would be quite helpful to perform radiographic images of the normal contralateral limb to establish the normal ranges of the values.

The baseline values for the four radiographic parameters provide a starting point for further investigation of the canine distal radius and assessment of when indirect reduction of distal radius fractures is appropriate in dogs.

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CYTOPATHOLOGICAL FINDINGS IN PLEURAL EFFUSIONS DETERMINED BY PRIMARY AND SECONDARY NEOPLASMS IN DOMESTIC CARNIVORES

SANDU I., DINESCU G., MILITARU M.

University of Agricultural Sciences and Veterinary Medicine Bucharest, Faculty of Veterinary Medicine, 050097, Splaiul Independentei 105, Bucharest, Romania
E-mail: anaoi.udnas@gmail.com

Summary

The cytopathological diagnosis is an area of interest of clinical pathology, which is a very useful, cheap and time worthing tool used in the assessment of patients with pleural effusion. The veterinary emergency and critical care literature sees the cytopathological approach of the patient with pleural effusion as a bridge between the pathologist and the clinician and as a very important source of information which leads to a correct therapeutic management. A pleural effusion, of unknown origin, discovered during clinical examination, is considered a life threatening condition that needs a rigorous mathematical approach protocol, using thoracocentesis as a method of therapy and diagnosis.

Keywords: pleural effusion, tumors, clinical pathology, cytopathology

Pleural effusion is defined as an abnormal accumulation of fluid in the pleural space. Primary thoracic neoplasia (6, 8) and secondary thoracic metastases may be associated with pleural effusions (7, 12, 18).

Tumor-associated effusions show a high variability in their composition. An effusion may be classified as tumor-associated, when tumor cells can be identified during microscopic examination (1, 4, 8). However, not all tumor-associated effusions yield a sufficient number of tumor cells. In those cases, diagnostic imaging may be a better option to establish a diagnosis. Tumors might have different pathological mechanisms: they can cause transudation (a) by obstructing venous or lymphatic drainage; exudation (b) by inciting an inflammatory reaction or rupture of an internal organ or causing hemorrhage (c). Also they may act by a mix of mechanisms (1, 4, 21, 23).

Cytology is an excellent but not overall sensitive tool to identify tumor-associated effusions. Sensitivity and specificity of cytology were 65% and 99% for dogs; 61% and 100% for cats (9, 13).

Mediastinal lymphoma, as primary neoplasm associated with pleural effusion, is commonly found in cats; the metastasis of the mammary adenocarcinoma, which could be associated with pleural effusion, is classified as a secondary tumor (1, 8, 22).

Mesothelioma and lymphoma are the most common primary intrathoracic tumors in canine patients; the metastasis of the mammary adenocarcinoma associated with pleural effusion, is classified as a secondary tumor (4, 6).

The research was performed in order to identify by cytopathological approach the most important morphological features of the cells that may be found in neoplasia associated effusions. It highlights the importance of the integrated interpretation of diagnostic imaging findings and cytology and refines cytologic diagnosis when obtaining a histology sample is not feasible.

Materials and methods

Pathologic samples, represented by fluid samples, were taken from cats and dogs with pleural effusion. All effusions were identified by thoracic ultrasound/ Roentgen examinations or CT scans. Fourteen samples were taken in work from seven cats and seven dogs. The samples were obtained by ultrasound guided thoracentesis. A volume of 2-3 ml of fluid was collected on sterile EDTA tubes in order to preserve the cells for cytological exam. The samples were centrifuged for 10 minutes at 4000 rotations/minute and the smears were obtained from the sediment. Standard methods of staining, Diff-Quick or May-Grunwald Giemsa were used to stain the samples.

Results and discussions

The fluid samples from the two groups were analyzed using cytopathological techniques. In all cases, except two of them, primary and secondary tumors which were confirmed by diagnostic imaging. There were cases where the cytopathological results were supported by the histopathological results. There is one case in which a necropsy was performed, revealing that the pleural and peritoneal effusions were caused by the local and systemic changes determined by the site of metastasis, vascular modifications and the mechanisms of metastasis secondary to a primary melanoma (case 5 from Table 2).

The first group of samples consisted of the samples obtained from the cats group with pleural effusion. The results are summarized in Table 1. In this group, the results were: four cats with primary mediastinal lymphoma, one cat with primary oral melanoma and two cats with primary mammary adenocarcinoma. All cases had diagnostic imaging performed in order to identify metastasis and pleural effusion.

Regarding the four cats diagnosed by cytopathological examination with lymphoma, three of the smears presented high numbers of a monomorphic population of exfoliating lymphoblasts with a scant to moderate amount of basophilic cytoplasm, round to variably shape eccentric nuclei, finely stippled nuclear chromatin and prominent nucleoli (Fig. 1). Only in one case, the population was made of small lymphocytes with a moderate amount of basophilic cytoplasm and round nuclei.

Table 1

Description of signalment, diagnostic imaging findings, suspected primary / secondary cancer, cytopathological and histopathological findings in cats. M = male; F = female

No	Breed	Sex	Age (years)	Imaging findings	Primary / Secondary neoplasia	Cytopathological/ Histopathological Findings
1	Domestic short hair	M	10	Pleural effusion	Oral melanoma	Primary oral melanoma with pulmonary metastasis
2	Domestic short hair	F	7	Pleural effusion with 2 masses at M1 and M2	Mammary adenocarcinoma	Primary mammary adenocarcinoma with pulmonary and pleural metastasis
3	Birman	F	14	Pleural effusion with masses covering the left mammary glands	Mammary adenocarcinoma	Primary mammary adenocarcinoma with pulmonary metastasis
4	Domestic short hair	F	12	Pleural effusion, enlargement of the mediastinal space, high opacity in the cranial mediastinum	Lymphoma	Lymphoma confirmed by cytopathological examination. No histological examination was made
5	Domestic short hair	F	7	Pleural effusion, enlargement of the mediastinal space, high opacity in the cranial mediastinum	Lymphoma	Lymphoma confirmed by cytopathological examination. No histological examination was made
6	Domestic long hair	M	11	Pleural effusion, enlargement of the mediastinal space, high opacity in the cranial mediastinum	Lymphoma	Lymphoma confirmed by cytopathological examination. No histological examination was made
7	Ragdoll	M	2	Pleural effusion, enlargement of the mediastinal space, high opacity in the cranial mediastinum	Mediastinal lymphoma	Primary mediastinal lymphoma. Additional PARR exam showing a B cell population.

A polymerase chain reaction for antigen receptor rearrangement (PARR) examination was performed and the result consisted in a monomorphic neoplastic population of B-cells (case 7 in Table 1).

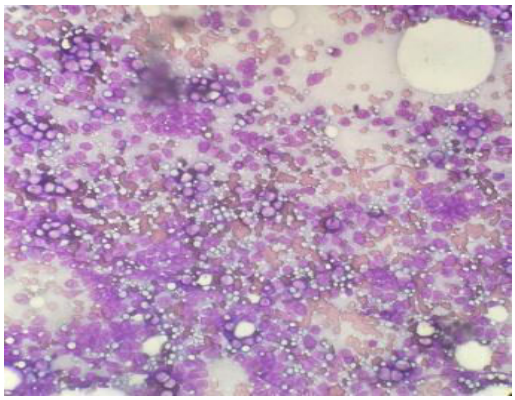

Fig. 1. Mediastinal lymphoma (case 4)
Effusion consisting of monomorphic population of lymphoblasts tumorales
(Diff-Quick, 200X)

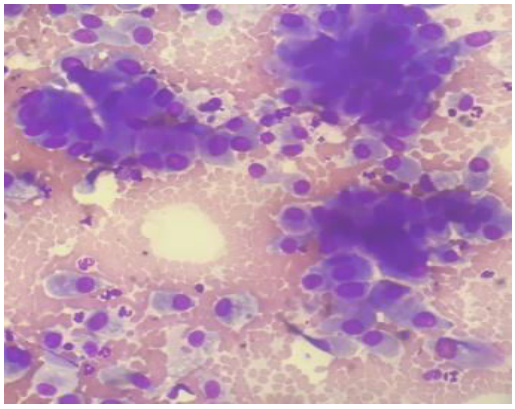

Fig. 2. Mammary adenocarcinoma (case 2)
Effusion consisting of clusters of epithelial cells (Diff-Quick, 400X)

Two samples were confirmed as adenocarcinoma associated pleural effusions. The cytopathological exam showed clusters of epithelial cells with eccentric nuclei and a mild amount of basophilic cytoplasm (Fig. 2).

One sample consisted of a melanoma associated effusion in a cat with primary oral melanoma and secondary pulmonary metastasis. The cytopathologic examination revealed the presence of round to spindle-shaped cells with abundant basophilic cytoplasm. Many of these cells contained black intracytoplasmic

granules. The cells had round to oval nuclei with a fine chromatin pattern and one to two prominent, large nucleoli (Fig. 3).

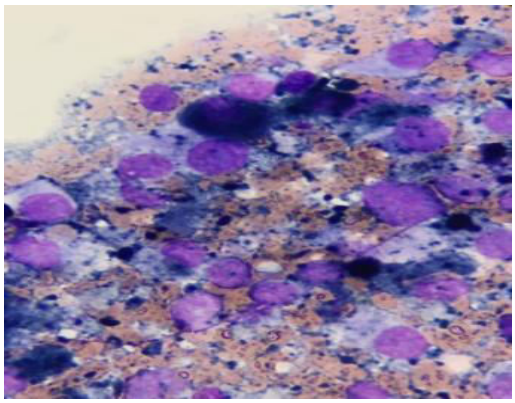

Fig. 3. Melanoma (case 1)
Population of round to spindle-shaped cells with abundant basophilic cytoplasm and black intracytoplasmic granules (May-Grunwald Giemsa, 400x)

The second group of samples consisted of those obtained from the dogs with pleural effusion. The results are summarized in Table 2. In this group, the results were the following: three subjects were diagnosed with primary mesotheliomas, one with sarcoma associated effusion, one with mast cell associated effusion, one with lymphoma associated effusion and the last one with a melanoma associated effusion.

The cytopathological features found on the smears from the first three dogs with mesothelioma associated effusion consisted of a large population of cells with extreme macrocytosis, marked anisokaryosis, large variably shaped nucleoli, large numbers of mitotic figures, and aberrant mitoses. Only in one case an immunocytochemistry examination was made. The cells were positive to vimentin and desmin and a primary mesothelioma diagnosis was made.

A population of mesenchymal cells which was diagnosed as sarcoma associated effusion was found in one of the samples. Slides contained many neoplastic cells, mostly individualized and occasionally embedded in scant light pink material reminiscent of chondroid/osteoid (Fig. 5). Criteria of malignancy included moderate anisocytosis and anisokaryosis, rare binucleation, prominent nucleoli, and occasional mitoses (5 per 10 fields at 400x magnification). The cytologic findings were highly suggestive of a chondrosarcoma/osteosarcoma associated effusion.

One of the samples was diagnosed as mast cell associated effusion. The smear consisted of round to ovoid cells with large numbers of metachromatic cytoplasmic granules (Fig. 4).

One mediastinal lymphoma featuring large cells, with a scant to moderate amount of basophilic cytoplasm, round to variably shape eccentric nuclei, finely stippled nuclear chromatin and prominent nucleoli was found by cytopathological examination of slides. Further investigations sustained the cytopathological diagnosis of mediastinal lymphoma (Fig. 6).

Table 2

Description of signalment, diagnostic imaging findings, suspected primary/secondary cancer, cytopathological and histopathological findings in dogs. F = female; M = male

No	Breed	Sex	Age (years)	Imaging findings	Primary/ Secondary neoplasia	Cytopathological/ Histopathological findings
1	Mixed breed	M	10	Pleural effusion, no masses observed at full body CT	Mesothelioma	Primary mesothelioma diagnosed by immunocytochemistry
2	Mixed breed	M	14	Pleural effusion	Mesothelioma	Suspected mesothelioma
3	Mixed breed	F	8	Pleural effusion, no mass observed at thoracic and abdominal X-rays	Mesothelioma	Suspected mesothelioma
4	Boxer	M	12	Pleural effusion with one mass on the left side of the thorax	Sarcoma	Chondrosarcoma with pulmonary and pleural metastasis
5	German Shepherd	M	12	Pleural and peritoneal effusion	Melanoma	No primary tumor was identified, multiple metastases were found consisted with a diagnosis of melanoma
6	Labrador Retriever	M	9	Pleural effusion	Mast cells tumor	Scapular mast-cell tumor with secondary metastasis in the lymph nodes and viscera
7	Golden Retriever	F	7	Pleural effusion, enlargement of the mediastinal space, high opacity in the cranial mediastinum	Mediastinal lymphoma	Primary mediastinal lymphoma. Additional PARR exam showing a B cell population.

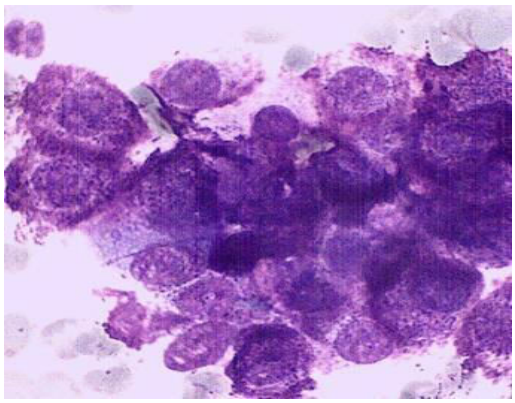

Fig. 4. Mast cell tumor (case 6)
Round to ovoid cells with large numbers of metachromatic cytoplasmic granules (May-Grunwald Giemsa, 1,000x)

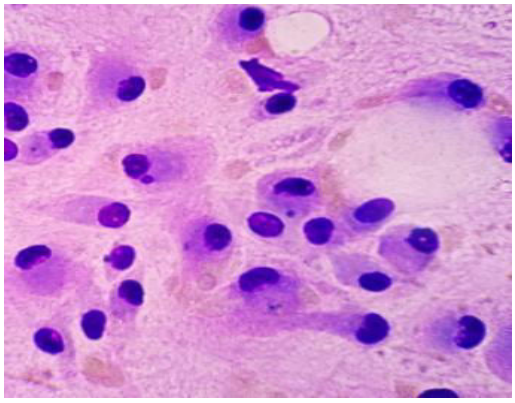

Fig. 5. Sarcoma (case 4)
Population of mesenchymal cells with eosinophilic cytoplasm, with large nuclei and one to two visible nucleoli; lakes of bright pink, smooth or slightly granular material, in which cells are embedded (May-Grunwald Giemsa, 400x)

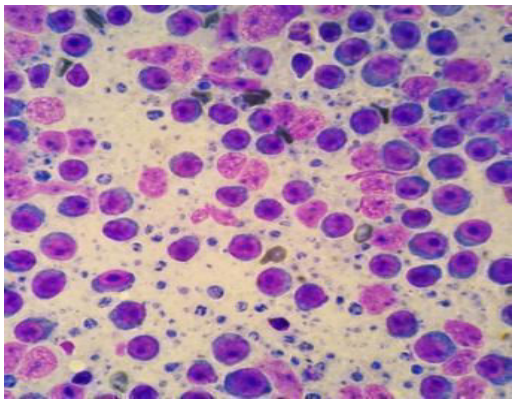

Fig. 6. Lymphoma (case 7)
Large cells, with a scant to moderate amount of basophilic cytoplasm, round to variably shaped eccentric nuclei, finely stippled nuclear chromatin and prominent nucleoli (Diff-Quick, 400x)

Melanoma associated effusion was found in a 12 years German shepherd. In this particular case, a necropsy was performed and confirmed the presence of a large amount of a dark fluid in the pleural and peritoneal cavities. On the cytopathological examination, round to spindle-shaped cells with abundant basophilic cytoplasm and black intracytoplasmic granules were observed.

The main finding in this research was the presence of neoplastic cells in the pleural effusion. Cavitary effusions may be caused by disturbances of hydrostatic or oncotic pressure, inflammation, impaired lymphatic drainage, hemorrhage, organ rupture, or neoplasia (9, 17, 24). Achieving a definitive diagnosis using a fluid sample during therapeutic thoracentesis, avoiding invasive procedures such as thoracotomy, and pleural or pulmonary biopsy would be ideal, but it is often not possible. Cytology is an excellent, but not overall sensitive tool to identify tumor-associated effusions. Sensitivity and specificity of cytology were 65% and 99% for dogs; 61% and 100% for cats (3, 4, 9).

Cytological evaluation of pleural fluid obtained from the first group (Table 1) lead to the following results: lymphoma associated effusion, melanoma associated effusion and mammary adenocarcinoma associated effusion. Mediastinal masses may be suspected based on the physical and radiographic findings. Most cases of lymphoma in dogs and cats are high-grade tumors composed predominantly of large blastic lymphoid cells if large, blastic lymphoid cells constitute greater than 50% of the cells in a highly cellular smear from lymphoid tissue containing mostly intact cells. The cases included in this research were diagnosed by cytological analysis of the pleural fluid which is an important factor in identifying underlying disease.

Mammary gland tumors are common in both dogs and cats; however, the biologic behavior of the tumors varies greatly between these species, in cats it tends to be more aggressive leading to pleural effusion (13). Carcinomas and adenocarcinomas may often be diagnosed by cytologic evaluation of effusions on the basis of significant numbers of exfoliating cells and numerous criteria of malignancy (3, 4). That was the case of our smears, where cells featured large, bizarre, or angular nucleoli, multiple nucleoli, nuclear molding, high nucleus-to-cytoplasm ratios, multinucleation.

Essentially, any tumor may metastasize to the lungs, but tumors more likely to do so include oral and nail bed melanoma, thyroid carcinoma, osteosarcoma, mammary carcinoma, and high-grade soft tissue sarcoma (10, 24). Melanocytic neoplasms are infrequently described in the cat and knowledge is limited (5, 11, 14). Morges et al. (16) described a unique case in the literature of a pleural effusion that contained malignant melanocytes, consistent with metastatic spread. These findings were available in the smears obtained in our research from a cat with primary oral melanoma. The author thinks that this biological behavior was possible judging the comportment of melanic tumors; they used to metastasize in the lymph nodes and viscera. Mast cell tumors tend to achieve initiative behavior in cats (4, 12).

Cytological evaluation of pleural fluid obtained from the second group (Table 2) lead to the following results: three cases of mesothelioma associated effusion, one of a sarcoma, one of mast cell associated effusion, one of a lymphoma and one of a melanoma.

Mesotheliomas are uncommon tumors in domestic species and are difficult to diagnose cytologically because of the pleomorphism exhibited by reactive mesothelial cells. If an effusion contains mesothelial cells where significant cytologic criteria of malignancy is not evident, it is often impossible to differentiate mesothelial reactivity or hyperplasia from mesothelioma. When mesothelial cells exhibit marked criteria of malignancy: extreme macrocytosis, marked anisokaryosis, large variably shaped nucleoli, large numbers of mitotic figures, and aberrant mitosis it can be difficult to differentiate mesothelioma from carcinoma or adenocarcinoma (1, 15, 20). In the latter cases, only one case was confirmed by immunocytochemistry, the two others were diagnosed as suspected mesotheliomas and no further investigations were made. Sawa at el. (20) based on the fact that mesothelial cells exhibit dual expression of cytokeratin and vimentin, where able to identify between epithelial, mesenchymal and mesothelial cells.

Sarcomas involving intracavity organs often do not exfoliate neoplastic mesenchymal cells into effusions and are rarely diagnosed on fluid analysis alone. The features found in our case and the medical record history, listed the effusion as being a chondrosarcoma associated effusion. Extraskeletal chondrosarcomas are extremely rare in both humans and domestic animals (7). Canine extraskeletal chondrosarcomas have been reported to occur in the mitral leaflet, right atrium, aorta, larynx, lung, and omentum and metastases were found in the eye (19, 25).

Mast cell tumors within body cavities (nodal, hepatic, splenic, gastrointestinal) may cause effusions and frequently exfoliate large numbers of mast cells into the effusion. In the case mentioned in this research, it was a metastasis. Cartagena et al. reported two cases of primary intrathoracic mast cell tumor and the literature (2) cites cases of mast cell tumor associated effusion.

Conclusions

The present study was able to demonstrate the most important morphological features of the cells that may be found in neoplasia associated effusions. It highlights the importance of the integrated interpretation of diagnostic imaging findings and cytology and the scope of cytologic diagnosis when obtaining a histology sample is not feasible. The cases taken in work resemble new cytological features and also showed rare biological behavior of some tumors.

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RESEARCHES REGARDING THE HEPARIN SIDE EFFECTS IN DOGS UNDERGOING HEMODIALYSIS

VIŢĂLARU B.A., ŞTEFĂNESCU A., POPESCU C.

University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Veterinary Medicine, 050097, Independentei Street No 105, District 5, Bucharest, Romania
E-mail: alexandrumv@yahoo.com

Summary

The paper aimed to present the best heparin dose usage in dogs undergoing hemodialysis in order to prevent side effects such as massive bleedings around the central venous catheter or thrombosis in the extracorporeal circuit during hemodialysis. Anticoagulation protocols in routine intermittent hemodialysis typically consist of the systemic administration of a standard dose of heparin as a bolus 5 minutes before starting the dialysis treatment, followed by a maintaining dose during the whole session. Unfractioned heparin is the most common anticoagulant used in dogs undergoing hemodialysis. This study was conducted on 15 patients, belonging to different breeds and having different ages. The 15 patients were divided in 3 equal batches. First batch received a dose of 50 U/kg heparin (n=5), the second one a dose of 25 U/kg heparin (n=5) and the third one received a dose of 10 U/kg heparin (n=5). The proper heparin dose usage in all patients undergoing hemodialysis, regardless of the age, appears to be 10-25 U/kg, in order to avoid side effects.

Keywords: hemodialysis, anticoagulation, heparin, thrombosis, bleeding

In veterinary medicine, the most common anticoagulant used in patients undergoing hemodialysis is unfractionated heparin (2).

In order to deliver a safe and effective dialysis treatment, an appropriate level of anticoagulation must be achieved to prevent thrombosis of the extracorporeal circuit without causing excessive bleeding in the patient (2).

Anticoagulation protocols in routine intermittent hemodialysis typically consist of the systemic administration of a standard dose of heparin (10-50 U/kg) as a bolus five minutes before starting the dialysis treatment. Adequate anticoagulation is then maintained with continuous infusion of heparin (10-50 U/kg/h) into the arterial limb of the circuit. The heparin infusion or bolus administration may be discontinued up to 30 minutes before the end of the treatment or continued throughout the treatment, depending on the patient's bleeding risk and the degree of clothing in the extracorporeal circuit (1).

The complications that appear after administrating anticoagulants in hemodialysis, are represented by thrombosis in the extracorporeal circuit, when the patient receive an under dose of heparin and the opposite, bleedings, when it was administrated an overdose (3).

Protamine sulphate is the antidote used in heparin overdose; 1 mg manages to neutralize 100 U.I of heparin (4).

Materials and methods

This study was conducted on 15 patients, with ages between 2 and 13 years, that belong to 10 different breeds (Abruzzo shepherd, Amstaff, Beagle, Chow-chow, German shepherd, Golden Retriever, Mixed breed, Rottweiler, Shar-pei, Siberian Husky), males and females. None of the patients that were submitted to hemodialysis were diagnosed with warfarin or any other anticoagulant poisoning. The 15 patients were divided in 3 equal batches.

All the patients received an initial dose of 50 U/kg heparin, 5 minutes before starting the dialysis treatment. During the hemodialysis session, to maintain the anticoagulation, each batch received a different dose of heparin.

First batch received a dose of 50 U/kg heparin (n=5), the second one a dose of 25 U/kg heparin (n=5) and the third one received a dose of 10 U/kg heparin (n=5).

In patients that experienced heparin overdose, protamine sulphate was administered to counter the side effects.

Results and discussions

Table 1

Patients from Batch no. 1 = maintaining dose of 50 U.I./kg

P.	Patient data	Weight (kg)	Heparin systemic dose (U.I./kg)	Maintaining dose (50 U.I./kg)	Complication
1.	Beagle, 13 years, male	19	50	950	B+++
2.	German shepherd, 10 years, male	30	50	1500	B++
3.	Shar-pei, 5 years, male	25	50	1250	B+
4.	Rottweiler, 7 years, male	40	50	2000	B++
5.	Golden Retriever, 13 years, male	35	50	1750	B+++

Table 2

Patients from Batch no. 2 = maintaining dose of 25 U.I./kg

P.	Patient data	Weight (kg)	Heparin systemic dose (U.I./kg)	Maintaining dose (25 U.I./kg)	Complications
6.	Abruzzo shepherd, 5 years, male	54	50	1350	N
7.	Half-breed, 11 years, male	30	50	750	B+
8.	Golden retriever, 6 years, female	40	50	1000	N
9.	Golden retriever, 9 years, male	36	50	900	B+
10.	Siberian Husky, 2 years, female	19	50	475	N

Table 3

Patients from Batch no. 3 = maintaining dose of 10 U.I./kg

P.	Patient data	Weight (kg)	Heparin systemic dose (U.I./kg)	Maintaining dose (10 U.I./kg)	Complications
11.	Amstaff, 8 years, female	30	50	300	N
12.	German shepherd, 3 years, female	28	50	280	N
13.	Chow-chow, 7 years, female	24	50	240	T
14.	Golden retriever, 5 years, female	32	50	320	N
15.	Rottweiler, 8 years, female	45	50	450	T

Legend:
B+++: massive bleeding around the catheter during hemodialysis;
B++: moderate bleeding around the catheter during hemodialysis;
B+: minimum bleeding around the catheter during hemodialysis;
N: no complications;
T: thrombosis in the extracorporeal circuit.

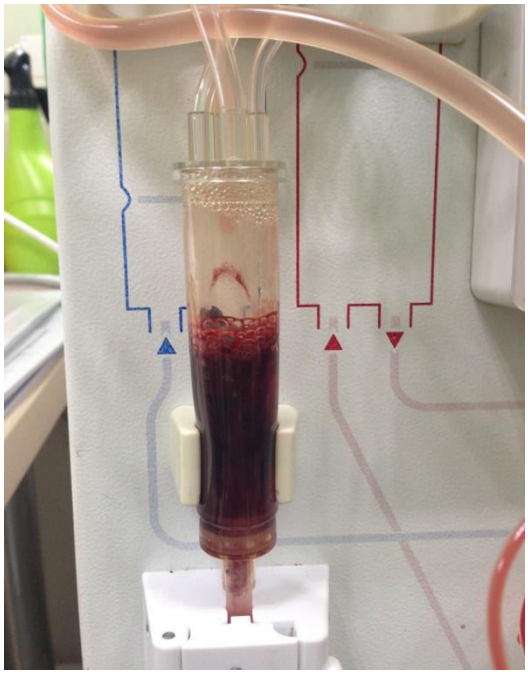

Fig. 1. Thrombosis in the extracorporeal circuit
(venous chamber) (orig.)

In the first batch, (=no.1), with the maintaining dose of 50 U.I./kg, 2 patients experienced massive bleeding around the central venous catheter (p. 1 and p. 5), 2 patients experienced moderate bleeding around the catheter (p. 2 and p. 4), and only one experienced minimum bleeding (p. 3), (Table 1).

In the second batch (=no. 2), with the maintaining dose of 25 U.I./kg, 2 patients experienced minimum bleeding around the catheter (Fig. 2), (p. 7 and p. 9) and the rest of them had no complications (p. 6, p. 8, p. 10), (Table 2).

In the third batch (=no. 3), with the maintaining dose of 10 U.I./kg, only 2 patients (p. 13 and p. 15) experienced thrombosis in the extracorporeal circuit during hemodialysis (Fig. 1), the rest of them (p. 11, p. 12, p. 14) had no complications (Tab. 3).

Patient p. 1, p. 2, p. 4, p. 5 from the first batch received a dose of 1mg

protamine sulphate per 100 U.I. of heparin after the hemodialysis session, in order to counter the heparin side effects.

Fig. 2. Minimum bleeding around the central venous catheter (orig.)

Conclusions

After receiving an initial dose of 50 U.I./kg before hemodialysis, geriatric patients that had the maximum maintaining dose (50 U.I./kg) experienced massive bleedings.

The lowest maintaining dose of heparin (10 U.I./kg) led to thrombosis in the extracorporeal circuit during hemodialysis, also in geriatric patients.

The proper heparin dose usage in all patients undergoing hemodialysis, regardless of the age, appears to be 10-25 U.I./kg, in order to avoid heparin side effects.

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OSCILLATION OF BLOOD PRESSURE IN DOGS WITH KIDNEY INJURY UNDERGOING HEMODIALYSIS

VIȚĂLARU B.A., MARCU M., ȘTEFĂNESCU A.

Faculty of Veterinary Medicine Bucharest, Splaiul Independenței 105, Bucharest, Romania
E-mail: alina_stefanescu9@yahoo.com

Summary

Hypertension related to kidney injury and fluid overload is commonly encountered in the dialysis patient population. It is estimated that approximately 30-55% of dogs diagnosed with chronic kidney disease (CKD) are impacted by systemic hypertension. One of the most common complications of hemodialysis is hypotension and hypovolemia as a result of ultrafiltration and large extracorporeal blood volume during the sessions. The aim of the present study is to determine the blood pressure variations in patients undergoing hemodialysis in order to adjust the hypertensive therapy. The study was conducted on 10 canine patients diagnosed with chronic kidney disease and hypertension. After every hemodialysis session, a reduction of BP observed in all patients. After observing the blood pressure variations, it is safe to say that hemodialysis has a role in the management of hypertension in dogs with renal function impairment and it can be a complementary therapy to classical hypotensive drugs in those patients.

Keywords: hemodialysis, hypertension, kidney, dogs, therapy

Systemic arterial hypertension is described as a sustained elevation in blood pressure (BP). Hypertension related to kidney injury and fluid overload is commonly encountered in the dialysis patient population. It is estimated that approximately 30-55% of dogs diagnosed with chronic kidney disease (CKD) are impacted by systemic hypertension (3).

The International Renal Interest Society (IRIS) recommends that anti-hypertensive treatment should be initiated in patients with persistently elevated systemic arterial BP (≥160 mmHg) (Table 1). The reasoning behind this is that these patients are at an increased risk for the development of target-organ damage (6).

Table 1

IRIS scheme for sub-staging CKD based on BP

Systolic BP mmHg	Blood pressure sub-stage	Risk of future target organ damage
<140	**Normotensive**	Minimal
141-159	**Pre-hypertensive**	Low
160-179	**Hypertensive**	Moderate
≥180	**Severely hypertensive**	High

In patients with CKD the reduction of BP should be considered an intermediate to long-term target (1).

The drugs of choice for the treatment of hypertension in dogs usually are Angiotensin-converting enzyme inhibitors and Dihydropyridine calcium channel blockers (5).

In addition to treating azotaemia and relieving the kidneys of their function, hemodialysis lowers the arterial blood pressure by eliminating the excess fluid (2).

One of the most common complications of hemodialysis is hypotension and hypovolemia as a result of ultrafiltration and large extracorporeal blood volume during the sessions (4).

Materials and methods

For this study, 10 dogs where observed, with age ranging from five to 13 years, from different breeds (Shar-pei, German Shepherd, Rottweiler, Beagle, Chow Chow, Golden Retriver and one of a mixed breed). All 10 dogs were diagnosed by a cardiologist with hypertension prior to the start of hemodialysis therapy, and they were on amlodipine therapy the entire time of the study. Each patient underwent four hemodialysis sessions in which the variation of blood pressure was studied (Table 2).

In order to assess the oscillations of blood pressure in dogs with kidney injury undergoing hemodialysis, a doppler-ultrasonic technique was used. Hair was clipped proximal to the palmar metacarpal pad of the right forelimb on all 10 patients, and the superficial palmar arterial arch was used for blood pressure measurement. An occluding cuff sized accordingly to each patient was placed mid-radius. Ultrasonic coupling gel was placed on the Doppler transducer and then it was held in place manually where an audible pulse signal was obtained. The cuff was inflated to a pressure approximately 40 mm Hg above the audible cut-off point of the signal. For each patient three systolic pressure measurements where determined before the start of the hemodialysis session, at each hour during the session and at one hour after the end of the hemodialysis session. The mean value of each three measurements was noted and taken into account as the mean systolic pressure at the given time (Table 3-12).

Results and discussions

Table 2

Representation of the patients undergoing hemodialysis and their BP at the moment of diagnosis and 1 week after discharge

Crt. No.	Patient Breed, Age, Sex	Diagnosis	BP at the moment of diagnosis	BP one week after discharge
1.	XENA CHINESE SHAR-PEI, 6 YO, F	Severely hypertensive	192 mmHg	170 mmHg
2.	MISSY CHINESE SHAR-PEI, 8 YO, F	Severely hypertensive	210 mmHg	160 mmHg
3.	SHAGGY GERMAN SHEPHERD, 9 YO, M	Severely hypertensive	210 mmHg	141 mmHg
4.	RICKY ROTTWEILER, 7 YO, M	Severely hypertensive	191 mmHg	143 mmHg
5.	PUPICEL CHINESE SHAR-PEI, 5 YO, M	Severely hypertensive	210 mmHg	125 mmHg
6.	HUMPHREY BEAGLE, 13 YO, M	Severely hypertensive	183 mmHg	130 mmHg
7.	ADOLF MIXED BREED, 13 YO, M	Severely hypertensive	199 mmHg	150 mmHg
8.	FOXY CHOW CHOW, 8 YO, M	Hypertensive	175 mmHg	133 mmHg
9.	FREDDY GOLDEN RETRIVER, 7 YO, M	Hypertensive	166 mmHg	122 mmHg
10.	SARAH GOLDEN RETRIVER, 11 YO, F	Severely hypertensive	198 mmHg	153 mmHg

Table 3

Representation of BP for patient No. 1

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	192	180	177	177	175	175
2	185	183	180	180	175	172
3	180	175	170	170	168	165
4	175	173	173	173	170	170

Table 4

Representation of BP for patient No. 2

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	210	200	190	185	185	185
2	200	195	190	183	179	170
3	192	180	175	170	170	170
4	180	175	170	168	168	166

Table 5

Representation of BP for patient No. 3

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	210	190	185	181	179	175
2	190	181	178	176	177	170
3	182	180	173	168	160	160
4	177	170	166	160	160	155

Table 6

Representation of BP for patient No. 4

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	191	180	173	169	160	158
2	170	159	150	153	152	151
3	166	134	134	132	133	148
4	150	125	126	126	119	137

Table 7

Representation of BP for patient No. 5

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	210	198	180	175	162	161
2	168	133	138	135	135	160
3	151	127	130	128	127	150
4	147	130	131	129	130	138

Table 8

Representation of BP for patient No. 6

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	183	77	75	80	87	113
2	161	75	79	81	83	117
3	147	69	79	88	87	115
4	145	81	88	85	89	120

Table 9

Representation of BP for patient No. 7

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	175	101	97	111	109	148
2	162	103	107	119	120	145
3	158	100	113	114	109	142
4	146	99	113	121	115	132

Table 10

Representation of BP for patient No. 8

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	199	186	169	171	164	160
2	177	171	168	166	160	155
3	172	160	160	158	157	150
4	163	150	145	143	140	156

Table 11

Representation of BP for patient No. 9

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP5
1	166	111	117	115	117	132
2	150	113	111	112	120	133
3	137	109	113	111	119	124
4	137	118	117	111	108	119

Table 12

Representation of BP for patient No.10

No. Of HD session	BP before session	BP 1	BP 2	BP 3	BP 4	BP 5
1	198	69	90	94	88	180
2	187	79	84	95	91	168
3	171	110	95	99	89	160
4	168	101	115	94	92	150

Legend:
BP 1 - blood pressure after the 1st hour of hemodialysis;
BP 2 - blood pressure after the 2nd hour of hemodialysis;
BP 3 - blood pressure after the 3rd hour of hemodialysis;
BP 4 - blood pressure after the 4th hour of hemodialysis;
BP 5 - blood pressure 2 hours after the hemodialysis session;

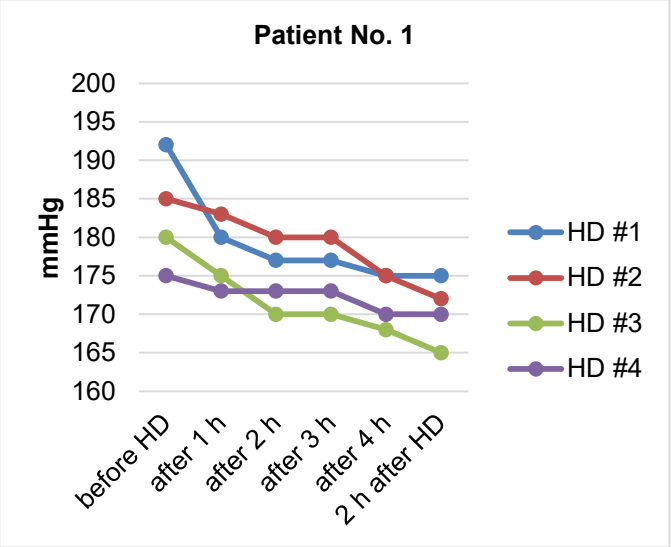

Fig. 1. Graphic representation of BP changes during HD sessions in patient no. 1

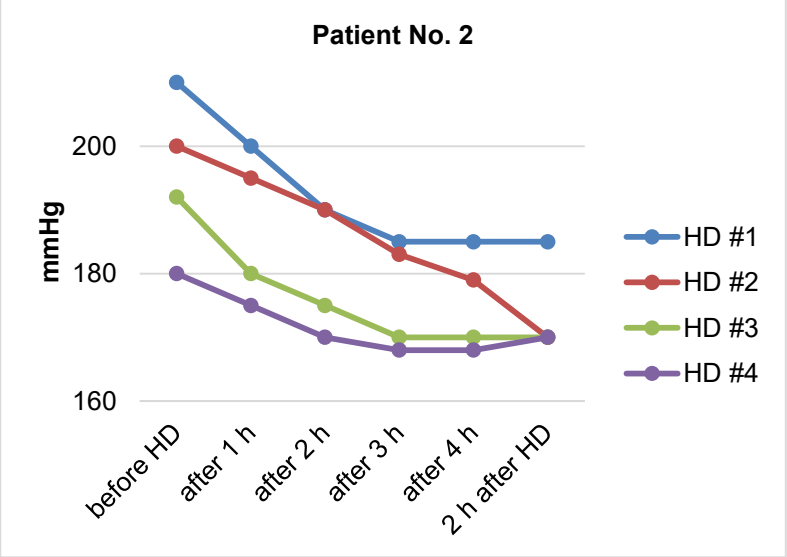

Fig. 2. Graphic representation of BP changes during HD sessions in patient no. 2

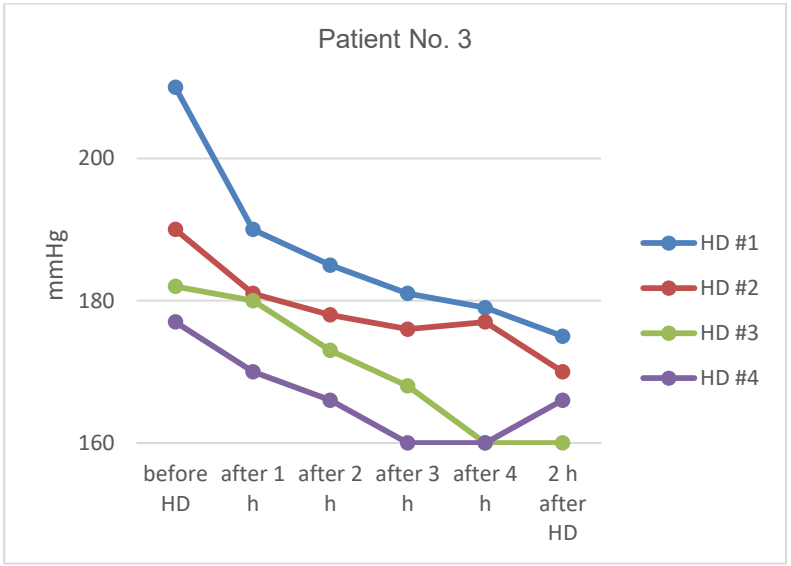

Fig. 3. Graphic representation of BP changes during HD sessions in patient no. 3

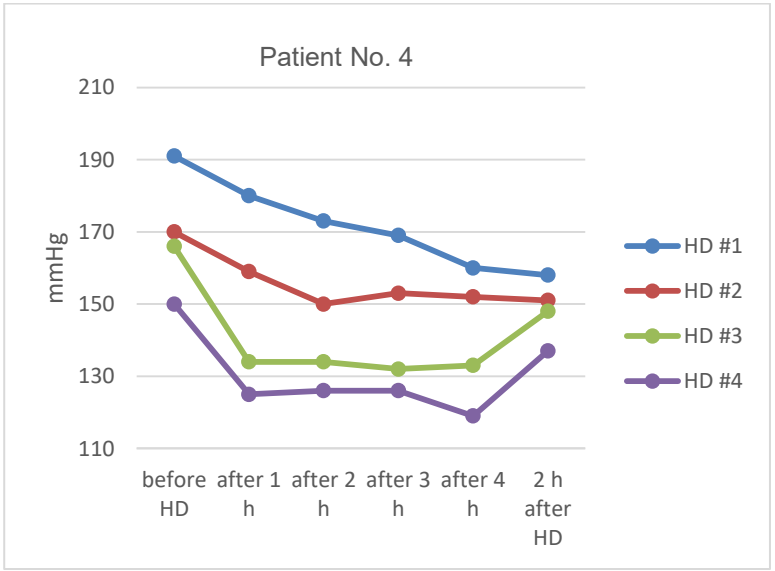

Fig. 4. Graphic representation of BP changes during HD sessions in patient no. 4

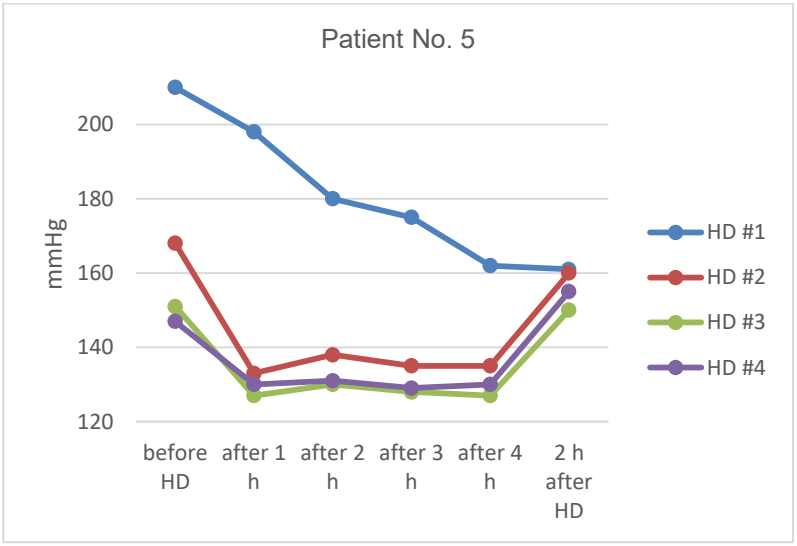

Fig. 5. Graphic representation of BP changes during HD sessions in patient no. 5

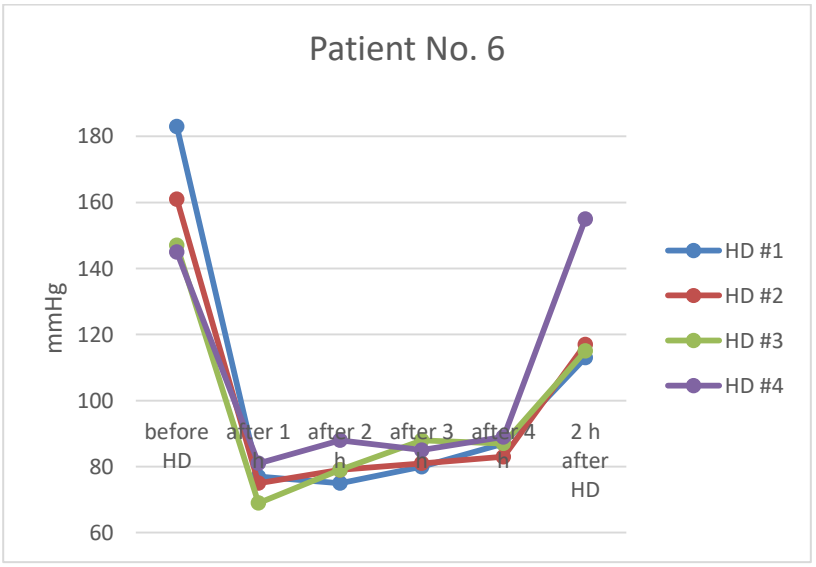

Fig. 6. Graphic representation of BP changes during HD sessions in patient no. 6

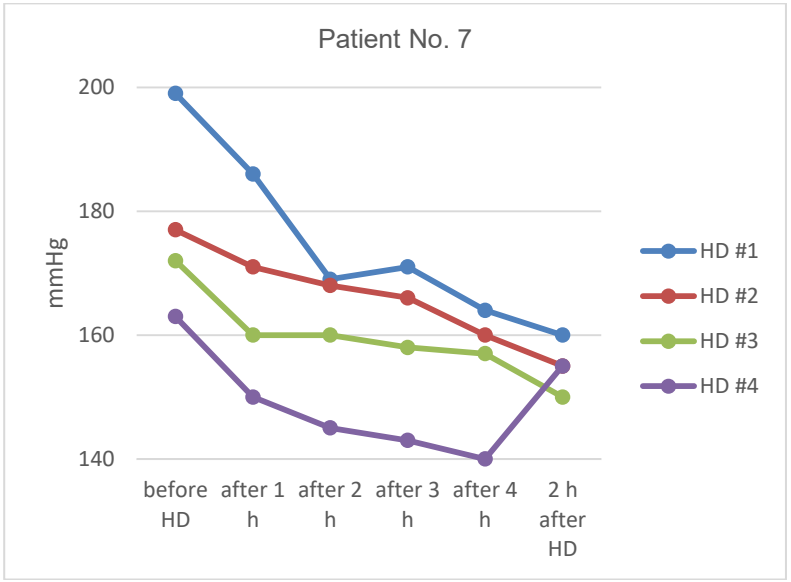

Fig. 7. Graphic representation of BP changes during HD sessions in patient no. 7

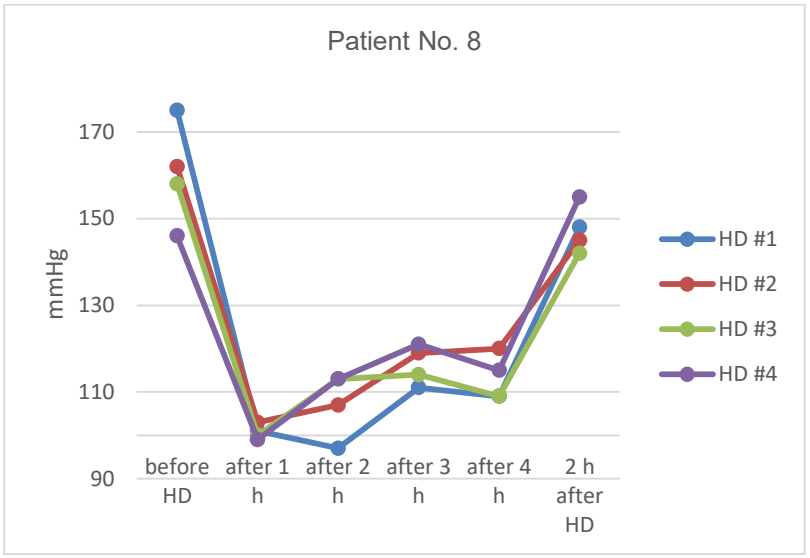

Fig. 8. Graphic representation of BP changes during HD sessions in patient no. 8

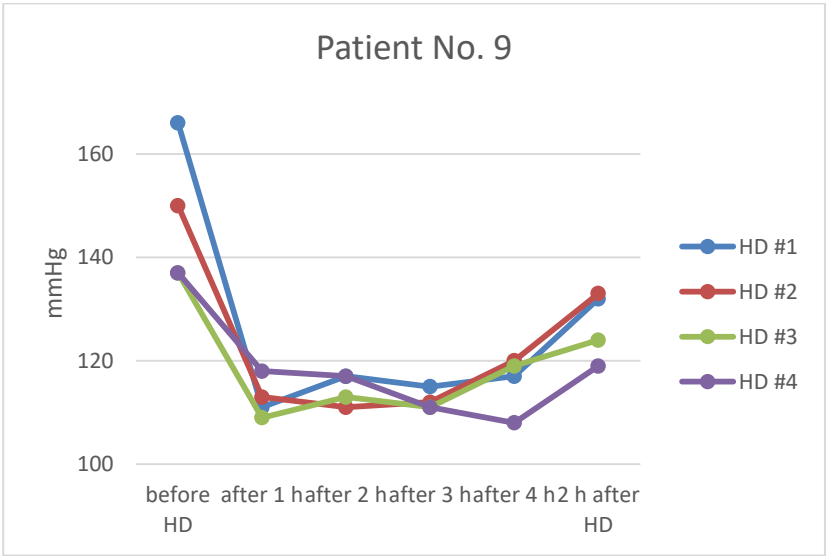

Fig. 9. Graphic representation of BP changes during HD sessions in patient no. 9

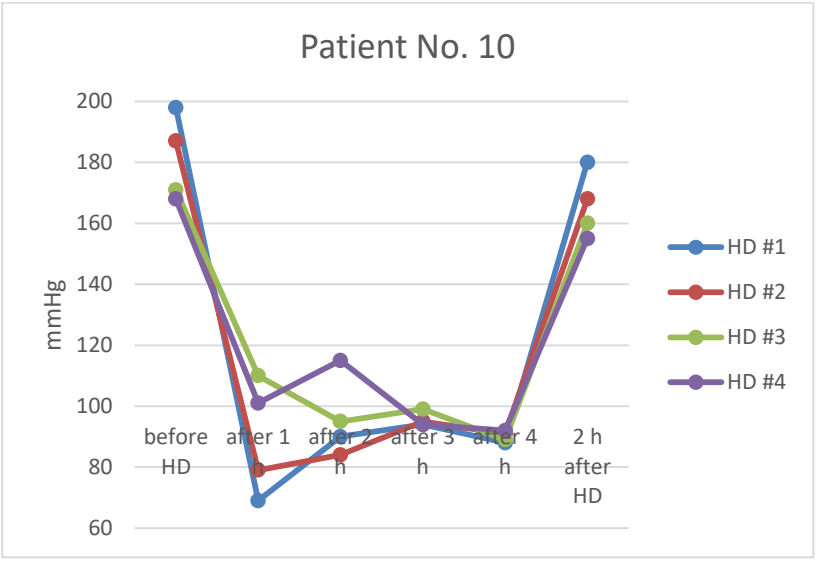

Fig. 10. Graphic representation of BP changes during HD sessions in patient no. 10

Legend:

The vertical axis represents the scale of BP in mmHg.

The horizontal axis represents the time at which the BP was measured (before the HD session, after every hour of the HD session and 2 hours after the end of HD session).

Each trend line is a different colour and each colour represents the number of the HD session (blue for the 1st session, red for the 2nd session, green for the 3rd session and purple for the 4th session).

The dots represent the BP value at a given time during one of the HD sessions (each value was selected from the tables presented above).

The trend lines represent the trend in which BP changes in a patient during a HD session.

All 10 patients experienced a decrease in BP more or less evident during the session of hemodialysis (Fig. 1-10).

From 10 patients, two were hypertensive (p. 8 and p. 9) and eight of them were severely hypertensive (p. 1, p. 2, p. 3, p. 4, p. 5, p. 6, p. 7 and p. 10) at the time of diagnosis (Fig. 1-10).

Of the eight patients that were severely hypertensive at the end of the 4th HD session, two (p. 1 and p. 2) became hypertensive, three (p. 3, p. 7 and p. 10) became pre-hypertensive and three (p. 4, p. 5 and p. 6) became normotensive. One week after discharge, two remained hypertensive (p. 1 and p.2), four remained pre-hypertensive (p. 3, p. 4, p. 7 and p. 10) and two remained normotensive (p. 5 and p. 6).

Of the two patients that were hypertensive (p. 8 and p. 9) at the time of diagnosis, at the end of the 4th HD session, both became normotensive and remained that way one week after discharge.

Patients 6 and 9 became hypotensive during one or more HD sessions.

The lowest changes in BP were observed in patient 1. The lowest difference between the highest BP and lowest BP during a hemodialysis session was observed in the 4th session, where the BP dropped down with a maximum of 5 mmHg from the beginning of the session. The highest difference in this patient was observed during the 3rd HD session, where the BP dropped down 15 mmHg. The average difference of BP during all four HD sessions was 10 mmHg.

The highest changes in BP were observed in patient 10. Its highest BP drop was 129 mmHg during his 1st HD session. The lowest drop in BP was recorded during its 4th HD session at 76 mmHg. The average difference of BP during all four HD sessions was 98.75 mmHg.

The average of BP drops in all patients during all of their HD sessions was 42.85 mmHg.

Conclusions

At the discharge, the acute phase of chronic kidney disease or the acute kidney injury was over and the patients required only renal and hypotensive medication to maintain the renal function.

With a reduction of BP observed in all patients, it is safe to say that hemodialysis has a role in the management of hypertension in dogs with renal function impairment and it can be a complementary therapy to classical hypotensive drugs in those patients.

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PRELIMINARY RESULTS OF THE SERIC CALCIUM VARIATIONS IN CANINE CHRONIC KIDNEY PATIENTS ACCORDING TO ORAL INTAKE OF CALCIUM PHOSPHORUS BINDERS

VIȚĂLARU B.A., ȘTEFĂNESCU A.

Faculty of Veterinary Medicine Bucharest, Splaiul Independenței 105, Bucharest, Romania
E-mail: alina_stefanescu9@yahoo.com

Summary

This study was performed in the Faculty of Veterinary Medicine's Clinic during 12 months (January 2016 - January 2017), on 100 patients with clinical signs of chronic renal disease and calcium levels lower than 7.9 mg/dL. The purpose of this study is to determine the efficacy of oral phosphorus binders on increasing the calcium levels in chronic kidney patients. Four batches of 25 dogs were created: the first batch (no. 1 = 25), received calcium carbonate binders once a day, in the morning with meal; the second batch (no. 2 = 25) twice a day, with meal; the third batch (no. 3 = 25) once a day, in the morning, one hour before meal; the forth batch (no. 4 = 25) twice a day, one hour before meal. All patients received a dose of 100 mg/kg. The results shown that the most important and coherent increase in calcium levels has accomplished in batch number 2 and the poorest results in batch number 3.

Keywords: calcium, hypocalcemia, binders, renal, phosphorus

Chronic kidney disease (CKD) is a common disease in dogs in which severe impairment of renal function gradually develops. During the late stages of CKD many disorders may be observed, including disturbances in calcium and phosphorus metabolism (3, 5, 7).

Most of the body's calcium is found in the skeleton as hydroxyapatite. Serum calcium concentration consists of three fractions: (1) ionized calcium (55%), which is the biologically active form, (2) protein-bound (35%), a storage form generally bound to albumin, and (3) complexed calcium (10%), which is bound to citrate, lactate, bicarbonate, or phosphate in serum. Serum total calcium concentration (including all three fractions) is most commonly measured, but measurement of serum ionized calcium concentration is becoming more readily available in practice settings (2, 7).

Disturbances of serum calcium concentration may occur in renal impairment for several reasons. An acute decrease in glomerular filtration rate (GFR) may lead to an abrupt increase in serum phosphorus concentration, causing a decrease in serum calcium concentration by the law of mass action. The decrease in serum ionized calcium concentration stimulates parathyroid hormone synthesis and release, which act to increase the calcium concentration back to normal. On the other hand, CKD may cause parathyroid hyperplasia which can lead to hypercalcemia (2, 6).

Metabolic acidosis increases the ionized calcium fraction, but more than 50% of dogs with CKD and metabolic acidosis are hypocalcemic (4).

Based on serum ionized calcium concentration, 36% to 56% of dogs with CKD are hypocalcemic, 20% to 55% are normocalcemic, and 9% to 24% are hypercalcemic. Based on serum total calcium concentration, 8% to 19% are hypocalcemic, 60% to 76% are normocalcemic, and 16% to 22% are hypercalcemic. The concordance between serum ionized calcium and serum total calcium concentrations is poor, especially in dogs with CKD (1, 8, 9, 10).

In patients with CKD, hypercalcemia based on total serum calcium concentration usually is mild and associated with normal serum ionized calcium concentration (1).

Metabolic acidosis usually develops as a consequence of the low excretion of acid radicals (protons) and phosphate and sulfate compounds through the kidneys, as well as because of low renal absorption of bicarbonate and a decrease in renal ammoniagenesis. Metabolic acidosis itself can cause anorexia, vomiting, weakness, muscle wasting, weight loss, and malnutrition, and a calcium disturbance that is related to urinary calcium loss, an increase of bone calcium reabsorption, and a deficit in calcitriol synthesis (1, 2, 7, 9, 10).

If serum ionized calcium concentration is low, dextrose is preferred to bicarbonate because alkalemia exacerbates hypocalcemia (4).

Calcium administration increases the risk of soft tissue mineralization if hyperphosphatemia is present (2).

In canine patients with hyperphosphatemia, calcium is usually administered with meals to improve phosphate binding (4).

The aim of the study is to determine the efficacy of oral phosphorus binders on increasing the calcium levels in chronic kidney patients, and to establish if long term calcium based phosphorus binders affects the values of serum calcium in dogs with chronic kidney disease.

Materials and methods

The present study was conducted over one-year (2016-2017) in the Dialysis Center in the Faculty of Veterinary Medicine Bucharest Romania, on 100, both males (n=50) and females (n=50) dogs diagnosed with chronic kidney disease in different stages. Patients belonged to eight different races (German Shepherd, Golden Retriever, Siberian Husky, Poodle, Shi-tzu, Chow-Chow, Caniche and Mixed breed) and had ages between 4 and 10 years old. All 100 were random divided into four batches of 25 patients receiving calcium carbonate binders but with 4 different administration protocols during two years. Every batch was randomly created and it was mixed, having both males and females from all eight races. The first batch (no. 1 = 25), received calcium carbonate binders once a day, in the morning with meal; the second batch (no. 2 = 25) received calcium carbonate binders twice a day, with meal; the third batch (no. 3 = 25) received calcium carbonate binders once a day, in the morning, one hour before meal; the forth batch (no. 4 = 25) received calcium carbonate binders twice a day, one hour before meal. All patients received a dose of 100 mg/kg of calcium carbonate binders. Serum calcium normal range values are noted between 7.9 - 12.0 mg/dl. None of these

patients were given supplements or other treatments involving calcium.

Serum calcium values were determined each month for 12 months, in all four batches. Therefore, we could monitor the patient's status better and, if needed, stop the therapy.

Results and discussions

After the administration of phosphorus binders according to the four different protocols, we obtained the following results.

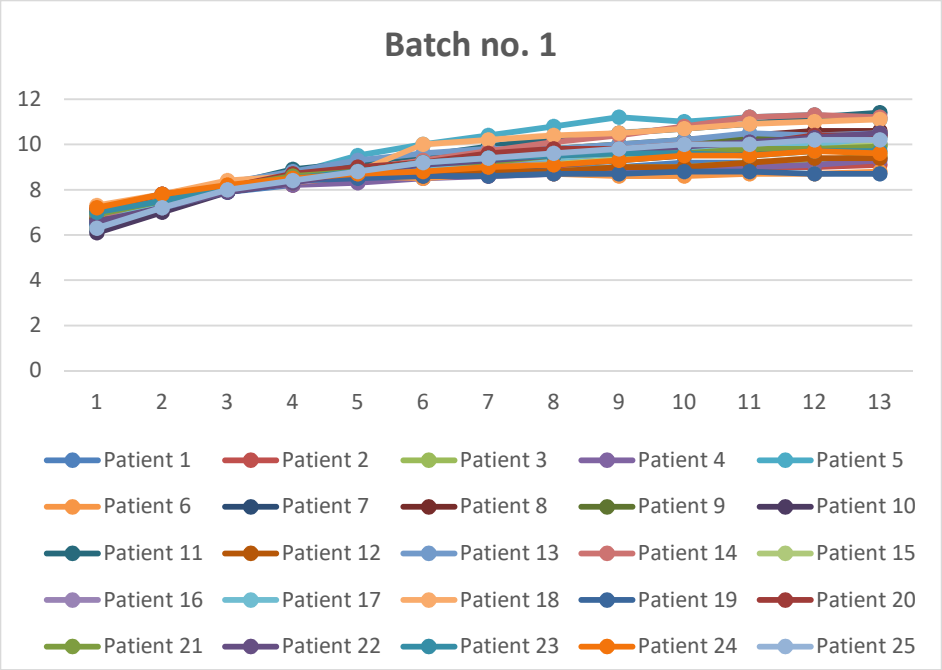

Fig. 1. Representation of the first batch (no. 1 = 25), with calcium carbonate binders administered once a day, in the morning with meal

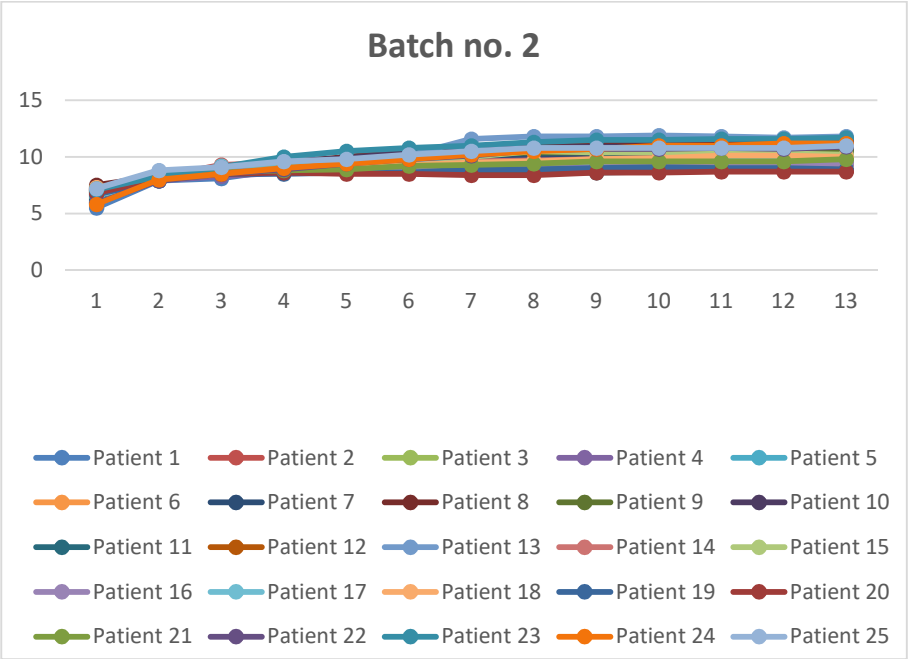

Fig. 2. Representation of the second batch (no. 2 = 25), with calcium carbonate binders administered twice a day, with meal

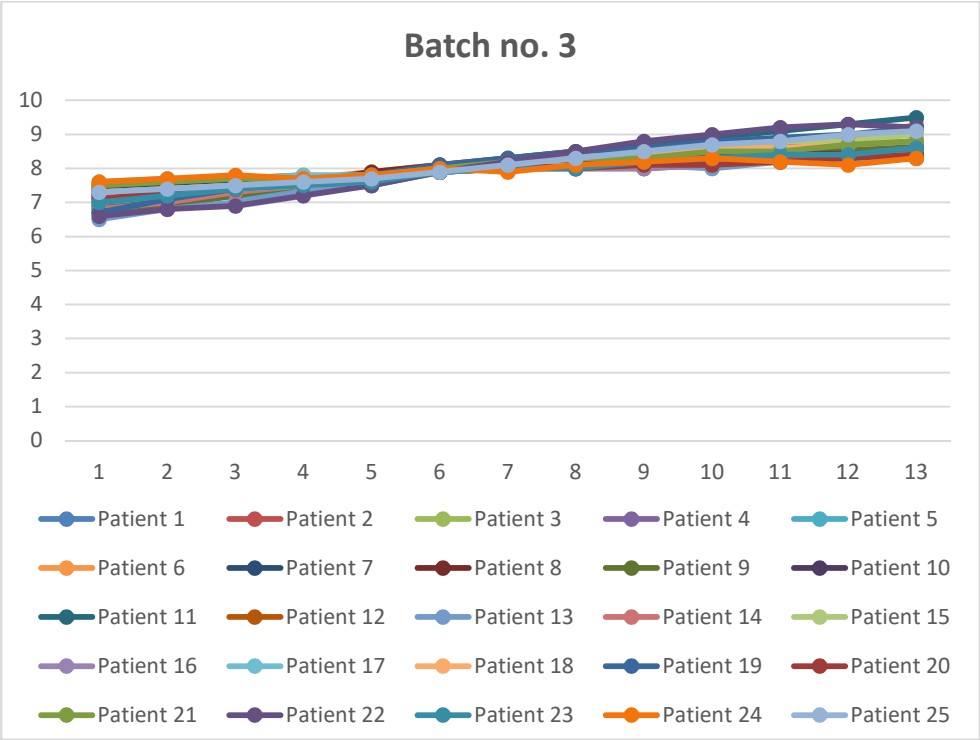

Fig 3. Representation of the third batch (no. 3 = 25), with calcium carbonate binders administered once a day, in the morning, one hour before meal

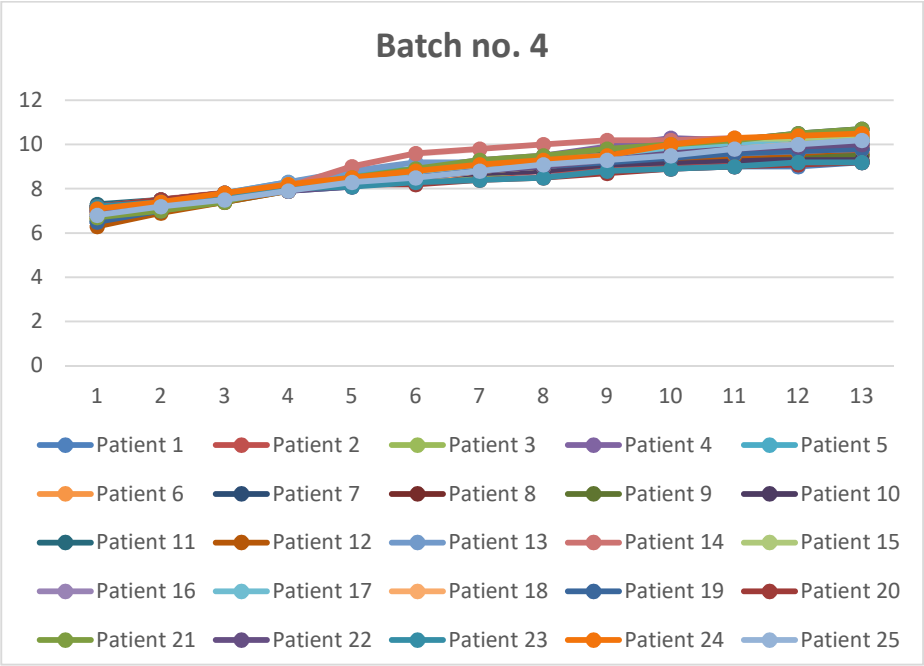

Fig 4. Representation of the fourth batch (no. 4 = 25), with calcium carbonate binders administered twice a day, one hour before meal

Legend:
OY ax – represents the serum calcium value;
OX ax – represents the period of time.

In the first batch (no. 1 = 25), after the serum calcium evaluation, patients had normal values (7.9 - 12.0 mg/dl.), from the 2nd month (Fig. 1).

In the second batch (no. 2 = 25), after the serum calcium evaluation, patients had normal values (7.9 - 12.0 mg/dl.), from the 1st month (Fig. 2).

In the third batch (no. 3 = 25), after the serum calcium evaluation, patients had normal values (7.9 - 12.0 mg/dl.), from the 5th month (Fig. 3).

In the fourth batch (no. 4 = 25), after the serum calcium evaluation, patients had normal values (7.9 - 12.0 mg/dl.), from the 3rd month (Fig. 4).

The results shown that the most important and coherent increase in calcium levels has been accomplished in batch number two in which patients received calcium carbonate binders twice a day, with meal; and the poorest results in batch number three in which patients received calcium carbonate binders once a day, in the morning, one hour before meal.

The results of this study are partial, serum calcium was monitored and determined during 12 months in each patient. The evolution of calcium values with

the same dose (100 mg/kg) was not yet determined for a longer period of time, and the influence of the calcium based binders on serum calcium is yet not known for more than 12 months.

Conclusions

The orally administration of calcium based phosphorus binders in patients diagnosed with chronic kidney disease is essential.

In the management of chronic kidney disease, in order to achieve the best results, calcium based oral phosphorus binders must be administered twice a day with meal.

If the calcium based phosphorus binders are administrated before meal, the influence upon the increase of calcium levels is poor.

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PRELIMINARY RESULTS OF THE SERIC PHOSPHORUS VARIATIONS IN CANINE CHRONIC KIDNEY PATIENTS ACCORDING TO ORAL INTAKE OF CALCIUM PHOSPHORUS BINDERS

VIȚĂLARU B.A., ȘTEFĂNESCU A.

Faculty of Veterinary Medicine Bucharest, Splaiul Independenței 105, Bucharest, Romania
E-mail: alina_stefanescu9@yahoo.com

Summary

Hyperphosphatemia is among the most common metabolic complications of renal patients. This study was performed in the Faculty of Veterinary Medicine's Clinic during 12 months (January 2016 - January 2017), on 100 patients who presented clinical signs of chronic renal disease and phosphorus level higher than 6.8 mg/dL. The purpose of this study was to determine the efficacy of oral phosphorus binders on decreasing the phosphorus levels in chronic kidney patients. Four batches of 25 patients were created: the first batch (no. 1 = 25), received calcium carbonate binders once a day, in the morning with meal; the second batch (no. 2 = 25) twice a day, with meal; the third batch (no. 3 = 25) once a day, in the morning, one hour before meal; the forth batch (no. 4 = 25) twice a day, one hour before meal. All patients received a dose of 100 mg/kg. The results shown that the most important and coherent reduction in phosphorus levels has accomplished in batch number 2 and the poorest results in batch number 3.

Keywords: phosphorus, binders, calcium, renal, chronic

Hyperphosphatemia is a universal complication of chronic kidney disease and it is accompanied by hypocalcemia and low serum levels of vitamin D. Without any treatment, this deficiency usually leads to severe secondary hyperparathyroidism, which in turn may lead to painful fractures and generalized osteopenia. Dietary restriction of phosphate has long been the cornerstone of therapy, but this measure is usually not sufficient to control hyperphosphatemia (11, 12).

The kidney normally filters large amounts of inorganic phosphorus and then reabsorbs >90% of this load in the tubules, so that excretion is less than 10% of the filtered load. Early kidney dysfunction and reduced glomerular filtration decreases the filtered load of phosphorus, but tubule reabsorption of phosphorus also decreases so that urinary phosphorus excretion continues to match gastrointestinal absorption. This compensating reaction is largely because of secondary hyperparathyroidism – increased parathyroid hormone (PTH) levels marked reduce renal tubule phosphorus reabsorption. Equality between phosphorus input and output, with only slight changes in serum inorganic phosphorus concentration, may be maintained for a period of time. However, as renal function deteriorates further, homeostatic mechanisms fail, phosphorus

balance becomes positive, and progressive hyperphosphatemia usually develops. As a result, oral phosphate binders are used in over 90% of patients with kidney failure. Hyperphosphatemia is associated with significant pathophysiology in chronic kidney disease (CKD). This pathophysiology contributes to the high rates of mortality observed in CKD (1, 2, 4, 9, 10).

A four-hour hemodialysis treatment may remove about 1000 mg of phosphorus, but this is generally inadequate to restore normal phosphorus levels and phosphorus balance (with the exception of slow, daily, and/or nocturnal hemodialysis, which removes much greater quantities of phosphorus). Therefore, reestablishment of normal phosphorus levels and balance requires a major reduction in phosphorus absorption. This can only be partially accomplished by dietary restriction because most foods contain abundant amounts of phosphorus. Consequently, various phosphorus binding compounds are usually required to normalize phosphorus levels and balance (3, 4, 7).

The history of phosphorus binders can be generally divided into three overlapping eras. The first began in the early 1970s, when the importance of phosphorus control was first emphasized, and was characterized by the pervasive use of alkaline aluminum salts. This continued until the early 1980s, when the toxicity of aluminum became widely recognized. The second, the era of calcium salts, started in the early 1980s and has continued through the present time. We are currently in the third phosphorus binder era, represented by introduction of nonmetallic phosphorus binding resins and other novel agents (1, 4).

If the main effect of phosphorus binders is on dietary phosphorus, binders would presumably be more effective if ingested with meals. If, however, their main effect is to trap endogenous phosphorus, then the timing of ingestion may be less critical. If altering the schedule of administration can improve the efficacy of phosphorus binders, lower doses can be used and toxicity due to absorption of binders' cations can be minimized (4, 12, 13).

Calcium salts such as calcium carbonate are used as phosphorus binders. Calcium carbonate decreases intestinal phosphorus absorption in normal and uremic patients. Nausea, constipation, and hypercalcemia are potential side effects of calcium-containing phosphate binders. Phosphorus binders are most effective when given with meals. Ingestion of a meal also decreases the absorption of calcium from the calcium binder (5, 6, 7, 8, 13).

All patients who are part of this study, are chronic kidney patients, not submitted to hemodialysis, that have a calcium:phosphorus ratio lower or equal to 1:2. In all patients, the purpose of phosphorus binders administration was to maintain the calcium:phosphorus ratio to 1:2 or/even 1:3.

Materials and methods

The present study was conducted over two years (2018-2019) in the Dialysis Center in the Faculty of Veterinary Medicine Bucharest Romania, on 100,

both males (n=50) and females (n=50) patients diagnosed with chronic kidney disease in different stages.

All 100 patients were random divided into four batches of 25 patients receiving calcium carbonate binders but with four different administration protocols during two years.

The first batch (no. 1 = 25), received calcium carbonate binders once a day, in the morning with meal; the second batch (no. 2 = 25) received calcium carbonate binders twice a day, with meal; the third batch (no. 3 = 25) received calcium carbonate binders once a day, in the morning, one hour before meal; the forth batch (no. 4 = 25) received calcium carbonate binders twice a day, one hour before meal.

All patients received a dose of 100 mg/kg.

Serum phosphorus normal range values are noted between 2.5-6.8 mg/dl.

None of these patients were intoxicated with organophosphates, and none of these patients were given supplements or other treatments involving phosphorus

Results and discussions

After the administration of phosphorus binders according to the four different protocols, we obtained the following results:

In the first batch (no. 1 = 25), after the serum phosphorus evaluation, patients had normal values (2.5-6.8 mg/dl.), from the 2nd month (Fig. 1).

In the second batch (no. 2 = 25), after the serum phosphorus evaluation, patients had normal values (2.5-6.8 mg/dl.), from the 1st month (Fig. 2).

In the third batch (no. 3 = 25), after the serum phosphorus evaluation, patients had normal values (2.5-6.8 mg/dl.), from the 5th month (Fig. 3).

In the fourth batch (no. 4 = 25), after the serum phosphorus evaluation, patients had normal values (2.5-6.8 mg/dl.), from the 3rd month (Fig. 4).

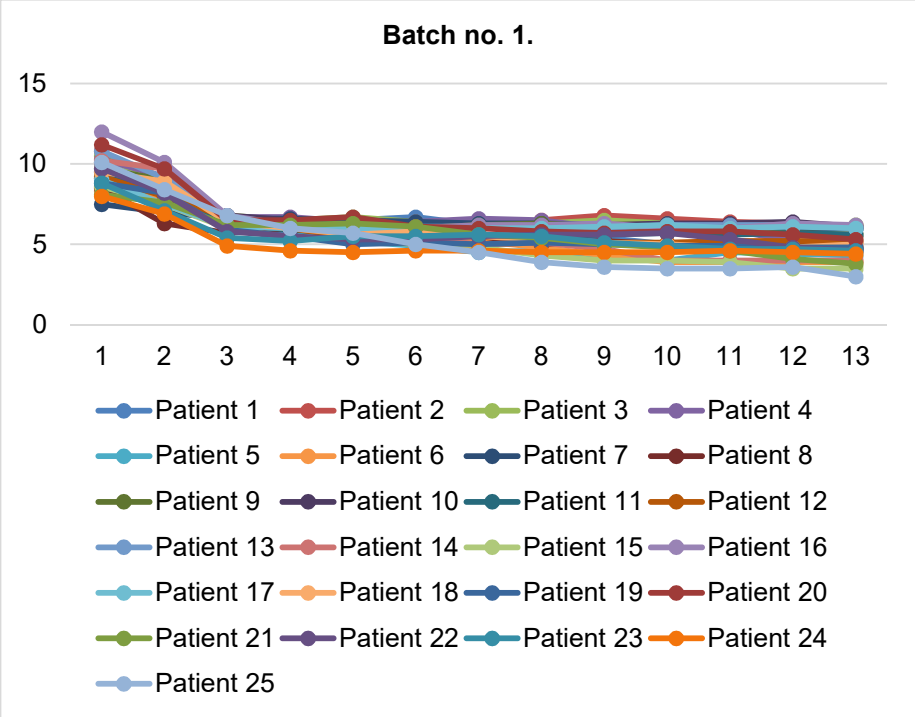

Fig. 1. Representation of the first batch (no. 1 = 25), in which patients received calcium carbonate binders once a day, in the morning with meal

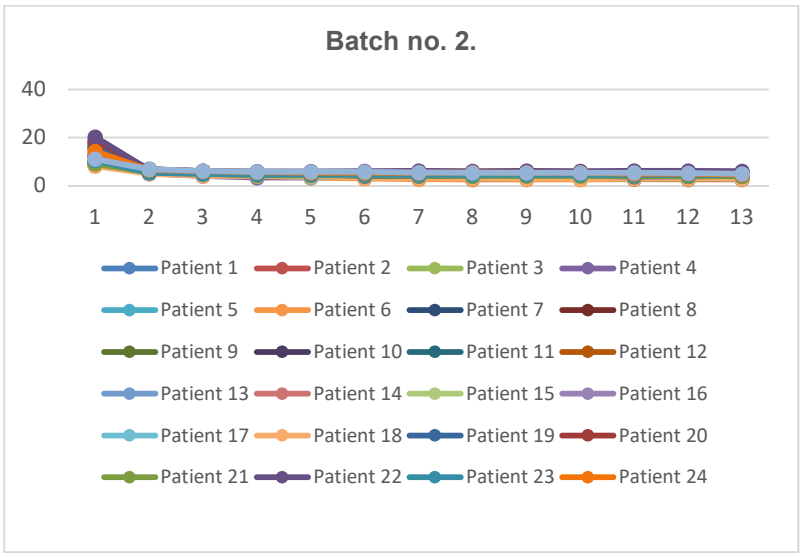

Fig. 2. Representation of the second batch (no. 2 = 25), in which patients received calcium carbonate binders twice a day, with meal

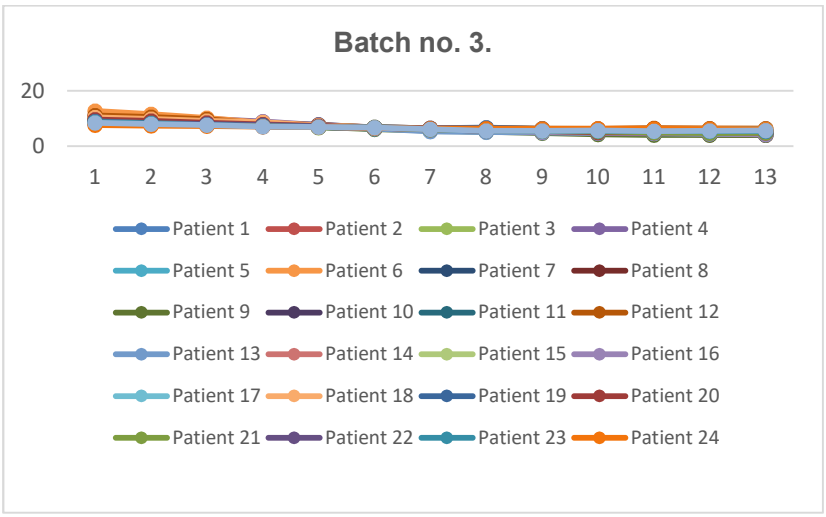

Fig. 3. Representation of the third batch (no. 3 = 25), in which patients received calcium carbonate binders once a day, in the morning, one hour before meal

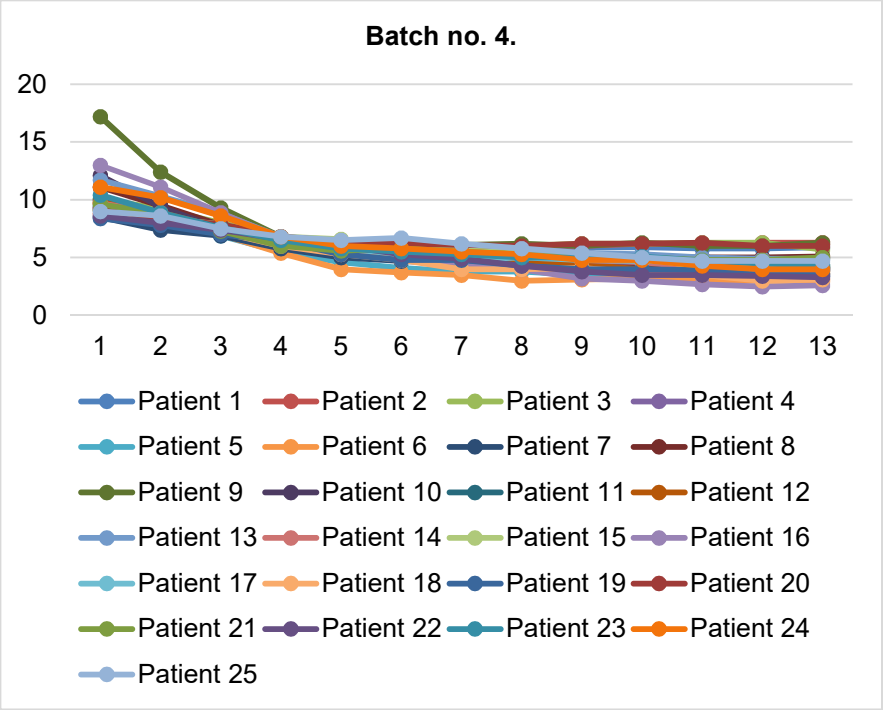

Fig. 4. Representation of the fourth batch (no. 4 = 25), in which patients received calcium carbonate binders twice a day, one hour before meal

Legend:
OY ax – represents the serum calcium value;
OX ax – represents the period of time.

The results shown that the most important and coherent reduction in phosphorus levels has been accomplished in batch number two in which patients received calcium carbonate binders twice a day, with meal; and the poorest results in batch number three in which patients received calcium carbonate binders once a day, in the morning, one hour before meal.

The results of this study are partial; serum phosphorus was monitored and determined during 12 months for each patient. The evolution of phosphorus values with the same dose (100 mg/kg) was not yet determined for a longer period of time, and the influence of the calcium based binders on serum calcium is yet not known for more than 12 months.

Conclusions

The orally administration of calcium based phosphorus binders in patients diagnosed with chronic kidney disease is essential.

In the management of chronic kidney disease, in order to achieve the best results, calcium based oral phosphorus binders must be administered twice a day with meal.

If the calcium based phosphorus binders are administrated before meal, the influence upon the increase of calcium levels is poor.

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RESEARCH REGARDING THE EFFECTS OF THE DIALYZER ON NEUTROPHILS FOR DOGS WITH KIDNEY INJURY UNDERGOING HEMODIALYSIS

VIȚĂLARU B.A., ȘTEFĂNESCU A., ASANDEI A., PETRE T.G.

University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Veterinary Medicine, 050097, Independentei Street No 105, District 5, Bucharest, Romania
E-mail: alina_stefanescu9@yahoo.com

Summary

Haemodialysis (IHD) is a renal replacement treatment that is defined by short, efficient haemodialysis sessions with the goal of removing endogenous or exogenous toxins from the bloodstream. The neutropenia occurring at the initiation of haemodialysis is generally thought to result from activation of complement cascade, generated by the contact of plasma with the dialyser membranes, which subsequently promotes aggregation and sequestration of granulocytes in the pulmonary bed. The aim of this study is to determine if the neutropenia is consistent after the haemodialysis session. A dramatic transient and rapidly reversible neutropenia occurs during the first minutes of treatment in patients undergoing haemodialysis. The neutropenia is followed by an increase in the number of band neutrophils and the return to near normal circulating leukocyte levels after dialysis session.

Keywords: neutrophils, haemodialysis, dialyzer, dog, kidney

Haemodialysis (IHD) is a renal replacement treatment that is defined by short, efficient haemodialysis sessions with the goal of removing endogenous or exogenous toxins from the bloodstream. Common indications for IHD include drug or toxin ingestion, acute or acute-on-chronic kidney injury, and chronic kidney disease (CKD) (1).

In veterinary medicine, dialysis is used most commonly for acute kidney injury (AKI), and is usually reserved until standard medical management has been attempted. Dialysis is a method of treating kidney disease and certain types of toxicities, and includes haemodialysis (HD) and peritoneal dialysis (PD). During the extracorporeal treatment, blood is transported out of the body and through the artificial tubes and capillaries of the machine, subjecting the components of the blood to a different environment (5).

The main forces used during HD are diffusion, convection, and adsorption. The magnitude of exchange of fluids and solutes is determined by the characteristics of the solute as well as the pore size and structural characteristics of the dialyzer membrane. Diffusion is best at removing molecules with low molecular weight (<500 Da) from the blood, including blood urea nitrogen (BUN) and creatinine, sodium, potassium, phosphorus, and magnesium. Convective modalities allow for the removal of small and medium-sized molecules, whereas diffusive modalities are limited to smaller molecules. Adsorption is saturable and therefore plays only a minor

role in clearance unless the filter is changed more frequently than every 18 to 24 hours (1, 2).

Haemodialysis impairs neutrophil function and may contribute to the increased risk of infection in uremic patients (3).

The ability of different dialysis membranes to activate polymorphonuclear neutrophil oxygen radical production was investigated with chemiluminescence, all six membranes were able to interact with neutrophils and stimulate their oxygen radical production (6).

A severe, transient granulocytopenia occurs shortly after the beginning of haemodialysis in the dog, as has been previously shown in man (4).

In blood, the five major categories of leukocytes are neutrophils, eosinophils, basophils, lymphocytes and monocytes. Neutrophils represents the majority of the leukocytes and are a part of the innate immune system, the first line of defense against bacterial infections. Release of neutrophils from the margination pool occurs in response to corticosteroids, which downregulate adhesion molecules responsible for margination (or patrolling of the endothelium via selectin-mediated rolling) and has the potential to double the mature neutrophil count. The earliest identifiable specific neutrophil precursor is a myelocyte, which differentiates into a metamyelocyte, then a band neutrophil, and finally to a mature segmented neutrophil (2).

Materials and methods

The present study was conducted on 10 dogs belonging of different breeds, that were treated in the Faculty of Veterinary Medicine Bucharest (Labrador Retriever, Shar-Pei, Golden Retriever, White Swiss Shepherd, Beagle). The patients age ranged between four and 13 years. All dogs underwent three haemodialysis treatment sessions and none of them received any steroid drugs. In order to observe and determine the changes made to the number and the morphology of the neutrophils in circulation, blood was drawn twice from the central venous catheter (CVC) of the patients undergoing dialysis treatment - once before and once after the haemodialysis session. The blood was collected into complete blood count (CBC) tubes containing ethylenediaminetetraacetic acid (EDTA). For the structural and morphological aspects of the cells, an optical microscope was used as well as blood smears coloured with May Grunwald Giemsa method.

Results and discussions

The values presented in the tables consist of a mean numeric value of the neutrophils identified in ten microscopic fields for each patient (Tab. 1, 2).

Table 1

Neutrophil values before and after the dialysis treatment for patients 1-5

Time of analysis	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Before 1st session	7.9	6.4	5.4	12.3	8.4
After 1st session	6.1	5.4	4.3	11.6	7.6
Before 2nd session	6.8	6.3	5.6	12.5	5.9
After 2nd session	5.3	5.2	4.9	11.4	4.3
Before 3rd session	5.7	6.7	4.7	11.7	5.6
After 3rd session	4.5	5.8	3.9	10.3	4.9

After studying the neutrophil values before and after each haemodialysis sessions for the 10 patients, we can see an overall decrease of the neutrophil values. This could be explained by the nature of the haemodialysis technique. By its very principle, it exposes the blood plasma and cells to a controlled, yet artificial environment (Tab. 1, 2).

Table 2

Neutrophil values before and after the dialysis treatment for patients 6-10

Time of analysis	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Before 1st session	5.4	3.2	4.1	7.5	7.3
After 1st session	4.5	2.8	3.9	4.3	6.9
Before 2nd session	3.5	4.2	-	6.8	5.7
After 2nd session	2.4	3.8	-	5.7	4.6
Before 3rd session	5.2	5.0	-	6.5	6.7
After 3rd session	4.5	4.8	-	4.9	5.4

Patient 1: Labrador Retriever, F, 8 years old, Dirofilariasis positive.

Patient's neutrophil count slightly decreases during each treatment session, final value being approximately 4/7 (57%) of the original (Fig. 1).

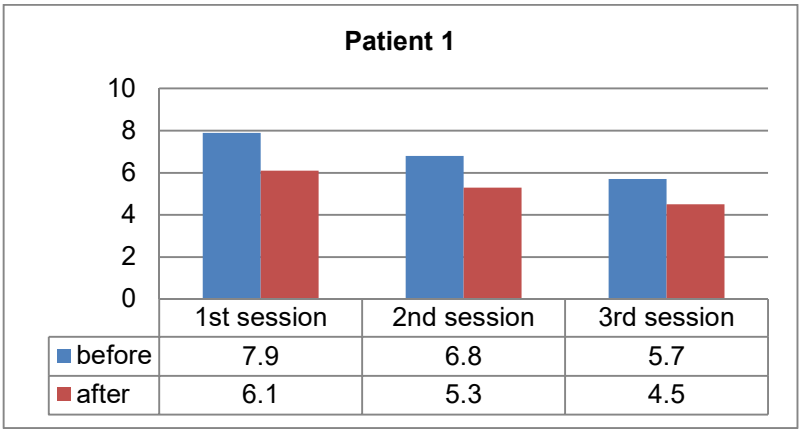


Fig. 1. Graphic representation of the neutrophil count during the treatment of patient 1

Patient 2: Labrador Retriever, M, 10 years old, Dirofilariasis positive.

Patient's neutrophil count temporarily decreases after every treatment, but the value keeps to the same levels (Fig. 2).

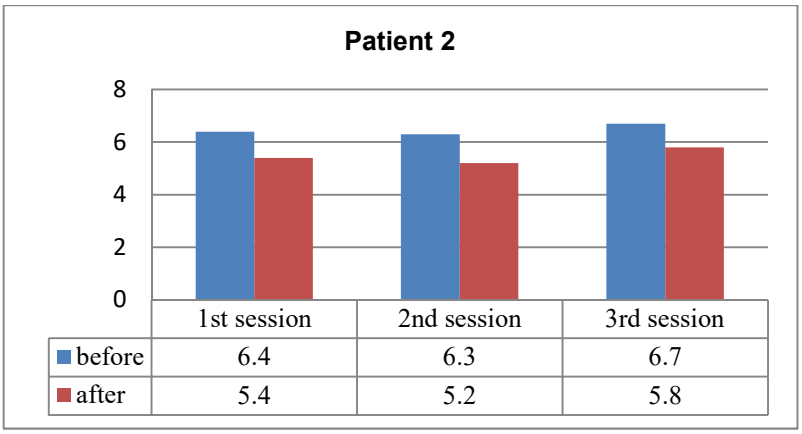


Fig. 2. Graphic representation of the neutrophil count during the treatment of patient 2

Patient 3: Labrador Retriever, F, 10 years old, NSAID intoxication.

Patient's neutrophil count temporarily decreases after each treatment; the value remains rather constant until the last session that brings a lower value (Fig. 3).

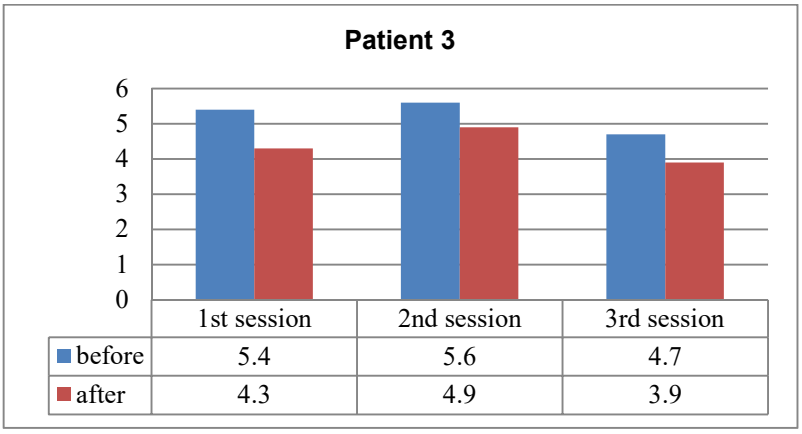


Fig. 3. Graphic representation of the neutrophil count during the treatment of patient 3

Patient 4: Shar Pei, M, 7 years old, Babesiosis, positive.

The patient starts the treatment with a high neutrophil count that persists during the hospitalization but each post treatment analysis shows a slight decrease in numbers that eventually is corrected by the organism's response (Fig. 4).

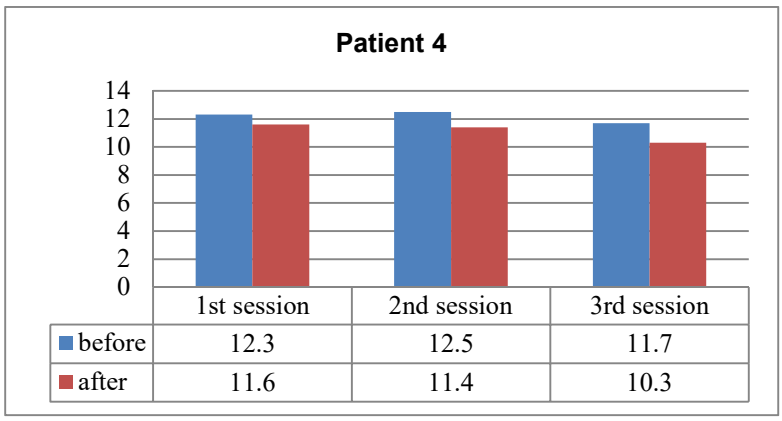


Fig. 4. Graphic representation of the neutrophil count during the treatment of patient 4

Patient 5: Golden Retriever, F, 6 years old, Babesiosis positive.

The patient presents a decrease of numbers during each session and a significant decrease during the entire hospitalisation period, the final measured value being close to a half of the original one (Fig. 5).

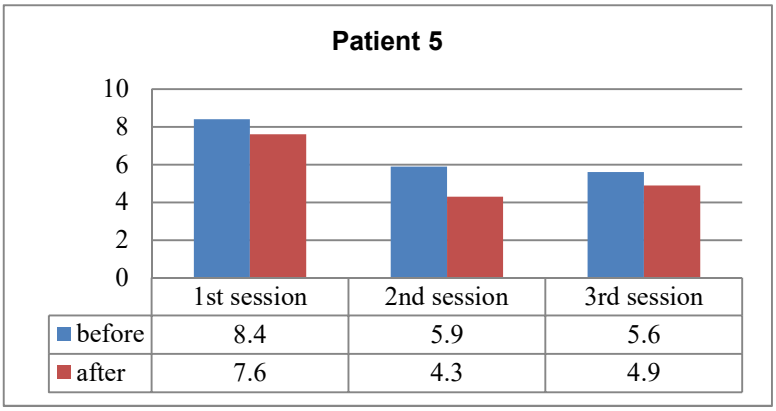
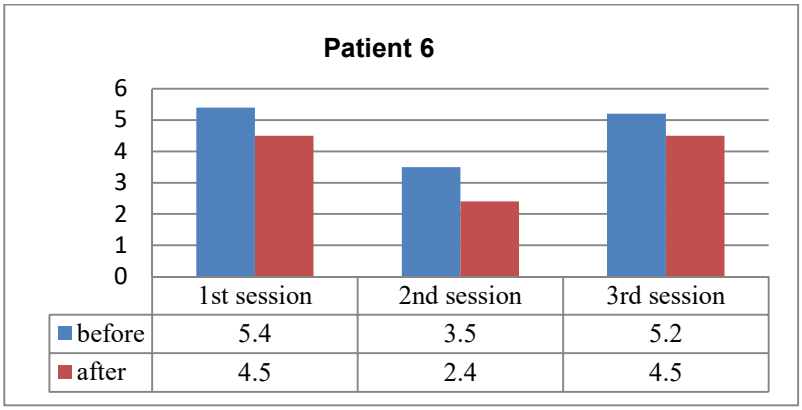


Fig. 5. Graphic representation of the neutrophil count during the treatment of patient 5

Patient 6: White Swiss Shepherd, F, 7 years old, antibiotics intoxication, exitus.

Patient shows a decrease in neutrophil numbers during each session. The second session appears to present lower values that are eventually compensated during the final part of the monitoring period (Fig. 6).

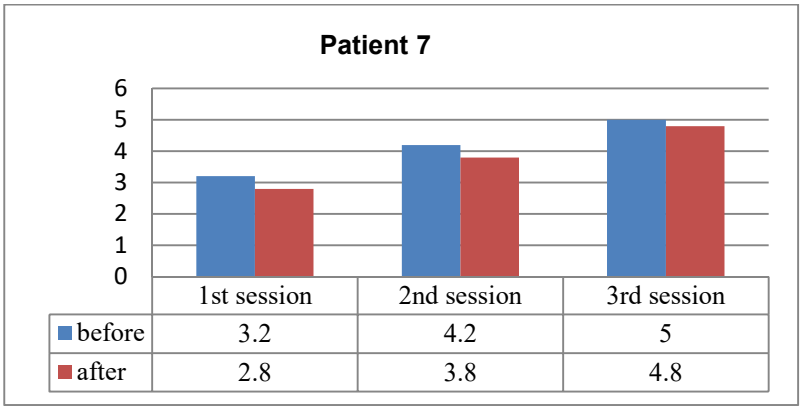


	1st session	2nd session	3rd session
before	5.4	3.5	5.2
after	4.5	2.4	4.5

Fig. 6. Graphic representation of the neutrophil count during the treatment of patient 6

Patient 7: Shar Pei, F, 7 years old, Babesiosis, positive evolution.

The patient shows and overall increase of neutrophil numbers during the hospitalisation and therapy period, while being consistent with the decrease pattern during the individual session time (Fig. 7).



	1st session	2nd session	3rd session
before	3.2	4.2	5
after	2.8	3.8	4.8

Fig. 7. Graphic representation of the neutrophil count during the treatment of patient 7

Patient 8: Cross Breed, M, 4 years old, ethylene glycol intoxication, negative evolution - exitus.

The patient shows a decrease in neutrophil numbers during the first session. The patient passed before the second session (Fig. 8).

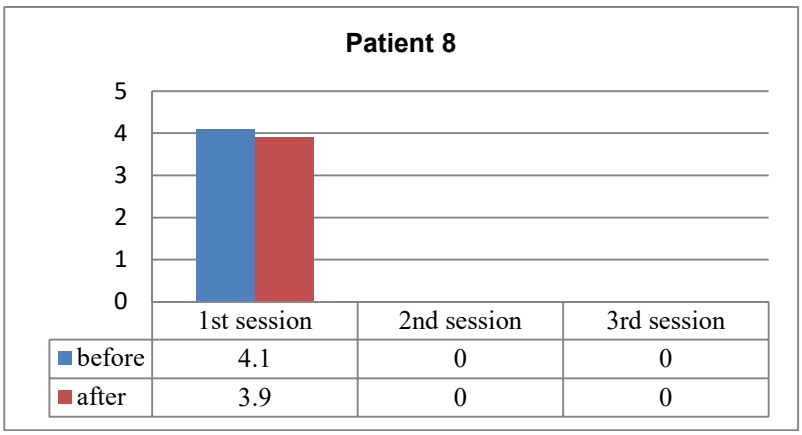


Fig. 8. Graphic representation of the neutrophil count during the treatment of patient 8

Patient 9: Labrador Retriever, M, 6 years old, Ehrlichiosis positive.

Patient shows a drastic decrease after the first session, that seems to be corrected after. The decrease taking place during session time is present (Fig. 9).

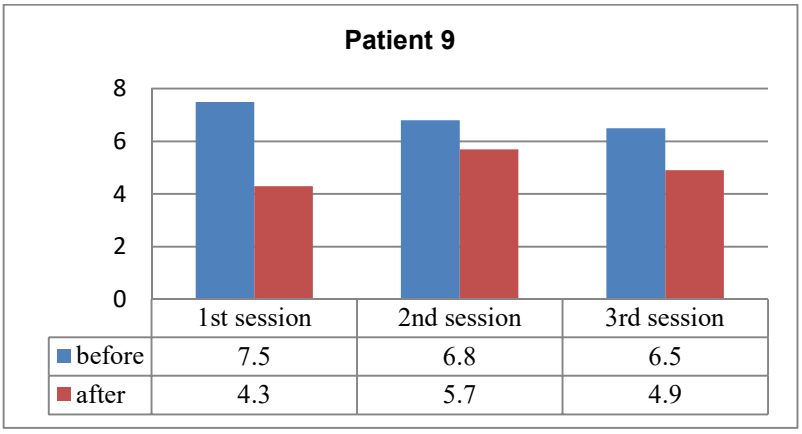


Fig. 9. Graphic representation of the neutrophil count during the treatment of patient 9

Patient 10: Beagle, M, 13 years old, Ehrlichiosis, positive evolution.

The values remain rather normal during the hospitalisation. The same decrease pattern can be observed during each treatment session, the first one being rather mild, the process being intensified in the two following sessions (Fig. 10).

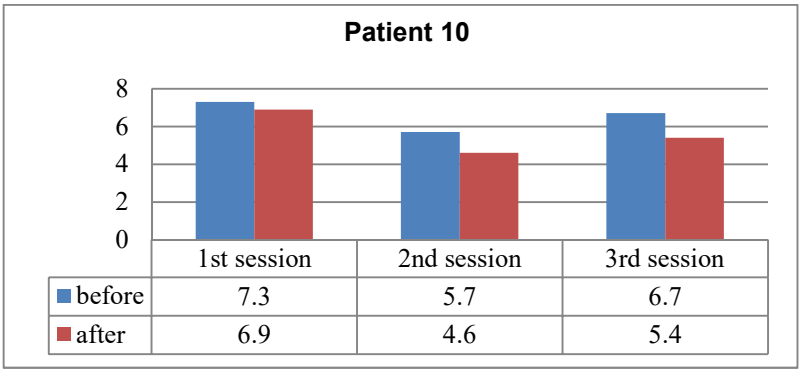


Fig. 10. Graphic representation of the neutrophil count during the treatment of patient 10

The values of all patients can be observed from a statistical perspective. It might seem as though the numeric means of neutrophils remain rather constant from one session to the other. The values are decreasing after the treatment, and then increasing almost to the original level in the time between each session. Those observations account for all values and do not take into consideration the individual changes.

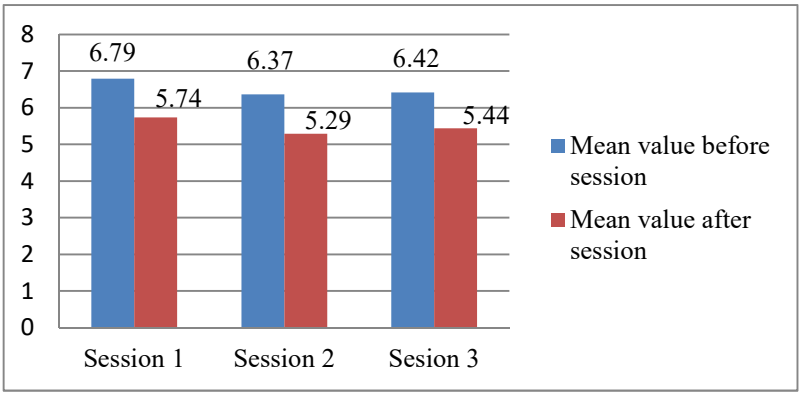


Fig. 11. Evolution of the mean values before and after dialysis for each session of treatment

Figure 11 shows the general tendency of the cellular losses during the dialysis treatment for the 10 patients observed. By taking into account the mean value of the cell counts before dialysis for all the patients, and that of the cell counts after, we can see how this treatment induces a decrease in the count.

Considering that the decrease in numbers seems to take place during the dialysis session, we can try to observe the gap taking into account a blood analysis using a sample taken 10 minutes into therapy. The analysis, performed on five selected patients from the 10 that were under observation, shows a drastic decrease in the neutrophil count, comparing the values to those before and after the actual treatment session.

Those numbers could suggest that the passage of blood outside the natural medium modifies the immune response of the organism.

The numbers start increasing after that critical point, which suggests that the organism responds to the apparent neutropenia caused by the extracorporeal passage, depending on one's individual immune competence.

The values of the neutrophil count analysed 10 minutes after the start of the treatment can be observed in Table 3.

Table 3

Neutrophil values after 10 minutes in the first dialysis session treatment for 5 patients

Time of analysis	Patient 1	Patient 4	Patient 6	Patient 9	Patient 10
After 10 minutes	3.4	4.3	2.1	2.9	2.3

These changes can be observed in relation to one another in Figure 12, which shows the decrease, lowest point, and then increase of neutrophils back to a more normal value.

Cytomorphological changes of neutrophils after dialysis sessions highlight a left shift. When the need for neutrophils is greater than the bone marrow can supply, band neutrophils are produced. Consequently, the patient responds dramatically to the immune suppression.

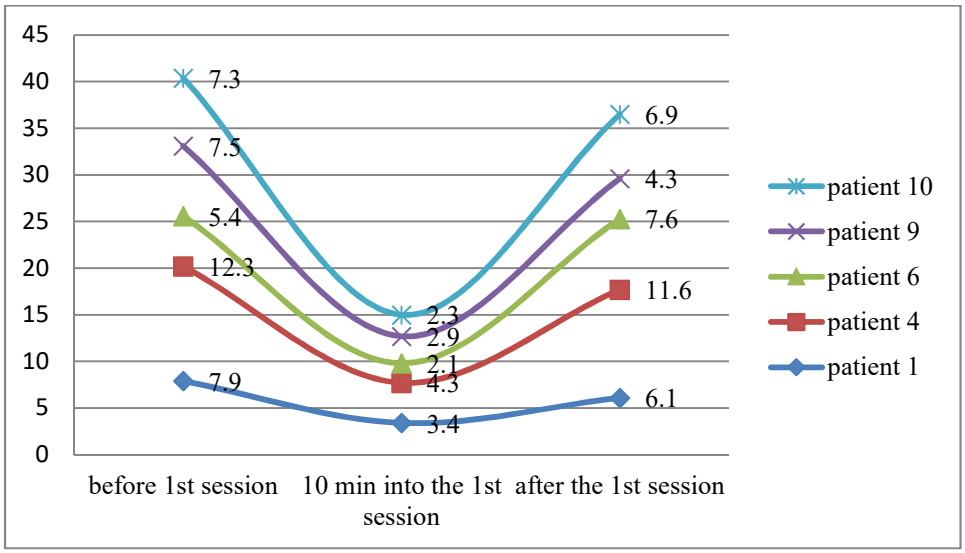


Fig. 12. Comparison between different stages of treatment session, observing the dramatic neutropenia

Conclusions

The decrease of neutrophil counts observed in the analysis performed during the dialysis treatment shows a constant pattern of quick decrease in immune response capabilities due to lack of cell numbers. The defect is quickly compensated by the organism's response. The phenomenon is thought to be of immune response origin, responding to the extracorporeal treatment conditions. Considering the presence of band neutrophils after the treatment, the organism clearly responds by releasing young immune cells into circulation, hence compensating the ones that have been lost during the treatment as result of non-specific responses, including adhesion and aggregation. The value differences in between the sessions are not consistent with a pattern, which shows that this is caused by individual patient differences (age, sex, race, pathology).

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AUTHORS INDEX

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