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Evaluation of T regulatory lymphocytes and serum concentration of selected cytokines in dogs with perianal tumors

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ABSTRACT

The purpose of this study was to determine concentrations of IL-2, IL-10, TGF-β1 in serum and T regulatory cell (Treg) percentage in peripheral blood of dogs with perianal tumours. Investigations were conducted on 32 male dogs of mixed breed. The animals were divided into 4 experimental groups and control group. The groups were established depending on the tumour malignancy degree and the type of dominant hormones. All measurements of serum cytokine concentrations were conducted by the use of commercial diagnostic ELISA kits. Treg lymphocyte percentage was measured by flow cytometry. In both groups with benign tumours cytokine levels decreased during therapy, whilst in groups with malignant tumors, in spite of applying anti-tumour therapy, concentrations of cytokines in serum markedly increased. The mean percentage of Treg lymphocytes in dogs with benign tumours (group I and II) was significantly lower than the mean percentage of these cells in control group at all time points, but after applying of anti-hormonal therapy, the significant increase of Treg percentage was observed compared to baseline values. By contrast, in both groups with malignant tumours (group III and IV), the mean percentage of Treg lymphocytes was significantly higher at the beginning of the experiment comparing with the control group as well as both groups with benign tumours and this percentage increased during anti-tumour therapy.

The results of this study suggest that monitoring changes in cytokine serum concentrations and Treg percentage in the bloodstream during anti-hormonal therapy may constitute a subsidiary marker in the monitoring of therapy effectiveness, in prognosis the outcome of a disease or in differentiating tumour degree of malignancy.

1. Introduction

The majority of canine perianal tumours originate from glands situated in perianal area (Withrow and McEwen, 2001; Hernandez-Aguirre et al., 2010). The most common of them are perianal gland tumours and they are diagnosed mainly in aged animals, usually in males (Goldschmidt and Shofer, 1992; Bray, 2011). There is some evidence showing that oncogenesis of perianal glands may be induced both by estrogens and androgens and these hormones stimulate neoplastic processes by influencing cells with estrogen or androgen receptors. The presence of these receptors has been detected inside the perianal tumours inner mass and in normal perianal gland tissue, which could be a proof that perianal glands are prone to estrogenic and androgenic regulation (Hayes and Wilson, 1977; Withrow and McEwen, 2001; Pisani et al., 2006). To date, most of the canine perianal tumour therapies are based on patient neutering and surgical excision of a tumour. A purpose of the neutering is to eliminate androgens and estrogens influence on the process of perianal gland oncogenesis. From the literature and our own experience, it is known that neutering alone is not enough for regression of neoplastic processes, especially in malignant tumours. Moreover, a perianal area is bothersome for surgical procedures, because tumour excision with the margin of healthy tissue in this region is in most cases impossible (Bennet et al., 2002; Emms, 2005; Hernandez-Aguirre et al., 2010). Therefore there is a strong

Abbreviations: IL-2, interleukin 2; IL-6, interleukin-6; IL-8, interleukin 8; IL-10, interleukin 10; TDSFs, tumour-derived soluble factors; TGF-β, transforming growth factor β; Treg, T regulatory lymphocytes; VEGF, vascular endothelial growth factor

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necessity to elaborate new conservative treatment methods for this body area, which could be applied as an alternative therapy for surgery. Currently, trials are being made in implementing conservative therapies in hormone-dependent neoplasms of a perianal area with the use of anti-androgen and anti-estrogen drugs. There are suggestions that this therapeutic procedure may be beneficial in the treatment of perianal tumours (Hayes and Wilson, 1977).

Tumour cells possess immunomodulatory influence on leukocyte functions because they secrete a large number of substances called tumour-derived soluble factors (TDSFs), which decrease or block local immune response. Several examples of TDSFs are IL-6 (interleukin-6), IL-10 (interleukin-10), TGF-β (transforming growth factor β) and VEGF (vascular endothelial growth factor). The influence of these substances is expanding from the tumour area to collateral lymphatic organs and peripheral blood vessels (Kaufman and Disis, 2004; Poggi et al., 2005; Kim et al., 2008). Besides TDSFs tumours secret chemokines with chemotactic properties which attract T regulatory lymphocytes (Huye and Dotti, 2010). That is why monitoring changes in serum level of selected cytokines may possess an immense importance in tumour diagnostics. In case of many neoplasms, the increase in product'ion and secretion of IL-6 is unfavourable prognostic factor (Galizia et al., 2002; Balwit et al., 2011). An increased concentration of IL-6 in sera of dogs with liver tumours was observed, similarly as in bitches with mammary gland tumours in which an increase of IL-8 (interleukin-8) concentration was noted. These findings may be an evidence of advanced stage of tumourigenesis, lymph nodes involvement, and relapse of a disease with increased risk of decease (Gelaleti et al., 2012; Neumann et al., 2012). Investigating the percentage of Treg lymphocytes simultaneously with selected cytokine serum concentrations is crucial because not only tumour cells are able to secrete anti-inflammatory cytokines such as IL-10 or TGF-β, but also T CD4+Foxp3+ regulatory lymphocytes (Atherton et al., 2016).

At present, several subpopulations of T regulatory cells have been characterized, and among them, Treg lymphocytes that express intracellular Foxp3 protein are the most important regarding tumourigenesis. This protein plays a crucial role in Treg cells functioning and is necessary for their normal differentiation (Biller et al., 2007; Gallimore and Simon, 2008; Ryba and Myśliwska, 2010; Mougiakakos, 2011).

In canine in vitro investigations, it has been proven that peripheral Treg CD4+Foxp3+ lymphocytes possess regulatory and suppressive function similarly as in humans (Pinheiro et al., 2010; Garden et al., 2011) and moreover they play a significant role in tumour immunology. One of the most significant mechanisms which promote neoplastic progression is impeding cellular immune response by the increased subpopulation of Treg cells during tumourigenesis. It has been proven that Treg cells significantly decrease the activity of T CD4+, T CD8+ lymphocytes and NK cells inside the tumour mass (Biller et al., 2007; Xiao-Feng, 2009; Biller et al., 2010; Lisiecka and Kostro, 2016).

On the basis of numerous clinical trials conducted in human medicine, it has been concluded that increased number of Treg cells, particularly within the tumour milieu and collateral lymph nodes is adverse prognostic factor in many types of tumours (Curiel, 2007; Yang and Ansell, 2009; Du et al., 2012; Tang et al., 2014).

In some cases, by estimating Treg number, patient survival time and response to treatment could be predicted in a more precise manner than by traditional prognostic methods such as tumour size assessment and the stage of a neoplastic process (Biller et al., 2007).

That is the reason for conducting trials on blocking the activity of immunosuppressive cells such as Treg lymphocytes. It was proven in mice that diminishing the amount of Treg cells in patients with neoplastic diseases simultaneously with the application of proper therapeutic drugs, results in increased anti-tumour immunity and higher survival rates (Orentas et al., 2006; Curiel, 2007; Yang and Ansell, 2009). Moreover, the extremely important fact is that canine cancers provide a suitable model for the disease in humans. When comparing to rodent tumours, companion animal tumour models more accurately reflect the features of human neoplasms (Atherton et al., 2016). That is why monitoring the changes of immunological parameters such as cytokine concentrations and T regulatory cells percentage could give additional prognostic value during anti-tumour therapy. Investigating interactions between cytokines, immune cells and neoplastic cells broadens possibility to elaborate new methods of immunodiagnostics and immunotherapy.

The aim of the present study was to investigate changes in the percentages of Treg lymphocytes and in serum levels of selected cytokines (IL-2, IL-10, and TGF-β1) during anti-hormonal therapy of malignant and benign perianal tumours. Moreover, our intention was to observe if any differences occur depending on tumour grade and tumour type and if these indices could be applicable to monitor the treatment effect in dog perianal tumours.

2. Material and methods

2.1. Experimental animals

Blood samples were collected from 32 male dogs of mixed breed aged above 9 years with naturally occurring perianal tumours, obtained from private owners. These animals were patients from the Department and Clinic of Animal Surgery, Faculty of Veterinary Medicine, University of Life Sciences in Lublin between May 2011 and June 2013. Physical examinations with evaluation of the size of the neoplasm and presence or absence of the ulceration were done for each of the dogs. To exclude dogs with concurrent diseases additional tests were performed including complete blood cell count, serum biochemical profile and urinalysis (with bacterial culture if needed). Tumour classification was made on the basis of tumour size, lymph node involvement, and metastasis. For detection of distant metastases, radiography was carried out. Biopsies collected from the tumor masses were evaluated to determine histological type and degree of malignancy (Brodzki et al., 2014a, b), and on the basis of histopathological examination the animals were divided into four experimental groups (group I, II, III and IV) and control group (group V). Group I comprised of 6 dogs with benign perianal tumours and increased serum concentrations of estrogen, group II was composed of 6 dogs with benign perianal tumours and increased serum concentrations of testosterone. Whereas group III and IV involved dogs with malignant perianal tumours, dogs from group III with increased estrogen concentrations and dogs from group IV with increased testosterone concentrations, respectively. The control group (group V) was composed of 8 clinically healthy male dogs at the same age as investigated animals. For the control group, eligibility criteria included dogs with normal blood tests, regular serum biochemical profile, and urinalysis.

In experimental groups, the anti-hormonal therapy was applied, depending on the increase of particular hormone concentration. Dogs with increased estrogen level (group I and III) were treated with Tamoxifen (Sandoz GmbH, Kundl, Austria) at a dose of 1 mg/kg of body weight during a period of one month, whereas dogs with increased testosterone concentrations (group II and IV) received Androcur (Bayer Schering Pharma, Berlin, Germany) at a dose of 5 mg/kg of body weight. Dogs from the control group were given a placebo instead of therapeutic drugs during the experimental period. The anti-hormonal therapy was applied in cases when the surgical procedures were extremely risky for an animal or when the owners refused standard surgical treatment. This kind of therapy is not an invasive method and it is especially advisable for older dogs to prevent the frequent complications after surgery. For the present study, dogs with perianal tumors with increased levels of estrogen or testosterone were chosen, in which surgical tumor extinction was not recommended. Response to the treatment was evaluated by assessing the tumour volume (reduction or enlargement) and by estimating the intensity of bleeding (diminished or increased).

Blood samples for serum analysis and cytometric procedures were

collected prior to any treatment, after 1 month of treatment and after 3 months of treatment. In both groups with malignant tumours the lack of data from the third time point after 3 months of treatment is caused by increased mortality and by the fact that most of these dogs were euthanatized.

All procedures used in this study were approved by the Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland.

2.2. Flow cytometry

Blood samples for flow cytometry were obtained by venipuncture of saphenous vein (*vena saphena*) into EDTA vacutainer tubes. Cytometric analysis was performed within 4 h of blood sampling. Lymphocyte immunophenotyping was performed with Epics XL flow cytometer (Beckman Coulter, Florida, USA). Lymphocytes were gated according to their size and granularity using FSC and SSC parameters. Lymphocytes from peripheral blood were stained with PE-conjugated anti-canine CD4 mAb (rat anti-dog CD4: RPE, clone YKIX302.9, Serotec Immunological Excellence Oxford, England). For staining of intracellular Foxp3 protein, peripheral blood cells were fixed and permeabilized using IntraPrep permeabilization reagent (Beckman Coulter, Florida, USA) following a set protocol. The samples were incubated with FITC labeled anti-murine Foxp3 mAb (clone FJK-16 s, eBioscience, San Diego, CA, USA) for 30 min. Cross-reactivity of this antibody has been confirmed by Biller et al. (2007) and Horiuchi et al. (2007). Before the experiment, optimal dilutions of antibodies were established by titration. Erythrocytes were removed from analysis using ammonium chloride lysing solution. At least thirty thousand leukocytes were collected per tube. Within the lymphocyte subpopulation, we determined CD4 + Foxp3 + cells by Quadrant analysis (Fig. 1). Appropriate controls were applied to assist gating decisions. Controls were run under exactly the same conditions as experimental samples. Validation procedures were conducted using Flow Check fluorospheres (Beckman Coulter, Florida, USA) and Immuno-Troll cells (Beckman Coulter, Florida, USA). All analyses were conducted on the same, unchangeable protocol, the same instrumental settings, and with the same voltages applied. Daily compensation procedures were applied for each sampling point. All samples were run at „low" flow rate.

2.3. Serum cytokine concentration assessment

Blood samples for ELISA tests were obtained by venipuncture of saphenous vein (*vena saphena*) into the serum separator tubes. Samples were left for 2 h to clot and after that time centrifuged at 3000 × *g* for 20 min. The serum was collected into the Eppendorf tubes, frozen and stored at −70 °C until analysis. Serum cytokine Il-2, Il-10, and TGF-β1 concentrations were assessed by the use of commercial diagnostic kits designed for detection of these cytokines in sera of dogs (ELISA kit for Interleukin 2, ELISA kit for Interleukin 10 and ELISA kit for Tumor Growth Factor beta, USCN Life Sciences, Hubei, China). The assay was performed according to the manufacturer's instructions. Optical density measurements were carried out by the use of an automated plate reader Elx800 Microplate Reader (BioTek Instruments, Inc., Winooski, USA), applying KC Junior programme (BioTek Instruments) for reading and gathering data. The optical density was measured at a wavelength of 450 nm. Each measurement was carried out in triplicate and readings were averaged for the analyses. A standard curve was created for each measurement, based on the results for the diluted standards. The concentration of each probe was calculated according to the standard curve. Each sample was diluted 1:2 in PBS prior to the assay to obtain optimal concentration. The optical densities of some samples were not within the range of individual standard curve and the samples were further diluted 1:20 (IL-2) and 1:10 or 1:100 (TGF-β1). After the measurements concentrations were multiplied by the final dilution factor accordingly.

Results for cytokines and Treg lymphocyte percentage are presented as mean values with standard deviations. The student's *t*-test was used to evaluate differences between two subgroups. The Mixed Model with repeatable measurements was used to evaluate differences between more than subgroup and interactions between quality variables. Statistical analysis was performed using Statistica 12.5 (StatSoft) and Graph Pad Prism 7 (Graph Pad Software).

3. Results

3.1. Percentages of Treg lymphocytes

The representative results of intracellular Foxp3 staining are given in Fig. 1, and the mean percentage of CD4 + Foxp3 + lymphocytes in experimental and control dogs is presented in Fig. 2. In dogs with benign tumours (group I and II), the mean percentage of lymphocytes with CD4 + Foxp3 + phenotype was significantly lower than the mean percentage of these cells in control group at all time points. However, after applying of anti-hormonal therapy, a significant increase of mean values of CD4 + Foxp3 + percentage was observed when compared to baseline values. By contrast, in both groups with malignant tumours (group III and IV), the mean percentage of CD4 + Foxp3 + lymphocytes was significantly higher at the beginning of the experiment compared with the control group as well as both groups with benign tumours. After applying anti-tumour therapy further increases were noted for the mean percentage of CD4 + Foxp3 + lymphocytes in groups III and IV.

3.2. Serum cytokine concentrations

The sequential changes in TGF-β1 levels in blood obtained from experimental and control dogs are given in Fig. 3. The concentrations of TGF-β1 in sera of dogs from the group I and II were less than 1 μg/ml, yet these values were significantly higher on the first and second occasion of sampling than the mean value of control group (0,45 μg/ml). On the last occasion of sampling, the mean values of TGF-β1 concentrations were similar to control. However in groups with malignant tumours (group III and IV), the concentrations of TGF-β1 significantly higher than in samples from control group and groups I and II. During the course of the therapy, these concentrations have further significantly increased.

From the data in Fig. 4 it becomes clear that at the first occasion of sampling, IL-2 concentrations in sera of dogs from all four groups were significantly higher compared with control group. After applying anti-hormonal therapy the mean values of IL-2 concentrations decreased significantly both in group I and II, and in the last stage of investigation, these concentrations reached values similar to control group. The mean values of IL-2 concentrations in dogs with malignant tumours (group III and IV) also decreased, but they still remained above the results from the group I, II and control group at each time point.

The results concerning changes in concentrations of serum IL-10 in investigated and control dogs are presented in Fig. 5. In groups I and II (benign tumours), after applying causative therapy, serum IL-10 concentrations decreased significantly comparing to initial values and reached significantly lower concentrations than controls. However, in groups III and IV, the mean values of IL-10 concentration were significantly increased compared with the group I, II and the control group, both at the first and the second time point. After applying therapy significant decrease of IL-10 concentrations was noted, yet these concentrations were still significantly higher than in the control group.

4. Discussion

The present study revealed that after applying therapy in dogs with benign perianal tumours the Treg CD4 + Foxp3 + percentage reached similar or significantly lower values than control, which may indicate

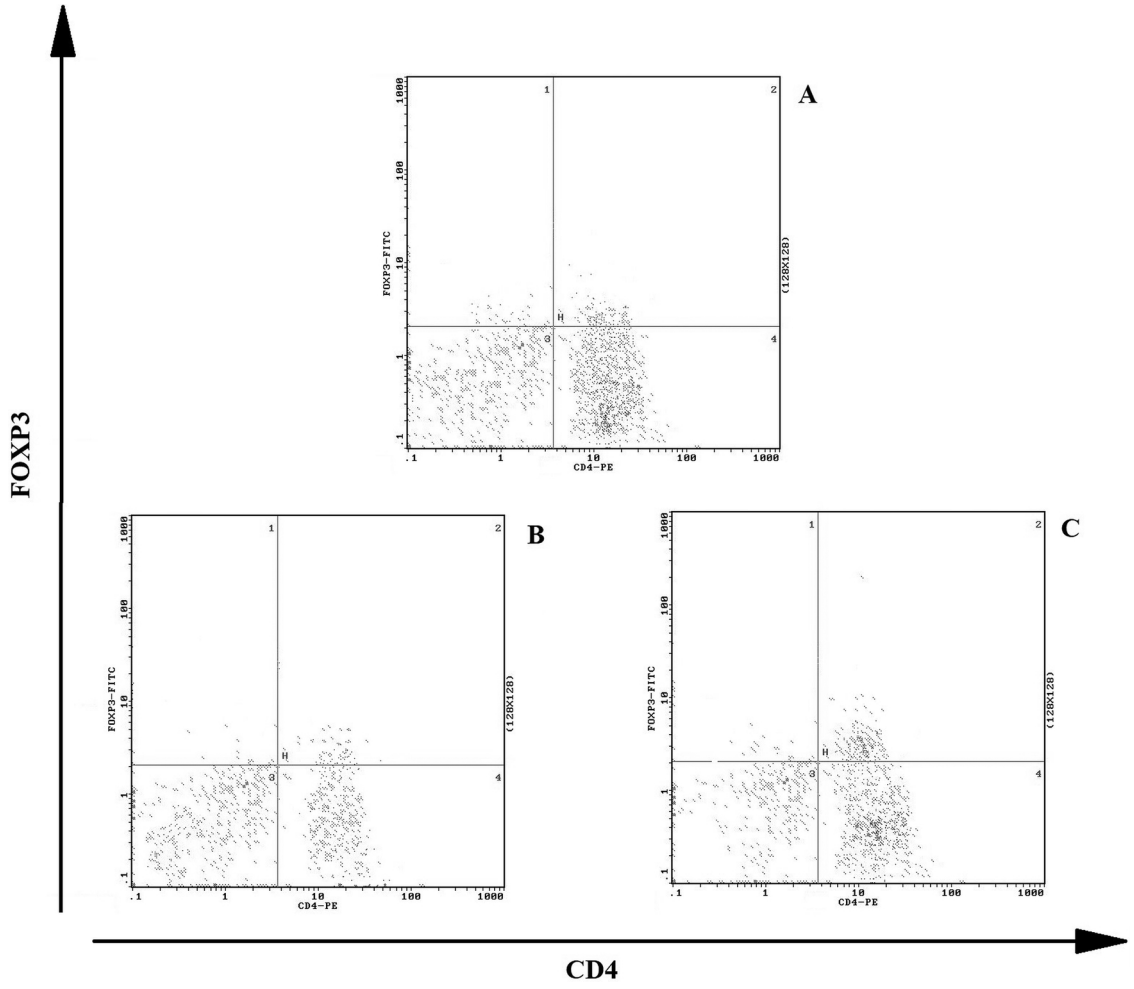

Fig. 1. Intracellular staining of Treg in peripheral blood from tumour bearing dogs – representative dot plots of intra-cellular staining. Peripheral blood lymphocytes from a healthy dog (A), a dog with benign perianal tumour (B), and a dog with malignant perianal tumour (C).

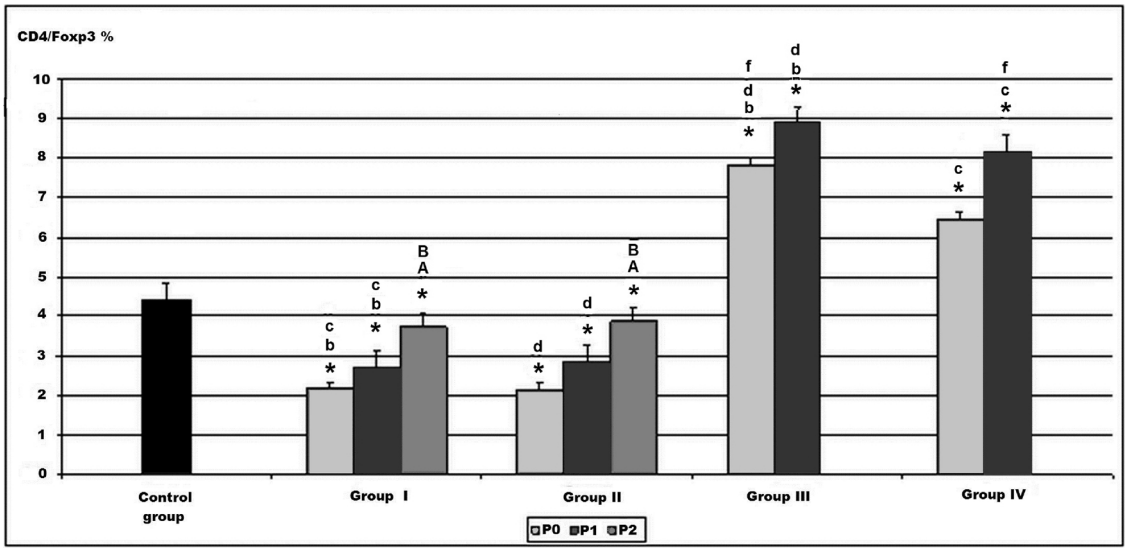

Fig. 2. Mean values of CD4 + Foxp3 + lymphocytes percentage in peripheral blood of dogs with benign and malignant tumours of diverse hormonal background and control group.

Mean values of CD4 + Foxp3 + lymphocytes percentage before therapy (P-0); after a month therapy (P-1); after three months therapy (P-2). Statistically significant differences at $P < 0.05$ between: definite investigated groups and control group (*); definite investigated group and baseline value (A); groups I and II between P1 and P2 (B); group I and II at individual time points (a); group I and III at individual time points (b); group I and IV at individual time points (c); group II and III at individual time points (d); group II and IV at individual time points (e); group III and IV at individual time points (f)

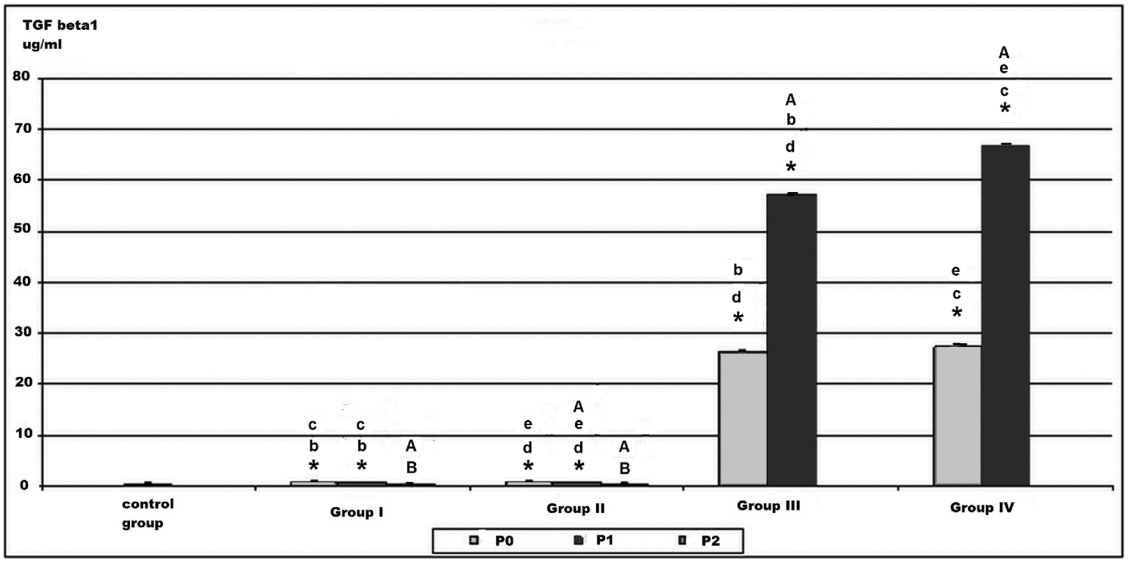

Fig. 3. Mean values of TGF β1 concentrations in sera of dogs with benign and malignant tumours of diverse hormonal background and control group.
Mean values of TGF β1 concentrations before therapy (P-0); after a month therapy (P-1); after three months therapy (P-2). Statistically significant differences at $P < 0.05$ between: definite investigated groups and control group (*); definite investigated group and baseline value (A); groups I and II between P1 and P2 (B); group I and II at individual time points (a); group I and III at individual time points (b); group I and IV at individual time points (c); group II and III at individual time points (d); group II and IV at individual time points (e); group III and IV at individual time points (f)

changes of immunological profile and elimination of immunosuppression triggered by neoplastic processes. By contrast, in dogs with malignant tumours, the percentage of Treg CD4 + Foxp3 lymphocyte subpopulation was significantly higher than in control group and undergone further increase during the course of the therapy, which was connected with therapy ineffectiveness. The results of the present study are confirmed by data obtained by Biller et al. (2007) that suggests that Treg lymphocytes could have significant influence on the course and regulation of malignant neoplastic processes in dogs. According to Biller et al. (2007), in healthy dogs, the mean percentage of Treg in peripheral blood amounts to 4,3%, and in dogs with diverse tumour types, it comes to 7,5%. Moreover, it has been proven in murine models that increase of the Treg number induces development of many types of tumours, but decrease in their number impedes neoplastic processes. It could be concluded, that further significant increase of the Treg CD4 + Foxp3 + percentage in dogs from groups III and IV after applying therapy, deepened suppression of the mechanisms of cellular anti-tumour immune response conducing to the process of tumourigenesis. Excessive production and release of transforming growth factor β1 (TGF-β1) which possess typical immunosuppressive properties, by Treg CD4 + Foxp3 + lymphocytes exerted significant influence on unfavourable prognosis of the course of a neoplastic disease in dogs from group III and IV (Yang and Ansell, 2009). TGFβ suppresses activity of cytotoxic TCD8 + lymphocytes, which are one of the main effector mechanisms in anti-tumour immune response (Piccirillo and Shevach, 2001; Somasundaram et al., 2002; Chen et al., 2005). Significant

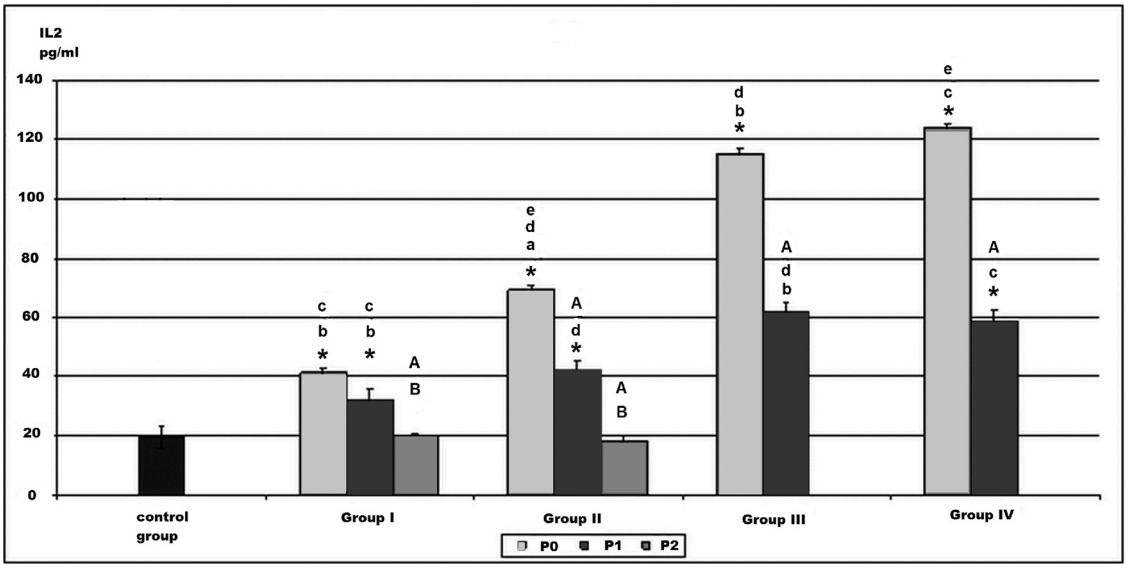

Fig. 4. Mean values of IL-2 concentrations in sera of dogs in with benign and malignant tumours of diverse hormonal background and control group.
Mean values of IL-2 concentrations before therapy (P-0); after a month therapy (P-1); after three months therapy (P-2). Statistically significant differences at $P < 0.05$ between: definite investigated groups and control group (*); definite investigated group and baseline value (A); groups I and II between P1 and P2 (B); group I and II at individual time points (a); group I and III at individual time points (b); group I and IV at individual time points (c); group II and III at individual time points (d); group II and IV at individual time points (e); group III and IV at individual time points (f)

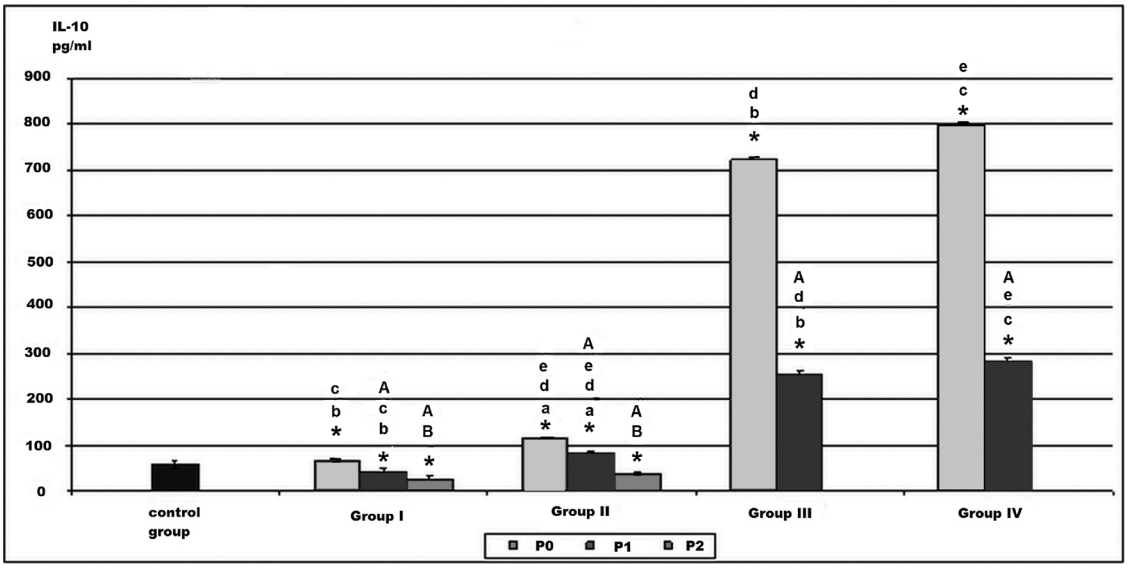

Fig. 5. Mean values of IL-10 concentrations in sera of dogs with benign and malignant tumours of diverse hormonal background and control group.
Mean values of IL-10 concentrations before therapy (P-0); after a month therapy (P-1); after three months therapy (P-2). Statistically significant differences at $P < 0.05$ between: definite investigated groups and control group (*); definite investigated group and baseline value (A); groups I and II between P1 and P2 (B); group I and II at individual time points (a); group I and III at individual time points (b); group I and IV at individual time points (c); group II and III at individual time points (d); group II and IV at individual time points (e); group III and IV at individual time points (f)

differences in the percentage of Treg CD4 + Foxp3 + lymphocytes were ascertained in dogs depending of type and the degree of malignancy of perianal tumours. These differences indicate that Treg participation in immune response may be dependent on the tumour type. In case of benign tumours of different hormonal background, there were no significant differences between group I and II concerning Treg percentage, which gives evidence that hormonal background in canine benign perianal tumours may not exert significant influence on alterations in peripheral blood Treg percentage. However, significant differences in the mean Treg percentages were observed in the case of malignant tumours. In dogs with malignant tumours with increased level of estrogen, the mean percentage of Treg was significantly higher than in dogs with increased level of testosterone. It is agreeable with the results of other researchers, which shows that Treg lymphocytes express estrogen receptors and this hormone exerts a strong effect on Treg function (Mjösberg et al., 2010). Moreover, the main cause of increase in peripheral Treg percentage in dogs with perianal tumours, particularly in dogs with malignant neoplasms, could be migration of these cells from collateral lymph nodes (Tominaga et al., 2010). The present study revealed that in dogs before applying anti-tumour therapy, serum cytokine concentrations of IL-2, IL-10, and TGF-β were significantly increased in all of four experimental groups compared to control, yet the mean values were different, depending on the type of tumour and degree of malignancy. Significant increase of IL-2 concentration during tumour development in experimental groups before implementing therapy gives evidence of stimulation of anti-tumour cellular immune response. IL-2 is an immunomodulatory cytokine which is produced by activated T lymphocytes. This cytokine exerts an indirect anti-tumour effect by stimulation and differentiation of lymphocyte subsets into lymphokine-activated killer (LAK) cells which have the capacity to recognize and kill different tumor cells irrespective of the histocompatibility expression status. It is known that TGF-β has a significant influence on IL-2 immunomodulatory properties (Schwinger et al., 2005). Significant increase in the concentration of this factor, particularly in dogs with malignant tumours may exert influence on the intensity of cellular immune response in these animals before and after the therapy. Whereas significant decrease of mean IL-2 concentrations in dogs from group I and II after applying the therapy may be an evidence of the change in immune response profile and gradual inflammation extinction. However in dogs with malignant tumours regardless of significant decrease of IL-2 concentration, its mean values remained on high level comparing to dogs from groups I and II which could be a result of maintaining high concentrations of TGF-β1 in sera of these animals. In the present study, it was concluded that serum concentration of IL-10 in dogs with perianal tumours varied depending on tumour type and degree of malignancy. The highest mean values of IL-10 concentrations, both before and after applying anti-tumour therapy, were observed in groups of dogs with malignant tumours (group III and IV), which is the evidence that an increase of Th2 type cytokines level in these animals may be one of the causes of tumour immune escape (Horiuchi et al., 2007; Andres de et al., 2013). The obtained results remain consistent with the fact that the type of immune response engaged depends on the tumour type and degree of malignancy. It has been evidenced that when the applied therapy proved to be successful in patients with renal cancers, Th1 type cellular immune response prevailed, whereas lack of positive response to the treatment was connected with the presence of Th2 humoral immune response and IL-10 production (Kaufman and Disis, 2004). Moreover, significant differences in serum TGF-β1 concentrations between groups with benign (group I and II) and malignant tumours (group III and IV) of perianal area were observed. In groups III and IV, TGF-β concentrations were significantly higher than in the control group and groups I and II. Furthermore in dogs with malignant tumours increasing TGF-β1 concentrations were connected with the rise of Treg CD4 + Foxp3 + percentage. This observation remains in agreement with literature data concerning the influence of this cytokine on Treg effector functions and it indicates a significant role of Treg cells in canine tumourigenesis. Biller et al demonstrated the increase in Treg cells number in dogs with various types of tumours, especially in case of malignant tumours (Biller et al., 2007). Tumour cells produce great amounts of TGF-β, in the presence of which lymphocytes transform into regulatory Treg CD4 + Foxp3 + cells. The presence of TGF-β in the tumour environment may be one of the main factors responsible for regulatory cell expansion throughout the tumour site. Treg cells impede the proliferation of tumour specific cytotoxic T lymphocytes through direct interaction with these cells activated by tumour antigens and by TGF-β secretion (Kaufman and Disis, 2004; Mougiakakos, 2011). However, the further increase of TGF-β concentrations during anti-hormonal therapy both in

group III and group IV may be an evidence of therapy ineffectiveness and could be a useful prognostic factor in this type of tumour.

5. Conclusion

In the accessible global literature, there is still a lack of data concerning changes of selected immune parameters in dogs with perianal tumours during non-invasive treatment. A large number of tumours are diagnosed in a highly advanced stage which limits the probability of effective treatment and patient recovery. That is why it is fully justified to look for new and more sensitive diagnostic factors which allow detecting the presence of tumour cells in early stages of a disease. Getting knowledge of the mechanisms which enable inversion of tumour escape from immune surveillance, which could be caused by increased numbers of Treg cells, could be the first step into introducing new and more efficient methods of medical treatment with diminished toxicity. Whether these factors can be used as diagnostic and prognostic indices should be evaluated in further studies. Investigations on the profile and intensity of immune response in dogs with malignant and benign tumours lead to the understanding of the complicated mechanisms and interactions which coexist within the immunological system during tumourigenesis in these animals. Profound investigations in the field of tumour immunooncology may facilitate the elaboration of new therapeutic methods directed more precisely into neoplastic cells and moreover, this kind of research may broaden possibilities of creating new immunotherapy methods and implementing innovative strategies in canine perianal tumour therapy. Application of immunotherapy, reinforcement and improvement of the immunological mechanisms in order to precisely recognize and successfully destroy tumour cells, is a hope for the future.

Ethics approval

All procedures used in the research were approved by the resolution nr 49/2006 of The Second Local Ethics Committee at the University of Life Sciences in Lublin on 12.12.2006.

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