UNIVERSITÀ DI PARMA

Dipartimento di Scienze Medico-Veterinarie
Corso di Laurea Specialistica a ciclo unico in Medicina Veterinaria

AUTOIMMUNE POLYENDOCRINE SYNDROMES IN DOGS

SINDROMI AUTOIMMUNI POLIENDOCRINE NEI CANI

Relatore: Chiar. mo Prof. Fausto Quintavalla

Correlatore: Dr. Andrea Corsini, PhD. EBVS

Laureanda:
Alessandra Pellegrino

ANNO ACCADEMICO 2021/2022

SUMMARY

ABSTRACT

Autoimmune diseases develop when the immune system attacks self-antigens of any organ or tissue leading to its destruction. The endocrine system appears to be particularly vulnerable to this kind of insult, indeed, some endocrine diseases are autoimmune in origin. Multiple glands can be affected in the same individual as part of the Autoimmune Polyendocrine Syndrome (APS). Different types of APS can be identified, but the only one documented in dogs, although very rare, is APS type 2. APS-2 is characterized by the presence of Addison's disease (AAD) associated with hypothyroidism (AITD) or type 1 diabetes mellitus (DM1) (Schmidt's syndrome) or both (Carpenter's syndrome). In general, autoimmune diseases are multifactorial involving genetic predisposition and environmental factors. Indeed, although some mutations and polymorphisms of MHC-encoding genes (HLA in humans, DLA in dogs) have been identified in association with many of the autoimmune endocrinopathies, their presence alone is not sufficient for the development of clinical manifestations. The clinical presentation of APS is related to the reduced functionality of the affected glands. Diagnosis and treatment of APS do not differ much from individual diseases, but attention should be paid to the interactions between hormones in the diagnostic processes and in the application of the therapies, which are simply given by the replacement of deficient hormones. Recent advances in understanding the pathogenetic mechanisms of autoimmunity are leading to the development of new therapies to replace the currently in use poorly effective ones, but further studies will be needed to establish their real efficacy and reduce any adverse effects. To reduce the mortality given by the possible complications of undiagnosed or untreated endocrinopathies, it is important for Veterinary Doctors to recognize the characteristics of APS in time and perform appropriate diagnostic investigations if they suspect it.

INTRODUCTION

The Autoimmune Polyendocrine Syndromes (APS) or Polyglandular Deficiency Syndromes (PDS) are characterized by the concurrence of at least two autoimmune-mediated endocrinopathies. They are also known by the more appropriate term Multiple Autoimmune Syndromes (MAS) since other autoimmune disorders of non-endocrine organs and tissues may be also found (Betterle et al., 2021).

Up to 2.3% of dogs diagnosed with endocrine disease are diagnosed with multiple endocrinopathies. While in some dogs it is clear that their concurrent endocrine disorders do not share an immune-mediated etiology (e.g., diabetes mellitus and hyperadrenocorticism), in others a common immune-mediated etiology similar to human APS has been proposed (Kuijlaars et al., 2021).

Organ-specific autoimmune diseases generally do not cluster casually but reveal preferential associations. According to the last proposed classification (Table 1), it is possible to identify 6 different types of APS differing in terms of: epidemiology; incidence; gender; main and minor combined diseases; age of onset; genetic associations; immunological features; prognosis; survival, mortality, and therapy (Betterle et al., 2021).

The purpose of this dissertation is to report the current state of the art of these rare syndromes, which have been poorly described in Veterinary Medicine, with particular regard to APS/MAS-2, the only one observed in the dog so far.

The choice of the topic stems from the interest sparked during my practical internship at the University Veterinary Teaching Hospital of the University of Parma and, in my opinion, the need for practitioners to be aware of the possible presence of additional endocrine and nonendocrine autoimmune pathologies in patients with endocrinopathy. This can help in diagnosing the syndrome early and treating patients before serious and irreversible complications occur.

Table 1: New proposed classification of APS/MAS (adaptive from Betterle et al., 2021)

APS/MAS-1 or APECED (Autoimmune-polyendocrine-candidiasis-ectodermal-dystrophy)	**Chronic mucocutaneous candidiasis** *And/or* **Chronic hypoparathyroidism** *And/or* **Autoimmune Addison's disease** *(at least two diseases present)*
APS/MAS-2	**Autoimmune Addison's disease (AAD)** *(Always present)* *And* **Autoimmune thyroid diseases (AITD)** *And/or* **Type 1diabetes mellitus (DM-1)**
APS/MAS-3	**Autoimmune thyroid diseases (AITD)** *And* **Other autoimmune endocrine diseases** *(Excluding Addison's disease)* **Other autoimmune gastrointestinal, hepatic, or pancreatic diseases** **Other autoimmune diseases of the skin, central nervous system, or hematopoietic system** **Other autoimmune rheumatic and cardiovascular diseases or vasculitis**
APS/MAS-4	**Any other autoimmune disease combination not included in the previous classifications**
IPEX syndrome	**Immune dysregulation, polyendocrinopathy, enteropathy, X-linked**
POEMS syndrome	**Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes**

1 IMMUNE SYSTEM AND SELF TOLERANCE

Immunity is the result of both non-specific (innate immunity) and specific (acquired immunity) responses that work in concert to protect the organism from infectious agents such as bacteria, viruses, fungi, and parasites, and the development of neoplastic processes. Innate immunity lacks memory and provides non-selective protection. Adaptive immunity, instead, can supply memory and offers wider protection against potential pathogens (Snyder, 2018). There are two types of adaptive immunity, named humoral immunity and cell-mediated immunity. Humoral immunity is mediated by proteins, the antibodies, produced by B lymphocytes. Antibodies prevent infection by eliminating microbes and microbial toxins present in the blood and the lumen of mucosal organs, but they do not have access to microbes inside cells. Cell-mediated immunity, instead, is mediated by T lymphocytes which provide protection against intracellular microbes, eliminating the entire infected cell (Abbas et al., 2004).

Figure 1: The principal mechanisms of innate and adaptive immunity (adapted from Abbas et al., 2004).
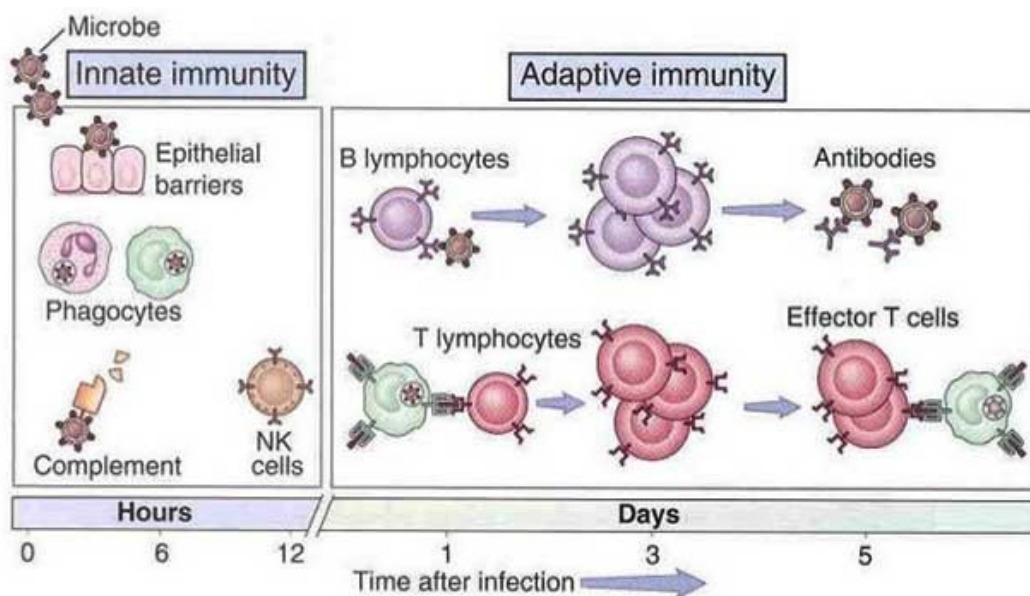
Cells of the immune system employ receptors present on their surface to recognize antigens, that is any substance capable of stimulating an immune response. These cells then deliver signals determining antigens' fate, either immunization for non-self-antigens or tolerance for self-antigens. In the case of self-antigens, the immune system shows a

7

specific type of response termed "immune tolerance", which is not simply the lack of immune response, but it is an active physiological process, defined as the loss of the immune system's ability to respond to a specific antigen after previous exposure to that antigen. Tolerance towards self-antigens is established at two levels: the form of tolerance occurring in the primary lymphoid organs is often referred to as "central", whilst that occurring in lymphocytes, once they have migrated out of these sites, is commonly referred to as "peripheral". The development of autoimmunity can simply be described as an escape from these mechanisms that regulate self-tolerance (Waldmann, 2015).

1.1 CENTRAL TOLERANCE

During the embryonic and neonatal stages of development, T cells and immature B cells acquire tolerance to self-antigens through selection processes to become effector cells (Amendt et al., 2022). Effector T cells are characterized by possessing T Cell Receptors (TCRs) on their surface which recognize self-peptides in association with Major Histocompatibility Complex (MHC) molecules on an Antigen Presenting Cell (APC).

T cells begin their development in the bone marrow as precursor cells. These cells migrate to the thymus during the so-called "thymic seeding", where they undergo two selection steps to become mature effector cells (Fig 2). However, the majority of thymocytes (95-99%) perish throughout these processes, and only relatively few escape the thymus as mature effector cells (Mehr et al., 1995).

These selection processes are:

- Positive selection: it occurs in the thyroid cortex. It is the clonal expansion of cells whose TCRs bind antigen in association with self-MHC-molecules. T cells that do not recognize either major histocompatibility complex MHC-I or MHC-II molecules degenerate through apoptosis (non-selection).
- Negative selection: it occurs in the thyroid medulla. It is the clonal deletion of those cells expressing TCRs with high affinity to MHC- I, II, or I/II self-peptide complex. A similar negative selection process has been observed during B cell development in the bone marrow. The immature B-cells expressing surface IgM

that recognize ubiquitous self-cell-surface antigens are induced to programmed cell death by a process known as clonal deletion or clonal anergy

(Amendt, 2022).

Figure 2: Central T-cell tolerance mechanism (adaptive from Ahmad et al., 2022).
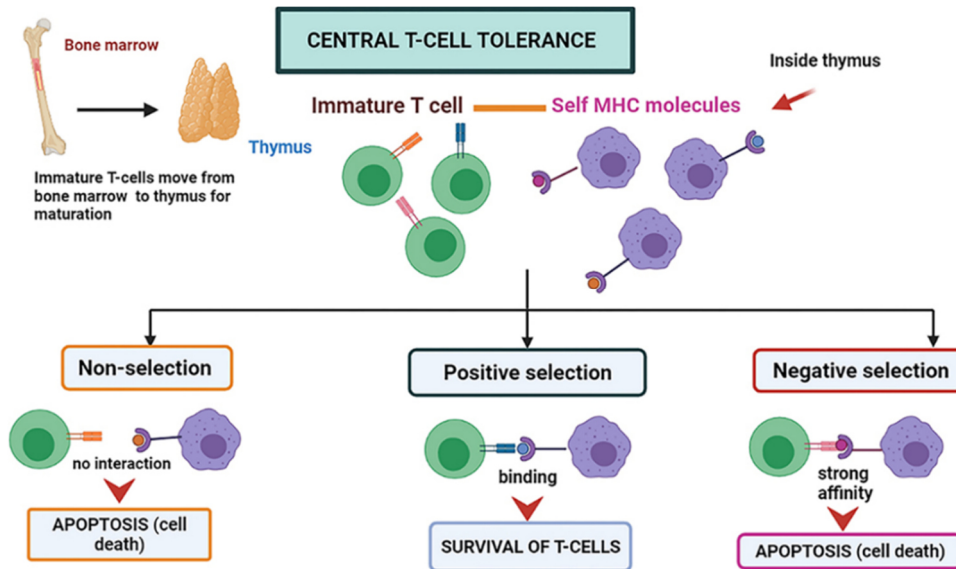
As a result, developing T and B lymphocytes expressing high-affinity receptors for self-antigens undergo an apoptotic process, forming a peripheral effector cell population free of self-reactive cells. The exact molecular pathways that trigger apoptosis are unknown. The expression of peripheral antigens in the thymus is assumed to be partially mediated by a protein called the autoimmunity regulator AIRE, which is thought to be essential for the deletion of immature autoreactive T lymphocytes (Snyder, 2017).

1.2 PERIPHERAL TOLERANCE

This process mainly occurs in adults. In peripheral tissues, there are mechanisms that prevent the activation of autoreactive T lymphocytes that have not been eliminated by the negative selection process in the thymus and averts the immune system to overreact against environmental factors. These mechanisms are implemented as part of a normal immune response to an antigen and involve the same signals required for the activation of lymphocytes during an immune response: mechanisms of anergy,

suppression by regulatory T cells, clonal deletion through cell death induced by activation, and antigen sequestration (Fig 3). Mature B cells are also under the control of peripheral tolerance (Snyder, 2018).

Figure 3: Peripheral T-cell tolerance (adapted from Ahmad et al., 2022).
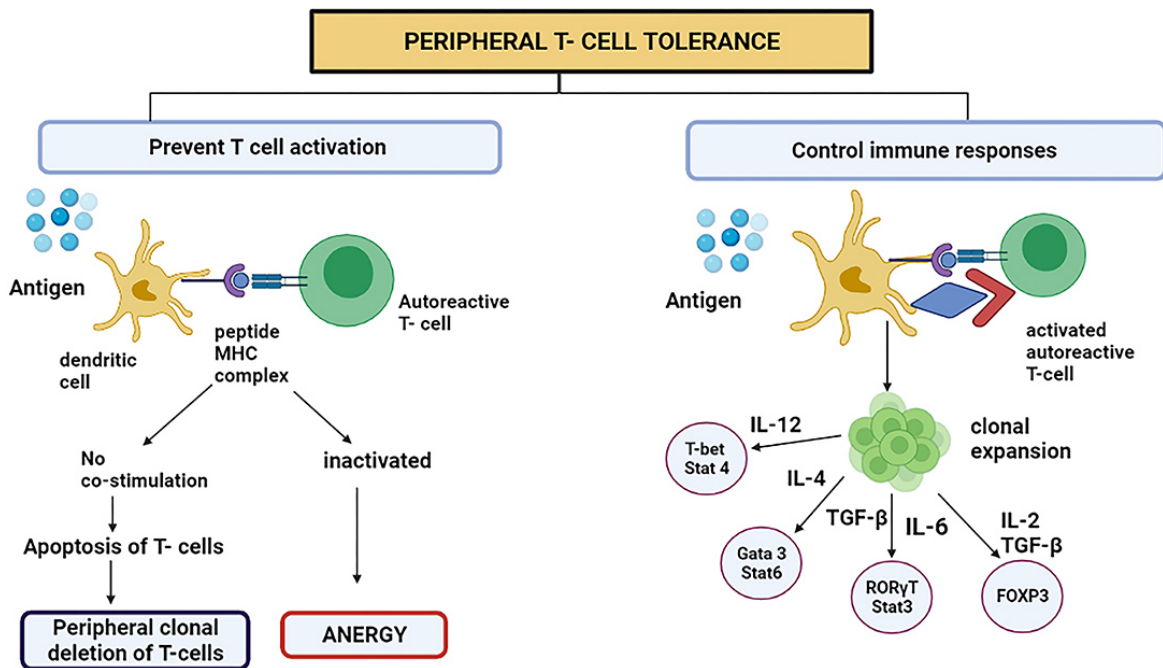
It is important to note, however, that even under the strict vigilance of central and peripheral tolerance, small numbers of potentially self-reacting lymphocytes can still 'leak out' into the periphery, even in otherwise healthy individuals. The existence of these potentially self-reactive T or B lymphocytes or both, and the ability of these cells to produce autoantibodies, does not necessarily lead to pathology (Salinas et al., 2013). Accordingly, autoimmunity can sometimes be classified as "physiological" and "pathological" autoimmunity (Avrameas et al., 2013). Physiological autoimmunity is usually transient without evidence of clinical disease. This is exemplified by the existence of so-called natural autoantibodies (Panda et al., 2015), which help eliminate degraded self- and foreign antigens in order to maintain homeostasis. When immune tolerance is broken and autoantibodies and self-reactive lymphocytes become involved in inflammation, classical or pathological autoimmunity develops which finally leads to tissue damage (Wang et al., 2015). Furthermore, many self-antigens, known as sequestered antigens, are not present in the thymus or bone marrow, and thus they do

not come into contact with developing lymphocytes (e.g., myelin basic protein, lens protein, and sperm protein), so these proteins may be released as a result of infection or trauma, triggering an immunologic response against these tissues (Snyder, 2018).

2 AUTOIMMUNE DISEASES (AID)

2.1 DEFINITION

Whenever self-tolerance is lost, immunocompetent cells attack self-antigens inducing a pathological state called autoimmunity (Manzari, 2003), characterized by normal or excessive activity of immune effector cells reacting against self-antigens. (Snyder, 2018). The endocrine system appears to be especially prone to this sort of insult, indeed, many endocrine disorders, including lymphocytic thyroiditis, hypoadrenocorticism, and type 1 diabetes mellitus, are autoimmune in nature (Greco, 2020). Notably, almost every endocrine and non-endocrine organ is a potential target for an autoimmune attack (Fig 4), and several may be targeted at the same time as part of a polyendocrine syndrome (Oftedal, 2012).

Figure 4: Pathogenesis of Autoimmunity (adapted from Snyder, 2018)
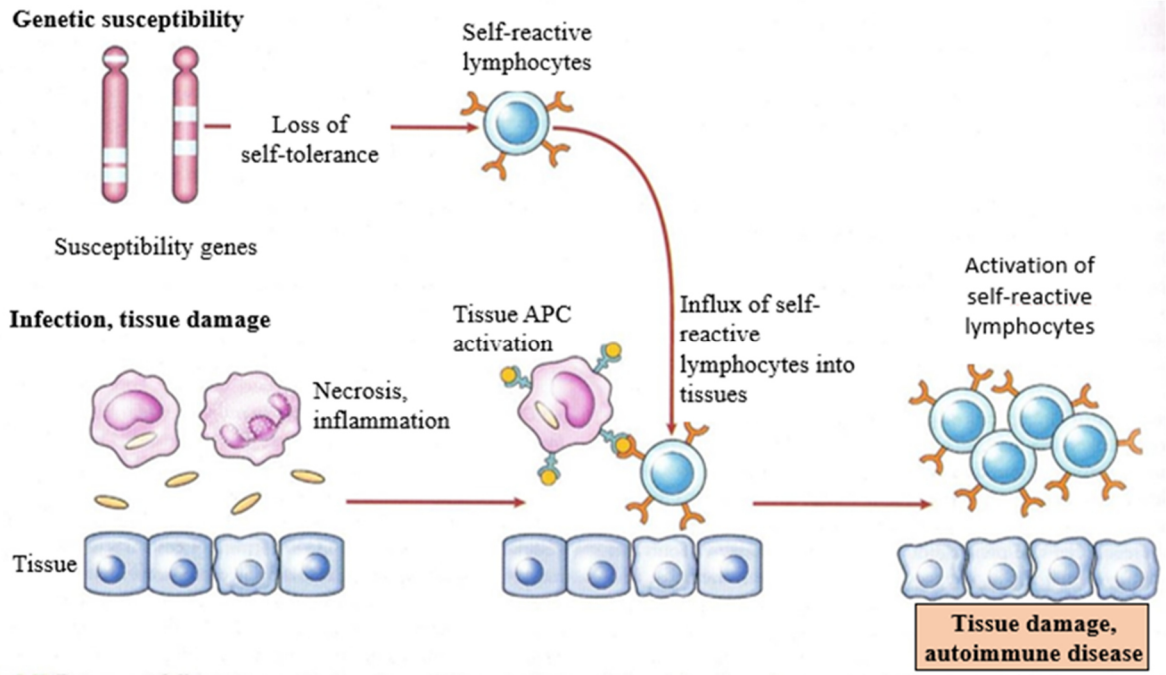
2.2 ETIOLOGY

Most autoimmune disorders have an unknown origin since they are multifactorial and include hereditary and environmental components (Snyder, 2018). Indeed, under the stimulation of environmental factors, organ-specific autoimmune disorders develop in genetically susceptible individuals. Consequently, these individuals produce specific humoral and cell-mediated immune responses against the constituents of the body's own tissues which may involve one or more organs (Betterle, 2004).

2.2.1 GENETIC COMPONENT

Most autoimmune diseases in humans have a significant genetic predisposition (Snyder, 2018). MHC molecules, which are essential for lymphocyte development and the control of peripheral effector lymphocytes, are the most studied genetic component. However, neither polymorphisms and mutations of MHC-coding genes nor the presence of autoreactive T lymphocytes, are sufficient by themselves to trigger an autoimmune disease. This suggests that the expression of an autoimmune phenotype is not the result of a single genetic flaw and that additional genetic variables, such as genes coding for proteins involved in other aspects of the immune response, or genes involved in the inflammatory response or scarring, may be implicated. Among domestic species, examples of autoimmune diseases with a familial tendency have been documented in some specific dog breeds and the mechanism is partly attributed to some MHC alleles (Snyder, 2018).

2.2.2 ENVIRONMENTAL COMPONENT

The identification of specific environmental factors has critical importance for understanding individual susceptibility, but there are very few agents that clearly have a role, and identification of generic risk factors remains elusive. These environmental factors in humans include nutrition, the microbiota, infectious processes, and xenobiotics, such as tobacco smoke, pharmaceutical agents, hormones, ultraviolet light, silica solvents, heavy metals, vaccines, and collagen/silicone implants (Colafrancesco et al., 2014). Infectious agents are the most well-studied environmental factors (Fig 5). Some viral infections, whether induced experimentally in specific strains of mice or naturally in humans, have been linked to the development of an autoimmune disease.

Peripheral anergy should regulate potentially autoreactive lymphocytes but infectious pathogens can disrupt this anergy towards self-molecules in two different ways: induction of co-stimulators on Antigen Presenting Cells (APCs) or molecular mimicry (selected antigenic determinants of microorganisms can potentially resemble host epitopes and therefore elicit an autoimmune response) (Snyder, 2018).

Figure 5: Potential role of microbial infections in the pathogenesis of autoimmunity (adapted from Snyder, 2018).
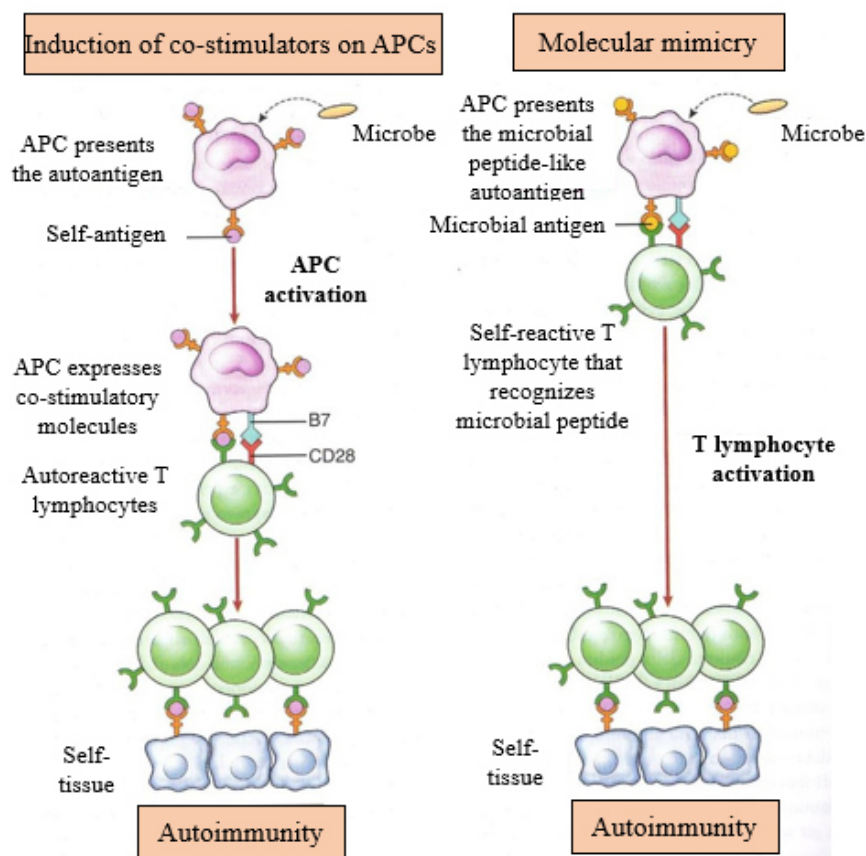
2.3 PATHOGENESIS

Depending on the autoimmune disease, tissue destruction can be divided into a variety of effector pathways (Fig 6). Antigens are recognized by antigen-presenting cells (APCs), which subsequently activate innate immune cells, that is dendritic cells (DCs), macrophages, and natural killer cells (NKs). APCs process antigens producing immunogenic peptides that are 'presented' to uncommitted T helper (Th0) lymphocytes,

which then differentiate into Th2, T follicular helper (Tfh), Th17, Th1 and T regulatory cells (Tregs). Th2 and Tfh cells promote B-cell activation, maturation, and differentiation into plasma cells and ultimately autoantibody production. The presence of autoantibodies is a common feature of autoimmune diseases (Damoiseaux et al., 2015), and a high fraction of serum antibodies are directed against the functional structures of the cell. Hence, they not only play a central role in diagnosis and classification but may also be involved in tissue damage through different mechanisms. One of the best-established pathogenic effects of autoantibodies is the cytotoxic destruction of cells by cell surface binding and lysis. In this process, the most common modes of destruction are complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC) or both (Ohishi et al., 1995). Another important pathogenic mechanism is immune complex-mediated damage. Autoantibodies may also interact with cell surface receptors, which can both activate (antithyroid-stimulating hormone for Graves' disease) and inhibit selectively. Another mechanism involves binding to extracellular molecules where autoantibodies are directed against b2-glycoprotein I in plasma (Lleo et al., 2010)

Th1 cells promote the development of cytotoxic T lymphocytes (CTLs). Autoreactive CTLs identify a target cell by binding the T-cell receptor (TCR) to the proper MHC-I and autoantigen-derived peptides combination. Then, a complex of MHC-I and autoantigen-derived peptides directly kills target cells via several mechanisms: secretion of cytotoxic granules (perforin and granzyme B) resulting in disintegration of the cell membrane and induced apoptosis; activation of Fas–Fas ligand, which induces apoptosis; and release of cytokines (such as TNF-α and interferon-c), leading to tissue injury (Davidson et al., 2001). Increased Th17 has also been reported to correlate with the progression of autoimmunity. Decreased Tregs, which negatively regulate innate and adaptive immunity, facilitate the loss of tolerance in several autoimmune diseases. (Wang et al., 2015).

Figure 6: Pathogenesis of autoimmunity (adapted from Wang et al., 2015).
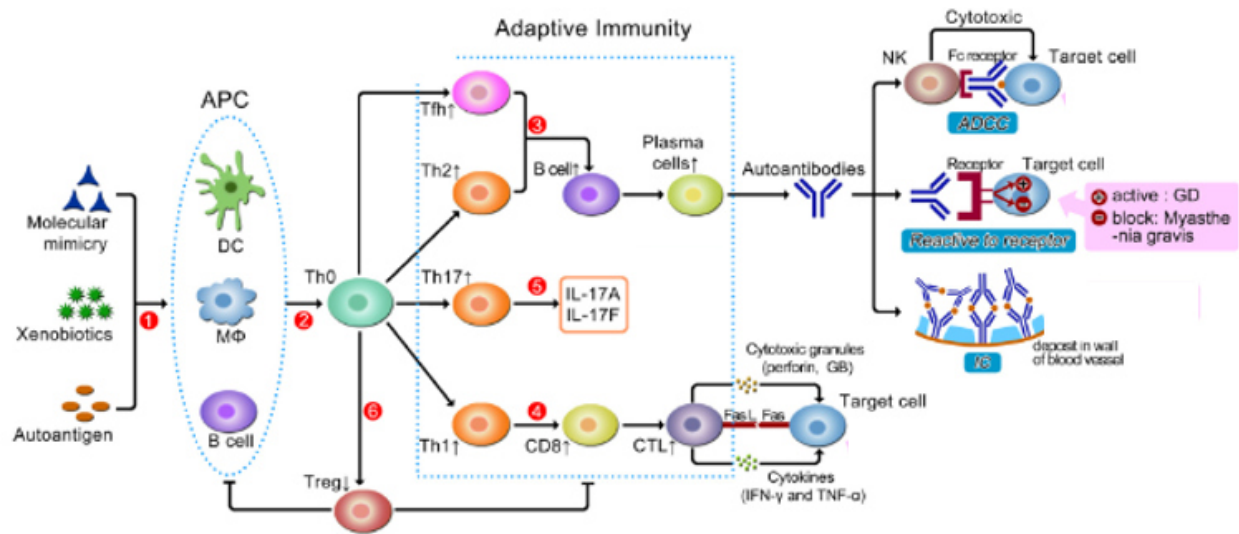
2.4 CLASSIFICATION

Autoimmune diseases can be classified into two groups. Organ-specific autoimmune diseases are characterized by the presence of autoantibodies and T cells reacting to self-antigens localized in a specific tissue/ organ (e.g., Hashimoto's thyroiditis [autoantibodies against thyroglobulin], Grave's disease [autoantibodies binding TSH receptors], autoimmune hemolytic anemia [autoantibodies against RBC], type 1 diabetes mellitus [autoantibodies against insulin-producing β cells]). Systemic autoimmune diseases are characterized by the presence of autoantibodies against antigens spread throughout various tissues, which leads to the formation of immune complexes that, by depositing in various districts of the body, induce organ/tissue damage (e.g., Lupus Erythematosus; rheumatoid arthritis) (Manzari, 2003).

Numerous classifications have been proposed for autoimmune diseases, even for the same autoimmune disease, and these generally depend upon clinical features, serology, and histopathology (Wang et al., 2015).

2.5 SYMPTOMS

Autoimmune diseases include a wide range of symptomatologic manifestations, differing in severity and depending on the most affected organs. (Oftedal, 2012). Most autoimmune diseases tend to be characterized by cyclic episodes of clinical illness alternating with periods of convalescence and/or apparent well-being (Snyder, 2018).

2.6 DIAGNOSIS

Some criteria that ideally should be fulfilled in order to define a disease as autoimmune in origin include:

- Direct evidence, such as transmissibility of the disease through cells or autoantibodies.
- Indirect evidence consisting of antigen identification, subsequent isolation of the homologous antigen, and replication of the disease by administering that antigen in experimental animal models.
- Isolation of autoreactive antibodies or autoreactive T lymphocytes; of note, they can also be found in normal individuals.
- Circumstantial evidence such as, association with other autoimmune diseases in the same individual or the same family, the presence of lymphocyte infiltrate of the target organ, association with particular HLA-haplotypes or aberrant expression of HLA class II antigens on the affected organ, and clinical improvement following immunosuppressive therapy (Rose et al., 1993).

Autoantibodies are important biomarkers used for confirming the diagnosis of autoimmune disease. Disease-specific autoantibodies can be detected at a very early stage when typical clinical symptoms are not present in the patient, allowing prediction of the disease several years before the symptoms are visible. Diagnosis at an early stage is essential to decrease morbidity, disability, and mortality caused by autoimmune diseases. Detection of autoantibodies, specific to particular phenotypes, helps to define these disorders as well as facilitate diagnosis, prognosis, and monitoring (Chauhan et al., 2019).

2.7 THERAPY

For many years, the treatment of autoimmune diseases has been based on non-selective immunosuppressant or cytotoxic drugs with minimal clinical success. The classical therapies for most autoimmune diseases consist of disease-modifying agents (e.g. methotrexate, leflunomide, azathioprine, sulphasalazine, or cyclophosphamide). Corticosteroids are usually administered for a short amount of time, during disease relapses or exacerbations. Intravenous immunoglobulin therapy is used in some cases. Drugs like methotrexate are frequently used in more than one disease, whereas other treatments are considered specific. Current disease-modifiers include some limitations such as a slow onset of action, a moderate efficacy that declines after several years of treatment, and side effects, the most common being hepatotoxicity, myelosuppression, or general immunosuppression (Balague´ et al., 2009).

The objective of treating people with autoimmunity is clear: find a drug that can totally reverse, if not cure, the condition. This approach needs to include a strategy directed at restoring immunological self-tolerance while preserving the immune response against invading pathogens (Balague´ et al., 2009). At present, this does not yet exist for any autoimmune disease. Although this is achievable in selected mouse models of autoimmunity, despite several attempts employing immunotherapy, including stem cell treatments, it has yet to be demonstrated effective in people (Zhao et al., 2014).

Recent advances in immunology and molecular biology have led to a considerably greater understanding of the pathogenetic pathways of autoimmune diseases, paving the way for the development of new rationally-based therapies (Sebastiani et al., 1992) through a massive number of molecular studies investigating not just genetic factors, but also the role of epigenetics (Zhao et al., 2014), the environment, infection, and the microbiota. Furthermore, there have been improvements in laboratory testing methods, including standardization of serology and development of new autoantibody tests (Wang et al., 2015). New biotherapies for the treatment of systemic autoimmune diseases have been developed throughout the previous decade in which all the steps of the immune response are targets (Amoura et al., 2006). The introduction of biological therapies in humans in the late nineties opened a new era in the treatment of autoimmunity, exerting a considerable influence on disease progression and patient quality of life. Biologicals

have a faster onset of action and higher efficacy than the existing therapies, but they are more expensive, some patients do not respond properly, and they can cause severe adverse effects. All these drugs offer a significant improvement in the quality of life of patients, but their risk/benefit must be carefully weighed and frequently reassessed. Safety is emphasized in both drug combinations and early disease intervention, and this is an unmet need that novel therapies should address. One of the most attractive approaches to treating autoimmune diseases appears to be cytokine inhibition. Preventing tissue damage remains an unmet medical need that could be achieved by inhibiting the migration of inflammatory cells, ideally at the early stage of the disease. Targeting Th17 cells appear to be a promising approach to prevent aggressive neutrophil and macrophage tissue infiltration. The discovery of the microRNAs that control the genetic programs of regulatory and effector lymphocytes is an exciting field that will increase our understanding of the immune system shortly (Balague´ et al., 2009).

3 AUTOIMMUNE POLYENDOCRINE SYNDROMES (APS/MAS)

Almost every organ can be involved by an autoimmune reaction, but a predilection for endocrine glands subsists. Indeed, many primary endocrine disorders are autoimmune in nature and are characterized by different impacts and severity, according to the organs involved. The endocrine organs more commonly susceptible to autoimmunity are the thyroid (e.g., Hashimoto's thyroiditis and Graves' disease), the pancreas (type 1 diabetes mellitus), and the adrenal cortex (Addison's disease). While hypophysitis, adrenalitis, POF (premature ovarian failure), and hypoparathyroidism represent quite rare conditions in humans. As already mentioned, several endocrine organs may be targeted at the same time in the same individual as part of the so-called Autoimmune Polyendocrine Syndrome (APS) but autoimmune endocrinopathies can also be accompanied by other non-endocrine autoimmune disorders (e.g., connective tissue, skin, or gastrointestinal diseases), hence the more appropriate term Multiple Autoimmune Syndromes (MAS) (Betterle et al., 2021).

In general, organ-specific autoimmune diseases do not cluster randomly but rather show preferential correlations. According to the most recent classification, and previously reported (Table 1), it is possible to identify 6 different types of APS differing in terms of: epidemiology; incidence; gender; main and minor combined diseases; age of onset; genetic associations; immunological features; prognosis; survival; mortality; and therapy (Betterle et al., 2021).

Up to now, APS type 2 is the only one that has been described in dogs (Greco, 2020).

3.1   AUTOIMMUNE   POLYENDOCRINE   SYNDROME TYPE 2 (APS-2)

Autoimmune Polyglandular syndrome type II, also known as Schmidt's syndrome, is a well-known disease, characterized by the presence of autoimmune-induced hypoadrenocorticism (Addison's disease) in combination with at least one other autoimmune endocrine disorder affecting either the thyroid gland (primary hypothyroidism), the pancreatic beta cells (type I diabetes mellitus) or both (Carpenter's syndrome). Other occurrence disorders in humans may be primary hypogonadism (premature ovarian failure, immune-mediated orchitis), myasthenia gravis, megaesophagus, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia (ITP), hypoparathyroidism, hypopituitarism, and celiac disease (Greco, 2020).

In humans, APS/MAS-2 is a rare syndrome, although not as rare as APS/MAS-1. Multiple endocrine disorders in dogs have only been documented in a few cases, just single reports, and case series. Among these, some cases were similar to APS/MAS 2 and common immune-mediated etiology was proposed (Furukawa et al., 2021) (Hwang et al., 2021) (Kooistra et al., 1995) (Kuijlaars et al., 2021) (Blois et al., 2011). Concurrent multiple endocrinopathies are poorly described in cats, with the most commonly reported associations being diabetes mellitus and hyperthyroidism or diabetes mellitus and hyperadrenocorticism. However, the disorders reported in the study are not thought to have an immune-mediated basis in cats (Blois et al., 2010).

3.2 ETIOLOGY

It is generally understood that AID stems from combinations of different genetic, epigenetic, environmental, and endogenous factors (Balague´ et al., 2009). However, it is unclear why a patient can develop a single disease or an APS/MAS. One hypothesis to explain APS/MAS is that tissues deriving from the same germ layer may share similar germ-layer-specific antigens, which would serve as targets for autoimmune responses. This hypothesis can explain APS/MAS-3 since the thyroid and the stomach derives from the same endodermal germ layer, but not APS/MAS-2 because the thyroid and the pancreas derive from the endoderm, while the adrenal glands are from the mesoderm.

Furthermore, according to this hypothesis, the APS/MAS should appear at the same time, but this rarely happens (Tadmor et al., 1992).

Many enzymes, hormones, and receptors have been identified as targeted autoantigens in organ-specific autoimmune diseases in humans (Table 2) (Song et al., 1996). The involvement of these autoantigens is thought to be critical in initiating and maintaining the autoimmune response. All the studies, aimed at identifying the antigen-antibody reactions, improved autoimmune diseases diagnosis and enabled the early detection of individuals at risk of developing organ-specific autoimmune diseases in the future (Betterle et al., 2004).

Table 2: Main organ-specific autoimmune diseases and recognized relevant autoantigen targets in humans (adapted from Betterle et al., 2004)

Target organ	Disease	Autoantigen
Thyroid	Grave's disease	TSH-receptor
	Hashimoto's thyroiditis	Thyroid peroxidase
	Idiopathic myxoedema	Thyroglobulin
Adrenal cortex	Addison's disease	21-hydroxylase
Gonads	Gonadal failure	P450 side-chain cleavage enzyme
		17 alpha-hydroxylase
Parathyroid	Hypoparathyroidism	Calcium-sensing receptor
Endocrine pancreas	Type 1 Diabetes mellitus	Glutamic acid decarboxylase
		Tyrosine- phosphatase like
		Insulin
Stomach	Body chronic atrophic gastritis	H+/K+ pump ATPase
	Pernicious anemia	Intrinsic factor
Intestine	Celiac disease	Transglutaminase
	Idiopathic malabsorption	Tryptophan hydroxylase
Liver	Chronic autoimmune hepatitis	P450 (IID6, IA2)
Pituitary	Lymphocytic hypophysitis	68, 49, 43 kD from human
	Infundibuloneurohypophysitis	Pituitary membrane (?)
Skin	Vitiligo	SOX9, SOX10 Tyrosinase
	Alopecia	Tyrosine hydroxylase
Muscle	Myasthenia gravis	Acetylcholine receptor

The genetic of APS has been studied extensively in human beings. APS-2 is inherited as an autosomal dominant trait in humans, associated with the presence of MHC, known as the human leukocyte antigens system (HLA) (Greco, 2020). The HLA molecules play a key role in the recognition of self and non-self proteins, as well as in the development of autoimmune diseases in some individuals. As previously stated, during the early stages of the autoimmune process, proteins are degraded in antigen-presenting cells (APCs) to produce peptides that bind to the MHC class II molecules, which are subsequently transported to the cell surface for presentation to the T lymphocyte via its receptor. A costimulatory signal provided by the interaction between the ligand B7 (on the APC) and CD28 (on the T lymphocyte) promotes T lymphocyte activation, differentiation, and proliferation. Antigen recognition, which is more likely to occur in peripheral lymph nodes close to the tissue of origin (for example the beta cell in type 1 diabetes and the thyrocyte in autoimmune thyroid disease), leads to infiltration of the tissue by activated lymphocytes. MHC variation, and hence antigen binding affinity are likely to play a critical role in the T lymphocyte activation and the development of autoimmune disease. Therefore, it is not unexpected, that consistent associations have been documented between alleles of the MHC-HLA class II immune response genes and many of the autoimmune endocrinopathies (Tait et al., 2002).

For many years, the MHC class II human leukocyte antigen (HLA) DR and DQ subtypes have been linked to, or associated with, the genetic risk of developing of diabetes mellitus type 1 (DM-1), autoimmune thyroid disease (AITD), and Addison's disease in humans (Redondo et al., 2002) (Baker, 1997). The location of risk to the MHC class II region emphasizes the importance of the T cell response in the development of these diseases, although the exact mechanism by which these structures confer risk remains unknown (Anderson, 2002). There are also non-MHC risk genes, such as the cytotoxic T lymphocyte antigen 4 (CTLA-4) gene, which has recently been identified as a risk factor in several association studies for all three of the major autoimmune endocrine disorders (Badenhoop, 2000), and the AIRE gene as the causative gene for APS type I (APECED Consortium, 1997).

In dogs, there may be different Dog Leukocyte antigens (DLA) depending on breed. One study showed that a single DLA class II three-locus haplotype, determined by sequence-based typing, was associated with an increased risk for Addison's Disease

(AD) (DLA-DRB1*009:01/DQA1*001:01/DQB1*008:02) in bearded collies with a distinct three-locus risk haplotype (DLA-DRB1*001:01/DQA1*001:01/DQB1*002:01) associated with AD in the West Highland White Terrier and Leonberger. These same two-locus haplotypes are also found in risk haplotypes associated with diabetes mellitus and hypothyroidism across different dog breeds, suggesting that this two-locus model serves as a good indicator for susceptibility to multiple organ-specific autoimmune diseases in the canine population. However, many dogs carrying these haplotypes never develop clinical autoimmune disease, making it clear that additional genes are necessary for actual disease expression (Gershony et al., 2019).

3.3 CLINICAL PRESENTATION

In most cases, the immune response to the target cell progressively destroys the endocrine gland, and the clinical manifestations are primarily the result of glandular hypofunction leading to inadequate hormone production. Thus, the immune process in these disorders begins long before the onset of clinical disease (Baker, 1997).

In humans, the main clinical manifestations of APS/MAS-2 are Autoimmune Addison's Disease (AAD) (always present) associated with an Autoimmune Thyroid Diseases (AITD) such as Chronic Thyroiditis (CT), or Graves' disease (GD), and with Type 1 Diabetes Mellitus (DM-1) or a combination of them. Other minor autoimmune disorders (e.g., POF, vitiligo, alopecia, celiac disease, immune-mediated thrombocytopenia, and multiple sclerosis) may also occur (Betterle et al., 2021).

In dogs, according to a study of 35 dogs with APS (Blois et al., 2011), the most common comorbidities found are DM-1 associated with AAD (57.1%), AAD associated with AITD (22.9%), and DM-1 with AITD (28.6%).

3.3.1 AUTOIMMUNE ADDISON'S DISEASE (AAD)

Hypoadrenocorticism (Addison's disease) is the most common initial endocrinopathy observed in APS-2 in dogs. Dogs are usually diagnosed with a second endocrinopathy approximately one year after the first endocrinopathy (Greco, 2020). This disease is characterized by adrenal cortical insufficiency leading to mineralocorticoid (produced by zona glomerulosa) and glucocorticoid (produced by zona reticularis and fasciculata) deficient production.

Hypoadrenocorticism can develop in any breed but a specific genetic disposition in certain breeds has been detected. Breeds with a known genetic predisposition include the Nova Scotia Duck Tolling Retriever, Standard Poodle, Bearded Collie, and Portuguese Water Dog (Oberbauer et al., 2002) (Famula et al., 2003). Other breeds commonly affected include the Great Dane, West Highland WhiteTerrier, Saint Bernard, WheatenTerrier, Leonberger, and Rottweiler (Scott-Moncrieff, 2015). Young to middle-aged female dogs are predisposed to Addison's disease, although dogs at any age may be affected. Dogs with other immune-mediated endocrinopathies, such as hypothyroidism and diabetes mellitus, are at increased risk (Lathan, 2013).

The majority of naturally occurring hypoadrenocorticism in dogs is idiopathic, likely due to immune-mediated destruction of the adrenal cortex with a probable genetic basis supported by breed predisposition. Less common causes of primary hypoadrenocorticism include infiltrative fungal organ or granulomatous disease, neoplasia, amyloidosis, infarction, hemorrhage due to trauma or coagulopathy, or iatrogenic causes resulting from the abrupt cessation of chronic glucocorticoid therapy or overdose or misuse of mitotane or trilostane (Van Lanen et al., 2014).

Hypothalamus release Corticotropin-Releasing Hormone (CRH) which acts on the pars distalis of the pituitary gland to promote the production of Adreno Cortico Tropic Hormone (ACTH), which, in turn, stimulates glucocorticoid secretion from the adrenal glands. Glucocorticoids have negative feedback on ACTH and CRH release and ACTH has a negative feedback on CRH production. This comprises the hypothalamic-pituitary-adrenal axis (Fig 7). Cortisol is the prevalent glucocorticoid produced. Glucocorticoid functions include stimulation of gluconeogenesis and erythropoiesis, lipid and protein

catabolism, suppression of the inflammatory response, maintenance of gastrointestinal mucosal integrity, contrast stress effects, and help maintaining blood pressure and body temperature. Aldosterone release, the major mineralocorticoid, is regulated by the renin-angiotensin system and potassium concentration. Renin release from the juxtaglomerular apparatus in the kidneys is stimulated by hypotension, reduced glomerular filtration rate, or renal blood flow. Aldosterone facilitates the conservation of sodium, chlorine, and water and the excretion of potassium and hydrogen ions (acid) from the distal renal tube.

Figure 7: Hypothalamic-pituitary-adrenal axis (adapted from Sjaastad, 2010).
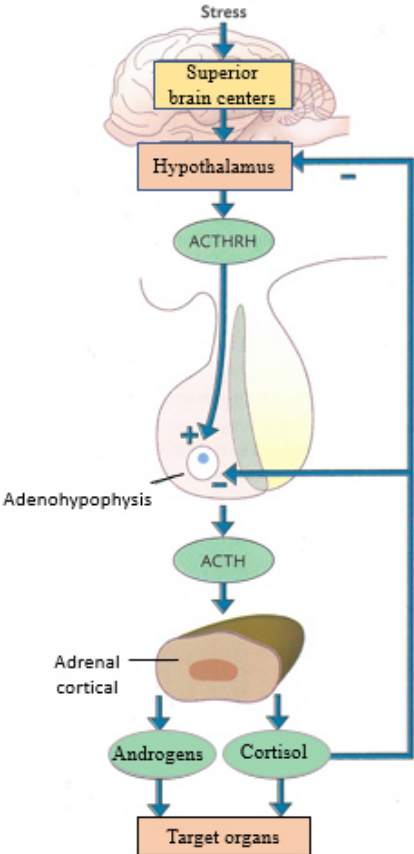
Clinical signs of primary hypoadrenocorticism occur when approximately 85-90% of all three layers of the adrenal cortex are destroyed. Clinical manifestation, detected through a collection of medical history, clinical signs, and physical examination, is highly variable with some dogs manifesting with chronic subtle signs and others presenting acutely in "Addison crisis"; they are vague and non-specific and frequently amplified by

stressful events, with improvement following fluid or steroid administration. Cortisol and mineralocorticoids deficiency may lead to weight loss, lethargy, weakness, collapse, anorexia, depression, gastrointestinal signs with vomiting, diarrhea, and inappetence, gastrointestinal bleeding with melena or hematochezia, hematemesis, abdominal pain, seizures, hypotension, bradycardia, weak pulses, dehydration, polyuria, polydipsia. Generalized or hindlimb muscle weakness, megaesophagus with regurgitation, muscle cramps, hair loss, or change in hair coat color are less common clinical symptoms.

In terms of diagnosis, complete blood counts (CBCs) in dogs with hypoadrenocorticism theoretically lack the typical stress leukogram characterized by neutrophilia, lymphopenia, monocytosis, and eosinopenia, but CBC can be diversified. In accordance with one study (Seth et al., 2011), an absolute lymphocyte count >2000 cells/mcL is about 58% sensitive and 85% specific as a screening tool for Addison. Mild normocytic, normochromic nonregenerative anemia is also common and may be due to chronic disease, gastrointestinal blood loss, or bone marrow suppression (Melián, 1996). Serum biochemistry's most common abnormalities include hyperkalemia and hyponatremia. According to one study (Adler et al., 2007), a cutoff of sodium-potassium ratio can be used to decide whether to pursue and recommend an ACTH stimulation test in dogs suspected of AAD, but different cutoffs have different sensitivity (Se) and specificity (Table 3), so a clinician should choose a cutoff depending on whether a higher Se or Sp is desired. Other possible abnormalities may be: pre-renal azotemia (caused by decreased renal perfusion and glomerular filtration rate due to hypovolemia and dehydration), hypercalcemia (according to some theories (Gow et al., 2009), caused by hemoconcentration, decreased calciuresis from glucocorticoid deficiency, excessive intestinal and bone calcium absorption due to glucocorticoid inhibition of vitamin D, hyperproteinemia, or acidosis), hypoglycemia (due to glucocorticoid deficiency and increased insulin sensitivity, resulting in decreased gluconeogenesis and increased utilization of glucose; it may be severe enough to cause seizures), hypoproteinemia and hypoalbuminemia (can occur from gastrointestinal hemorrhage, protein-losing enteropathy, or decreased albumin synthesis), hypocholesterolemia, elevated alanine aminotransferase or aspartate aminotransferase (most likely due to poor tissue perfusion and liver hypoxia), and metabolic acidosis.

Table 3: Sensitivity and specificity of sodium to potassium (Na: K) ratio as a diagnostic cutoff in dogs with hypoadrenocorticism (adapted from Adler et al., 2007)

| Na: K Ratio	%Sensitivity	%Specificity	Percent Dogs Correctly Classified as Diseases or Not Diseased
<32	100 (95-100)	62 (54-68)	72
<30	100 (95-100)	84 (80-90)	88
<29	97 (91-100)	90 (86-95)	92
<28	93 (85-98)	96 (92-98)	95
<27	89 (80-95)	97 (93-99)	95
<24	79 (67-86)	100 (97-100)	94
<23	74 (61-82)	100 (97-100)	92
<15	8(3-16)	100 (98-100)	75

Ultrasonographic evaluation of the adrenal glands should not be used to assess their function, but a measurable reduction in size can be detected in AAD dogs due to atrophy, usually <3 mm (Hoerauf et al., 1999). Many dogs affected with hypoadrenocorticism have one or more thoracic and abdominal radiographic abnormalities (i.e., small size of the heart, cranial lobar pulmonary artery, caudal vena cava, or liver) which can be attributed to systemic volume depletion (Melian et al., 1999). The megaesophagus is a rare and reversible finding (Whitley, 1995).

An electrocardiogram and blood pressure measurement may be useful in the Addison crisis as severe hyperkalemia and hypoxia (from hypotension or hypovolemia) may result in bradycardia, atrial standstill, prolonged P-R interval, increased T-wave amplitude, widened QRS complex, decreased R-wave amplitude, and ventricular fibrillation or asystole (Tag et al., 2008).

According to one study (Lennon et al., 2007), basal (or resting) serum or plasma cortisol level has been documented as a reliable screening test for dogs with symptoms compatible with hypoadrenocorticism. Resting cortisol levels <2 mcg/dL were 100% sensitive and 78.2% specific for detecting dogs with hypoadrenocorticism. Therefore, dogs with a high resting cortisol level (>2 mcg/dL) are unlikely to have hypoadrenocorticism, whereas those with a low resting cortisol level (<2 mcg/dL) require ACTH stimulation testing to diagnose or rule out hypoadrenocorticism. The

determination of the urinary corticoid: creatinine ratio (UCCR) seems to be a valuable and reliable screening test for AAD in dogs, with a sensitivity of 100% and specificity of 97,3%, using a cut-off of 1.4x 10^-6. Hence, a UCCR value >1.4x10^-6 could be useful in excluding AAD in dogs (Del Baldo et al., 2022). Confirming a diagnosis of AAD requires testing adrenal gland function. The ACTH stimulation testing (ACTHst) is considered the gold standard. It is performed by measuring serum cortisol concentrations before and 1 hour after giving 5 mcg/kg 4 synthetic ACTH, IV (Lathan et al., 2008). A dog with normal adrenal function should respond to ACTH injection increasing his serum cortisol concentration (from basal 0.5-6 mcg/dL to >2 mcg/dL after injection). In most dogs with AAD, serum cortisol concentrations do not change or stay low (<1 mcg/dL) (Fig 8).

Figure 8: Plasma cortisol concentrations before and after exogenous ACTH stimulation from normal dogs and those with hypoadrenocorticism (adapted from Hess, 2017)**.**
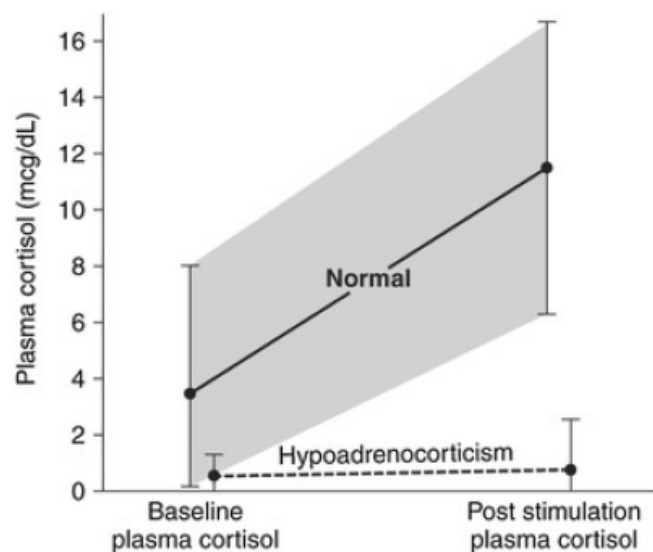
However, a few healthy dogs may also have basal serum cortisol concentrations <1 mcg/dL. Thus, this test alone cannot be used to diagnose AAD (Bovens et al., 2014). In these cases the Cortisol Endogenous ACTH ratio (C:eACTH) can be performed, being significantly lower in AAD dogs than in healthy patients (Javadi et al., 2006).

Long-term therapy consists of life-long glucocorticoid and mineralocorticoid supplementation to replace the missing cortisol and aldosterone. Glucocorticoids can be

supplemented with prednisone or prednisolone at an initial dose of 0.1-0.2 mg/kg/d PO (Schaer, 2001) with the necessary dose adjustment depending on the presence of adverse effects and exposure to stressful events. Mineralocorticoids can be supplemented with deoxycorticosterone pivalate (DOCP) at a recommended initial dose of age 2.2 mg/kg SC or IM every 25 days (Bates et al., 2013), or with fludrocortisone acetate, which contains both glucocorticoids and mineralocorticoids, given at 0.01-0.02 mg/kg/d PO divided into two doses (Kintzer et al., 1997). Any mineralocorticoid dose adjustment depends on serum Na and K concentrations. Thus, frequent follow up are necessary for dose adjustment.

## 3.3.2 AUTOIMMUNE THYROID DISEASES (AITD)

Hypothyroidism results from a deficiency of thyroid hormones, namely thyroxine (T4) and triiodothyronine (T3). It can be caused by a malfunction in one of the hypothalamic-pituitary-thyroid axis steps (Fig 9). Most cases of hypothyroidism in dogs are primary, resulting from thyroid gland failure or idiopathic follicular atrophy. Primary hypothyroidism can also be congenital due to iodine deficiency, thyroid dysgenesis and dyshormonogenesis, or iatrogenic following the administration of anti-thyroid drugs, surgical thyroidectomy, or radioactive iodine treatment (Parry, 2013). The less common secondary hypothyroidism arises from thyroid-stimulating hormone (TSH) deficiency caused by pituitary disease, such as neoplasia. The rare tertiary hypothyroidism result from a thyroid-releasing hormone (TRH) reduction (only one case of pituitary neoplasm invading the hypothalamus (Shiel et al., 2007).

Hypothyroidism can affect dogs of any age, breed, or gender. However, it occurs predominantly in middle-aged-to-older animals and the average age of presentation is around 7 years, with spayed females and neutered males at higher risk than sexually intact animals (Dixon et al., 1999). Since it is an autoimmune disorder, genetics undoubtedly plays a major role, especially given the increased incidence of this disorder in certain breeds, such as Beagles and Borzoi (Conaway et al., 1985) English Setter, Golden Retriever, Rhodesian Ridgeback, Cocker Spaniel and Boxer (Graham et al. 2007). Thyroiditis has also been linked to certain major histocompatibility complex DLA class II haplotypes in Doberman Pinchers, English Setters, Rhodesian Ridgebacks, and Giant Schnauzers (Kennedy et al., 2005).

The hypothalamic-pituitary-thyroid axis regulates thyroid function (Fig 9). Thyroid-releasing hormone (TRH), produced by the hypothalamus, stimulates the anterior pituitary gland's production of thyroid-stimulating hormone (TSH), which regulates the synthesis and release of T3 and T4 stored in the thyroid gland. TSH and TRH production are both regulated by negative feedback from circulating thyroid hormone, namely T3. Because all circulating T3 and T4 are transported bound to carrier proteins (thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), albumin, and certain plasma lipoproteins), only a small fraction of free hormone can enter cells to produce its effects by binding to nuclear receptors to change gene expression. Thyroid hormones serve a variety of purposes. They regulate all metabolism aspects, including synthesis, mobilization, and degradation of carbohydrates, lipids, vitamins, minerals, and all other hormones. They are also necessary for fetal growth and promote calorigenesis. Furthermore, they have significant chronotropic and inotropic effects on the heart; increase the number and affinity of beta-adrenergic receptors; enhance the response to catecholamines; are required for the normal hypoxic and hypercapnic drive to the respiratory centers; stimulate erythropoiesis and bone turnover (Greenspan, 2001).

Figure 9: Hypothalamic-pituitary thyroid axis (adapted from Sjaastad, 2010)
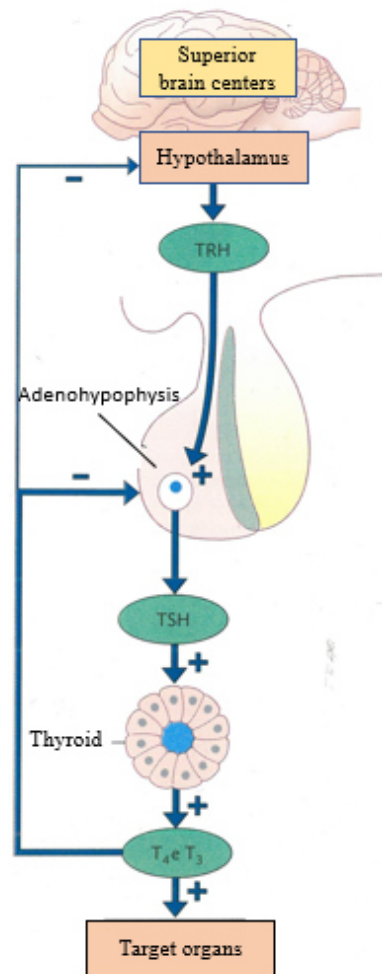
The most prevalent cause of naturally occurring thyroid failure in adult dogs is acquired primary hypothyroidism, which has two histologic: lymphocytic thyroiditis and idiopathic atrophy, which some belief is the terminal stage of severe lymphocytic thyroiditis. Lymphocytic thyroiditis is an immune-mediated disorder. Indeed, it may sometimes develop in conjunction with other immune-mediated endocrine deficiency syndromes. During the inflammatory phase of lymphocytic thyroiditis, antibodies are released into the circulation. In dogs, the predominant antibody that arises is directed against Tg. Antibody binding to the follicular cell, colloid, or Tg antigens is thought to trigger the complement cascade, antibody-dependent cell-mediated cytotoxicity, or both, resulting in follicular cell destruction. The cell-mediated immune system may also play an important, if not predominant, role in the development and maintenance of lymphocytic thyroiditis (Scott-Moncrieff, 2015). There was a significant association between the

number of CD4+ cells and the concentration of Tg in the cultures, suggesting that a loss of self-tolerance of CD4 + cells is important in the pathogenesis of canine thyroiditis (Tani et al., 2005). Also, a strong association between thyroiditis and certain major histocompatibility complex DLA class II haplotypes has been demonstrated in some breeds (Kennedy et al., 2006) (Kennedy et al., 2005).

Lymphocytic thyroiditis is slowly progressive, causing signs of hypothyroidism after about 80% of the gland has been destroyed. As a result, physical abnormalities are variable, depending on the severity of the disease and the body systems involved. The most consistent clinical signs of hypothyroidism in adult dogs are those caused by decreased cellular metabolism (lethargy, mental dullness, exercise intolerance, obesity or weight gain, cold intolerance) and dermatologic manifestations (bilaterally symmetric or asymmetric non-pruritic alopecia in areas of friction and pressure, rat tail, dry hair coat, skin hyperpigmentation, otitis externa, and secondary bacterial infection like pyoderMalasseziaezia, myxedema with the so-called "tragic facial expression"). Additional clinical signs may affect the cardiovascular system (bradycardia, cardiac arrhythmias), neuromuscular system (polyneuropathy/myopathy, vestibular signs, facial nerve paralysis, laryngeal paralysis, megaesophagus, seizures, disorientation/circling, myxedema coma, myasthenia gravis), gastrointestinal system (esophageal hypomotility, diarrhea, constipation), blood system (anemia, hyperlipidemia), and reproductive system (prolonged parturition, periparturient mortality, low birth weight puppies, female infertility, inappropriate galactorrhea or gynecomastia).

If the history and clinical signs are suggestive of hypothyroidism, further diagnostic testing is necessary. Although most of the alterations are generic and seen in a variety of other disorders, their presence might help to confirm the diagnosis. At CBC, normocytic, normochromic, and nonregenerative anemia can be detected, likely due to decreased erythropoietin synthesis and a lack of thyroid hormones stimulatory action on bone marrow (Green et al., 1986). Hypothyroidism has been linked to increased platelet counts and small platelet size, a result of an inverse relationship between thrombopoiesis and erythropoiesis. Evaluation of red blood cell morphology may reveal increased concentrations of leptocytes, a direct result of concomitant hypercholesterolemia and hypertriglyceridemia (Sullivan et al., 1993). Other findings may be increased creatine kinase (CK), high fructosamine concentrations, mild

hypercalcemia, and a modest increase in liver enzyme activity, possibly due to mild hepatic deposition (Parry, 2013). Urinalysis is usually normal; however, proteinuria can occur in dogs with lymphocytic thyroiditis due to concomitant immune-complex glomerulonephritis (Mansfield et al., 2006). Regarding ultrasonographic evaluation, in hypothyroid dogs the thyroid lobes tend to be round or oval in shape on the transverse plane, hypoechoic compared to surrounding muscle, have a smaller volume and cross-sectional area relative to body size, and are not homogenous but irregular in outline. One study reported a diagnostic specificity of 96% and sensitivity e of 98% (Reese et al., 2005)), although thyroid gland abnormalities progress over time, and in early hypothyroidism, the thyroid lobes may seem normal on ultrasound examination. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) have limited usefulness; however, scintigraphy is effective for evaluating the size, shape, and location of thyroid tissue. Adult dogs with primary hypothyroidism typically have a low or non-detectable accumulation of radioisotope by the thyroid gland, and the thyroid gland may seem smaller than normal (Kintzer et al., 1991).

There are several diagnostic tests available for the assessment of thyroid function in dogs with a wide variety of recommendations for their use and interpretation. Tests that measure the serum total free T4 concentrations, in addition to serum free T4 and TSH concentration, are currently suggested. The TT4 test is the recommended test, and is a useful initial screening test, measuring bound (>99%) and unbound (<1%) fractions of circulating thyroxine. Dogs with hypothyroidism will have reduced TT4 levels. However, levels can be reduced in dogs with other illnesses too, so if low levels of TT4 levels are detected, additional investigation is recommended, while a diagnosis can usually be ruled out if the T4 is in the upper half of the reference range (Parry, 2013). Hence this is an extremely sensitive but not a specific test. Because fT4 reflects the hormone fraction that is available to enter cells, its measurement provides a more consistent evaluation of thyroid gland function than TT4. Measuring fT4 by modified equilibrium dialysis (MED) offers the highest specificity and sensitivity. A low fT4 level is consistent with hypothyroidism since it is less susceptible to non-thyroidal illness than TT4. Decreasing levels of fT4 cause an increased output of TSH from the pituitary gland. It has poor specificity because of an overlap in results between hypothyroid dogs and euthyroid dogs with concurrent illness (e.g., hypoadrenocorticism) (Reusch et al., 2017). Thus, TSH measurement is only useful if used in combination with TT4 and fT4 (Parry, 2013).

Anti-thyroglobulin antibodies (ATAs), detectable through a simple Enzyme-Linked ImmunoSorbent Assay (ELISA) test, are a sensitive indicator of canine thyroiditis since they are detected in approximately 50% of hypothyroid dogs (Graham et al., 2007). The presence of Tg autoantibodies implies the presence of thyroiditis but does not give any information about the severity or progressive nature of the inflammatory response or about thyroid function; thus, it cannot be used alone to diagnose hypothyroidism. Dogs with confirmed hypothyroidism can have negative Tg autoantibody concentrations, and euthyroid dogs can be positive for Tg autoantibodies. The prevalence of Tg autoantibodies varies with the breed, so their measurement can be a screening method to ultimately eliminate heritable forms of thyroiditis (Nachreiner et al., 2002). T3 and T4 autoantibodies may interfere with serum T3 or T4 concentrations test, causing false elevations in serum fT4 concentration, but this does not occur if fT4 is measured using an assay that includes a dialysis step (MED assays), because autoantibodies cannot pass through the dialysis membrane and interfere with the assay.

Hypothyroid dogs require thyroid hormone replacement for life. Synthetic T4 (levothyroxine) is the drug of choice. The generally recommended initial dose is around 0.02 mg/kg PO q12h (Graham, 2009).

3.3.3 TYPE 1 DIABETES MELLITUS (DM-I)

Type 1 diabetes mellitus is characterized by irreversible destruction of ß-cells in the islets of the endocrine pancreas leading to a deficit in insulin production, which results in chronic hyperglycemia, and an absolute requirement for exogenous insulin to maintain glycemic control and avoid ketoacidosis; in fact, it is also known as insulin-dependent diabetes mellitus (IDDM).

Most diabetic dogs are between 4 and 14 years of age, females are at increased risk compared with males, neutered male dogs are at increased risk compared with intact male dogs (Marmor et al., 1982), mixed-breed dogs are at increased risk compared with pure breeds, and dogs weighing less than 22.7 kg were at increased risk compared with larger dogs (Guptill et al., 2003). Popularity and geographical locations impact breed predispositions. For example, Irish Setter, Poodle, Yorkshire Terrier, and English Setter are among the breeds with the highest risk for diabetes in Italy (Fracassi et al., 2004).

The endocrine pancreas is structured into small aggregates of cells scattered throughout the gland forming the so-called islets of Langerhans. Among these cells are ß-cells which produce insulin in response to various stimuli (increased plasma concentrations of glucose, amino acids, some intestinal hormones, or parasympathetic activity). Insulin has an anabolic-type action on carbohydrate, lipid, and protein metabolism in all body cells (Fig 10).

Figure 10: Insulin effects (adapted from Jennings, 2017; Sjaastad, 2010).
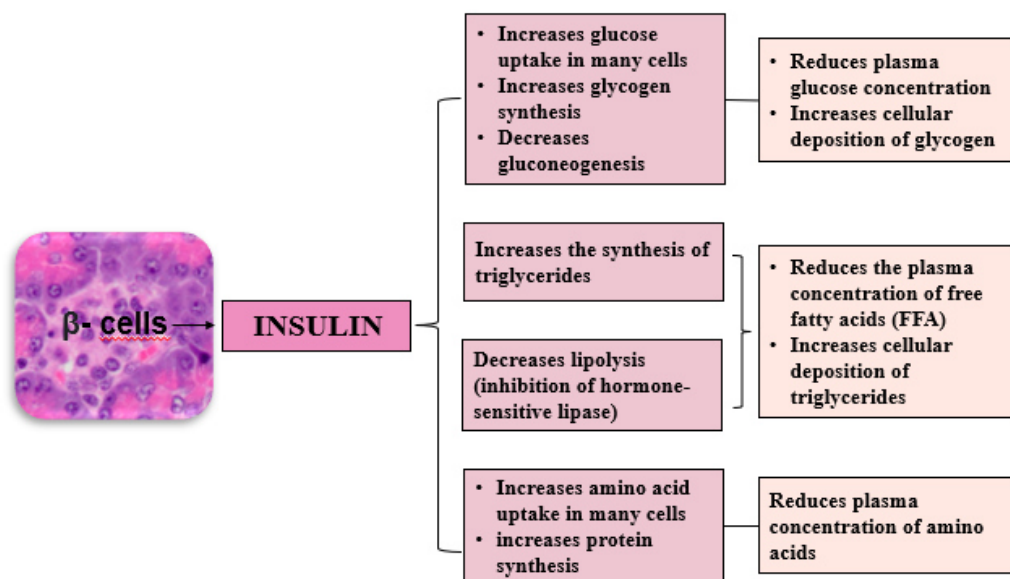
The etiology of DM is multifactorial (Table 4). Diabetes mellitus in dogs has been linked to MHC II genes, namely dog leucocyte antigen (DLA), with similar haplotypes and genotypes being identified in the most susceptible breeds. Some alleles in the canine insulin gene have been associated with susceptibility or resistance to DM in a breed-specific manner (Catchpole et al., 2013). In one study, five dog breeds were identified as having one or more DLA haplotypes associated with diabetes mellitus susceptibility or protection. Four DM-associated haplotypes were identified in the Cocker Spaniel breed (one DLA haplotype associated with increased risk of DM and three haplotypes associated with protection from disease), suggesting that the MHC plays an important role in determining an individual's susceptibility to DM in this breed. In the three breeds known to be at the highest risk of DM included in the study (Samoyed, Tibetan Terrier, and Cairn Terrier), no DLA haplotypes were found to be associated with DM. As a

result, novel DLA associations with DM in specific dog breeds provide further evidence that immune response genes contribute to susceptibility to this disease in some cases. It is also apparent that DLA may not be contributing to obvious or strong risk for DM in some breeds (Denyer et al., 2020).

In some dogs an immune-mediated component is suggested for the destruction of beta-cells, perhaps induced by environmental factors (Nerup et al., 1994). In immune-mediated insulitis cases of diabetic dogs, antibodies directed against islet cells, insulin, proinsulin (Davison et al., 2011), intracellular glutamic acid decarboxylase 65 (GAD65), and insulinoma antigen 2 (IA-2) (Davison et al., 2008) have been identified. Autoimmune processes in combination with genetic and environmental variables, insulin-antagonistic disorders and drugs, obesity, and pancreatitis may all be responsible for initiating and progressing diabetes in dogs. The final result is a loss of ß-cell function.

Table 4: Potential factors involved in the etiopathogenesis of diabetes mellitus in dogs (adapted from Nelson, 2015).

• Genetics
• Immune-mediate insulitis
• Pancreatitis
• Obesity
• Concurrent hormonal disease
Hyperadrenocorticism
Diestrus-induced excess of growth hormone
Hypothyroidism
• Drugs
Glucocorticoids
Progestogens
• Infection
• Concurrent illness
Renal insufficiency
Cardiac disease
• Hyperlipidemia

In diabetes, insulin deficiency leads to impaired utilization of glucose, amino acids, and fatty acids, as well as increased hepatic glycogenolysis and gluconeogenesis, resulting in hyperglycemia. When the blood glucose concentration exceeds the renal tubular cell's ability to resorb glucose, glycosuria occurs. Glycosuria causes osmotic diuresis, resulting in polyuria and compensatory polydipsia to prevent dehydration. The reduced utilization of glucose by peripheral tissues results in weight loss. Without insulin, glucose cannot access the satiety center to inhibit the feeding center, therefore dogs become polyphagic despite hyperglycemia. These are the classical signs of DM, and their severity is directly related to the severity of hyperglycemia. The most severe result is diabetic ketoacidosis (DKA). Other clinical findings might be lethargy, dry brittle, and lusterless hair coat, hepatomegaly due to hepatic lipidosis, cataract, anterior uveitis, and keratoconjunctivitis (Nelson, 2015).

Diagnosis of DM needs simply proof of persistent hyperglycemia and glycosuria, in addition to the previously mentioned clinical signs. In the uncomplicated DM, other laboratory findings might include: CBC that is usually normal; biochemistry panel that might show hyperglycemia, hypercholesterolemia, hypertriglyceridemia, mild increase of liver enzymes activity; urinalysis that may reveal high urine-specific gravity, glycosuria, proteinuria, bacteriuria, and ketonuria in case of DKA; and other additional tests depending on the case. It is worth noting that mild hyperglycemia in a suspected DM patient can be caused by other factors such as a recent meal of easily digestible carbohydrates; stressed, hyperactive, aggressive, or extremely nervous dogs; in the early stages of DM development; and in the presence of disorders or drugs causing insulin resistance. In general, any concurrent inflammatory, infectious, hormonal, neoplastic, or organ system problem can cause insulin resistance and impair insulin therapy's efficacy (e.g., hyperadrenocorticism, hypothyroidism, glucocorticoids, diestrus, chronic pancreatitis). In these circumstances, insulin therapy should not be initiated before diagnosis and treatment of these factors (Nelson, 2015).

The goals of treatment are to reduce or eliminate clinical symptoms and to prevent short-term complications (e.g., hypoglycemia, DKA) by reducing blood glucose concentration fluctuations and maintaining near-normal glycemia. This can be accomplished with exogenous insulin therapy, an appropriate diet, exercise, and prevention or control of any possible concomitant conditions. Currently, the feeding of

high fiber and high complex carbohydrate diet at a caloric intake targeted is suggested to treat obesity and maintain ideal body weight. Several small meals should be fed throughout the time of insulin activity, to help minimize postprandial changes in blood glucose concentration (Nelson, 1992). Physical activity has a glucose-lowering effect by increasing blood and lymph flow and increasing mobilization and absorption of insulin from its injection site. It also stimulates the translocation of glucose transporters in muscle cells increasing glucose disposal despite basal insulin concentrations (Nishida et al., 2001). The amount and timing of exercise each day should be as consistent as the timing of meals and insulin. Long-term DM-1 treatment involves the use of several types of insulin. They are classified according to the duration of action and potency and include intermediate-acting (Lente, Neutral Protamine Hagedorn [NPH]) and long-acting insulins (Protamine Zinc [PZI], Insulin Glargine, Insulin Detemir) (Table 5).

Table 5: Commonly used preparations for treating uncomplicated diabetes mellitus in dogs (adapted from Fracassi, 2017).

INSULIN	PRODUCT	CONCEN-TRATION (U/mL)	DURATION OF EFFECT (hours)	FREQUENCY OF ADMINISTRATION	STARTING DOSAGE (U/Kg/injection)
Lente	Vetsulin / Caninsulin	40	8-14	q12h	0.25
NPH	Humulin N Novolin N	100	4-10	q12h	0.25
PZIcorticoid	ProZinc	40	10-16	q12h	0.25-0.5
Glargine	Lantus	100	8-16	q12h (q24h)	0.3
Detemir	Levemir	100	8-16	q12h (q24h)	0.1

NPH, Neutral Protamine Hagedorn
PZI, protamine zinc

Appropriate dose adjustment requires adequate blood glucose monitoring (through blood glucose curves or continuous glucose monitoring).

3.4 DIAGNOSIS

In most dogs with APS, each endocrinopathy typically appears separately; hence, the diagnosis of supplementary disorders is delayed (Furukawa et al., 2021)

3.4.1. ADDISON'S DISEASE AND HYPOTHYROIDISM

In dogs diagnosed with hypoadrenocorticism as the initial endocrinopathy, in case of poor response to mineralocorticoid therapy, persistent hyponatremia or bradycardia, hypercholesterolemia, or other supportive clinical signs, hypothyroidism should be suspected. (Melendez et al., 1996). TSH is increased in hypothyroidism but in some dogs, it can also be increased, despite normal T4 concentrations, in untreated AD (Topliss et al., 1980). As TSH will normalize after glucocorticoid supplementation, the increase has been explained by a lack of the inhibitory effect of cortisol on the secretion of TSH (Husebye et al., 2014). Therefore, in untreated dogs with AD, to avoid misdiagnosis of hypothyroidism, thyroid function should be evaluated in light of the cortisol concentration, better after diagnosis and treatment with glucocorticoids for minimum 2 weeks to normalize TSH concentrations (Reusch et al., 2017). Nevertheless, these two disorders might coexist in the APS-2 requiring both hormone therapy replacement.

3.4.2. HYPOTHYROIDISM AND DIABETES MELLITUS

Hypothyroidism has been recognized as an uncommon cause of insulin resistance in diabetic dogs (Ford et al., 1993) along with increased concentrations of IGF-1 and GH, which can be documented in hypothyroidism (Diaz-Espiñeira et al., 2009). However, recognizing hypothyroidism in dogs with poorly controlled diabetes mellitus may be challenging, since clinical signs (e.g., lethargy and weakness) and abnormalities in clinical pathologic values (e.g., hyponatremia, lipemia, and hypercholesterolemia) may be present in both disorders. Therefore, thyroid testing should be undertaken in diabetics dogs with poor glucose regulation and the interpretation of thyroid test findings (serum concentrations of T4, fT4, and TSH) must take into account the degree of success achieved in managing hyperglycemia. Furthermore, hypothyroid dogs have increased fructosamine concentrations due to decreased metabolic rate and resultant

decreased protein turnover, which may complicate the assessment of glycemic control in hypothyroid diabetic dogs (Reusch et al., 2002).

### 3.4.3. DIABETES MELLITUS AND ADDISON'S DISEASE

According to one study describing the first clinical case of hypoadrenocorticism diagnosed in a diabetic dog, clinicians should consider the possibility of coexistence of hypoadrenocorticism in dogs with diabetes mellitus presenting with steady weight loss; also, hypoalbuminemia, hypocholesterolemia, and a relatively low blood glucose level with frequent and severe hypoglycemic reactions may be important clues for early diagnosis (Furukawa et al., 2021). Thus, in dogs with type 1 diabetes, a decrease in insulin requirement could be the first sign of Addison's disease. On that account, before modifying insulin dosage, it is prudent to investigate the possible coexistence of an underlying adrenal insufficiency (Schatz et al., 2002).

3.4.4. HISTOPATHOLOGICAL FINDINGS

Studies on histopathology of the target organs involved in Type 2 APS have yielded results similar to those seen in isolated autoimmune forms. Adjacent organs or tissues non directly targeted by the autoimmune reaction are typically spared by the autoimmune attack (Betterle et al., 2004).

No relevant case histories are available in bibliographies describing the underlying histopathologic abnormalities in dogs with hypoadrenocorticism, most likely because the majority of hypoadrenocorticism cases are either successfully treated or the dog dies without a necropsy being performed. In most confirmed cases of hypoadrenocorticism, the typical findings are severe lymphoplasmacytic inflammation and adrenocortical atrophy involving all layers of the adrenal cortex. The pattern of infiltration of adrenals in Addison's disease at the onset is characterized by a widespread mononuclear cell infiltrate consisting of lymphocytes, plasma cells, and macrophages. Residual cortical nodules of regenerating cells may be seen secondary to high levels of corticotropin (ACTH), but in the advanced stages of the disease, fibrosis, and atrophy greatly predominate. In contrast to autoimmune thyroiditis and Type 1 diabetes, there has been no phenotypic characterization of infiltrating lymphocytes (McNicol, 1994).

Figure 11: Left, adrenal cortex free of cellular infiltration or degeneration; ZG, zona glomerulosa; ZF, zona fasciculata. Right, adrenal gland with AD and severe cortical atrophy. The adrenal cortex is devoid of cortical cells and consists of macrophages and numerous lymphocytes in the collapsed stroma (adapted from Frank et al., 2013).
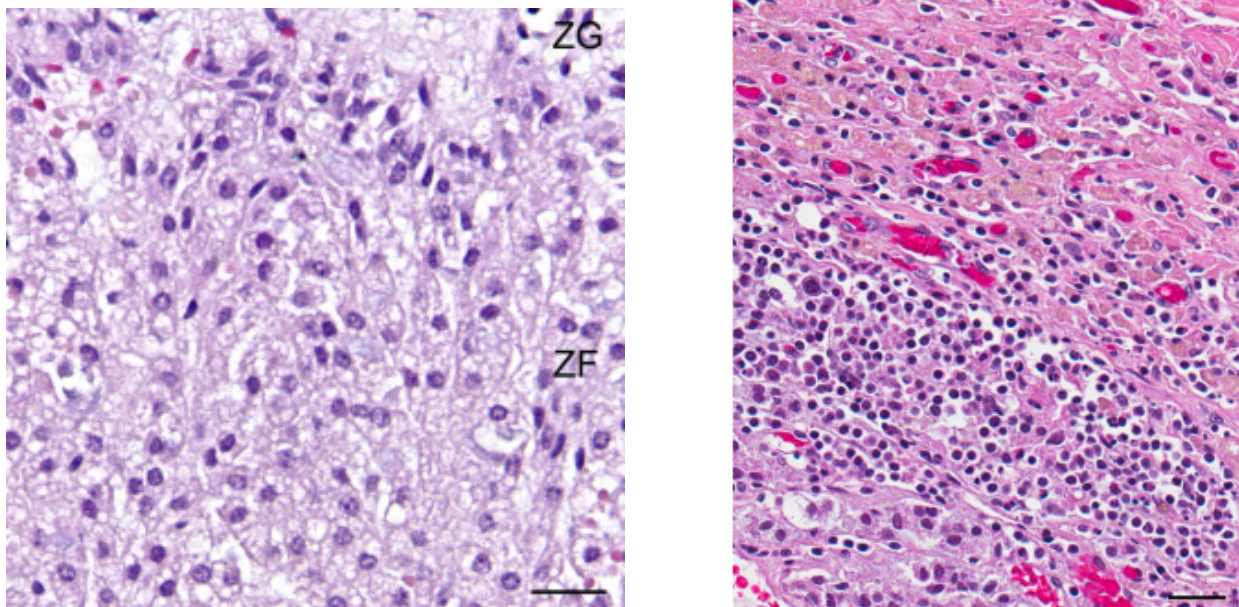
Common histologic abnormalities in dogs include a reduction in the number and size of pancreatic islets, a decrease in the number of beta cells within islets, and beta cell vacuolation and degeneration (Nelson, 2015). The characteristic pathological lesion in the insulitis is the presence of mononuclear immune cells around and within the islets. This infiltration is dominated by T lymphocytes, in particular T cytotoxic/suppressor phenotype and T helper with a few B cells and macrophages (Gepts, 1965). The destructive process is limited to the β-cells, all other endocrine cells of the islets are spared (Peakman, 2011). In the advanced phases, acinar cell atrophy is usually found.

Figure 12: Left, islets of Langerhans (center) in a physiologic pancreas (adapted from Jennings, 2017). Right, Pancreas, islet of Langerhans. Islets show a massive lymphocytic infiltration associated with decreased and lost endocrine cells (adapted from Jouvion et al., 2006)
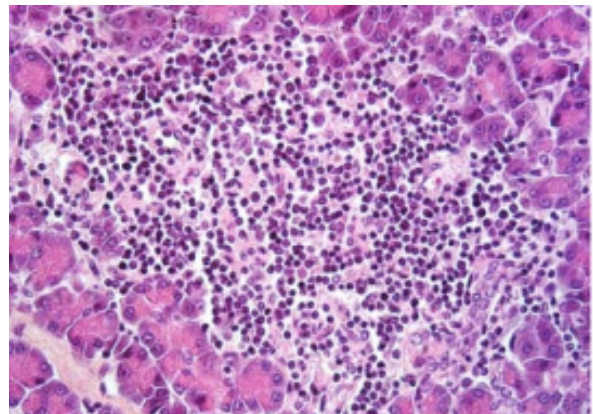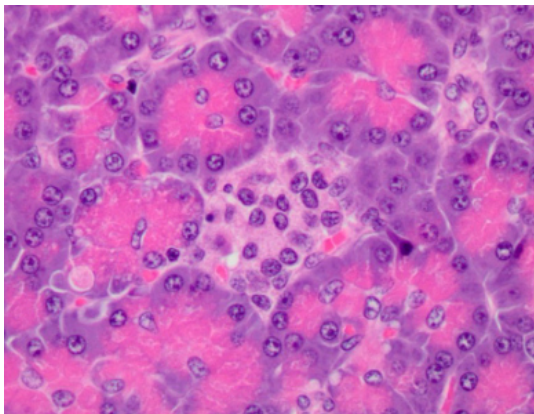
Histologically, lymphocytic thyroiditis is characterized by multifocal to diffuse infiltration of lymphocytes, plasma cells, and macrophages into the thyroid gland, with the formation of some lymphoid nodules and destruction of follicles (Gosselin et al., 1981). Frequently, lymphocytes are organized into well-developed germinal centers. Thyroid follicles are of reduced size and variable degrees of fibrosis are present. The infiltrating T cells are mainly CD8+, but also CD4+ T cells are present, many of which are activated as they express HLA class II molecules (Weetman et al., 1994).

Figure 13: Left, physiologic thyroid gland (adapted from Jennings, 2017). Right, a large number of lymphoplasmacytic infiltrate in the interstitium and thyroid follicles. A lymph follicle with a germinal center (arrow) is found. Thyroid follicles in the inflamed area are small with a decrease in colloid, whereas those in the non-inflamed area are morphologically normal (adapted from Doi et al., 2017).
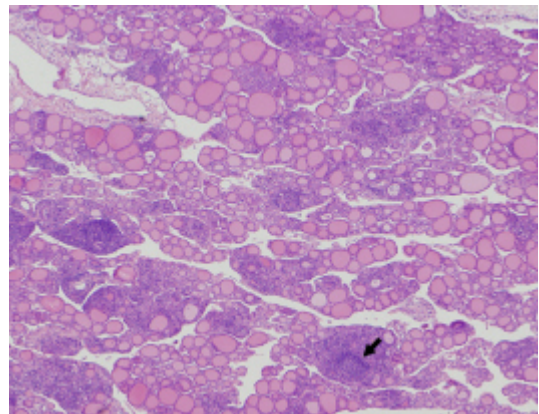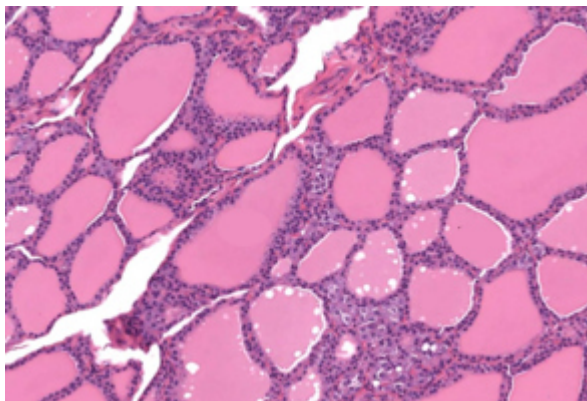
3.5 THERAPY

The therapies regarding the different components of Type 2 APS are similar whether they occur as single or in multiple associations with other autoimmune diseases. Hence, the hormonal therapy of APS/MAS 2 consists of a single treatment for every AID, which is the specific replacement of the hormones produced by the damaged endocrine organ (Betterle et al., 2004). However, in patients with APS, physiologic interactions of hormones need to be considered.

3.5.1. ADDISON'S DISEASE AND HYPOTHYROIDISM

It is worth remembering that for dogs with concurrent hypothyroidism and hypoadrenocorticism, treatment with fludrocortisone or deoxycorticosterone pivalate (DOCP) should be initiated first, followed by levothyroxine therapy. That's because, since thyroxine enhances hepatic corticosteroid metabolism, in hypothyroidism, the low serum thyroxine concentrations reduce the rate of clearance of cortisol and cholesterol, and when thyroid supplementation is initiated, cortisol clearance increases suddenly without a compensatory increase in cortisol synthesis because of undiagnosed adrenal insufficiency. As a result, thyroid therapy may precipitate an adrenal crisis (Canbay et al., 2000) (Patel et al., 2020). Hence, in animals with suspected multiple endocrinopathies, the adrenal function should be assessed prior to initiation of levothyroxine therapy, and glucocorticoids replacement should precede thyroxine therapy. In addition, some patients with Addison's disease show a normalization in thyrotropin levels following glucocorticoids therapy, regardless of the presence of thyroid autoantibodies, which is related to the loss of the inhibitory effects of glucocorticoids on thyrotropin secretion (Eisenbarth et al., 2004).

3.5.2. HYPOTHYROIDISM AND DIABETES MELLITUS

Since hypothyroidism can cause insulin resistance in diabetic dogs, its treatment can increase insulin sensitivity, reducing the dosage of insulin required to treat diabetes. Hence, blood glucose concentration should be carefully monitored when thyroid supplementation is initiated. How much to decrease the insulin dosage is variable and dependent, in part, on the severity of insulin resistance, the amount of insulin being administered, and the expected rapidity of improvement in insulin resistance after treatment of the disorder. In one study of three hypothyroid-diabetic dogs, hypoglycemia was documented within 2 weeks of starting sodium levothyroxine administration. The insulin dosage was decreased by 60 to 62% during the ensuing months and good glycemic control was obtained at these lower insulin dosages in all dogs (Ford et al., 1993).

3.5.3. DIABETES MELLITUS AND ADDISON'S DISEASE

DOCP may be useful in treating dogs with diabetes mellitus complicated with hypoadrenocorticism, since it has a stronger mineralocorticoid effect, while fludrocortisone may increase insulin resistance for its glucocorticoid activity (Furukawa et al., 2021). Additionally, since supplementation with glucocorticoids and DOCP in dogs with hypoadrenocorticism could negatively affect insulin sensitivity, a gradual increase in insulin dosage could be required if diabetes is concomitant (Greco, 2020).

Because of the complexity of hormonal therapy, patients must be instructed and informed about these interactions in order to avoid complications when taking the drug and/or side effects (Kahaly et al., 2018).

4 CONCLUSIONS

In conclusion, multiple endocrine deficiencies occur with a low incidence in dogs, with hypoadrenocorticism, hypothyroidism, and diabetes mellitus type 1 among the most common disorders involved. This syndrome appears to be similar to APS 2 in human beings and they are all potentially immune-mediate in origin and may be genetically related. APS should be suspected when multiple endocrine gland failure is identified in a dog. Even just the presence of one endocrinopathy should alert a veterinarian to the possibility of a patient developing a poly-endocrinopathy. Practitioners should also be aware that additional autoimmune non-endocrine organ involvement may occur, resulting in highly variable disease manifestations and clinical presentation. (Kuijlaars et al., 2021)

Even if each disorder is diagnosed separately, it is important to consider the effects that one endocrine disorder may have on the tests used to diagnose another disorder (e.g., untreated diabetes mellitus suppresses circulating thyroid hormone concentrations) (Scott-Moncrieff, 2015). In particular, veterinarians should be aware of some of the features of APS. For example, hypothyroidism should be suspected in dogs with hypoadrenocorticism if there is a poor response to mineralocorticoid therapy; and diabetic dogs that exhibit inadequate glycemic control should be tested for hypothyroidism as well. The same applies to the treatment. It is critical to consider the effects that treating one endocrine disorder may have on the treatment of concurrent endocrine disorders (e.g., initiation of thyroid supplementation may dramatically improve insulin sensitivity in a diabetic animal; thyroid supplementation may precipitate an Addisonian crisis in hypoadrenocorticism) (Scott-Moncrieff, 2015).

Although none of these disorders can be reliably predicted or prevented, organ-specific autoantibody screening in patients with monoglandular autoimmune endocrinopathies would undoubtedly aid in the identification of those at risk of developing a future APS. Early detection and treatment of another autoimmune endocrine disease may be critical, even lifesaving. Currently, these diseases can only be managed with pharmacological replacement therapy. However, progress in understanding the inner immunological mechanisms involved in these conditions should allow for common treatments aimed at preventing or at least slowing the progression to irreversible multiple organ damage

(Betterle et al., 2004). Immunosuppressive drug therapy is not indicated in these syndromes and may actually create problems (e.g., insulin resistance or thyroid suppression with high-dose glucocorticoid therapy) (Scott-Moncrieff, 2015).

Additional genetic and clinical studies are required to fully investigate the existence of autoimmune polyendocrine disorders in dogs.

BIBLIOGRAPHY

Abbas A.K., Lichtman A.H. «Introduzione al sistema immunitario. Nomenclatura, proprietà generali e componenti del sistema immunitario.» In Abbas A.K., Lichtman A.H., *Le basi dell'immunologia. Fisiopatologia del sistema immunitario,* 2nd ed*.,* Milano: Elsevier, 2006: 1-20.

Adler J.A., Drobatz K.J., Hess R.S. «Abnormalities of Serum Electrolyte Concentrations in Dogs with Hypoadrenocorticism.» *Journal of Veterinary Internal Medicine*, 2007: 21(6):1168-1173.

Ahmad H.I., Jabbar A., Mushtaq N., Javed Z., Hayyat M.U., Bashir J., Naseeb I., Abideen Z.U., Ahmad N., Chen J. «Immune Tolerance vs. Immune Resistance: The Interaction Between Host and Pathogens in Infectious Diseases.» *Frontiers in Veterinary Science*, 2022: 9:827407.

Amendt T., Jumaa H. «Adaptive tolerance: protection through self-recognition.» *BioEssays*, 2022: 44(3):e2100236.

Amoura Z., Piette J.C. «Nouvelles approches thérapeutiques des maladies auto-immunes.» *Revue Neurologique*, 2006: 162:7-11.

Amr B.S., Mamillapalli C. «Achalasia in a patient with polyglandular autoimmune syndrome type II.» *Case Reports in Gastroenterology*, 2015: 9(2):160-164.

Anderson M.S. «Autoimmune endocrine disease.» In *Current Opinion in Immunology*, 2002: 14(6):760–764.

Avrameas S., Selmi C. «Natural autoantibodies in the physiology and pathophysiology of the immune system.» *Journal of Autoimmunity*, 2013: 41:46-49.

Badenhoop K. «CTLA4 variants in type 1 diabetes: some stirrups serve better backing endocrine autoimmunity.» *Clinical Endocrinology*, 2000: 52(2):139-140.

Baker J.R.Jr «Autoimmune Endocrine Disease.» *Jama*, 1997: 278(22):1931-1937.

Balague´ C., Kunkel S.L., Godessart N. «Understanding autoimmune disease: new targets for drug discovery.» *Drug Discovery Today*, 2009: 14(19-20):926-934.

Bates J.A, Shott S., Schall W.D. «Lower initial dose desoxycorticosterone pivalate for treatment of canine primary hypoadrenocorticism.» *Australian Veterinary Journal*, 2013: 91(3):77-82.

Betterle C., Lazzarotto F., Presotto F. «Autoimmune polyglandular syndrome Type 2: the tip of an iceberg?» *Clinical and Experimental Immunology*, 2004: 137(2):225-233.

Betterle C., Sabbadin C., Scaroni C., Presotto F. «Autoimmune Polyendocrine Syndromes (APS) or Multiple Autoimmune Syndromes (MAS).» *Polyendocrine Disorders and Endocrine Neoplastic Syndromes*, 2021: 1-50.

Bjørklund G., Pivin M., Hangan T., Yurkovskaya O., Pivina L. «Autoimmune polyendocrine syndrome type 1: Clinical manifestations, pathogenetic features, and management approach.» *Autoimmunity Reviews*, 2022: 21(8):103-135.

Blois S.L., Dickie E.L., Kruth S.A., Allen D.G. «Multiple endocrine diseases in cats: 15 cases (1997-2008).» *Journal of Feline Medicine and Surgery*, 2010: 12(8):637-642.

Blois S.L., Dickie E., Kruth S.A., Allen D.G. «Multiple endocrine diseases in dogs: 35 cases (1996-2009).» *Journal of the American Veterinary Medical Association*, 2011: 238(12):1616-1621.

Bovens C., Tennant K., Reeve J., Murphy K.F. «Basal serum cortisol concentration as a screening test for hypoadrenocorticism in dogs.» *Journal of Veterinary Internal Medicine*, 2014: 28(5): 1541-1545.

Canbay A., Gieseler R., Ella R., Fink H., Saller B., Mann K. «Manifestation einer
Nebennierenrindeninsuffizienz nach Gabe von Levothyroxin bei einem Patienten
mit polyglandulärem Autoimmunsyndrom Typ II (Schmidt-Syndrom)» *Der
Internist*, 2000: 41(6):588-591.

Catchpole B., Adams J.P., Holder A.L., Short A.D., Ollier W.E.R., Kennedy L.J.
«Genetics of canine diabetes mellitus: Are the diabetes susceptibility genes
identified in humans involved in breed susceptibility to diabetes mellitus in
dogs?» *The Veterinary Journal*, 2013: 195(2):139-147.

Chauhan R., Raina V., Nandi S.P. «Prevalence of Autoimmune Diseases and Its
Challenges in Diagnosis.» *Critical Reviews in Immunology*, 2019: 39(3):189-201.

Colafrancesco S., Perricone C., Priori R., Valesini G., Shoenfeld Y. «Sjogren's
syndrome: another facet of the autoimmune/inflammatory syndrome induced by
adjuvants (ASIA).» *Journal of Autoimmunity*, 2014: 51:10-16.

Conaway D.H., Padgett G.A., Nachreiner R.F., Prieur D.J. «The Familial Occurrence of
Lymphocytic Thyroiditis in Borzoi dogs.» *American Journal of Medical Genetics*,
1985: 22(2):409-414.

Damoiseaux J., Andrade L.E., Fritzler M.J., Shoenfeld Y. «Autoantibodies 2015: from
diagnostic biomarkers toward prediction, prognosis, and prevention.»
Autoimmunity Reviews, 2015: 14(6):555-563.

Davidson A., Diamond B. «Autoimmune diseases.» *New England Journal of Medicine*,
2001: 345(5):340-350.

Davison L.J., Weenink S.M, Christie M.R., Herrtage M.E., Catchpole B. «Autoantibodies
to GAD65 and IA-2 in canine diabetes mellitus.» *Veterinary Immunology and
Immunopathology*, 2008: 126(1-2):83-90.

Davison L.J., Herrtage M.E., Catchpole B. «Autoantibodies to recombinant canine proinsulin in canine diabetic patients.» *Research in Veterinary Science*, 2011: 91(1):58-63.

Del Baldo F., Gerou Ferriani M., Bertazzolo W., Luciani M., Tardo A.M., Fracassi F. «Urinary cortisol-creatinine ratio in dogs with hypoadrenocorticism.» *Journal of Veterinary Internal Medicine*, 2022: 36(2):482-487.

Denyer A.L., Massey J.P., Davison L. J., Ollier W. E. R., Catchpole B., Kennedy L. J. «Dog leucocyte antigen (DLA) class II haplotypes and risk of canine diabetes mellitus in specific dog breeds.» *Canine Medicine and Genetics*, 2020: 7(1):1-13.

Diaz-Espiñeira M.M., Mol J.A., Rijnberk A., Kooistra H.S. «Adenohypophyseal function in dogs with primary hypothyroidism and nonthyroidal illness.» *Journal of Veterinary Internal Medicine*, 2009: 23(1):100-107.

Dixon R.M. Reid S.W., Mooney C.T. «Epidemiological, clinical, hematological and biochemical characteristics of canine hypothyroidism.» *Veterinary Records*, 1999: 145(17):481-487.

Doi T., Tomonari Y., Kawasako K., Yamada N., Tsuchitani M. «Lymphocytic adrenal medullitis and lymphocytic thyroiditis in a laboratory beagle dog.» *Journal of Veterinary Medical Science*, 2017: 79(2): 255–257.

Eisenbarth G.S., Gottlieb P.A. «Autoimmune Polyendocrine Syndromes.» *New England Journal Medicine*, 2004: 350(20):2068-2079.

Famula T.R., Belanger J.M., Oberbauer A.M. «Heritability and complex segregation analysis of hypoadrenocorticism in the standard poodle.» *Journal of Small Animal Practice*, 2003: 44(1):8-12.

Finnish-German APECED Consortium. «An autoimmune disease: APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains.» *Nature Genetics*, 1997: 17(4):399-403.

Ford S.L., Nelson R.W., Feldman E.C., Niwa D. «Insulin resistance in three dogs with hypothyroidism and diabetes mellitus.» *Journal of the American Veterinary Medical Association*, 1993: 202(9):1478-1480.

Fracassi F., «Canine Diabetes Mellitus.» In Ettinger S.J., Feldman E.C., Cote E., *Textbook of Veterinary Internal Medicine*, 8th ed, St. Louis: Elsevier, 2017: 4290.

Fracassi F., Pietra M., Boari A., Aste G., Giunti M., Famigli-Bergamini P. «Breed distribution of canine diabetes mellitus in Italy.» *Veterinary Research Communications*, 2004: 1:339-342.

Frank C.B., Valentin S.Y., Scott-Moncrieff J.C.R., Miller M.A. «Correlation of Inflammation with Adrenocortical Atrophy in Canine Adrenalitis.» *Journal of Comparative Pathology*, 2013: 149(2-3):268-279.

Furukawa S., Meguri N., Koura K., Koura H., Matsuda A. «A Case of Canine Polyglandular Deficiency Syndrome with Diabetes Mellitus and Hypoadrenocorticism.» *Veterinary Sciences*, 2021: 8(3):43.

Gepts W. «Pathologic anatomy of the pancreas in juvenile diabetes.» *Diabetes*, 1965: 14(10):619-633.

Gershony L.C., Belanger J.M., Short A.D., Le M., Hytönen M.K., Lohi H., Famula T.R., Kennedy L.J., Oberbauer A.M. «DLA class II risk haplotypes for autoimmune diseases in the bearded collie offer insight to autoimmunity signatures across dog breeds.» *Canine Genetics and Epidemiology*, 2019: 6(1):1-13.

Gosselin S.J., Capen C.C., Martin S.L. «Histologic and Ultrastructural Evaluation of Thyroid Lesions.» *Veterinary Pathology*, 1981: 18(3):299-309.

Gosselin S.J., Capen C.C., Martin S.L., Targowski S.P. «Biochemical and immunological investigations on hypothyroidism in dogs.» *Canadian Journal of Comparative Medicine*, 1980: 44(2):158-168.

Gow A.G., Gow D.J., Bell R., Simpson J.W., Chandler M.L., Evans H., Berry J.L., Herrtage M.E., Mellanby R.J. «Calcium metabolism in eight dogs with hypoadrenocorticism.» *Journal of Small Animal Practice*, 2009: 50(8):426-430.

Graham P.A., Refsal K.R., Nachreiner R.F. «Etiopathologic Findings of Canine Hypothyroidism.» *Veterinary Clinics of North America: Small Animal Practice*, 2007: 37(4):617-631.

Graham P. «Canine hypothyroidism: diagnosis and therapy.» *Companion Animal Practice*, 2009: 31(2):77-82.

Greco D. «*Canine Autoimmune Polyglandular Syndromes.*» In Bruyette D.S*., Small Animal Internal Medicine*, Vol.1, Hoboken, New Jersey: John Wiley & Sons, Inc, 2020: 113-116.

Green S.T., Ng J.P. «Hypothyroidism and anemia.» *Biomed Pharmacother*, 1986: 40(9):326-331.

Greenspan F.S., «The thyroid gland.» In Greenspan F.S., Gardner D.G., *Basic and clinical endocrinology*, 6th ed., New York: McGraw-Hill Education, 2001: 201.

Guptill L., Glickman L., Glickman N. «Time Trends and Risk Factors for Diabetes Mellitus in Dogs: Analysis of Veterinary Medical Data Base Records (1970–1999).» *The Veterinary Journal*, 2003: 165(3):240-247.

Hess R.S., «Hypoadrenocorticism.» In Ettinger S.J., Feldman E.C., Cote E., *Textbook of Veterinary Internal Medicine*, 8th ed, St. Louis: Elsevier, 2017: 4431.

Hoerauf A., Reusch C. «Ultrasonographic evaluation of the adrenal glands in six dogs with hypoadrenocorticism.» *Journal of the American Animal Hospital Associaton*, 1999: 35(3):214-218.

Husebye E.S., Allolio B., Arlt W., Badenhoop K., Bensing S., Betterle C., Falorni A., Gan E.H., Hulting A.L., Kasperlik-Zaluska A., Kämpe O., Løvås K., Meyer G., Pearce S.H. «Consensus statement on the diagnosis, treatment, and follow-up of patients with primary adrenal insufficiency.» *Journal of Internal Medicine*, 2014: 275(2):104-115.

Hwang S.Y., An J.H., Lee J.H., Park S.M., Kyu Chae H., Kim K.B., Song W.J., Youn H.Y. «Autoimmune polyendocrine syndrome with hypoadrenocorticism and diabetes mellitus in a dog: A rare case.» *Veterinary Medicine and Science*, 2021: 7(6):2120-2123.

Jacob F., Polzin D.J., Osborne C.A., Neaton J.D., Kirk C.A., Allen T.A., Swanson L.L. «Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure.» *Journal of Small Animal Practice*, 2005: 226(3):393-400.

Javadi S., Galac S., Boer P., Robben J.H., Teske E., Kooistra H.S. «Aldosterone-to-renin and cortisol-to-adrenocorticotropic hormone ratios in healthy dogs with primary hypoadrenocorticism.» *Journal of Veterinary Internal Medicine*, 2006: 20(3):556-561.

Jennings R. «Pancreas: Exocrine and Endocrine.», in Premanandan C., Jennings R., *Veterinary histology*, Ohio State University, 2017.

Jennings R. «Thyroid gland.», in Premanandan C., Jennings R., *Veterinary histology*, Ohio State University, 2017.

Jouvion G., Abadie J., Bach J.M., Roux F., Miclard J., Deschamps J.Y., Guigand L., Saï P., Wyers M. «Lymphocytic Insulitis in a Juvenile Dog with Diabetes Mellitus.» *Endocrine Pathology*, 2006: 17(3):283-290.

Kahaly G.J., Frommer L. «Polyglandular autoimmune syndromes.» *Journal of Endocrinological Investigation*, 2018: 41(1):91-98.

Kennedy L.J., Huson H.J., Leonard J., Angles J.M., Fox L.E., Wojciechowski J.W., Yuncker C., Happ G.M. «Association of hypothyroid disease in Doberman Pinscher dogs with a rare major histocompatibility complex DLA class II haplotype.» *Tissue antigens*, 2005: 67(1):53-56.

Kennedy L.J., Quarmby S., Happ G.M., Barnes A., Ramsey I.K., Dixon R.M., Catchpole B., Rusbridge C., Graham P.A., Hillbertz N.S., Roethel C., Dodds W.J., Carmichael N.G., Ollier W.E. «Association of canine hypothyroidism with a common major histocompatibility complex DLA class II allele.» *Tissue antigens*, 2006: 68(1):82-86.

Kintzer P.P., Peterson M.E. «Thyroid scintigraphy in small animals.» *Seminars Veterinary Medicine and Surgery of Small Animal*, 1991: 6(2):131-139.

Kintzer P.P., Peterson M.E. «Treatment and long-term follow-up of 205 dogs with hypoadrenocorticism.» *Journal of Veterinary Internal Medicine*, 1997: 11(2):43-49.

Kooistra H.S., Rijnberk A., van den Ingh T.S. «Polyglandular deficiency syndrome in a boxer dog: Thyroid hormone and glucocorticoid deficiency.» *Veterinary Quarterly*, 1995: 17(2):59-63.

Kuijlaars M., Yool D.A., Ridyard A.E. «Autoimmune polyendocrine syndrome in a standard poodle with concurrent non endocrine immune-mediated diseases.» *Veterinary Record Case Reports*, 2021: 9(3):e90.

Lathan P., Moore G.E., Zambon S., Scott-Moncrieff J.C. «Use of low-dose ACTH stimulation test for diagnosis of hypoadrenocorticism in dogs.» *Journal of Veterinary Internal Medicine*, 2008: 22(4):1070-1073.

Lathan P. «Hypoadrenocorticism in dogs.» In Behrend E., Gunn-Moore D., Campbell-Ward M., Rand J., *Clinical Endocrinology of Companion Animals*, Hoboken, New Jersey: John Wiley & Sons, 2013: 1-21.

Lennon E.M., Boyle T.E., Hutchins R.G., Friedenthal A., Correa M.T., Bissett S.A., Moses L.S., Papich M.G., Birkenheuer A.J. «Use of basal serum or plasma cortisol concentrations to rule out a diagnosis of hypoadrenocorticism in dogs: 123 cases (2000-2005).» *Journal of American Veterinary Medicine Association*, 2007: 231(3):413-416.

LiVolsi V.A. «The pathology of autoimmune thyroid disease: a review.» *Thyroid*, 1994: 4(3):333-339.

Lleo A., Invernizzi P., Gao B., Podda M., Gershwin M.E. «Definition of human autoimmunity–autoantibodies versus autoimmune disease.» *Autoimmunity Reviews*, 2010: 9(5):259-266.

Mansfield C., Mooney C.T. «Lymphocytic-plasmacytic thyroiditis and glomerulonephritis in a boxer.» *Journal of Small Animal Practice*, 2006: 47(7):396-399.

Manzari V. «Autoimmunità.», in Manzari V., *Immunologia ed immunoematologia*, 2nd ed., Padova: Piccin, 2003:143-151.

Marmor M., Willeberg P., Glickman L.T., Priester W.A., Cypess R.H., Hurvitz A.I. «Epizootiologic patterns of diabetes mellitus in dogs.» *Journal of Veterinary Research*, 1982: 43(3):465-470.

McNicol A.M. «Histopathology of adrenal glands in Addison's disease.» In Bhatt H.R., James V.H., Bessere G.M., Bottazzo G.F., Keen H., *Advances in Thomas Addison's diseases,* vol 2. Bristol, UK*: Journal of Endocrinology*, 1994: 4:5-11.

Mehr R., Globerson A., Perelson A.S. «Modeling Positive and Negative Selection and Differentiation Processes in the Thymus.» *Journal of Theoretical Biology*, 1995: 175(1):103-126.

Melendez L.D., Greco D.S., Turner J.L., et al. «Concurrent hypoadrenocorticism and hypothyroidism in 10 dogs.» *Journal of Veterinary Internal Medicine*, 1996: 10:182.

Melián C., Peterson M.E. «Diagnosis and treatment of naturally occurring hypoadrenocorticism in 42 dogs.» *Journal of Small Animal Practice*, 1996: 37:(6):268-275.

Melián C., Stefanacci J., Peterson M.E., Kintzer P.P. «Radiographic findings in dogs with naturally-occurring primary hypoadrenocorticism.» *Journal of the American Animal Hospital Association*, 1999: 35(3):208-212.

Nachreiner R.F., Refsal K.R., Graham P.A., Bowman M.M. «Prevalence of serum thyroid hormone autoantibodies in dogs with clinical signs of hypothyroidism.» *Journal of American Veterinary Medicine Association*, 2002: 220(4):466-471.

Nelson R.W., «Canine Diabetes Mellitus.» In Feldman E.C., Nelson R.W., Reusch C.E., Scott-Moncrieff J.C.R, Behrend E.N., *Canine and feline endocrinology and reproduction, 4th ed.,* St Louis: Elsevier Saunders, 2015: 213-257.

Nelson R.W. «Dietary management of diabetes mellitus.» *Journal of Small Animal Practice*, 1992: 33(5):213-217.

Nerup J., Mandrup-Poulsen T , Helqvist S , Andersen H.U., Pociot F., Reimers J.I., Cuartero B.G., Karlsen A.E., Bjerre U., Lorenzen T. «On the pathogenesis of IDDM.» *Diabetologia*, 1994: 37(2):82-89.

Nishida Y., Higaki Y., Tokuyama K., Fujimi K., Kiyonaga A., Shindo M., Sato Y., Tanaka H. «Effect of mild exercise training on glucose effectiveness in healthy men.» *Diabetes Care*, 2001: 24(6):1008-1013.

Oberbauer A.M., Benemann K.S., Belanger J.M., Wagner D.R., Ward J.H., Famula T.R. «Inheritance of hypoadrenocorticism in bearded collies.» *American Journal of Veterinary Research*, 2002: 63:(5)643-647.

Oftedal B.E.V. «Autoimmune polyendocrine syndrome type I. Novel diagnostic assays and immune regulation.» Dissertation for the degree phiosophiae doctor (PhD) at the University of Bergen, 2012.

Ohishi K., Kanoh M., Shinomiya H., Hitsumoto Y., Utsumi S. «Complement activation by cross-linked B cell-membrane IgM.» *Journal of Immunology*, 1995: 154(7):3173-3179.

Panda S., Ding J.L. «Natural antibodies bridge innate and adaptive immunity.» *Journal of Immunology*, 2015: 194(1):13-20.

Parry N.M.A. «Hypothyroidism in dogs: laboratory findings.» *Companion animal*, 2013: 18(3):101-105.

Parry N.M.A. «Hypothyroidism in dogs: pathophysiology, causes, and clinical presentation.» *Companion animal*, 2013: 18(2):34-38.

Patel D.M., Gurumikhani J.K., Patel M.V., Patel M.M., Patel S.Y., Patel P.N. «Adrenocortical Crisis Triggered by Levothyroxine in an Unrecognized Autoimmune Polyglandular Syndrome Type-2: A Case Report with Review of the Literature.» *Current Drug Safety*, 2020: 16(1):101-106.

Peakman M., «Immunology of type 1 diabetes.» In Wass J.A.H, Stewart P.M., Amiel S.A., Davies M.J., *Oxford textbook of endocrinology and diabetes*, 2nd ed., Oxford: Oxford University Press, 2011.

Peterson M.E., Kintzer P.P., Kass P.H. «Pretreatment clinical and laboratory findings in dogs with hypoadrenocorticism: 225 cases (1979-1993).» *Journal of American Veterinary Medicine Association*, 1996: 208(1):85-91.

Raber W., Gessl A., Nowotny P., Vierhapper H. «Thyroid ultrasound versus anti-thyroid peroxidase antibody determination: a cohort study of 451 subjects.» *Thyroid*, 2002: 12(8):725-731.

Redondo M.J., Eisenbarth G.S. «Genetic control of autoimmunity in Type I diabetes and associated disorders.» *Diabetologia*, 2002: 45(5):605-622.

Reese S., Breyer U., Deeg C., Kraft W., Kaspers B. «Thyroid sonography as an effective tool to discriminate between euthyroid sick and hypothyroid dogs.» *Journal of Veterinary Internal Medicine*, 2005: 19(4):491-498.

Reusch C.E., Fracassi F., Sieber-Ruckstuhl N.S., Burkhardt W.A., Hofer-Inteeworn N., Schuppisser C., Stirn M., Hofmann-Lehmann R., Boretti F.S. «Altered Serum Thyrotropin Concentrations in Dogs with Primary Hypoadrenocorticism before and during Treatment.» *Journal of Veterinary Internal Medicine*, 2017: 31(6):1643-1648.

Reusch C.E., Gerber B., Boretti F.S. «Serum fructosamine concentrations in dogs with hypothyroidism.» *Veterinary Research Community*, 2002: 26(7):531-536.

Rosato E., Pisarri S., Salsano F. «Current strategies for the treatment of autoimmune diseases.» *Journal of Biological Regulators and Homeostatic Agents*, 2010: 24(3):251-259.

Rose N.R., Bona C. «Defining criteria for autoimmune diseases (Witebsky's postulates revisited).» *Immunology Today*, 1993: 14(9):426-430.

Ruggeri R.M., Giuffrida G., Campennì A. «Autoimmune endocrine diseases.» *Minerva Endocrinology*, 2018: 43(3):305-322.

Salinas G.F., Branza F., Brouard S., Tak P.P., Baeten D. «The role of B lymphocytes in the progression from autoimmunity to autoimmune disease.» *Clinical Immunology*, 2013: 146(1):34-45.

Schaer M. «The treatment of acute adrenocortical insufficiency in the dog.» *Journal of Veterinary Emergency Critical Care*, 2001: 11(1):7-14.

Schatz D.A., Winter W.E. «Autoimmune polyglandular syndrome II. Clinical syndrome and treatment.» *Endocrinology and Metabolism Clinics of North America*, 2002: 31(2):339–352.

Scott-Moncrieff J.C.R., «Hypoadrenocorticism.» In Feldman E.C., Nelson R.W., Reusch C.E., Scott-Moncrieff J.C.R, Behrend E.N., *Canine and feline endocrinology and reproduction, 4th ed,* St Louis: Elsevier Saunders, 2015: 485-520.

Scott-Moncrieff J.C.R., «Hypothyroidism.» In Feldman E.C., Nelson R.W., Reusch C.E., Scott-Moncrieff J.C.R, Behrend E.N., *Canine and feline endocrinology and reproduction, 4th ed,* St Louis: Elsevier Saunders, 2015: 77-135.

Sebastiani G.D., Passiu G. «Attuali prospettive nella terapia delle malattie autoimmuni.» *Annali Italiani di Medicina Interna*, 1992: 7(2):95-101.

Seth M., Drobatz K.J., Church D.B., Hess R.S. «White blood cell count and the sodium to potassium ratio to screen for hypoadrenocorticism in dogs.» *Journal of Veterinary Internal Medicine*, 2011: 25(6):1351-1356.

Shiel R.E., Acke E., Puggioni A., Cassidy J.P., Mooney C.T. «Tertiary hypothyroidism in a dog.» *Irish Veterinary Journal*, 2007: 60(2):88-93.

Sjaastad O.V., Sand O., Hove K. «Il sistema endocrino.», in Sjaastad O.V., Sand O., Hove K., *Fisiologia degli animali domestici*, 2nd ed., Rozzano, Milano: Casa Editrice Ambrosiana, 2010: 212-250.

Skopek E., Patzl M., Nachreiner R.F. «Detection of autoantibodies against thyroid peroxidase in serum samples of hypothyroid dogs.» *American Journal of Veterinary Research*, 2006: 67(5): 809-814.

Snyder P.W. «Malattie immunitarie.» In Zachary J.F., *Patologia generale Veterinaria*, 6th ed., Milano: Edra S.p.a., 2018: 254-299.

Song Y.H., Li Y., Maclaren N.K. «The nature of autoantigens targeted in autoimmune endocrine diseases.» *Immunology Today*, 1996:17(5):232-238.

Sullivan P., Gompf R., Schmeitzel L. «Altered platelet indices in dogs with hypothyroidism and cats with hypothyroidism.» *American Journal of Veterinary Research*, 1993: 54(12):2004-2009.

Tadmor B., Putterman C., Naparstek Y. «Embryonal germ-layer antigens: target for autoimmunity.» *Lancet*, 1992: 339(8799):975-978.

Tag T.L., Day T.K. «Electrocardiographic assessment of hyperkalemia in dogs and cats.» *Journal of Veterinary Emergency and Critical Care*, 2008: 18(1):61-67.

Tait K.F., Gough S.C. «The genetics of autoimmune endocrine disease.» *Clinical Endocrinology*, 2003: 59(1):1-11.

Tani H., Nabetani T., Sasai K., Baba E. «Proliferative Responses to Canine Thyroglobulin of Peripheral Blood Mononuclear Cells from Hypothyroid Dogs.» *Journal of Veterinary Medical Science*, 2005: 67(4):363-368.

Topliss D.J., White E.L., Stockigt J.R. «Significance of thyrotropin excess in untreated primary adrenal insufficiency.» *Journal of Clinical Endocrinology and Metabolism*, 1980: 50(1):52-56.

Van Lanen K., Sande A. «Canine Hypoadrenocorticism: Pathogenesis, Diagnosis, and Treatment.» *Topics in Companion Animal Medicine*, 2014: 29(4):88-95.

Waldmann H. «Mechanism of immunological tolerance.» *Clinical Biochemistry*, 2016: 49(4-5):324-328.

Wang L, Wang F.S., Gershwin M.E. «Human autoimmune diseases: a comprehensive update.» *Journal of Internal Medicine*, 2015: 278(4):369-395.

Weetman A.P., McGregor A.M. «Autoimmune thyroid disease: further developments in our understanding.» *Endocrine Reviews*, 1994: 15(6):788-830.

Wenger M., Mueller C., Kook P.H., Reusch C.E. «Ultrasonographic evaluation of adrenal glands in dogs with primary hypoadrenocorticism or mimicking diseases.» *Veterinary Records.*, 2010: 167(6):207-210.

Whitley N.T. «Megaoesophagus and glucocorticoid-deficient hypoadrenocorticism in a dog.» *Journal of Small Animal Practice*, 1995: 36(3):132-135.

Zhao M., Liu S., Luo S., Wu H., Tang M., Cheng W., Zhang Q., Zhang P., Yu X., Xia Y., Yi N., Gao F., Wang L., Yung S., Chan T.M., Sawalha A.H., Richardson B., Gershwin M.E., Li N., Lu Q. «DNA methylation and mRNA and microRNA expression of SLE CD4+ T cells correlate with disease phenotype.» *Journal of Autoimmunity*, 2014: 54:127-136.