Contribution to the study of Feline Pemphigus Foliaceus

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Abstract

Pemphigus foliaceus is the most common auto-immune skin disease in cats. No age, breed or sex predisposition are reported.

A retrospective study of eleven cats diagnosed for pemphigus foliaceus over a period of three years in “ADVETIA” clinic was performed. Age at onset ranged from less than 1 year to 10 years, median 5 years. Clinical signs of the disease included crusts (n=11), paronychia (n=7), erosions (n=4) and alopecia (n=4). Lesions were most common on claw folds (n=7), outer pinnae (n=7) and/or inner pinnae (n=3), nasal planum (n=3) and muzzle (n=3). Cytologic evaluation revealed acantholytic keratinocytes in 7 cats. The histological examination of 4 biopsy specimens revealed acantholytic keratinocytes and neutrophilic crusts (n=2). Active acantholysis was not observed in 1 case. All cases were followed for 1 to 49 months. Most cats (n=9) achieved total remission with immunosuppressive treatment. Only one cat died due to systemic signs development during therapy.
Resumo

O pênfigo foliáceo é a doença de pele autoimune mais comum no gato, não estando descrita predisposição etária, genética ou sexual.

Neste trabalho, foi realizado um estudo retrospectivo de onze casos diagnosticados de pênfigo foliáceo na espécie felina, durante um período de três anos na clínica ADVENTIA. O início das lesões variou de menos de 1 ano a 10 anos de idade, mediana de 5 anos. Os sinais clínicos observados incluíram crostas (n=11), paroniquia (n=7), erosões (n=4) e alopécia (n=4). As lesões foram mais frequentes nas pregas ungueais (n=7), pavilhão auricular externo (n=7) e/ou pavilhão auricular interno (n=3), plano nasal (n=3) e nariz (n=3). A avaliação citológica em 7 casos demonstrou a presença de queratinócitos acantolíticos. No exame histológico de 4 biópsias observaram-se queratinócitos acantolíticos e crostas neutrofílicas (n=2). Num caso não foi observado acantólise activa. Todos os casos foram seguidos por 1 a 49 meses. A maioria dos casos (n=9) atingiram remissão completa com tratamento imunosupressor. Apenas um gato foi eutanasiado, devido ao desenvolvimento de sinais sistémicos durante a terapia.
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Abbreviations

ANA – Anti-nuclear Antibodies
AKs – Apoptotic Keratinocytes
CBC – Complete Blood Count
CsA – Ciclosporin A
DM – Diabetes Mellitus
DNA – Desoxyribonucleic acid
Dsc – Desmocollin
Dsg – Desmoglein
ELISA – Enzyme Linked Immunosorbent Assay
FAD – Flea Allergic Dermatitis
FeLV – Feline Leukemia Virus
FIV – Feline Immunodeficiency Virus
Gc(s) – Glucocorticoid(s)
H&E – Haematoxylin and Eosin
HVBV – Hospital Veterinário do Baixo Vouga
IBD – Intestinal Bowel Disease
IF – Immunofluorescence
IgG – Immunoglobulin G
IM – Intramuscular
IV – Intravenous
IVIg – Human Intravenous Immunoglobulin
Kg – kilogram
LE – Lupus Erythematosus
mg – milligram
ml – milliliters
PAS – Periodic Acid Schiff
PD – Polydipsia
PE – Pemphigus Erythematosus
PF – Pemphigus Foliaceus
PO – Per os
PU – Polyuria
PV – Pemphigus Vulgaris
TPMT - Thiopurine methyltransferase
® - Registered Trademark
™ - Unregistered Trademark
ºC – Degrees Celsius
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1. Literature review

1.1. Introduction

The immune-mediated skin disorders include a large group of inflammatory diseases that have in common the central participation of the immune system in their development. They are classified into primary or autoimmune, and secondary or immune-mediated (Table 1) (Torres, 2004).

<table>
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<th>Autoimmune</th>
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<tr>
<td>Pemphigus complex</td>
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<td>Exfoliative cutaneous lupus erythematosus</td>
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The pemphigus complex is a group of uncommon autoimmune diseases and described in humans, dogs, cats, horses and goats (Olivry and Chan, 2001; Bystryn and Rudolph, 2005; Olivry, 2006). In cats, the pemphigus group has been described from three decades (Thoday, 1981; Ackerman, 1985). It is characterized by the formation of autoantibodies against an autoantigen, creating vesicles in the skin. The clinical manifestations differences between pemphigus forms are based on which particular antigens are targeted by autoantibodies and in the distribution of these antigens in the different regions of the body (Torres, 2004; Waschake, 2008; Gershwin, 2010).

In cats, three different forms of pemphigus are distinguished: Pemphigus foliaceus (PF), Pemphigus erythematosus (PE), and Pemphigus vulgaris (PV). PF and PE are specific forms of superficial pemphigus, whereas PV is a deep form clinically distinct (Figure 1) (Scott et al., 2001; Paterson 2006; Tater and Olivry, 2010). PF is the most common form of pemphigus seen in cats, as well as the most common autoimmune dermatosis (Scott et al., 2001). PE may be a benign form of PF, with the skin lesions limited to the face, or a crossover between pemphigus and Lupus Erythematosus (LE) (Torres, 2004; Hnilika, 2011). The incidence of feline PF has been calculated as five per 1000 patients, per 10 years (Preziosi et al., 2003).
1.2. Epidemiology

A breed predisposition for PF has not been reported definitively in the feline species even though domestic short-haired cats were found to be most commonly affected with this disease. Many other breeds, including Orientals and long-haired and short-haired varieties were represented (Preziosi et al., 2003). PF has been reported in Siamese, Himalayan, Persian, Maine Coon, Somali, Ragamuffin, Scottish Fold and American Blue breeds (Angus, 2005).

No sex predisposition for development of PF in cats has been noted. Feline PF is most common in young adult cats, however the age of onset is highly variable, ranging from less than 1 year to greater than 17 years, with a median age of onset of 5 years (Preziosi et al., 2003).

1.3. Pathogenesis

The exact pathomechanism of PF lesions is not known. PF affects the epidermis, the outermost superficial skin layer and is a specific type of superficial pemphigus. About 85% of the epidermal cells are composed of tightly adherent keratinocytes. Four types of adhesion structures hold keratinocytes together: desmosomes, responsible for cell-to-cell adhesion, hemidesmosomes that bind the basilar epidermal keratinocytes to the basement membrane, adherens junctions and focal adhesions (Scott et al., 2001). Desmosomes occur predominantly in epithelial cells and their molecular components play an important role for cell-cell adhesion. The molecular basis of hiperadhesion is complex and incompletely understood, and more than one desmosomal component may be involved (Waschake, 2008; Garrod, 2010). Desmosomes contain two major transmembrane components of the cadherin-type: desmoglein (Dsg) and desmocollin (Dsc) (Amagai, 2009). The basis of pemphigus pathophisiology is that acquired autoantibodies attack these molecules in desmosomes and the resilience of epidermis is lost.
resulting in superficial blister formation (Scott et al., 2001; Fassihi et al., 2006; Amagai, 2009; Tater and Olivry, 2010). This loss of cohesion between epidermal cells is known as acantholysis. Free epidermal cells in the vesicles or bullae are called acantholytic keratinocytes and not acanthocytes, which are erythrocytes with membrane projections (Scott et al., 2001). Indeed, in a canine PF case, autoantibodies titles were related with disease activity, suggesting that PF is an antibody-mediated autoimmune disorder (Nishifuji et al., 2005).

Unfortunately, studies to characterize autoantibodies and antigens in feline species with PF are scarce and pathogenesis of cats is extrapolated from other species.

1.3.1. Autoantibodies involved

In dogs with PF, recent reports were made to determine whether antikeratinocyte IgG (Immunoglobulin G) antibodies are pathogenic (Olivry et al., 2008). It was detected high titles of antikeratinocyte antibodies of IgG4 subclass in 80% of the serum of PF-affected dogs, but only rarely in the serum of control dogs. Antikeratinocytes antibodies of IgG1 subclass were found in both affected and non-affected dogs and there was no statistical difference between groups. The other subclasses, IgG2 and IgG3, were uncovered at low titles. This study supports the importance of IgG4 in the pathogenesis of PF, as their titles decrease during treatment-induced reduction of disease severity and can induce subgranular blisters when transferred passively in neonatal mice. In human PF, patients produce IgG1 and IgG4 anti-Dsg1. However, IgG1 isolated from PF sera failed to induce lesions when injected in neonatal mice (Hacker et al., 2002). All these data support the idea that IgG4 is common between species with PF and cats probably have a similar pathogenesis.

1.3.2. Target antigen(s)

In human PF, autoantibodies recognize more than one antigen, but Dsg1 is known as the principal autoantigen targeted by autoantibodies (Amagai, 1999; Cotell et al., 2000; Karlihofer et al., 2003; Fassihi et al., 2006; Culton et al., 2008), and the same process has been proposed for domestic animals (Scott et al., 2001). Limited immunoblotting studies suggested that the main autoantigen in canine PF might also be Dsg1 (Iwasaki et al., 1997). However, subsequent studies did not suggest Dsg1 to be the major autoantigen in dogs with PF (Olivry et al., 2006; Olivry and Linder, 2009; Yabuzoe et al., 2009) suggesting that other desmosomal proteins may be involved, such as desmoplakin. Microscopy techniques show that the binding site of autoantibodies is the extracellular region of desmosomes (Yabuzoe et al., 2009). Very recent immunomapping studies are strongly suggestive that Dsc1 may be a major target autoantigen in canine PF (Bizikova et al., 2011a; Bizikova et al., 2011b), although a previous study suggests that extracellular domains of Dsc1 are not involved in canine PF (Aoki-Ota et al.,
Therefore, the results are not conclusive, and more research needs to be performed in order to identify major autoantigen(s) for this disease in dogs and cats. Once identified, it should be considered the suitability of immunological tests (e.g. ELISA (Enzyme Linked Immunosorbent Assay) and immunoblotting tests) employing recombinant antigen as an aid for the diagnosis, disease monitoring and the development of targeted and specific immunotherapies (Stanley et al., 2009).

Since canine PF appears to be clinically, histologically and immunologically heterogeneous, with rare PF sera autoantibodies targeting Dsg1, it has been proposed that PF may not be a single disease but a general term for all superficial pemphigus diseases, since all superficial pemphigus conditions are very similar (Olivry, 2006). Deep pemphigus conditions are clinical and immunological distinct from superficial pemphigus, and the term Pemphigus cannot be used alone as a diagnostic because it refers to a heterogeneous group of deep and superficial pemphigus.

1.3.3. Eosinophilic infiltrate and Apoptosis

To date, only one study investigates the importance of eosinophilic infiltrates in the pathogenesis of PF in dogs. There were no statistically significant differences in the occurrence of acantholytic cells or active acantholysis in dogs with or without an eosinophilic infiltrate (Vaughan et al., 2010). In cats with PF, eosinophils are rarely seen (Preziosi et al., 2003) and its importance in pathogenesis seems irrelevant. Further studies are warranted to confirm if there is a relationship between eosinophilic infiltrate and autoimmune diseases in domestic animals.

In humans, apoptosis contributes to the mechanisms by which autoantibodies induce acantholysis. Theoretically, the blockade of the caspase pathway could prevent apoptosis and acantholysis. This observation may be a promising therapeutic tool that can help in the treatment of pemphigus flare-ups (Schmidt and Waschake, 2009; Bektas et al., 2010; Pacheco-Tovar et al., 2011). Apoptosis is also seen in some cats with PF (Vogel et al., 2009), but it is not known if the activation of the apoptotic pathway can be an early consequence of the binding of autoantibodies to keratinocytes in this species.

A recent study indicates that inflammatory mediators released from eosinophils may be involved in triggering apoptosis in epidermal keratinocytes (Griffin et al., 2010). Further studies are needed to confirm and elucidate the inflammatory mediators involved in this process.

1.4. Triggering factors

The mechanisms leading to immune dysregulation and autoimmunity are complex and not fully understood. In humans both endogenous and exogenous factors have been implicated
in the pathogenesis of pemphigus (Culton et al., 2008). In domestic species it is unclear if several factors can exacerbate pemphigus, but they should be avoided whenever it is possible.

1.4.1. Ultraviolet light

Ultraviolet exposure may be a potential environment trigger for PF. However, such suspicion is referred only in dogs (Olivry, 2006), and no seasonal or environmental risk factors have been identified in cats. However, Griffin (1991) proposed a seasonal evolution for some cats.

1.4.2. Other skin disorders or other diseases

It is not clear if previous skin diseases are implicated in the development of feline PF. In a retrospective study, two of eleven cats with previous dermatological problems (including allergic dermatitis, indolent ulcer, otitis, feline acne, pyoderma and unspecified pruritic skin disease) had a history of chronic allergic skin disease for several years prior to developing PF, suggesting that chronic skin inflammation may be a trigger factor (Preziosi et al., 2003). A 3 year-old cat diagnosed with PF was treated before for an eosinophilic granuloma complex for one year, with prednisolone, essential fatty acids, hipoallergenic diet and ectoparasites control, but it is unlikely that eosinophilic granuloma complex leads to PF lesions (Chapelin et al., 2004). Previous skin allergy was also reported in canine PF (Scott et al., 2001). In dogs, PF is reported in patients with leishmaniasis (Ginel et al., 1993), thymoma (Day, 1997) and Systemic Lupus Erithematosus (Foster et al., 2000). Concurrent Leishmaniosis and PF have been described in a cat that lived in both Spain and Switzerland. It is not clear if Leishmania species infection had a causative relation with PF. However, the concurrence of both diseases seems to be rare (Rüfenacht et al., 2005). PF can be present in these patients by coincidence or these systemic diseases may produce autoantibodies against desmosomes.

1.4.3. Drugs

It is reported that in humans, some drugs may influence the development of PF. Some of them may activate proteolytic enzymes in the skin that alter desmosomes and results in biochemical acantholysis. They may also stimulate the development of autoantibodies against desmosomes resulting in immunologic acantholysis. These drugs include thiols compounds, containing sulfur groups (e.g. penicillamine) or compounds containing amide group (e.g. enalapril); or nonthiol drugs that contain sulfur and may undergo metabolic changes to form active thiol drugs (e.g. penicillins, cephalosporins) (Brenner et al., 1998; Brenner and Goldberg, 2011).
Drug eruptions resembling PF lesions have been reported in dogs and cats with antibiotics like ampicillin (Mason et al., 1987), cimetidine (McEwan et al., 1987), amoxicillin, enrofloxacin, sulfonamides and metronidazole (Willemse, 2000; Vaughan et al., 2010), cephalexin, trimethoprim-sulfadiazine and oxacillin (White et al., 2002). The administration of itraconazol, lime sulfur dip, methimazole and ipodate may also be implicated as potential triggers (Preziosi et al., 2003; Gross et al., 2005). More recently, the administration of a topical spot-on product containing metaflumizone and amitraz (Promeris-Fort Dodge Animal Health) has been associated with PF in 22 dogs (Oberkirchner et al., 2011). It is difficult to establish a relationship between a drug and PF because patients have often been exposed to multiple drugs and some drugs may have a prolonged latency period between exposure and onset of the disease. If any drug is suspected as a trigger of feline PF, it should be discontinued and avoided in the future.

1.4.4. Other factors

Genetic factors (Tron et al., 2005), food containing certain organic compounds, such as thiols and phenols (Brenner et al., 1997; Fedelels et al., 2010), viral infections (Brenner et al., 2002; Sagi et al., 2008) and skin infections caused by Staphylococcus aureus (Amagai, 2009) have also been associated with some cases of human PF, but it is unknown if any of these factors, especially the diet, are triggers in domestic species.

1.5. Clinical signs and lesions distribution

The signs of an attack on keratinocyte adhesion structures are clinically evident. Pemphigus is characterized by the development of intraepidermal vesicles or bullae which are rapidly infiltrated by leucocytes and so form pustules (Broek, 1991). Pustule is defined as a small, circumscribed elevation of epidermis that is filled with pus. The color is usually yellow, but may be green or red (Scott et al., 2001). Unfortunately, the thinness of feline epidermis promotes rupture of pustules and primary lesions are very difficult to find. Affected cats often present thick, yellowish to honey-colored adherent crusts with associated scale, alopecia and erosions (Figures 2-6) (Preziosi et al., 2003; Sparkes and Caney, 2005; Paterson, 2006; Peterson and McKay, 2010). Nikolsky’s sign (elicited by applying pressure on a vesicle, or at the edge of an ulcer or erosion or even on normal skin) may be positive, when the outer layer of the skin is easily rubbed off or pushed away, indicating poor cellular cohesion (Scott et al., 2001). Other clinical signs may include pruritus in variable degrees, lethargy, pyrexia, mild to moderate anorexia, tachycardia, dehydration, limb edema and apparent skin pain (Preziosi et al., 2003; Chapelin et al., 2004; Medleau and Hnilika, 2006; Foster et al., 2007; Peterson and McKay, 2010). Other systemic signs infrequently reported included weight loss,
lymphadenopathy, otitis externa, cystitis, seborrhea sicca and depression (Broek, 1991; Preziosi et al., 2003). The disease commonly has a waxing/weaning course. There may be hours to days when numerous new pustules form, followed by days to weeks of crusting during which few new lesions are found (Scott et al., 2001). Secondary bacterial infection can occur together with PF (Broek, 1991; Foster et al., 2007).

The initial distribution of feline PF lesions is often localized and mild, but most of them generalize to dorsum and ventrum (Preziosi et al., 2003). The most common affected area is the head (almost 80%), with lesions in the pinnae, face/head, nose, chin or periocular area (Figures 2-4) (Preziosi et al., 2003; Chapelin et al., 2004; Matousek, 2004; Sparkes and Caney, 2005; Friberg, 2006; Foster et al., 2007; Peterson and McKay, 2010).

Paws are the next most likely area of involvement, with feet (Preziosi et al., 2003; Chapelin et al., 2004; Peterson and McKay, 2010) or claw folds lesions, these with sterile paronychia with purulent exudate (Figures 5-9) (Preziosi et al., 2003; Matousek, 2004; Crow, 2009; Peterson and McKay, 2010). In some cases, purulent claw fold discharge may be the only

Figure 2. Alopecia, erosions and crusts developed on the face of a 1.5-year-old Siamese cat during a flare of PF (Olivry, 2006)

Figure 3. Yellowish crusts are present on the convex (a) or concave (b) aspects of the pinnae of two cats with PF (Olivry, 2006)

Figure 4. An adult Burmese cat with PF lesions on the muzzle (photo courtesy of Dr Pascal Prélaud)
sign (Torres, 2004). Often, PF lesions are confined to the face and paw pads. Nail changes seem to be rare, and onychomadesis was reported by Guaguere et al. (2000). Interdigital skin, pads and junction of pad with haired skin are also common (Angus, 2005). Hyperkeratosis is an uncommon finding (Paterson, 2006) but in some cats may be the only sign (Broek, 1991).

**Figure 5.** In feline PF, crusts can be seen around footpads and claws (a) or on pads paws (b) (Olivry, 2006).

**Figure 6.** Same cat as in figure 4. Footpad crusts, characteristic of feline PF (photo courtesy of Dr Pascal Prélaud).

**Figure 7.** Alopecia, erythema and erosions on the dorsum of the paw along with paronychia and crusting at the claw folds (Preziosi et al., 2003).

**Figure 8.** Palmer surface of the paw in a cat with PF. Thick crusts are seen at the margins of the pads as well as in the interdigital spaces along with scaling of the surface of the pads (Preziosi et al., 2003).

Other areas reported for initial involvement include the dorsum in 10%, area around the nipples, legs (Figure 10) or tail (Preziosi et al., 2003; Moriello, 2005). PF does not affect mucous membranes (Broek, 1991) although the mucocutaneous junctions and oral cavity has been reported as an unusual location for PF in cats (Gross et al., 2005; Rees, 2011).
1.6. Differential diagnosis

Differential diagnosis for feline PF, based in clinical findings (pustular or papular dermatosis) include bacterial folliculitis, notoedric mange, otodectic mange, cheyletiellosis, leishmaniasis, other autoimmune skin diseases (discoid and systemic lupus, PE), dermatophytosis (*Trichophyton mentagrophytes*), demodicosis, cutaneous epitheliotropic lymphoma, cutaneous adverse drug reactions, zinc responsive dermatosis, actinic dermatosis, dermatomyositis, eosinophilic pustulosis, superficial necrolytic migratory erythema and mosquito bite hypersensibility (Gross *et al*., 2005; Moriello, 2005; Medleau and Hnilika, 2006; Paterson, 2006; Peters *et al*., 2007; Nuttal *et al*., 2009).

When the lesions are localized in the face, feline miliary dermatitis, allergy (especially atopy), food intolerance and cowpox infection should be ruled out (Jackson and Foster, 2006; Paterson, 2006).

Differential for paronychial disease include Staphylococcal infection of the nail beds, yeast infection (*Malassezia* spp.) and neoplasia (metastatic bronchogenic carcinoma and squamous cell carcinoma of the lungs) (Paterson, 2006).

The frequent bilateral symmetry and the polycyclic pattern of lesions are important differentiating features (Gross *et al*., 2005).
1.7. Diagnosis

The most important diagnostic aspects are the history, physical examination, direct smears, histopathological findings and in rare cases direct Immunofluorescence (IF) (Scott et al., 2001; Tater and Olivry, 2010). Antinuclear antibody (ANA) test is not necessary for the diagnosis (Tater and Olivry, 2010). A history of acute onset of crusted lesions in cats is suggestive of PF (Peterson and McKay, 2010). Footpads hyperkeratosis may be very suggestive of feline PF (Broek, 1991).

Other diagnostic tests to rule out differentials include fungal cultures, bacterial culture, skin scrapings, hair plucks and impression smears (Moriello, 2005; Paterson, 2006; Crow, 2009). Due to the potential of serious secondary effects, the diagnosis of PF should be made before the administration of immunosuppressive drugs.

Complete Blood Count (CBC) and biochemical profile abnormalities detected in the remaining cats are mild and nonspecific (Preziosi et al., 2003; Peterson and Mckay, 2010), so they are not relevant for the diagnosis. Many cases can have moderate to marked leukocytosis and neutrophilia, mild nonregenerative anaemia, mild hypoalbuminemia and mild elevations in globulins. A hemogram and biochemical exams cannot diagnose PF, but they are helpful to detect any systemic disease, that may appear with immunosuppressive therapy.

1.7.1. Cytology

The diagnosis of PF in cats begins with impression smears, usually under newly formed crusts, since intact pustules are transient and rare in cats. If a pustule is present, the procedure is rupture with a fine needle and put the contents carefully on a slide, to prevent cellular damage (Tzanck preparation) (Forsythe, 2007; Foster et al., 2007; Rees, 2011). However, when these are rare, they should be preserved for histopathology. After drying and staining with Diff-Quick™ or Rapistain, cytologic preparations should be evaluated with 10x, 40x and 100x (oil immersion) objectives (Paterson, 2006; Forsythe, 2007). The structures that are seen in normal skin include squamous (angular, anuclear keratinocytes) and occasional nucleated keratinocytes. Free melanin granules can be seen eventually and should not be mistaken for bacteria. Melanin granules may also be found in keratinocytes.

In PF disease, pustules contain neutrophils and are sterile. Cytology is useful to find acantholytic keratinocytes, which are nucleated epithelial cells with rounded shape that have lost their intercellular adhesions. Their cytoplasm is normally stained or can be hypereosinophilic (Scott et al., 2001). Acantholytic keratinocytes admixed with nondegenerated neutrophils are very suggestive of PF (Figure 11) (Broek, 1991; Preziosi et al., 2003; Paterson, 2006; Forsythe, 2007; Crow, 2009; Peterson and McKay, 2010; Tater and Olivry, 2010), but acantholysis may be absent (Chapelin et al., 2004). However, they are not pathognomonic
since they may be seen in other pustular and inflammatory dermatoses, such as superficial pyoderma and dermatophytosis (Torres, 2004; Forsythe, 2007; Nuttal et al., 2009). Acantholytic keratinocytes exhibit either microscopic characteristics of normal differentiated spinous or granular layer epithelial cells, or they present signs of apoptosis with eosinophilic cytoplasm, condensed chromatin or karyorrhexis. Occasionally, neutrophils can be seen in close apposition to detached keratinocytes (Olivry, 2006). Eosinophils can be present, but it is not common (Forsythe, 2007).

Figure 11. Cytology from under crusts around claw folds, from the cat of Figure 3, stained with Diff-Quick. A first evaluation with 10x objective shows acantholytic cells with numerous neutrophils (a). With 40x objective, isolated acantholytic keratinocytes (red arrows) surrounded by non-degenerated neutrophils were seen (b) and rare eosinophils were present too (black arrows) (c). Bacterial infection was not observed (photos courtesy of Dr Pascal Prélaud)

Cytologic study is helpful to discover bacteria, yeasts and fungi. When secondary infection is present, bacteria can be seen and neutrophils tend to be more degenerated, due to bacterial toxins exposition. An appropriate antibiotic can be administrated during 2 to 3 weeks, and then repeat cytology (Paterson, 2006; Forsythe, 2007; Tater and Olivry, 2010). If the lesions are resolved only with antimicrobial treatment, the condition is not pemphigus, but probably a bacterial pyoderma.
1.7.2. Histopathology

Histological examination of lesional skin is usually diagnostic of PF (Yager and Wilcock, 1988; Broek, 1991; Preziosi et al., 2003; Chapelin et al., 2004; Peterson and McKay, 2010; Hnilika, 2011). Anti-inflammatory agents can dramatically affect the histological appearance of PF. Cats that receive some form of corticosteroid at the time of biopsy had a significant reduction of samples that contain adequate diagnostic criteria (Preziosi et al., 2003). The administration of such agent should optimally be stopped for 2 to 3 weeks before biopsy. Secondary bacterial pyoderma often obliterate the histopathological features of PF and it is imperative to eliminate these secondary infections with appropriate antibiotic therapy before biopsies are performed, to increase the chances of a clear diagnosis from histological examination (Scott et al., 2001; Crow, 2009; Tater and Olivry, 2010). Ideally, samples should include an intact pustule and adjacent normal skin. The pustule should be centered in the biopsy specimen. However, most of times this is not possible and newly developed crusts are the second choice (Broek, 1991; Scott et al., 2001; Paterson, 2006; Forsythe, 2007). The crust often contains diagnostic elements and should be included as part of the sample (Torres, 2004). Other possibility is to hospitalize the patient and check every 2 to 4 hours if primary lesions develop (Paterson, 2006). The clinician should take multiple samples and obtain specimens from a variety of lesions (Scott et al., 2001).

Technique

Sedation and local anesthesia is usually adequate before the excision. The local anesthetic should not contain vasoconstrictor properties, once vasoconstriction affects the histopathological pattern. Lidocaine 1% to 2% injected subcutaneously can be used for this purpose. Do not surgically prepare the skin, because it removes diagnostic lesions. A 4-6mm biopsy punch is placed perpendicular to the skin surface and the incision is made with unidirectional downward and rotational movement, to minimize shearing artifacts. In some body areas, like ear pinnae or around the nipples, a scalpel blade is more appropriate to do the biopsy. The samples must be performed very carefully, should be fixed in 10% neutral buffered formalin and sent to a laboratory, with a complete history, physical examination findings, a list of differential diagnoses and previous treatment (Broek, 1991; Torres, 2004; Moriello, 2005; Paterson, 2006; Forsythe, 2007; Foster and Foil, 2007).

Histological examination

Haematoxylin and eosin (H&E) stain is the most widely used routinely for skin biopsies. Due to acanthosis, the epidermis of most cases it is hyperplastic in various degrees, and may or may not present hypergranulosis (Preziosi et al., 2003). The stratum corneum exhibits
orthokeratotic hyperkeratosis, with focal parakeratosis in some cases (Preziosi et al., 2003; Gross et al., 2005).

The dermis has perivascular to interstitial infiltrate in most cases and it is accompanied by edema, vascular ectasia and congestion (Gross et al., 2005). Neutrophils predominate but almost all samples have mast cells in the dermis and in some samples, the mast cells can be the prominent cell type in the dermis (Gross et al., 2005; Peterson and McKay, 2010). The mast cells may be present in epidermis too, but it is uncommon. As an explanation for this findings, it was suggested a possible association with allergic dermatoses. Eosinophils are usually present, in the dermis or marginating dermal blood vessels, but they are not the predominant cell type (Figure 13) (Gross et al., 2005). Apparently, there is no significant difference in the type or intensity of dermal infiltrate in the biopsies of cats that had a prior diagnosis of allergic dermatitis or acute inflammatory dermatitis (Preziosi et al., 2003).

Brightly, eosinophilic, contracted Apoptotic Keratinocytes (AKs) may also be present, surrounded by neutrophils and sometimes a few lymphocytes or histiocytes (Gross et al., 2005). AKs undergo a series of morphologic changes: the cells shrink, become denser and more eosinophilic and lose its normal contacts. Nuclear changes include pyknosis, margination of

Figure 12. Feline epidermis. An active area of acantholysis located within the stratum spinosum is forming beneath an older recomified pustule (x10) (a). Acantholytic cells can be seen "springing" up from the stratum spinosum (x20) (b). Staining with H&E (Preziosi et al., 2003).

Figure 13. Feline dermis. A diffuse dermal infiltrate consisting predominately of mast cells and neutrophils are seen in the superficial dermis. Staining with H&E (Preziosi et al., 2003).
chromatin and karyorrhexis (Scott et al., 2001). In a recent retrospective study, only in 24% of skin biopsies from cats with PF were found AKs. Besides, AKs are found also in other feline inflammatory dermatoses, thus other histopathological findings are needed for accurate diagnosis (Vogel et al., 2009).

Intact pustules may be found, but it is more common the presence of overlying degenerating pustules, especially in samples of paw pads. Recornification or newly reformed stratum corneum at the base of neutrophilic pustules is very suggestive of PF (Figure 15) (Preziosi et al., 2003). Caution is warranted because re-epithelialization may cause subepidermal location. Re-epithelialization is usually recognized as a single layer of elongated basal epidermal cells at the base of the vesicle or pustule (Scott et al., 2001). Acantholysis also may occur at the level of stratum spinosum or granulosum (Figure 12), and some samples may present both subcorneal and intracorneal pustules. Rafts of acantholytic cells clinging or adhered to the overlying stratum corneum are characteristic (Preziosi et al., 2003; Gross et al., 2005). The pustules content are predominantly neutrophils and acantholytic cells and some samples may present eosinophils. Neutrophils may encircle and cling to individual acantholytic cells (Figure 14 and 15). Pustules may span various follicular hairs, depending on the density of hair and size of the pustule (Preziosi et al., 2003; Chapelin et al., 2004; Gross et al., 2005; Foster and Foil, 2007).

Some samples should be stained with Periodic Acid Shiff (PAS), to evaluate the presence of pustular dermatophytosis.
1.7.3. Immunopathology

Biopsy specimens can be stained with anti-IgG fluorescein to demonstrate the deposition of antibody (Gershwin, 2010), which may be helpful for diagnosis (Figures 16 and 17). Detection of pemphigus antibody by IF or immunohistochemical testing are not routinely recommended, due to the costs, technical problems and poor diagnostic sensitivity and specificity (Scott et al., 2001). These techniques are more available for research and to distinguish the forms of subepidermal vesiculobullous diseases (Torres, 2004).

![Figure 16. Canine PF. Direct IF reveals intercellular epidermal IgG in the stratum spinosum and granulosum (Olivry, 2006).](image1)

![Figure 17. Canine PF. Indirect IF demonstrates that only rare sera from dogs with PF recognize Dsg1. These results indicate that Dsg1 is a minor antigen in canine PF (Olivry et al., 2006).](image2)

In a recent review, three criteria for the diagnosis of PF in animals with a skin disease were suggested by Olivry (2006): 1- clinical examination: transient pustules that rapidly evolve to erosions and crusts, predominantly on the face and feet; 2- histopathology: superficial epidermal or follicular pustules, with non-degenerated neutrophils and acantholytic keratinocytes; 3- differential diagnoses: rule out other acantholytic neutrophilic pustular diseases, such as bacterial skin infections and pustular dermatophytosis.

1.8. Treatment

To date, this disease is chronic and not curable. PF is managed with drugs that suppress the immune system, affecting autoreactive B cells and decreasing autoantibody production, and therefore blister formation. There are many treatments available, however it is not known which is the most effective or safest treatment option, or which is the best combination for PF, both in humans (Martin et al., 2009) and in domestic species (Rosenkrantz, 2009). The therapy's goal is suppress disease, maintaining the quality of life and minimizing
drug side effects. If secondary infection is concurrent with PF, an antimicrobial therapy combination should be considered.

Treatment options should be chosen based on the severity of clinical presentation, after careful evaluation of the benefits and side effects, in the context of the individual’s other medical conditions, like Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV), initial response and response to treatment. Owner compliance and financial resources are important too (Dunn, 1998). It is very important that owners are fully informed of the prognosis and medication side effects before starting treatment (Tater and Olivry, 2010).

A division in three treatment categories of treatment is suggested by Rosenkrantz (2004): common therapeutics, current alternative therapeutics and additional alternative therapeutics.

1.8.1. Common therapeutics

Actually, the most common immunosuppressive agents used in domestic animals consist in the powerful drugs of the steroid group and cytotoxic drugs.

1.8.1.1. GCs (Glucocorticoids)

Localized forms of PF can be treated with topical GCs, initially with a potent topical GC and then, if adequate response is seen, it is recommended to switch to a less potent topical GC. These cats may not need a systemic therapy later if topical preparation induces clinical remission of signs and no further development of new lesions (Rosenkrantz, 2004; Paterson, 2006). Suitable topical GCs for initial therapy are represented in Table 2. Persistent use of more potent topical GC can create skin atrophy, alopecia and localized pyoderma. If no positive response is seen within 14 days, the introduction of systemic therapy is recommended (Rosenkrantz, 2004; Paterson, 2006). Topical therapy can be used in conjunction with systemic therapy on more persistent focal areas that remain active despite systemic treatment (Rosenkrantz, 2004; Rosenkrantz, 2009).

Systemic GCs are actually the most common form of therapy used in PF lesions management, because of their rapid onset. The oral GCs of choice have been prednisolone or prednisone (Willems, 2000; Scott et al., 2001; Chandler et al., 2004; Merchant, 2007; Peterson and McKay, 2010). However oral prednisolone is a better choice than prednisone for feline patients, because of the lower bioavailability of prednisone compared with other GCs (Graham-Mize and Rosser, 2004). The majority of feline cases respond well and it is maintained in clinical remission with prednisolone monotherapy (Preziosi et al., 2003; Rosenkrantz, 2004). Some authors prefer methylprednisolone, due to the reduced mineralocorticoid effects and better response in some cases (Chapelin et al., 2004; Rosenkrantz, 2009). Cats are few sensitive to
the immunosuppressive effects of GCs and often require high doses for remission (Thacker, 2010) and if no improvement in clinical signs is evident within one or two weeks, the dose may be increased (Tater and Olivry, 2010). Oral triamcinolone (Preziosi et al., 2003) or oral dexamethasone are alternative GCs, 6 to 10 times more potent than prednisolone or prednisone (Rosenkrantz, 2009; Rees, 2011). If response is seen within 10 to 14 days, the dosage is reduced gradually on a weekly basis over 30 to 40 days and then lowered to an alternate day basis to the lowest effective dosage of 1 mg kg\(^{-1}\) q 48 hours or less (Rosenkrantz, 2009). The patient should be reevaluated every time that the dosage is adjusted. Induction and maintenance doses are detailed in Table 2. If the PF recurs when the GC is tapered, another immunosuppressive drug should be added as adjunctive therapy.

Nowadays, injectable GCs such as methylprednisolone acetate are not recommended for PF treatment (Tater and Olivry, 2010).

The most common GCs side effects include poor dull scaly hair coats, muscle atrophy, polyuria and polydipsia (PU/PD), polyphagia, weight gain, behavioral changes, panting and increased risk for infections. Bladder infections and skin infections, especially dermatophytosis, Malassezia infections and demodicosis, may develop due to chronic steroid usage (Paterson, 2006; Rosenkrantz, 2009). When secondary pyoderma is present, antibiotics according to bacteriogram or culture should be used (Peterson and McKay, 2010). Other skin changes include atrophic skin, calcinosis cutis, atrophic scars, comedones and miliary follicular cysts. Gastrointestinal ulcerations, diarrhea, pancreatitis, Diabetes mellitus (DM), adrenal gland suppression, iatrogenic hyperadrenocorticism and hipotiroidism can also occur (Rosenkrantz, 2009; Thacker, 2010).

Table 2. GC therapy for feline PF

<table>
<thead>
<tr>
<th>Topical therapy</th>
<th>Induction dosage</th>
<th>Maintenance dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone, dexamethasone, betamethasone fluocinonide, triamcinolone, mometasone</td>
<td>Applied every 12 hours</td>
<td>Taper to lowest effective dose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systemic therapy</th>
<th>Induction dosage</th>
<th>Maintenance dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone</td>
<td>2.0-4.0 mg kg(^{-1}) PO q12-24 hours, during 4 weeks</td>
<td>2.5-5.0 mg kg(^{-1}) PO q2-7 days</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>4.4-6.6 mg kg(^{-1}) PO q24 hours, during 14 days</td>
<td>1.1-2.2 mg kg(^{-1}) PO q48 hours</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>0.2-1 mg kg(^{-1}) PO q12-24 hours</td>
<td>0.5-1 mg kg(^{-1}) PO q2-7 days</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.1-0.4 mg kg(^{-1}) PO q12-24 hours, during 14 days</td>
<td>0.05-0.1 mg kg(^{-1}) PO q48-72 hours</td>
</tr>
</tbody>
</table>

References: (Chandler et al., 2004; Rosenkrantz, 2004; Medleau and Hnilika, 2006; Paterson, 2006; Foster and Foil, 2007; Crow, 2009; Rosenkrantz, 2009; Hnilika, 2011; Rees, 2011).
1.8.1.2. **Chlorambucil**

Chlorambucil is an alkylating agent that affects the Deoxyribonucleic acid (DNA) synthesis. In cats whose PF lesions fail to respond to GCs, chlorambucil is the most commonly cytotoxic drug used and can be used in combination with GCs or as sole therapy. When combined with prednisolone, these drugs are given on alternating days to prevent gastrointestinal irritation. The onset of action is slow and results appear within 3 to 6 weeks. Therapy has to be given 4 to 8 weeks and then one should be able to stop chlorambucil and use GCs alone (Rosenkrantz, 2009; Tater and Olivry, 2010). Initial and maintenance dosages are detailed in Table 3.

Chlorambucil associated adverse effects include myelosuppression, hepatotoxicity in addition to vomiting, diarrhea and anorexia with weight loss. When bone marrow suppression occurs, the drug should be withdrawn until parameters return to normal (Chandler et al., 2004; Paterson, 2006; Rosenkrantz, 2009; Irwin et al., 2011).

1.8.2. **Current alternative therapeutics**

Current immunosuppressive drugs can be introduced when common therapeutics fail or have undesirable secondary effects (Rosenkrantz, 2009). Combination therapy can also be used as an initial treatment strategy (Paterson, 2006).

1.8.2.1. **CsA (Ciclosporin) and tacrolimus**

CsA is a potent inhibitor of cell-mediated immunity, and a less potent inhibitor of humoral immunity. CsA properties include immunosuppressive effects, anti-inflammatory effects, antiproliferative effects, inhibits antigen presentation and it is antiparasitic and lacrimomimetic (Robson, 2003b). CsA has been used successfully in veterinary medicine to treat several dermatologic diseases (Robson and Burton, 2003). Topical CsA and tacrolimus is not recommended in cats because of the potential toxicity and/or lack of efficacy and the cat’s tendency to ingest topical medication (Paterson 2006; Foster et al., 2007). The efficacy of transdermal formulation of CsA (Atopica – Novartis, Animal health) in cats was recently studied, due to the difficulties of oral administration in this species. The absorption via transdermal was inconstant therefore oral administration remains the most recommended (Miller et al., 2011). Initial and maintenance dosages are detailed in Table 3.

In dogs and cats, CsA is often used in conjunction with GCs. In human patients with pemphigus, apparently the combination treatment with GCs and CsA offers no advantage over treatment with GC alone (Ioannides et al., 2000).

Irwin et al. (2011) compared oral CsA with chlorambucil in management of feline PF. It's interesting that there was no significant difference in remission times or disease response
between CsA and chlorambucil, and the cats managed with CsA required significantly less GCs for remission induction and for maintenance therapy. It seems that CsA provides comparable efficacy for management of feline PF.

Few clinically relevant drug interactions with CsA have been reported in veterinary medicine. A very useful drug interaction is with ketoconazol, which inhibits CsA metabolism, resulting in CsA dose reduction and costs saving to the client (Robson, 2003a; Rosenkrantz, 2009; Katayama et al., 2010). Few adverse effects of CsA have been reported in cats. Diarrhea is the most frequently reported adverse reaction (Robson, 2003a; Rosenkrantz, 2004). The administration of gastric protectants, starting with lower dosages or more frequently dividing dosages may prevent gastrointestinal disturbances (Rosenkrantz, 2009). Gingival hyperplasia, hypertrichosis, pyoderma, self-limiting diarrhea, vomiting, nephrotoxicity and intermittent soft stools were reported too (Foster et al., 2007; Irwin et al., 2011).

1.8.3. Additional alternative therapy

1.8.3.1. Cyclophosphamid e

This drug is an alkylating agent and it is more toxic than chlorambucil. Induction dosage is 1.5-2.5 mg kg⁻¹ q 48 hours, and reduction of dosage and frequency is recommended once remission is achieved (Table 3) (Rosenkrantz, 2009).

Cyclophosphamide has no advantages over chlorambucil in cats in the treatment of pemphigus (Paterson, 2006). The use of cyclophosphamide has been associated with bone marrow suppression, hemorrhagic cystitis with hematuria and dysuria, nausea, vomiting and anorexia, infertility and teratogenic effects. It is potentially carcinogenic. Wound healing disturbance, alopecia and loss of whiskers can occur (Rosenkrantz, 2009).

1.8.3.2. Chrysotherapy

Chrysotherapy can be used alone or with systemic GCs. Remission can be successfully obtained with this protocol (Kofod, 1993; Rosenkrantz, 2009). Gold salts are available as oral (auranofin) formulations. Injectable forms (aurothiomalate or aurothioglucose) are quite effective in feline PF but are no longer available (Rosenkrantz, 2009). About 25% of cats that have not results to GC treatment respond to chrysotherapy (Willemse, 2000).

Side effects with gold therapy include myelosuppression, thrombocytopenia, oral ulcers, glomerulonephropathy, hepatotoxicity, stomatitis, sterile abscesses and cutaneous drug eruptions (Chandler et al., 2004; Foster et al., 2007).
1.8.3.3. IVIg (Human Intravenous Immunoglobulin) therapy

IVIg is a high-purified IgG preparation from human plasma. IVIg therapy may be useful in patients to whom conventional therapies have failed not only in humans (Ahmed and Sami, 2002) but also in animals (Rosenkrantz, 2009). This form of therapy is not completely evaluated in domestic species due to limited number of cases treated with IVIg, but appears to be safe. The animal’s response to IVIg is inconstant, showing 50% of efficacy. IVIg should be started at 0.01 ml kg⁻¹ minute intravenously and gradually increased every 30-60 minutes to a maintenance fluid rate not exceeding 0.08 ml kg⁻¹ minute. IVIg is administered over 6 to 12 hours. Anaphylaxis may be seen after the first treatment and concurrent GC therapy is recommended (Rosenkrantz, 2009).

Table 3. Alternative Immunosuppressive drugs recommended for feline PF

<table>
<thead>
<tr>
<th>Drug</th>
<th>Induction dosage</th>
<th>Maintenance dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorambucil</td>
<td>0.1-0.2 mg kg⁻¹ PO q 24-48 hours</td>
<td>0.1-0.2 mg kg⁻¹ PO q48 hours</td>
</tr>
<tr>
<td>Ciclosporin*</td>
<td>5-12.5 mg kg⁻¹ q12-24 hours</td>
<td>5 mg kg q2-3 days</td>
</tr>
<tr>
<td>Ciclophosphamide</td>
<td>50 mg m² / 1.5-2.5 mg kg⁻¹ PO q48 hours</td>
<td>25-50 mg m² / 0.75-1.5 mg kg⁻¹ PO q48h</td>
</tr>
<tr>
<td>Auranofin</td>
<td>0.1-0.2 mg kg⁻¹ PO daily</td>
<td>0.1-0.2 mg kg⁻¹ PO at lowest possible frequency</td>
</tr>
<tr>
<td>Aurothiomalate or aurothioglucose</td>
<td>1 mg kg⁻¹ IM q7d 6-12 weeks</td>
<td>1 mg kg⁻¹ IM q30-60 days</td>
</tr>
</tbody>
</table>

References: (Chandler et al., 2004; Rosenkrantz, 2004; Medleau and Hnilika, 2006; Paterson, 2006; Foster et al., 2007; Crow, 2009; Rosenkrantz, 2009; Hnilika, 2011; Rees, 2011).

*The ciclosporin dose for feline PF has not been established

1.8.4. Other immunosuppressive drugs not used in feline patients

1.8.4.1. Azathioprine

Azathioprine is a cytotoxic drug to T cells. Despite this drug was successful in PF remission in cats (Caciolo et al., 1984 cit. by Broek, 1991), it is contraindicated due to profound myelosuppression and potential fatal reactions in cats. This may be related to the low levels of activity in cats of Thiopurine methyltransferase (TPMT), the enzyme responsible for the metabolism of azathioprine, and therefore cats are more susceptible to azathioprine adverse effects (Foster et al., 2000; White et al., 2000). Other secondary effects include hepatotoxicosis, vomiting, diarrhea, panniculitis, drug eruption, alopecia and increased susceptibility to opportunistic infections (Chandler et al., 2004; Rosenkrantz, 2009).
1.8.4.2. **Niacinamide and tetracycline or doxycycline**

These drugs can be used in dogs and cats for PF treatment, however are not typically used in feline patients, due to the difficulty of oral administration.

1.8.4.3. **Mycophenolate mofetil**

This drug has been used limitedly in dogs (Rosenkrantz, 2009), but in cats it is unknown its efficacy for the treatment of autoimmune diseases.

1.8.4.4. **Dapsone**

Dapsone is avoided in cats, because this species commonly develop hemolytic anemia and neurotoxicity (Paterson, 2006).

There is a variation in dosage plan and combination of drugs used, and the response to treatment can vary between animals, which makes the choice of treatment schedule complex. It is important that clinicians do not underestimate the difficulty in managing this disease. Relapsing or refractory cases are very common and it is important to differentiate immunosuppression-induced pyoderma, demodicosis or dermatophytosis from an actual disease flare, otherwise lesions may worsen from immunosuppression (Paterson, 2006; Tater and Olivry, 2010; Hnilika, 2011).

Further studies are needed to determine the optimal treatment protocol and especially to assess the optimal GC dose and the role of adjuvant immunosuppressive drugs, to improve benefit results and decrease adverse effects.

A very recent experimental study in mice suggested that superficial expression of Dsg-2 activates multiple growth and survival pathways and can limit epidermal blister formation mediated by PF autoantibodies (Brennan et al., 2010). Thus, a drug that increases Dsg2 levels on the skin offers a potential therapeutic treatment.

Recently, rituximab has been used in refractory and severe human PF (Schmidt et al., 2009; Kasperkiewicz et al., 2010; Lipozenčić and Marinović, 2011). Rituximab is a monoclonal antibody that reduces the number of B lymphocytes and therefore autoantibodies production. However, efficacy in remission of PF lesions was not proved due to lack of patients and adjuvant therapy with other immunosuppressive drugs. In future, this drug may be studied and adapted for pemphigus treatment in animals.
1.9. Monitoring

Often cats require lifelong therapy to maintain remission, and under any immunosuppressive treatment, the patient should be monitored with physical examination, CBCs and chemistry profiles to monitor medication side effects, urinalysis and urine bacterial cultures to monitor occult urinary tract infections, especially during induction periods (Paterson, 2006; Rosenkrantz, 2009). Re-evaluation is recommended before and after each change in medication type or dose to help monitor clinical signs (Tater and Olivry, 2010). Monitoring of drug therapy is detailed in Table 4.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCs</td>
<td>Twice yearly CBC, chemistry profiles, urinalysis, and urine cultures</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Routine hematology (including platelet count), liver function tests every 2-4 weeks for 2 months, then 3-4 times yearly</td>
</tr>
<tr>
<td>CsA</td>
<td>CBC, Chemistry profiles and urinalysis every 3-4 months</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>CBC, urinalysis and chemistry profiles every 2 weeks for the first 8-12 weeks</td>
</tr>
<tr>
<td>Gold salts</td>
<td>Routine hematology (including platelet counts), biochemistry and urinalysis every 2-3 weeks for 4 months, then 2-4 times yearly.</td>
</tr>
</tbody>
</table>

Cutaneous side effects of immunosuppression, such as skin infection, demodicosis or dermatophytosis may mimic PF flares. It is important to rule out any possibility of these conditions instead of assuming that a new skin lesion is a PF flare, otherwise, lesions may worsen with immunosuppressive therapy, and refractory pemphigus may be erroneously diagnosed (Tater and Olivry, 2010).

The principles of treating PF are summarized in Table 5.

1.10. Prognosis

In most cases, the prognosis is fair to good, but some of them can have an unfavourable clinical evolution (Kofod, 1993; Chapelin et al., 2004). Some animals have significant improvement after discontinued immunosuppressive therapy, but most of them require lifelong therapy to maintain remission. Rare cases evolve unfavourable, provoking death or were euthanized due to disease or complications of therapy (Preziosi et al., 2003).
Chronic blistering can result in pain, dehydration, secondary infections and rarely, death (Preziosi et al., 2003; Chapelin et al., 2004). In several conditions, pemphigus may have a significant impact of the quality of life. Psychological impact on owners may be profound, due to chronicity of the disease, lifelong therapy, long-term costs of recheck examinations and test to monitor PF patients receiving therapy and side effects from treatment. The successful treatment of PF depends to a large extent on the client, who administers most prescribed therapies. It is important to make the owner aware of potential problems, the likely course of disease as well as the need for follow-up and therapeutic modifications. The owner’s compliance is a very important factor to obtain good results (Scott et al., 2001).

**Table 5. Principles of treating PF in dogs and cats** (Tater and Olivry, 2010)

- Control any concurrent secondary bacterial infection. Consider antibiotic selection based on bacterial culture and antimicrobial sensitivity results, especially with deep pyoderma.

- Select an immunosuppressive therapy after reviewing indications, dosages, administration regimens, and adverse effects. Immunosuppressive therapy should only be used in dogs and cats with a confirmed diagnosis.

- Recheck patients at regular intervals to monitor for recurrence of pemphigus lesions. Perform laboratory tests (CBC, serum chemistry profiles, urinalysis, and urine bacterial culture) to monitor for adverse effects.

- If lesions decrease in extent and severity, the dose or frequency or both, of immunosuppressive therapy should be decreased.

- If new cutaneous lesions occur during the treatment, first rule out bacterial skin infections, demodicosis, or dermatophytosis.

  If new cutaneous lesions are determined to be due to a flare-up of PF, adjust the dose or frequency, or both, of the medication. If systemic GCs are being used, adding another medication may enable GCs to be decreased and then discontinued in the future.

- Cases of PF that cannot be maintained in remission or develop adverse effects should be referred to a veterinary dermatologist.
Objectives

The purpose of this retrospective study is to increase the knowledge in feline PF, by describing the epidemiological, clinical features, cytological and histopathological findings of 11 cases of cats diagnosed for PF. The therapeutic options employed and their outcomes are also characterized. The results obtained are discussed, compared with the literature and conclusions about these cases are exposed.
2. Retrospective study

3.1. Material and Methods

Medical records of cats evaluated for PF between 2007 and 2010 at one specialty clinic (Clinique ADVETIA) were reviewed. The database listed eleven cats with a diagnosis of PF. During three years of clinical practice in Hospital Veterinário do Baixo Vouga (HVBV), there were not cases of PF or they were undiagnosed. Cats were included in the study if a definitive diagnosis of PF was made, based in history, clinical signs highly suggestive of PF, corticosteroid response, supportive cytology and/or histopathological findings.

Breed, gender and age of the cats were recorded. Seasonal onset was also registered. History of previous diseases (dermatological or other diseases), previous medications, and clinical signs when the disease was first noted and at presentation was reviewed. The relationship between all previous treatments and the onset of clinical signs of PF was recorded and a possible drug reaction considered. Two groups were formed based on initial administration of antibiotics, to see if concurrent use of antibiotics during the initial treatment phase significantly improved the remission rate and long-term survival. Skin lesions were categorized as crusts (formed when pustules burst and dried pus adhere to the surface), scales (an accumulation of loose fragments of the horny layer of the skin and loss of clusters in larger flakes), alopecia (loss of hair, due to inflammation or trauma), erosion (a shallow epidermal defect that does not penetrate the basal laminar zone) and/or paronychia (inflammation or infection of claw folds) (Scott et al., 2001). Whenever it was possible, the following information about the skin lesions was obtained: the date and age of onset, the initial specific body locations of lesions and the tendency of progression or regression. Possible factors affecting the clinical course were noted too.

Surface cytology and histopathology results were also evaluated, when they were available. The technique for collection of samples was also described. Cytologic samples were evaluated for the presence of acantholytic cells, neutrophils, eosinophils and the presence or absence of bacteria and/or fungi. All cytologic samples were stained with Diff-Quick™. Skin biopsy specimens were reviewed for the presence and location of pustules or crusts and their content. Epidermis abnormalities and cellular infiltrate of the dermis was described when observed. Bacteria, fungi or parasites were looked to rule out differentials. All samples were routinely fixed with 10% formalin and sent for routine histopathological evaluation. One sample was stained with PAS in order to evaluate the presence of dermatophytes. Other tests performed to rule out differential diagnoses were also registered. Immunosuppressive therapy used for initial treatment was determined, as well as the duration of treatment until initial improvement and complete remission. When occurred, time to relapses (return of clinical signs after a while of remission) was recorded. Drug adjustments were detailed and final treatment
outcomes were discussed. Final treatment outcomes were recorded as total remission of clinical signs, improvement (one or more small areas of crusts, erosions or scaling) or death. Adverse effects due to medication were also discussed.

Follow-up information was obtained by reviewing the records and calling the animal’s veterinarians.

Statistical analysis

It was performed a descriptive statistical analysis with the data obtained. Median and range or mean were used to describe continuous variables. Proportions for categorical variables were described using percentages. Categorical variables were not compared using statistical tests, due to the low number of cases.
3.2. Results

3.2.1. Incidence

The incidence at “ADVETIA” clinic of feline PF was calculated as 11 by 313 patients (all with dermatologic problems), in three years (3.5% of PF cases in three years).

3.2.2. History

Eleven cats were included in this retrospective study. Age of onset ranged from 5 months to 10 years with a median of 5 years (Graphic 1). Five males (45.5%) and six females (54.5%) were affected. Common european cats were the most affected 73.7% (8/11) and the other cats were of breeds Maine Coon (1/11), Persian (1/11) and Burmese (1/11). Initial lesions before reference to a specialist were known in six cats. In two cats (33.3%), the course of the disease was determined to be rapid (clinical signs developed within a period of < 1 month) and slow in four (66.6%) (clinical signs developed within a period ≥ 1 month). The average time from the onset of disease to presentation to a specialist was 3.7 months (median of 3.5 months and range 9 days to 8 months).

![Histogram of age at the onset of lesions](image)

Six of ten cats with PF (60%) included in this study first exhibited clinical signs in Summer, two in Fall (one of them at the end of September and the other in October), one in Spring and one in Winter (February) (Graphic 2). Only in six cats it is known the time of lesions onset, before referral to a specialist. As far as the other cats are concerned, it is known the date of the first consult at the ADVETIA veterinary clinic. In one cat, information about lesions onset was not recorded.
Three cats had previous skin diseases, not related with PF lesions, that included fibrossarcoma and dermatophytosis in one cat, flea allergic dermatitis (FAD) with milliar dermatitis in other, and the other one had pad paw infection. Other medical disorders observed included Intestinal bowel disease (IBD) in one cat, chronic hepatitis suspicion in one case and another cat had upper airway associated disease.

Three cats had skin biopsies two to six months prior to a definitive diagnosis of PF. In one case, eosinophilic infiltrate was noted but acantholysis was absent. However, the rounded shape of keratinocytes observed could be the first sign of acantholysis. Eosinophilic infiltrate is also very suggestive of PF (Gross et al., 2005). A second histological examination performed later confirmed the diagnosis. In the other two cases, the samples did not show any histological finding suggestive of PF. In one, a biopsy was made later, and PF diagnosis was also confirmed.

Nine cats received medications before referral. Antibiotics and/or antimicrobial were administered in seven cats. These drugs included cefovecin sodium (Convenia® - Pfizer) in 2, marbofloxacin (Marbocyl® - Vétoquinol) in 4, a topical antibiotic and antifungal with marbofloxacin, clotrimazole and dexamethasone (Aurizon® - Vétoquinol) in 1, Amoxicillin (Clavobay® - Bayer) in 1 and Doxycycline (Doxirobe - Pfizer) in 1 case. The cat with previous FAD received dexchlorfeniramine (Polaramine® - Schering-Plough). One cat received antifungal drugs including itraconazole (Itrafungol® – Janssen) and enilconazole (Imaveral® - Janssen). The onset of PF was thought to be related to the administration of itraconazole and/or enilconazole in one case, and to the administration of doxycycline in another case. Seven cats received GCs IV (2) and/or PO (6) that included methylprednisolone (Oromedrol - Pfizer or Vetacortyl® - Vétoquinol) in 2, Dexamethasone (Dexoral® - Virbac) in 1, dexamethasone and Triamcinolone acetonide (Panolog® - Novartis) in one, prednisolone (Megasolone - Mérial) in one and prednisone (Cortancyl® - Roussel Diamant) in one, at various dosages. In all cats, it was observed a positive response with GC administration, showing partial remission of clinical signs. Despite some authors do not consider a GC response as a diagnostic criteria, it should be considered as an aid for the diagnosis.

Figure 19. Seasonal onset for 10 cats with PF

![Seasonal onset for 10 cats with PF](image-url)
3.2.3. Clinical signs

The initial distribution and aspect of the lesions before referral to the veterinary dermatologist specialist was known in six cats. In the other five cases lesions were not specified before the first dermatological consult. The lesions observed were crusts in four cats (66.7%) and suppurative or crusted paronychia associated with lameness in two cases (33.3%). The initial distribution of lesions was located in the pinnae (n=2), claw folds (n=3), fingers (n=1), tempores, tail and dorsolombar region (n=1). Only one cat (16.7%) had multiple sites affected initially. In the other 5 cats (83.3%) the lesions were located but in all, lesions became generalized to other body regions.

At presentation to the clinic, yellowish to honey coloured, slightly adherent crusts were described in all cats (Table 6). Pustules, vesicles or bullae were not observed in any of the cats. Crusted or suppurative paronychia was observed in 7 cases and hyperkeratosis in one. Other skin disorders described included erosions (n=4), alopecia (n=4), pruritus (n=3), papules (n=2), erythema (n=1), dull coat (n=1) and discoloured coat (n=1). The degree of pruritus was not registered. Other reported signs included intense licking (n=1), pain (n=1) and hyperthermia (n=1). All cats presented good general condition and normal physical examination, excepting the cat with hyperthermia.

Table 6. Frequent dermatological lesions in cats with PF at diagnosis

<table>
<thead>
<tr>
<th>Lesions (n=10)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crusts</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Paronychia</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Erosions</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Papules</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>

The distribution of the lesions upon presentation to the veterinary dermatologist was known in 10 cats (see Table 7). One cat had no record available. Lesions involved multiple sites in 9 cats (90%). The most common site affected was the face/head noted in 9 cats (90%) that included inner and/or outer pinnae in 7 cases, dorsal nasal planum and/or muzzle in 6 cases, chin in 2 cases and periorcular region in 1 case (Figure 19). Focal disease affecting only the face and feet occurred in 6 cats (60%). Claw folds involvement was specified in 7 cases, these with paronychia. Other areas reported were the legs (n=2), around the nipples (n=2), dorsum (n=1) and neck (n=1). Some cats with foot involvement were reluctant to move. In all cases it was observed a symmetrical bilateral pattern of distribution. Mucous membranes were not affected,
although some authors referring feline PF lesions in this location (Gross et al., 2005; Rees, 2011).

Table 7. Locations of dermatological lesions in cats with PF at diagnosis

<table>
<thead>
<tr>
<th>Distribution of dermatological lesions (n=10)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claw folds</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Outer pinnae</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Nasal planum</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Inner pinnae</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Muzzle</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Chin</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Legs</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Nipples</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Dorsum</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Foot pads</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Periocular</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Temples</td>
<td>1 (10%)</td>
</tr>
</tbody>
</table>

Figure 20. Scheme of distribution pattern and the regional location of lesions in ten cats with PF.

3.2.4. Diagnostic findings

Diagnosis was based in corticosteroid response (in cats that received GC therapy before referral), cytology findings and biopsy.

CBC and biochemical profiles were performed in some cases, with mild and non specific abnormalities and were not relevant for diagnosis. The results were negative for the two cats
tested for FeLV and FIV. To exclude differential diagnosis, scrapings and fungal cultures were performed in five cats by either the referring veterinarian or the veterinary dermatologist. The results were also negative for both. Although negative results do not rule out the presence of Dermatophytes or Demodex (Scott et al., 2001), biopsy did not show any fungi or parasite, when performed.

3.2.4.1. **Cytology**

Cytologic findings are very useful for the diagnosis of PF, and to detect the presence of fungi or bacteria. All affected cats were submitted to a cytologic examination. There was not found any pustule and then multiple direct smears were collected removing crusts and pressing the microscope slide directly against the site to be examined. All samples were stained with Diff-Quick™. Results of skin surface cytology at first consult at Advetia clinic were recorded in seven cats. All of them showed high numbers of non-degenerated neutrophils and individual and/or clusters of acantholytic cells, indicating acantholysis. Eosinophils were noted in 3 samples.

One cat also presented degenerated neutrophils, however bacteria were absent. In this cat, cytology was very suggestive of a bacterial pyoderma. Before performing a skin biopsy to confirm the diagnosis, it was administered cefovecin sodium (Convenia® - Pfizer), an appropriate antibiotic to resolve the skin infection.

3.2.4.2. **Histopathology**

Histopathology was not performed in those cases whose history (acute onset, corticosteroid response), physical examination (aspect of the lesions and their distribution) and cytology samples (acantholytic cells with non-degenerated neutrophils) were diagnostic of PF, thus limiting unnecessary expenses to cat owners. Only four of eleven cats were submitted to skin biopsy. In one of them, the record from histopathological evaluation was not available. In cats in which a biopsy was not obtained to conclusively diagnose PF, the lesions responded to immunosuppressive doses of GCs, at the beginning of treatment.

As primary lesions were not found in any cat, all the biopsy samples consisted on adherent crusts, newly formed (not the old crusts). Multiple samples, from multiple lesions for each case were collected. All samples were sent to a histopathology laboratory and routinely processed. A periodic-acid-Schiff (PAS) stain was applied in one case to evaluate the presence of dermatophytes. The result was negative for arthrospores or fungal hyphae.

Intraepidermal pustules newly formed were described in one cat. The location of these pustules was within the stratum corneum, i.e. the pustule had acquired a cornified base. Neutrophilic crusts (or degenerative pustules) were described in three cases. These pustules or crusts were layered and expanded over multiple hair follicle openings. They contained
numerous nondegenerative neutrophils as well as acantholytic keratinocytes, often in rafts. Active acantholysis was not observed in one case, whose acantholytic keratinocytes were old (angular, acidophylic and pyknotic nuclei). Bacteria or Malassezia spp. were looked in order to evidence secondary infections, but they were not found in any biopsy sample.

In one case, the epidermis was discretely hyperplasic due to a regular acanthosis (an increased thickness of the stratum spinosum due to an increased number of epidermal cells). The stratum corneum presented moderated and diffuse orthokeratotic hyperkeratosis (an increased thickness of the stratum corneum).

The dermis presented eosinophilic infiltrate in two cases and mild perivascular infiltrate of inflammatory mononuclear cells, including mast cells and lymphoplasmacytic cells in one case.

3.2.4.3. Other tests

ANA test was performed in one cat, with negative results. Immunopathological tests were not performed.

3.2.5. Treatment and clinical follow-up

Of the 11 cats treated for PF, 9 went into remission with treatment, 1 improved greatly with treatment but still had mild focal lesions when was seen last time and 1 was euthanized (Graphic 3).

Initial therapy consisted of oral prednisolone (Megasolone - Mérial) in six cats, oral prednisolone (Megasolone – Mérial) and chlorambucil (Chloraminophene – Techni Pharma) in one cat, methylprednisolone in two cats, methylprednisolone (Oromedrol – Pfizer) and topical hydrocortisone (Cortavance® - Virbac) in one cat and topical hydrocortisone (Cortavance® - Virbac) in one cat (Table 8). Information regarding treatment for at least 1 month following diagnosis was available for all cats.

After diagnosis of PF, the initial treatment for most cats (54.5%) was oral prednisolone (Megasolone - Mérial) at immunosuppressive doses of 2-5 mg kg⁻¹ q24h (Table 8). Once the lesions were in remission, the treatment regimen was changed, slowly decreasing the prednisolone dosage to 1 mg kg⁻¹ q24h and discontinued when possible. Total remission of clinical signs without relapses occurred in 2/6 (33.3%) after prednisolone monotherapy was discontinued. For these cats the average time for remission was 112 days (14 days and 210 days). The cats that responded to prednisolone were not on a significantly higher dose than the cats that did not respond. One cat (1/6 (16.7%)) had been in remission for two years with prednisolone monotherapy, but developed clinical signs compatible of PF after treatment was
stopped. A biopsy was not performed to confirm a PF relapse, but the lesions respond to GC IV (not specified by the owner) once daily during three days. At the veterinary clinic, oral dexamethasone was prescribed (Dexoral® - Virbac) at 0.2 mg kg\(^{-1}\) q24h in combination with CsA (Atopica - Novartis) at 5.6 mg kg\(^{-1}\) q24h, PO. An antimicrobial treatment with clindamycin (Antirobe® - Pfizer) was associated to limit the risk of bacterial or parasitic infections. Despite cutaneous lesions improvement after 27 days with this protocol, with just some focal areas of involvement, the cat developed several changes in general condition that included hyperthermia (39.7ºC), anorexia with weight loss and behavioural changes. Immunosuppressive treatment was changed for the third time to prednisolone (Megasolone - Mérial) 1.1 mg kg\(^{-1}\) daily in combination with topical betamethasone (Célestène® - MSD France) and a non-steroidal anti-inflammatory drug, Meloxicam (Metacam® - Boehringer Ingelheim). However, the cat remained prostrated during one month and then it was euthanized, due to the chronicity of the disease, adverse effects from medication, severe disease that did not respond to treatment and poor quality of life.

Three of the six cats (50%) receiving prednisolone monotherapy obtained improvement of lesions but not complete remission. In these cats, other immunosuppressive drugs were added to the initial treatment or prednisolone was replaced by another immunosuppressive drug. In two cats, other immunosuppressive drugs to prednisolone were added. These included oral chlorambucil (Chloraminophene – Techni Pharma) in one cat after 28 days; and oral chlorambucil (Chloraminophene - Techni Pharma) and hydrocortisone topically (Cortavance® - Virbac) on other, after 56 days. The first cat achieved total remission with this protocol within 4 months. The other cat went into partial remission and prednisolone was replaced 19 days later by oral dexamethasone (Dexoral® - Virbac), an immunosuppressive drug more potent than prednisolone, (Rosenkrantz, 2009) at a dosage of 0.3 mg kg\(^{-1}\) q24h in combination with an antibiotic, enrofloxacin (Xeden - Sogeval), during one week. Then treatment was replaced by prednisolone at 1.4 mg kg\(^{-1}\) q12h during two weeks and then tapered to 0.7 mg kg\(^{-1}\) q24h during another two weeks. This regimen resulted in total remission of clinical signs in 8 months. During the treatment, this cat relapsed three times when therapy frequency or dose was decreased. At first time of relapse (64 days after a clinical remission), prednisolone dosage was increased again to 1.4 mg kg\(^{-1}\) q12h and chlorambucil was added to therapy, in a way to decrease GC dosage. Despite the cat achieved remission with combination therapy within 1 month, 60 days later the PF lesions reappeared. Clinical remission was achieved with prednisolone at 1.4 mg kg\(^{-1}\) q24h in one week.

One cat was treated with both daily oral prednisolone (Megasolone – Mérial) 1.5 mg kg\(^{-1}\) and chlorambucil (Chloraminophene – Techni Pharma) 0.15 mg kg\(^{-1}\) (Table 8). The PF lesions improved greatly after nine months of treatment, however therapy was changed due to undesirable secondary effects development (localized pyoderma in the chin, probably due to the
usage of GC therapy for a long-time). An antibiotic treatment with cefovecin sodium (Convenia® - Pfizer) during 2 weeks and local application of Hibiscrub® and Fucidine® was started. For the lesions of pemphigus, prednisone (Cortancyl® - Roussel Diamant) was prescribed at 0.8 mg kg⁻¹ q24h PO in combination with topical betamethasone (Diprosone® - Schering-Plough) daily during one week. The dosage was tapered for prednisone to 0.4 mg kg⁻¹ q24h PO and for betamethasone to q72h by one week. Remission was obtained with this treatment after 10 months.

Two cats were treated with methylprednisolone (Oromedrol - Pfizer) at 1.0 to 2.3 mg kg⁻¹ q24h PO and one was also treated with topic hydrocortisone (Cortavance® - Virbac) (Table 8). Once the lesions were in remission (2 to 5 weeks of treatment) the treatment regimen was changed and doses were tapered. One cat was still having the dosage of their drug decreased when was last seen. The other had been in remission for one year and 7 months and doing well with maintenance medication. Medication was discontinued when the patient was last seen. The cat treated with methylprednisolone and hydrocortisone achieved total remission in 2 months.

The cat that received only topical GC developed adverse reactions and the corticoid treatment was interrupted after 68 days. During the next 30 days, the patient did not receive any immunosuppressive drug and 30 days later oral therapy with oral prednisolone (1.6 mg kg⁻¹) alone was started. Forty-seven days later, the cat was cured, without relapses.

Table 8. Immunosuppressive drugs used for initial treatment

<table>
<thead>
<tr>
<th>Drugs used initially for the treatment</th>
<th>Number of cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone (2.0-5.0 mg kg⁻¹ q24h PO)</td>
<td>6</td>
</tr>
<tr>
<td>Methylprednisolone (1.0-2.3 mg kg⁻¹ q24h PO)</td>
<td>2</td>
</tr>
<tr>
<td>Prednisolone (1.5 mg kg⁻¹ q24h PO) and Chlorambucil (0.15 mg kg⁻¹ q 24h PO)</td>
<td>1</td>
</tr>
<tr>
<td>Methylprednisolone (2.0 mg kg⁻¹ q24h PO) and Hydrocortisone topically</td>
<td>1</td>
</tr>
<tr>
<td>Hydrocortisone topically</td>
<td>1</td>
</tr>
</tbody>
</table>

Adverse effects from the initial treatment occurred in two cats (18.2%) and prompted a change in therapy in one case (hydrocortisone was changed to methylprednisolone) and addition of other immunosuppressive drug (hydrocortisone) to the initial therapy of methylprednisolone in the other. Adverse effects reported in the cat receiving hydrocortisone topically included anorexia with weight lost, lethargy and prostration, alopecia, skin atrophy and skin elasticity loss. The cat that received methylprednisolone suffered lethargy and PU/PD.

In addition, three cats whose therapies were altered had also adverse effects. One cat was changed to dexamethasone (Dexoral® - Virbac) and CsA (Atopica - Novartis) and had anorexia with weight loss, behavioural changes, hyperthermia and skin atrophy with elasticity...
loss. Following a therapy change from prednisolone to prednisone/chlorambucil/hydrocortisone one cat experienced stress. The other cat had superficial pyoderma when receiving prednisone and betamethasone.

Seven cats (64%) received antimicrobials/antibiotics in combination with initial immunosuppressive treatment that included cefalexin (Cefaseptin® - Vetoquinol), cefalexin and neomycin (Rilexine® - Virbac), cefovecin sodium (Convenia® - Pfizer), amoxicillin and clavulanic acid (Clavaseptin® - Vetoquinol), clavulanic acid and potassium clavulanate (Synulox™ - Pfizer), marbofloxacin (Marbocyl® - Vetoquinol) and clyndamicine (Antirobe® - Pfizer). The other 4 cats received immunosuppressive therapy without antibiotics. Remission of clinical signs ranged 14 days to 10 months for the cats treated with antibiotics and 47 days to 31 months for the cats without antibiotics (one of them was still in remission when last seen). The cat euthanized was included in the group of cats treated with antibiotics. During immunosuppressive therapy, other drugs added included enrofloxacin (Xeden - Sogeval), clyndamicine (Antirobe® - Pfizer) and meloxicam (Metacam® - Boehringer Ingelheim).

3.2.6. Monitoring

Information about monitoring was available only for two cats. In one cat (with only 5 months of age) CBC was performed every month, during the three first months of therapy, to control adverse effects from immunosuppressive therapy. All exams performed were in the normal parameters. In the other cat, due to weight loss (1 kg lost in two months), lethargy and bowel thickening to abdominal palpation, CBCs, biochemical tests and urinary analysis were performed, all with normal results. Additional tests included thoracic radiographies, which revealed a widened mediastinum, and abdominal ultrasounds, which confirmed the bowel thickening.

![Figure 21. Treatment outcomes for the 11 cats included in the study.](image-url)
4. Discussion

Although PF is the most common autoimmune skin disease observed in cats, it is still rare. During three years of practice, it was not diagnosed any cat with PF at HVBV, a general veterinary hospital in Águeda, Portugal. In cats, PF lesions tend to be mild and the majority of cats do not present serious systemic signs and maybe the owners are not concerned in taking the animal to a veterinarian. Also in Portugal, a big portion of feline population is homeless and lack medical support when they need (in rural environment, less of 20% of the cats have veterinary support and 30% to 40% in urban areas, estimated by Pet Food Industry in 2009). One cat was suspected of being PF, however the cat was euthanized before a diagnostic confirmation, and for this reason it was not included in this study. In comparison with general veterinary clinics, the incidence of PF cases is supposedly higher at ADVETIA veterinary clinic because it has a dermatology service and receives dermatologic cases from other clinics, especially uncommon dermatologic diseases suspicions.

In this study 6 females and 5 males were affected by PF. Indeed, previous studies on feline PF did not relate any gender predilection. In this study, more domestic European short-haired cats were seen with PF (72.7%), in comparison with any other breed. The results are similar to a retrospective study made with 57 cases, in which 59.6% were domestic short-haired cats (Preziosi et al., 2003). This study revealed that feline PF can develop in any age, affecting cats with few months of life (5 months) or old cats (10 years). The lack of an age predisposition is in accordance with a previous study (Preziosi et al., 2003).

The vast majority of the cats in this study first exhibited clinical signs in the Summer season (60%). Also, in one case the owner specified that the skin lesions get worse in Summer and improved in Winter. Ultraviolet light is not referred as a triggering factor for the development of PF in cats, however the possibility of seasonality in some cats was already proposed by Griffin (1991). The importance and consistency of this finding will need to be confirmed with larger studies.

It is not clear what role, if any, previous skin disease plays in the development of PF in cats. It is reported a possible association with chronic allergic dermatitis with PF in some cats (Preziosi et al., 2003; Chapelin et al., 2004). In this study one cat was FAD 5 months prior to developing PF, however if this chronic inflammatory skin disease plays a role in PF development, this cannot be proven. It is unknown if previous diseases reported in the history of the cats included in this study play a role in inducing PF or if these diseases are together by coincidence.

As reported previously, drug induced PF can occur in cats. In one cat, with only five months, the disease onset was acute (lesions development in nine days) and occurred after administration of itraconazole and enilconazole, indicating a possible drug reaction. In fact, the administration of itraconazole was reported as a potential trigger of PF in a large feline
retrospective study (Preziosi et al., 2003). In another case, the administration of doxycycline could be considered a potential drug for PF development, because disease onset was also acute and clinical signs resolved upon discontinuation of the drug and with prednisolone therapy during 14 days. However, it was not reported a drug induced PF by the tetracycline group. In this two cases, lesions resolved without relapses when therapy was discontinued. However, the probability of association between the administration of medication and lesions onset using “adverse drug reaction probability scales”, such as the one developed by Naranjo et al. (1981) was not possible for evaluation in these cases. As far as the other drugs are concerned, any association with PF is not related. Another possibility is that the cat with only five months developed PF lesions due to an infection: in humans a possible association of viral infections and PF development has been reported (Brenner et al., 2002; Sagi et al., 2008).

PF is an exfoliative skin disease and commonly reported lesions of feline PF are thick, yellowish to honey-colored adherent crusts with associated scale, alopecia and erosions (Preziosi et al., 2003; Sparkes and Caney, 2005; Paterson, 2006; Peterson and McKay, 2010). The most consistent and prominent lesions observed were yellowish crusts (100%). In the study reported here, it was also seen paronychia (70%), erosions (40%), alopecia (40%) pruritus (30%) and papules (20%). Pustules were not seen, probably due to the thickness of the skin in this species. Other clinical signs reported in this study included dull coat, discoloured coat, intense licking and pain. Pruritus has been reported as variable in feline PF (Preziosi et al., 2003; Chapelin et al., 2004; Peterson and McKay, 2010), and some cats in this study were pruritic. However, many cats received GCs before presentation to the dermatologist veterinary specialist and it was not possible to determinate pruritus from the records. Cats can also be affected by systemic signs such as lethargy, anorexia and hyperthermia (Preziosi et al., 2003), as was seen in one case in this study.

The initial distribution of feline PF lesions is often localized and mild, but most of them generalize to dorsum and ventrum (Preziosi et al., 2003). In this study, despite most cats had a localized distribution of lesions when they were first noted, the majority of cats had a generalized distribution of lesions at presentation to the speciality clinic. In the current study most cats had lesions in the face/head (90%), particularly in the ear pinnae. This is also reported in literature (Preziosi et al., 2003; Chapelin et al., 2004; Matousek, 2004; Sparkes and Caney, 2005; Friberg, 2006; Foster et al., 2007; Peterson and McKay, 2010). In feline PF footpads and claw folds involvement is also frequent (Preziosi et al., 2003; Matousek, 2004; Crow, 2009; Peterson and McKay, 2010). In the current study, claw folds lesions, with sterile paronychia was specified in 70% of cases. Occasionally, the feet may be the only affected site (Preziosi et al., 2003), however exclusive footpad involvement was not observed in this study. Focal disease affecting only the face and feet occurred in 6 cats (60%). In all cases a symmetrical bilateral pattern of distribution was observed. This study found involvement of other
areas of the body (tail, dorsum, legs, nipples), as was founded in a retrospective study of 57 cats (Preziosi et al., 2003). Mucous membranes were not affected, although some authors refer to feline PF lesions in this location (Gross et al., 2005; Rees, 2011). Hyperkeratosis is an uncommon finding in cats with PF (Paterson, 2006), however it was reported in one case.

Direct cytology is very useful to diagnose PF, especially the cytological feature of acantholytic cells, either singly or in rafts. In the study reported here, 7 cytological specimens had a high number of non-degenerated neutrophils and individual and/or clusters of acantholytic keratinocytes. Microscopic evaluation of Tzanck preparations or impression smears is part of a routine diagnostic workup for pustular and crusty skin diseases. So, the number of cytological samples recorded (7/11) seemed very low. However, it was unknown how many cytological samples were obtained and not recorded. Despite the presence of acantholytic keratinocytes in cytological preparations from crusted lesions suggest PF, they are not pathognomonic. Superficial folliculitis (e.g. bacterial or caused by Trichophyton spp.) can occasionally result in acantholysis – most likely secondary to enzyme release from neutrophils or the organisms themselves.

In this study, two of three biopsy samples performed before a definitive diagnosis of PF did not exhibit any finding suggestive of PF. Curiously, the two cats were with GC use at the time of biopsy. Indeed, it is reported that GC use at the time of biopsy produced more nondiagnostic samples (Preziosi et al., 2003). To increase diagnostic yield, the use of GC at the time of biopsy should be avoided.

Pustules were not found and diagnosis was based on examination of crusts (degenerating pustules). Histopathology examination was performed in four cats, and all confirmed PF diagnosis with the presence of micropustules or crusts with non-degenerated neutrophils and acantholytic cells. Active acantholysis was not seen in one case, but old acantholytic keratinocytes were seen. Acantholysis absence was also registered in one case of feline PF (Chapelin et al., 2004). Hyperplasia of the epidermis and a perivascular infiltrate of a mixed population of cells, especially mast cells and eosinophils were also seen in other studies (Preziosi et al., 2003; Chapelin et al., 2004; Peterson and Mckay 2010).

Immunofluorescence or immunohistochemical evaluation may support the diagnosis but these tests are less sensitive than direct histopathological evaluation and they are not performed routinely because of technical or cost factors (Scott et al., 2001). None of the cats in the current study underwent immune testing. One cat was submitted for ANA test with negative results.

Secondary pyoderma is common in PF and may complicate the diagnosis (Scott et al., 2001). Initial treatment with antibiotics prior to or concurrent with the onset of immunosuppressive treatment may be associated with better outcomes. However, no study in feline PF refers this discrepancy. In this study differences between cats treated with or without
antibiotics, with regard to remission or euthanasia rates was not performed, because animal numbers in these treatment groups were too small to perform statistical analysis. However, both groups had a wide range for remission time.

At this time, the initial recommended treatment for PF is oral GCs, at immunosuppressive doses, which are tapered based on clinical improvement (Willemse, 2000; Scott et al., 2001; Chandler et al., 2004; Merchant, 2007; Peterson and McKay, 2010). Monotherapy with prednisolone was the initial treatment of choice for most cats. It was not possible to determine a significant difference in outcomes between therapies due to lack of cases. In this study, total remission of the clinical signs exclusively with GC therapy was observed in three cases (two with prednisolone and one with a combination therapy of methylprednisolone and topical hidrocortisone). Protocols were altered in seven cats due to lack of remission, side effects of treatment or because the dosage of the drugs used could not be decreased without causing a relapse. One case was still having the dosage of their drugs decreased when last seen (methylprednisolone).

Adverse effects observed were largely attributed to iatrogenic hyperadrenocorticism and due to the administration of prednisolone, hydrocortisone, methylprednisolone, prednisone/betamethasone, prednisone/chlorambucil/hidrocortisone and dexamethasone/CsA.

The majority of cats with PF achieve remission with therapy (Kofod, 1993; Preziosi et al., 2003; Chapelin et al., 2004). In this study, ten cats were alive and their disease controlled or in remission (one case) at their last recorded visit or phone contact. Only one cat was euthanized due to an inability to control the disease and from serious side effects development.
5. Conclusion

Despite the low number of cases in this retrospective study, it was evident that feline PF is characterized by yellowish crusts, scale, alopecia and paronychia, when claw folds are involved. Primary lesions (pustules) were rarely found and it was not seen in the cats included in this study. Lesions were more localized to the face/head and paws, but can affect any body region. Cytological evaluation was a rapid and highly informative diagnostic test and showed acantholytic keratinocytes and neutrophils. Despite cytology being very useful, definitive diagnosis is confirmed on skin histopathology. The mainstay of treatment was systemic GCs, which can be combined with other immunosuppressive medications. In most cats, the prognosis is favorable, however the difficulty in managing this disease should not be underestimated. Some cases may be refractory.

During my stage period I had the opportunity to see one cat with PF, included in the present study. I observed the appearance of the lesions and the typical distribution of clinical signs. I performed cytologic exams and their microscopic evaluation and I also followed the biopsy collection technique. The biopsy specimens from this case were sent to a laboratory and the PF diagnosis confirmed. An immunosuppressive treatment with methylprednisolone was started and the cat was in remission when I saw it for the last time. I also had the opportunity to see two of the cats included in this study, in total remission of PF lesions.
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What is Pemphigus Foliaceus?

**Pemphigus Foliaceus** is the most common autoimmune skin disease in cats and also in dogs. It occurs when autoantibodies are produced against the own body, causing illness.

At first your pet develops small red spots that rapidly form a pustule (pimple). In most cases you will find thick yellowish to honey coloured crusts, because pustules are very transient and rupture easily. Most of times, cats with **Pemphigus Foliaceus** present lesions on the head/face and/or paws (claw folds and/or pad paws). However, other body regions can also be affected.

Your pet may develop secondary skin infections, as a result of skin damage to the skin.

How does it develop?

The cause of Pemphigus Foliaceus is not known. Some theorize that genetics and/or environment play a role. Exposure to ultraviolet light can worsen the skin lesions. Rarely certain drugs may be related to the development of this skin disease.

Is it contagious?

Pemphigus Foliaceus also occur in humans, but it is not contagious.

How it is diagnosed?

Several tests are needed to confirm Pemphigus Foliaceus.

1. Skin cytology is a very simple exam, to see microscopically abnormalities of superficial cells from the skin and if bacteria and/or fungi are present.
2. Skin biopsy is needed to confirm the diagnosis of Pemphigus Foliaceus. Histopathology allows a microscopic evaluation of all the layer of the skin.
3. Blood tests and urine tests are needed to diagnose other medical conditions and to choose the appropriate medication. Many other skin diseases can look like Pemphigus Foliateus and your veterinarian may require multiple skin biopsies or other skin tests to confirm Pemphigus Foliateus and rule out other skin diseases.

**How is it treated?**
Actually the treatment consist in drugs that suppress the immune system (which is causing the disease), most commonly used are glucocorticoids. Once your cat responds to initial treatment, the dosage and frequency is gradually decreased over time to the lowest possible dose.

If secondary bacterial infections are present, antibiotics will be used. Your veterinarian will determine the optimal treatment plan for your cat’s condition.

It is required frequent reexaminations and tests to evaluate the remission of clinical signs and response to treatment.

**How serious is this disease?**
The majority of cats respond well to the initial treatment. However Pemphigus Foliateus is a skin disease with a wane and wane course. Some cats will fully recover with treatment and never again develop signs of Pemphigus Foliateus. More frequently, Pemphigus Foliateus becomes a chronic condition that requires life-long therapy and monitoring. Very rarely the treatment fails or serious side effects from medication can develop.