Outi Varpuluoma

DRUGS, DERMATITIS HERPETIFORMIS AND CELIAC DISEASE AS RISK FACTORS FOR BULLOUS PEMPHIGOID IN FINLAND
OUTI VARPULUOMA

DRUGS, DERMATITIS HERPETIFORMIS AND CELIAC DISEASE AS RISK FACTORS FOR BULLOUS PEMPHIGOID IN FINLAND

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 8 of Oulu University Hospital (Kajaanintie 50), on 29 March 2019, at 12 noon
Bullous pemphigoid (BP) is the most common autoimmune blistering disease. It mostly affects elderly patients and is characterized by intense pruritus and blistering or bullae. Treatment options include topical and systemic corticosteroids, other immunosuppressive drugs and doxycycline. Disease course may be chronic and relapses are common. The incidence of BP has been reported to have increased in the last few decades, but the reason for this trend is not known.

The aim of this thesis was to study the risk factors of BP. Firstly, the influence of the use of dipeptidyl peptidase (DPP-4) inhibitors was analyzed as a risk factor, and then those of other oral diabetes medications. This study also aimed to determine whether drugs used for psychiatric and neurologic conditions are risk factors for BP. Finally, previously diagnosed dermatitis herpetiformis (DH) and celiac disease (CD) were examined as potential risk factors for subsequent BP.

For this retrospective, matched case-control study, patient data were obtained from the Finnish Care Register for Health Care database, and data on reimbursed drugs from the Social Insurance Institution of Finland.

In the present study, prior use of DPP-4 inhibitors was found to increase the risk of BP twofold and in particular, vildagliptin increased the risk tenfold. The mean time between the initiation of vildagliptin and diagnosis of BP was 449 days. Metformin and other conventional diabetes drugs were not risk factors for BP. Several drugs used for neurological and psychiatric diseases were associated with an elevated risk for BP, but no pharmacological or chemical properties of these drugs emerged as candidates to explain the increased risk. A prior diagnosis of DH increased the risk of BP 22-fold and a diagnosis of CD doubled it. Dapsone had been used in the two years before BP diagnosis by 44% of patients whose BP was preceded by DH. The mean time between the diagnoses of DH and BP was 3.3 years.

This study confirms the view that DPP-4 inhibitors increase the risk for BP. No such association was found with other classes of diabetes drugs and therefore their use can be continued following a diagnosis of BP. Doctors treating patients with DH should be aware of the association between DH and BP, and be particularly vigilant if a DH patient’s skin symptoms change or become unresponsive to a gluten-free diet and/or dapsone.

**Keywords:** bullous pemphigoid, celiac disease, comorbidity, dermatitis herpetiformis, dipeptidyl peptidase-4 inhibitor, epidemiology, vildagliptin

Tämän tutkimuksen tavoite oli tutkia pemfigoidin riskitekijöitä Suomessa. Retrospektiivisessä tapaus-verrokkitutkimuksessa käytettiin aineistona Terveyden ja hyvinvoinnin laitoksen hoitotiloina poimittuja pemfigoidiopaitoja (N=3397) ja verrokkeina ihon tyvisolusyöpäpotilaat (N=12941). Tiedot korvattuja lääkekoostumukset saatiin Kelan lääkekorvausrekisteristä.


Pemfigoidin on kuvattu voivan puhtaa ihokeliakian jälkeen, mutta laajempia tutkimuksia näiden sairauksien yhteydestä ei oltu aiemminktehty. Tämän vuoksi samassa potilasaineistossa tutkittiin ihokeliakia ja keliakiaa pemfigoidin riskitekijöinä. Edeltäväihokeliakia lisäsi pemfigoidin toteamisen riskiä selvästi, jopa 22-kertaiseksi ja keliakia kaksinkertaiseksi. Huomattava osa potilaita oli ostanut ihokeliakian hoitoon käytettävää dapsonia edeltävän 2 vuoden aikana ennen pemfigoidin toteamista, mikä voi kertoa ihokeliakian oireiden aktiivisuudesta.

Tämä tutkimus vahvistaa näkemystä siitä, että DPP-4:n salpaajat ovat pemfigoidin riskitekijä. Muut tutkitut diabeteslääkkeet eivät lisänneet riskiä ja voidaan ajatella, että ne eivät edelleen hankaloita aiemmin todetun pemfigoidin oireita. Koska ihokeliakian todettiin olevan pemfigoidin riskitekijä, tulee näitä potilaata hoitavan lääkärin muistaa pemfigoidin mahdollisuus, jos ihokeliakian oireet muuttuvat tai hoitovaste menetetään.

Asiasanat: autoimmuunitautuidit, dipeptidyylipeptidaasi 4:n salpaaja, epidemiologia, ihokeliakia, keliakia, komorbiditeetti, rakkulainen pemfigoidi
Acknowledgements

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Oulu, January 2019

Outi Varpuluoma
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATC</td>
<td>Anatomical Therapeutic Chemical</td>
</tr>
<tr>
<td>BPDAI</td>
<td>Bullous Pemphigoid Disease Area Index</td>
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<tr>
<td>BMZ</td>
<td>basement membrane zone</td>
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<tr>
<td>C3</td>
<td>complement 3</td>
</tr>
<tr>
<td>CD</td>
<td>celiac disease</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CRHC</td>
<td>Care Register for Health Care</td>
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<td>DH</td>
<td>dermatitis herpetiformis</td>
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<tr>
<td>DIF</td>
<td>direct immunofluorescence</td>
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<tr>
<td>DLQI</td>
<td>Dermatology Life Quality Index</td>
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<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>DPP-4</td>
<td>dipeptidyl peptidase-4</td>
</tr>
<tr>
<td>DPP-4i</td>
<td>dipeptidyl peptidase-4 inhibitor</td>
</tr>
<tr>
<td>EBA</td>
<td>epidermolysis bullosa acquisita</td>
</tr>
<tr>
<td>EDF</td>
<td>European Dermatology Forum</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>e.g.</td>
<td>exempli gratia, for example</td>
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<tr>
<td>HD</td>
<td>hemidesmosome</td>
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<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IgE</td>
<td>immunoglobulin E</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>IIF</td>
<td>indirect immunofluorescence</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>LAD</td>
<td>linear IgA disease</td>
</tr>
<tr>
<td>MMP</td>
<td>mucous membrane pemphigoid</td>
</tr>
<tr>
<td>NC</td>
<td>non-collagenous</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>PD-1</td>
<td>programmed cell death protein 1</td>
</tr>
<tr>
<td>PD-L1</td>
<td>programmed cell death ligand 1</td>
</tr>
<tr>
<td>PG</td>
<td>pemphigoid gestationis</td>
</tr>
<tr>
<td>TG3</td>
<td>transglutaminase 3</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
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</table>
Publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


III Varpuluoma, O., Jokelainen, J., Försti, A., Timonen, M., Huilaja, L. & Tasanen K. (accepted for publication). Drugs used for neurological and psychiatric conditions increase the risk of bullous pemphigoid: a Finnish case-control study. *Journal of the American Academy of Dermatology.*

Contents

Abstract
Tiivistelmä
Acknowledgements
Abbreviations
Publications

1 Introduction

2 Review of the literature
  2.1 Clinical presentation of bullous pemphigoid (BP).............................. 17
  2.2 Pathophysiology of bullous pemphigoid............................................. 19
    2.2.1 Cutaneous basement membrane and hemidesmosomal proteins.................................................. 19
    2.2.2 Autoimmunization in bullous pemphigoid................................... 20
  2.3 Diagnosis of bullous pemphigoid and measurement of disease activity........................................................................................................ 21
  2.4 Treatment of bullous pemphigoid..................................................... 22
  2.5 Epidemiology of bullous pemphigoid.............................................. 24
    2.5.1 The incidence of bullous pemphigoid........................................... 24
    2.5.2 Age at bullous pemphigoid diagnosis and differences between sexes.................................................. 25
    2.5.3 Mortality in bullous pemphigoid................................................ 25
    2.5.4 The comorbidities of bullous pemphigoid................................. 25
  2.6 Risk factors for bullous pemphigoid............................................... 29
    2.6.1 Drugs as risk factors for bullous pemphigoid.............................. 29
    2.6.2 Other risk factors...................................................................... 31
  2.7 Other pemphigoid diseases............................................................ 32
    2.7.1 Pemphigoid gestationis............................................................. 32
    2.7.2 Linear IgA disease.................................................................... 32
    2.7.3 Mucous membrane pemphigoid ................................................ 33
    2.7.4 Anti-laminin γ1/p200 pemphigoid............................................. 34
    2.7.5 Epidermolysis bullosa acquisita.............................................. 34
  2.8 Other subepidermal autoimmune bullous diseases................................. 35
    2.8.1 Dermatitis herpetiformis........................................................ 35

3 Objectives of the study

4 Materials and methods
4.1 Populations and databases ................................................................. 39
4.2 Statistical analyses (I, II, III, IV) ...................................................... 41
4.3 Ethical issues and study permissions (I, II, III, IV) ............................. 41

5 Results 43
5.1 Subject characteristics (I, II, III, IV) ................................................. 43
5.2 Diabetes among study populations (I, II) .......................................... 43
5.3 DPP-4 inhibitor use and risk of bullous pemphigoid (I) ...................... 43
5.4 Other diabetes drugs and the risk of bullous pemphigoid (I, II) ......... 44
5.5 Use of drugs for psychiatric and neurologic diseases and the risk of bullous pemphigoid (III) ............................................................. 44
5.6 Dermatitis herpetiformis and celiac disease and the risk for bullous pemphigoid (IV) ................................................................. 45
5.7 The use of dapsone and DPP-4 inhibitors in study populations (IV) ................................................................................................. 45

6 Discussion 47
6.1 DPP-4 inhibitors increase the risk of bullous pemphigoid (I) .......... 47
6.2 Diabetes drugs other than DPP-4 inhibitors are not associated with an increased risk for bullous pemphigoid (II) ................................. 50
6.3 Drugs affecting nervous system may contribute to elevated bullous pemphigoid risk (III) ................................................................. 51
6.4 Dermatitis herpetiformis is a risk factor for bullous pemphigoid (IV) ................................................................................................. 52
6.5 Strengths of the study ....................................................................... 54
6.6 Limitations of the study ................................................................. 54
6.7 Future prospects .............................................................................. 55

7 Conclusions 57
References 59
Original publications 73
1 Introduction

Autoimmune diseases affect approximately 5–9% of the population in Western countries (Cooper, Bynum, & Somers, 2009; Davidson & Diamond, 2001). Although bullous pemphigoid (BP) is the most common autoimmune blistering disease, it is still rare, with incidence varying from 2.4 to 42.8 individuals per million per year (Amber, Murrell, Schmidt, Joly, & Borradori, 2018a). Mainly affecting elderly individuals, BP not only impairs quality of life (Kouris et al., 2016), but also increases mortality (Joly et al., 2012; Langan et al., 2008).

The detailed pathogenesis of many autoimmune diseases is not well understood but genetic susceptibility and environmental or other triggering factors are thought to be involved (Davidson & Diamond, 2001). The pathomechanism of BP is better known than those of many other autoimmune diseases. Its main autoantigen is the hemidesmosomal collagen XVII (BP180) but it is not known what initiates the cascade of events that leads to the loss of BP180 immunotolerance (Amber et al., 2018a). Unlike many other autoimmune diseases, BP manifests late in life, usually when the patient is aged almost 80 years (Amber et al., 2018a). Over the past few decades, many countries have seen an increase in the incidence of BP, but this cannot be explained solely by ageing populations (Kridin & Ludwig, 2018).

The partially unexplained increasing incidence of BP inspired the present study, which was designed to investigate the risk factors of BP. It was conducted utilizing two comprehensive Finnish registries, maintained by the National Institute for Health and Welfare, and that of the Social Insurance Institution. Data on both hospital inpatient care and outpatient visits give reliable information on diagnoses and cross-referencing these with data on reimbursed drugs offers an excellent overview of patterns of drug use prior to disease onset.
2 Review of the literature

2.1 Clinical presentation of bullous pemphigoid (BP)

The typical clinical appearance of BP is tense bullae end erythema (Fig.1) (Amber et al., 2018a; Schmidt & Zillikens, 2013). Urticarial plaques are also often seen. The characteristic skin manifestations are often preceded by several weeks or months of pruritus and/or non-specific skin symptoms such as eczematous, urticarial, papular or excoriated lesions. Therefore, the clinical appearance of BP can easily be confused with other, more common dermatoses especially before blistering appears (Amber et al., 2018a; Schmidt & Zillikens, 2013). The limbs and trunk are the most frequently affected areas (Della Torre et al., 2012).

Fig. 1. Typical bullae and erythema of bullous pemphigoid (Department of Dermatology, Oulu University Hospital).
In a prospective study of 117 patients with BP (Della Torre et al., 2012), 82.9% of patients had actual vesicles, postbullous erosions or blistering and the rest had erythematous papules and plaques, eczematous lesions, urticarial or erythema-multiforme-like lesions. In addition, mucosal involvement was noted in 14.5%. A retrospective French study of 502 patients reported that 79% had typical clinical features of BP and 8% had oral mucosa involvement (Joly et al., 2012). The skin findings of nonbullous pemphigoid (Fig. 2) have recently been reviewed by Lamberts et al. Erythematous, urticarial papules and plaques were seen in half of the cases, papules/nodules and excoriations in about one-fifth, and dermatitis herpetiformis-like lesions, ulcerations and erythroderma in only a few (Lamberts, Meijer, & Jonkman, 2017).

Although BP is mostly a disease of the elderly, it can also occasionally affect pediatric patients. Most (79% to 100%) infant BP patients have skin symptoms on their hands and feet (Schwieger-Briel et al., 2014; Waisbourd-Zinman et al., 2008), whereas in later childhood only 17% of patients have acral involvement (Waisbourd-Zinman et al., 2008).

Fig. 2. Excoriations and papules of non-bullous pemphigoid (Department of Dermatology, Oulu University Hospital).
2.2 Pathophysiology of bullous pemphigoid

2.2.1 Cutaneous basement membrane and hemidesmosomal proteins

The basement membrane zone (BMZ) connects the epidermis and the dermis (Fig. 3). Hemidesmosomes (HDs) are structures found on the basal surface of keratinocytes at the basal layer of the epidermis. They are composed of e.g. BP180 (also called BP antigen 2 or collagen XVII), BP230 (also called BP antigen 1), integrin α6β4, tetraspanin CD151 and plectin. Plectin is a high molecular weight protein that connects the HD to keratin filaments of basal keratinocytes and is important in the cytoskeleton. Integrin α6β4 is a transmembrane receptor that consist of two subunits, α6 and β4, and interacts with plectin by its intracellular domain. The connection between plectin and integrin α6β4 is essential for the stability of the HD. The extracellular portion of integrin α6β4 interacts with basement membrane components such as BP180. BP180 is a transmembrane collagen with 15 collagenous and 16 non-collagenous (NC) domains. The cytoplasmic domain of the BP180 interacts with integrin subunit β4, plectin and BP230. The extracellular domain of BP180 consist of collagenous domains that extend to the BMZ (de Pereda, Ortega, Alonso-García, Gómez-Hernández, & Sonnenberg, 2009; Giudice, Emery, & Diaz, 1992; McMillan, Akiyama, & Shimizu, 2003; Walko, Castañón, & Wiche, 2015). BP180 is cleaved within the NC16A domain and therefore BP180 appears in two different forms: as a transmembrane protein and as a soluble ectodomain (Schumann et al., 2000; Walko et al., 2015). BP230 is a cytoplasmic non-collagenous protein of the plakin family that links keratin filaments with HDs. Laminins are glycoproteins in the BMZ and located in the lamina densa. Laminins interact with collagen VII in upper papillary dermis (de Pereda et al., 2009; Walko et al., 2015).
2.2.2 Autoimmunization in bullous pemphigoid

The most important autoantigen in BP is BP180 and evidence for the pathogenic role of BP180 autoantibodies originates from clinical findings and experimental mouse models of BP (Liu et al., 2008; Nishie et al., 2007). The NC16A domain of BP180 is known to be the immunodominant region in BP (Nishie, 2014) but the majority of BP patients also have IgG autoantibodies against other epitopes of BP180 (Di Zenzo et al., 2008; Perriard et al., 1999). The other BP autoantigen is BP230, which has been reported to be recognized by about 70% of BP sera, the COOH-terminus of BP230 is the immunodominant region of BP230 (Skaria et al., 2000). Levels of IgG autoantibodies against BP180 have been shown to correlate with BP disease severity (Di Zenzo et al., 2011; Schmidt, Obé, Bröcker, & Zillikens, 2000; Tsuji-Abe et al., 2005), but the degree to which the level of IgG autoantibodies against BP230 correlates with disease severity is controversial (Di
It has been reported that in BP, autoimmunity against BP180 precedes the appearance of autoantibodies against BP230, and thus BP230 autoantibodies may result from epitope spreading (Di Zenzo et al., 2011). Taken together, the role of BP230 autoantibodies in the pathogenesis of BP has not been clearly elucidated.

Epitope spreading is a phenomenon in which an immune response is developed to one or more other epitopes in addition to the dominant epitope (Di Zenzo et al., 2011). Epitope spreading has been reported to occur in about half of BP cases, is most likely to happen in the early stage of the disease and is associated with more active and severe BP (Di Zenzo et al., 2011).

In the pathomechanism of BP, T cells recognize BP180 and activate B cells to produce IgG and IgE class autoantibodies (Lo Schiavo et al., 2013; Nishie, 2014). The binding of autoantibodies to their epitopes leads to complement activation, which is thought to be critical in the process leading to blister formation in BP (Lo Schiavo et al., 2013; Nishie, 2014). Complement activation induces degranulation of mast cells, but this can also be caused by IgE. Mast cell degranulation leads to the release of several mediators, including leukotrienes, tumor necrosis factor (TNF) α and other cytokines, which recruit neutrophils and eosinophils. Recruited neutrophils along the dermo-epidermal junction produce proteolytic enzymes that impair dermo-epidermal adhesion and this leads to blister formation. Eosinophils are also known to release proteolytic enzymes and contribute to blister formation (Lo Schiavo et al., 2013; Nishie, 2014).

2.3 Diagnosis of bullous pemphigoid and measurement of disease activity

As with other autoimmune blistering diseases, direct immunofluorescence (DIF) microscopy is the golden standard in diagnosis of BP (Amber et al., 2018a; Schmidt & Zillikens, 2013). A diagnosis of BP is based on DIF microscopy of perilesional skin, clinical criteria and serological studies. Linear deposits of IgG and/or C3 at the epidermal basement membrane are seen in BP. However, similar immunofluorescence findings may also be seen in some other autoimmune blistering diseases, so clinical presentation and serological tests must be taken into account for a differential diagnosis. Enzyme-linked immunosorbent assays (ELISA) are used to measure circulating antibodies against BP180 and BP230. The commercially available BP180 ELISA tests detect autoantibodies targeting the NC16A domain of BP180 (Amber et al., 2018a; Di Zenzo, Della Torre, Zambruno,
Indirect immunofluorescence (IIF) is preferably performed on salt-split human skin, and shows circulating IgG autoantibodies binding to the epidermal side of the artificial split (Di Zenzo et al., 2012; Schmidt & Zillikens, 2013). In laminin 332- mucous membrane pemphigoid, epidermolysis bullosa acquisita and anti-laminin γ1/p200 pemphigoid autoantibodies bind to the dermal side. Light microscopy of a BP bulla shows a subepidermal blister accompanied by dermal infiltration of eosinophils and neutrophils (Di Zenzo et al., 2012; Schmidt & Zillikens, 2013).

The activity of BP can be measured with the BPDAI (Bullous Pemphigoid Disease Area Index) which takes into account erosions, blisters, urticaria, erythema and damage at different sites of the body, as well as mucosal involvement (Murrell et al., 2012). The BPDAI has good inter-rater reliability and the ability to indicate clinically meaningful changes in clinical condition (Wijayanti et al., 2017). However, currently the BPDAI is mostly used for study purposes, not in everyday practice.

2.4 Treatment of bullous pemphigoid

There are European guidelines and national guidelines for treating BP (Bernard, Bedane, Prost, Ingen-Housz-Oro, & Joly, 2011; Cozzani et al., 2018; Eming et al., 2015; Feliciani et al., 2015; Venning, Taghipour, Mustapa, Highet, & Kirtschig, 2012) but Finland does not have its own national guidelines. The first treatment option recommended by the European Dermatology Forum (EDF) guidelines (Feliciani et al., 2015) for the treatment of localized/limited disease with mild activity is topical corticosteroid clobetasol propionate 10–20 grams per day. For extensive BP (with more than 10 new blisters per day or inflammatory lesions covering a large body surface area) either topical clobetasol propionate 30–40 grams per day divided into two applications or systemic prednisone 0.5–0.75 mg per kg body weight is recommended. Topical treatment can be combined with systemic therapy. High dose prednisone (1.0 mg per kg) is not recommended for initial treatment because of the potential for side-effects and higher mortality risk (Feliciani et al., 2015). According to the EDF guidelines, second-line choices or adjuvant therapy regimens for generalized disease include azathioprine, mycophenolates, tetracyclines combined with nicotinamide, methotrexate, chlorambucil, dapsone and cyclosporine (Feliciani et al., 2015). In choosing between these regimens, cost aspects, practical experience, specific contraindications and availability must all be considered. Third-line treatment
recommendations are mostly reserved for treatment-resistant BP and include intravenous immunoglobulins, immunoadsorption, anti-CD20 (rituximab) or anti-IgE antibodies (omalizumab), plasma exchange and cyclophosphamide (Feliciani et al., 2015). Novel third-line regimens have also been recently reviewed by Amber et al (Amber, Maglie, Solimani, Eming, & Hertl, 2018b). Small studies have shown rituximab to be effective, whereas with omalizumab, only limited data are available with variable results. Immunoabsorption and intravenous immunoglobulins have been reported to be effective but larger studies are lacking. In addition, some other monoclonal antibodies e.g. the IL-5 antibody mepolizumab and an eotaxin-1 antibody bertilimumab, are currently being studied for the treatment of BP (Amber et al., 2018b).

According to the EDF guidelines, discontinuation of BP treatment is done gradually by tapering the doses of the regimens used (Feliciani et al., 2015). In topical corticosteroid treatment, doses are tapered monthly and weekly maintenance therapy can be continued for up to eight months. In systemic corticosteroid therapy, the first tapering is done after 15 days of disease control (defined as the appearance of no new bullae and cessation of pruritus) and the objective is to find the minimal dose needed to keep the disease controlled during the 4-6 months. The optimal overall duration of treatment is recommended to be about 9–12 months (Feliciani et al., 2015).

The German guidelines note the practical aspect for applying topical corticosteroids twice a day to the entire body, this may not be practical for elderly patients (Eming et al., 2015). These German guidelines also mention doxycycline as an alternative for initiation of systemic therapy or adjuvant therapy (Eming et al., 2015). The British guidelines are mostly consistent with the EDF guidelines, although they recommend slightly higher doses of prednisone (0.75mg–1mg per kg) for patients with extensive disease (Venning et al., 2012). The newly published Italian guidelines are mainly based on the EDF guidelines and do not add anything new to the treatment options (Cozzani et al., 2018). The French guidelines are published only in French (Bernard et al., 2011).

Treatment of patients with extensive BP with prednisone at high doses of 1mg per kg has been associated with heightened mortality compared to topical corticosteroid treatment, while a 0.5mg per kg dose was not associated with such an effect (Joly et al., 2002). Considering the safety issues of oral corticosteroid therapy, doxycycline 200 mg daily was compared with oral prednisolone 0.5mg daily in a non-inferiority trial of 253 BP patients (Williams et al., 2017). At week 6, treatment success (defined as three significant blisters or fewer) was seen in 74%
of doxycycline-treated and 91% of prednisolone-treated patients. By the week 52, severe adverse effects were observed in 15% of patients in the doxycycline group and in 36% of the prednisolone group. Three treatment-related deaths were observed in the doxycycline group and 11 in the prednisolone group. The authors concluded that doxycycline is non-inferior to prednisolone but significantly safer (Williams et al., 2017). This study has been criticized for its definition of treatment success, which may have led to overestimation of the efficacy of doxycycline (Sladden & Hutchinson, 2017).

2.5 Epidemiology of bullous pemphigoid

2.5.1 The incidence of bullous pemphigoid

The highest incidence of BP, 42.8 per million person-years, has been reported in UK (Langan et al., 2008). However, since the study was population-based and diagnoses set by general practitioners, it is not known if all the cases were immunohistologically confirmed. In Northern Finland, the age-standardized incidence of BP was 14 per million person-years between the years 1985 and 2009 (Försti, Jokelainen, Timonen, & Tasanen, 2014). A Scottish study between the years 1991 and 2001 yielded the same incidence (14 per million per year) (Gudi et al., 2005) whereas in Switzerland the age-standardized incidence was 6.8 per million per year (Marazza et al., 2009) and in France 21.7 cases per million persons per year (Joly et al., 2012). The lowest incidence in Europe, 2.5 cases per million per year, was reported in Romania (Baican et al., 2010).

Several studies have reported an increase in the incidence of BP over the last few decades, ranging in magnitude from two- to almost five-fold (Försti et al., 2014; Joly et al., 2012; Langan et al., 2008). Since the incidence also seems to increase remarkably after the age of 70–80 years (Brick, Weaver, Lohse et al., 2014; Försti et al., 2014; Gudi et al., 2005; Joly et al., 2012; Langan et al., 2008; Marazza et al., 2009), part of the increase in incidence may be explained by the aging of the population. However, an increase in incidence has also been reported in studies that took into account the aging of the general population (Försti et al., 2014; Langan et al., 2008). In a French study that reported an increase in BP incidence, the demographic characteristics (including mean age and distribution of age categories) of the general population were similar those of a previous study that reported a lower incidence (Joly et al., 2012).
2.5.2 Age at bullous pemphigoid diagnosis and differences between sexes

In recent studies the mean age at BP diagnosis has varied between 69.2 and 82.6 years (Brick et al., 2014a; Cai et al., 2014; Försti, Jokelainen, Timonen, & Tasanen, 2016a; Gual et al., 2014; Joly et al., 2012; Langan et al., 2008; Lee & Kim, 2014; Marazza et al., 2009). In many studies, BP is shown to be slightly more common in women than in men (Bastuji-Garin et al., 2011; Brick et al., 2014a; Cortes et al., 2012; Försti et al., 2016a; Kibsgaard, Bay, Deleuran, & Vestergaard, 2015; Kridin & Bergman, 2017; Langan et al., 2008; Marazza et al., 2009; Sim et al., 2017). BP can occur also in childhood. Pediatric BP is a rare entity that can affect children at any age, but as many as half of pediatric cases occur during the first year of life (Waisbourd-Zinman et al., 2008).

2.5.3 Mortality in bullous pemphigoid

BP is associated with heightened mortality. A recent systematic review and meta-analysis of 25 studies yielded an overall 1-year mortality rate of 23.5% in patients with BP (Kridin, Shihade, & Bergman, 2018). The 1-year mortality varied between 6% and 41% in the included studies (Kridin et al., 2018). Polypharmacy is common among BP patients and it has been associated with higher mortality: the more drugs the BP patients received, the greater the mortality risk (Försti et al., 2016a). Advanced age at BP onset, comorbid diabetes and diagnostic delay have also been reported to be associated with increased 1-year mortality (Lee & Kim, 2014). In a French study of 341 patients, treatment of extensive BP with oral corticosteroids (1 mg per kg) was associated with heightened 1-year mortality (41%) compared to topical corticosteroid clobetasol propionate (24%) (Joly et al., 2002). Extensive disease was defined as > 10 new bullae per day.

2.5.4 The comorbidities of bullous pemphigoid

BP patients tend to be multimorbid: in a recent study 84% of subjects with BP also had at least two other chronic diseases (Sim et al., 2017). Commonly reported comorbidities include hypertension, psoriasis and diabetes (Jeon, Yun, Lee, Won, & Lee, 2018; Kibsgaard et al., 2017; Phan, Goyal, & Murrell, 2018; Sim et al., 2017). Reports of associations between BP and malignancies have yielded
inconsistent results. A recent meta-analysis on this topic included eight studies and did not find BP to be associated with overall cancer morbidity (Atzmony et al., 2017). However, a pooled analysis found that BP was associated with haematological malignancies (Atzmony et al., 2017). A large study of hospitalized in-patients with BP as either primary or secondary diagnosis (N= 2108 and N= 11 234, respectively), found several conditions that had a significant association with BP: the strongest associations were with Cushing syndrome, systemic lupus erythematosus, hidradenitis suppurativa, myocarditis and coeliac disease (Ren et al., 2017). The comorbid diagnoses of BP patients reported by the largest studies are summarized in Table 1.

Table 1. Comorbid conditions of bullous pemphigoid in different studies.

<table>
<thead>
<tr>
<th>First author</th>
<th>Study years</th>
<th>Data origin</th>
<th>Number of BP cases</th>
<th>Comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Parker et al., 2008)</td>
<td>1998-2003</td>
<td>Hospital data</td>
<td>223</td>
<td>Diabetes mellitus 11.7%, dementia 10.3%, hypertension 21.1%, hypothyroidism 4.5%, stroke 7.2%, other neurological disease 9.4%, cancer 7.2%, chronic renal failure 3.6%, chronic lung disease 4.9%</td>
</tr>
<tr>
<td>(Jedlickova et al., 2010)</td>
<td>1991-2006</td>
<td>1 hospital</td>
<td>178</td>
<td>Prostate hyperplasia 50.6%, hypertension 50.6%, neurologic disease 42.7%, ischemic heart disease 56.2%, diabetes mellitus 34.8%, recent malignant disease 14.6%, malignant disease (both recent and historical) 21.3%</td>
</tr>
<tr>
<td>(Bastuji-Garin et al., 2011)</td>
<td>2003-2007</td>
<td>11 hospitals</td>
<td>201</td>
<td>Severe cognitive impairment 42.7%, stroke 25.9%, Parkinson’s disease 14.3%, Unipolar or bipolar disorders 4.0%, Diabetes 6.0%, Psoriasis 4.5%, past fracture or joint prosthesis 21.7%</td>
</tr>
<tr>
<td>(Cortés, Marazza, Naldi, Combescure, &amp; Borradori, 2011)</td>
<td>2001-2002</td>
<td>All Swiss hospitals</td>
<td>115</td>
<td>Diabetes 8.2%, heart diseases 60.8%, liver diseases 7.3%, malignancies 13.7%, neurological diseases 33.3%</td>
</tr>
<tr>
<td>(Chen et al., 2011)</td>
<td>1997-2008</td>
<td>National Health Insurance Research Database</td>
<td>3485</td>
<td>Stroke 36.8%, dementia 17.7%, Parkinson’s disease 11.9%, epilepsy 5.8%, bipolar/unipolar disease 0.8%, schizophrenia 0.6%, paranoid state 0.06%, anxiety 6.1%, SLE 0.06%, systemic sclerosis 0.06%, rheumatoid arthritis 0.6%, Sjögren syndrome 0.06%, lichen planus 0.09%, psoriasis 2.1%, vitiligo 0.06%, alopecia areata 0.09%</td>
</tr>
<tr>
<td>First author</td>
<td>Study years</td>
<td>Data origin</td>
<td>Number of BP cases</td>
<td>Comorbidities</td>
</tr>
<tr>
<td>--------------------</td>
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<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(Li, Zuo, &amp; Zheng, 2013)</td>
<td>1991-2011</td>
<td>hospital data</td>
<td>140</td>
<td>Hypertension 30.7%, chronic lung disease 12.1%, heart disease 17.9%, neurologic disease 17.9%, diabetes 15.0%, chronic renal disease 5.7%, digestive system disease 8.6%, malignant neoplasms 7.1%</td>
</tr>
<tr>
<td>(Li, Zuo, Zheng, &amp; Qiu - Ning, 2013)</td>
<td>1992-2012</td>
<td>1 hospital</td>
<td>190</td>
<td>Chronic lung disease 12%, hypertension 31%, heart disease 16%, neurological disease 16%, diabetes 14%, malignancies 7%, chronic renal disease 5%, digestive system disease 8%</td>
</tr>
<tr>
<td>(Gual et al., 2014)</td>
<td>1990-2010</td>
<td>1 hospital</td>
<td>101</td>
<td>Hypertension 59.8%, dyslipidemia 17.5%, diabetes 22.7%, heart disease 26.6%, neurological disease 44.3%, lung disease 13.4%, kidney disease 9.3%, liver disease 5.2%, malignancies 9.3%</td>
</tr>
<tr>
<td>(Lee &amp; Kim, 2014)</td>
<td>1993-2013</td>
<td>1 hospital</td>
<td>168</td>
<td>Diabetes 34.8%, heart disease 6.7%, hypertension 32.1%, dementia 12.1%, stroke 17.0%, neurological disease 7.9%, cancer 5.5%, chronic renal failure 6.06%, lung disease 7.9%</td>
</tr>
<tr>
<td>(Cai et al., 2014)</td>
<td>2004-2009</td>
<td>1 hospital (national skin center)</td>
<td>359</td>
<td>Ischemic heart disease 22.0%, heart failure 5.6%, hypertension 59.3%, diabetes 32.6%, thyroid disease 4.2%, gastrointestinal disease 11.4%, liver disease 2.8%, chronic lung disease 6.4%, chronic renal disease 11.4%, stroke 40.4%, dementia 23.7%, Parkinson’s disease 10.3%, malignancies 13.4%</td>
</tr>
<tr>
<td>(Kibsgaard et al., 2015)</td>
<td>2006-2013</td>
<td>1 hospital</td>
<td>117</td>
<td>Cardiovascular disease 70%, neurological disorders 37%, lung diseases 18%, diabetes 15%, cancers 14%</td>
</tr>
<tr>
<td>(Försti et al., 2016a)</td>
<td>1985-2012</td>
<td>1 hospital</td>
<td>198</td>
<td>Cardiovascular diseases 76.3%, neurodegenerative diseases 40.9%, other skin conditions 37.4%, diabetes type 2 23.2%, malignant diseases 8.6%, other autoimmune diseases besides BP 3.5%</td>
</tr>
<tr>
<td>(Jeon et al., 2018)</td>
<td>2006-2013</td>
<td>1 hospital</td>
<td>103</td>
<td>Hypertension 48.5%, diabetes 32%, cardiac disease 10.7%, renal disease 10.7%, stroke 21.4%, neurologic disease 11.7%, pulmonary disease 6.8%, malignancy 11.7%, dementia 23.3%</td>
</tr>
</tbody>
</table>
Several studies have established an association between neurological diseases and BP: In many of studies, the neurological diseases preceded BP, typically by many years (Cordel et al., 2007; Försti et al., 2016b; Stinco, Codutti, Scarbolo, Valent, & Patrone, 2005). Parkinson’s disease (Bastuji-Garin et al., 2011; Brick, Weaver, Savica et al., 2014; Chen et al., 2011; Försti et al., 2016b; Kibsgaard et al., 2017; Langan, Groves, & West, 2011; Stinco et al., 2005), dementia (Brick et al., 2014b; Chen et al., 2011; Daneshpazhooh et al., 2017; Försti et al., 2016b; Langan et al., 2011; Taghipour et al., 2010; Teixeira, Cabral, Brites, Vieira, & Figueiredo, 2014), stroke (Chen et al., 2011; Daneshpazhooh et al., 2017; Försti et al., 2016b; Kibsgaard et al., 2017; Langan et al., 2011; Teixeira et al., 2014), epilepsy (Chen et al., 2011; Daneshpazhooh et al., 2017; Försti et al., 2016b; Langan et al., 2011) and multiple sclerosis (Daneshpazhooh et al., 2017; Försti et al., 2016b; Kibsgaard et al., 2017; Langan et al., 2011) are the neurological diseases most often reported to be associated with BP. The strongest association between neurological disease and BP has been shown in multiple sclerosis (Försti et al., 2016b; Kibsgaard et al., 2017);
The risk for neurological diseases like motor neuron diseases, epilepsy and dementias has also been reported to increase following a diagnosis of BP (Försti et al., 2016; Ong, Goldacre, & Taghipour, 2013; Taghipour et al., 2010).

Several psychiatric diseases are also reported to be associated with BP, including schizophrenia (Chen et al., 2011; Försti et al., 2016b), schizotypal and delusional disorders, personality disorders, neurotic, stress-related and somatoform disorders (Försti et al., 2016b) and unipolar and bipolar disorders (Bastuji-Garin et al., 2011; Försti et al., 2016b). The mean interval between the diagnoses of psychiatric diseases and BP varies between seven and 11 years (Chen et al., 2011; Försti et al., 2016b).

Interestingly, the main BP autoantigen BP180 is expressed in the human brain (Seppänen, 2013). The BP230 isoforms BPAg1a and BPAg1a2 are also expressed in the central and peripheral nervous systems (Künzli, Favre, Chofflon, & Borradori, 2016). Knowing the epidemiological association of BP and neurological diseases, it has been hypothesized that neurodegeneration or neuroinflammation may lead to cross-reaction of the immunoresponse to cutaneous and neural antigens (Försti, Huilaja, Schmidt, & Tasanen, 2017). BP180 autoantibodies have been shown to be more often detected in patients with dementia or Alzheimer’s disease than in control individuals without these neurological diseases (Foureur et al., 2006; Kokkonen et al., 2017). However, in an indirect immunofluorescence examination, the sera of patients with Alzheimer’s disease that were positive for BP180 did not bind to the skin BMZ (Kokkonen et al., 2017). When studying patients with Alzheimer’s disease and multiple sclerosis, autoantibodies seem to target other epitopes than those in BP and therefore they are not pathogenic (Tuusa et al., 2018).

### 2.6 Risk factors for bullous pemphigoid

#### 2.6.1 Drugs as risk factors for bullous pemphigoid

To the best of my knowledge, the first case of drug-induced BP was described in 1970 in an 11-year boy receiving salicylasulphapyridine (Bean, Good, & Windhorst, 1970). To date, more than 60 drugs have been reported to induce BP, including certain antibiotics, diuretics and other anti-hypertensive drugs, anti-TNF-α-drugs and vaccines (Stavropoulos, Soura, & Antoniou, 2014). However, few case-controlled studies have investigated the association between drugs and BP.
A French prospective study of 116 patients found an association between neuroleptics (Odds Ratio [OR] 2.0, 95% confidence interval [CI] 1.0–4.1) and aldosterone antagonists (OR 3.1, 95% CI 1.3–7.1) and BP (Bastuji-Garin et al., 1996). After adjustments for sex, age and the number of drug used, only the association between aldosterone antagonists and BP remained statistically significant. The study investigated drugs that patients had used for more than 3 months the year before BP onset and data on drugs were collected by patient questionnaire and interviews (Bastuji-Garin et al., 1996). Another study from the same authors included 201 BP patients and showed a heightened risk of BP with the use spironolactone (OR 1.90, 95% CI 1.06–3.40) and neuroleptics with an aliphatic side chain (OR 3.29, 95% CI 1.29–8.41) (Bastuji-Garin et al., 2011). These associations remained statistically significant in multivariate analysis. Data from the questionnaire included drugs used for more than 3 months within 6 months prior to BP onset (Bastuji-Garin et al., 2011). A case-controlled study of 86 patients reported an association between the use of loop diuretics and BP (Lloyd-Lavery et al., 2013). In contrast, a recent Singaporean case-control study of 105 patients found no associations between drug use and BP risk (Tan et al., 2017). However, DPP-4 inhibitors were not analysed in the study.

Immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) and programmed cell-death ligand 1 (PD-L1) used as cancer therapy have recently been reported to induce BP (Siegel et al., 2018). This retrospective study of 853 patients found BP in 0.8% of patients treated with PD-1 or PD-L1 inhibitors.

**Dipeptidyl peptidase-4 inhibitors**

The enzyme Dipeptidyl peptidase-4 (DPP-4) was first described by the Finnish dermatologist Väinö Hopsu-Havu and his American colleague George G. Glenner in 1966 (Hopsu-Havu & Glenner, 1966). Dipeptidyl peptidase-4 inhibitors (DPP-4i) are a class of drugs that were introduced in the mid-2000s to treat type 2 diabetes (Drucker & Nauck, 2006). DPP-4 is an enzyme that degrades glucagon-like peptide-1, which is an incretin hormone that stimulates insulin secretion, inhibits gastric emptying and reduces appetite. DPP-4 inhibitors are used as monotherapy or in combination with other blood glucose-lowering drugs (Drucker & Nauck, 2006). Sitagliptin, vildagliptin, saxagliptin, linagliptin and alogliptin were approved in this order in European Union including Finland. According to the Finnish Medicines Agency drug consumption statistics (www.fimea.fi) sitagliptin and vildagliptin were the most frequently prescribed DPP-4 inhibitors in Finland in
the year 2013: consumption in defined daily doses/1000 inhabitants/day were 1.23 for sitagliptin and 0.13 for vildagliptin.

In 2011, Skandalis et al reported five patients who developed BP after using a DPP-4i and metformin for 2–13 months (Skandalis, Spirova, Gaitanis, Tsartsarakis, & Bassukas, 2012). Since then, numerous reports and studies of DPP-4i-induced BP have been published originating from different countries and concerning all the available DPP-4i drugs (Aouidad et al., 2013; Attaway, Mersfelder, Vaishnav, & Baker, 2014; Bene et al., 2015; Benzaquen et al., 2018; Esposito, Moretta, Peris, & De Simone, 2017; Fania et al., 2017; García, Aranburu, Palacios-Zabalza, Lertxundi, & Aguirre, 2016; Haber, Fayad, Stephan, Obeid, & Tomb, 2016; Harada et al., 2017; Mendonça, Martin-Gutierrez, Rios-Martin, & Camacho-Martínez, 2016; Oya et al., 2017; Pasmatzis, Monastirli, Habeos, Georgiou, & Tsamboas, 2011; Sakai et al., 2017; Schaffer et al., 2017; Skandalis et al., 2012; Yoshiji et al., 2017). In 2016, reports from European and French pharmacovigilance databases showed a disproportionately high incidence of BP in patients treated with DPP-4is, with vildagliptin particularly singled out by both studies (Bene et al., 2016; García et al., 2016).

A Swiss study of 23 diabetic BP patients and 170 diabetic controls found the use of DPP-4is to be more frequent among diabetic BP patient than controls (39.1% and 33.5% respectively) although the difference was not statistically significant (Schaffer et al., 2017). A case-controlled study of 61 BP patients with diabetes and 122 diabetic controls recruited from an endocrinology department found that the use of DPP-4 inhibitors increased the risk of BP almost threefold, use of other DPP-4 inhibitors than vildagliptin did not increase the risk (Benzaquen et al., 2018).

### 2.6.2 Other risk factors

Several physical factors have been reported to trigger BP. Exposure to ultraviolet rays or other forms of radiation, surgical wounds and ostomies, burns, photodynamic therapy and several different types of mechanical trauma have all been reported to have triggered BP (Dănescu, Chiorean, Macovei, Sitaru, & Baican, 2016). In a review of published cases, the delay between exposure to the risk factor and the onset of BP varied widely from days to years but most often the triggering factor preceded BP by less than one month (Dănescu et al., 2016).

In infant BP, many of the case reports concern BP that has appeared 1 to 21 days after vaccination, most frequently after tetanus, polio, diphtheria and pertussis vaccination (Barreau et al., 2012; Brad, Deng, Flynn, & Suarez, 1998; de la Fuente

2.7 Other pemphigoid diseases

Diseases of the pemphigoid group share clinical features and some also have the same target autoantigens. The other pemphigoid diseases are therefore briefly reviewed below.

2.7.1 Pemphigoid gestationis

Pemphigoid gestationis (PG) is a rare pruritic disease of pregnancy. Its incidence varies between 1 in 50,000 and 1 in 60,000 pregnancies (Lipozenčić, Ljubojevic, & Bukvić-Mokos, 2012). The onset of PG is typically during the second and third trimester of pregnancy, but it can also appear during early pregnancy and during the puerperium (Huilaja, Mäkikallio, & Tasanen, 2014; Lipozenčić et al., 2012). The symptoms of PG most often begin at the abdomen. Its itch is intense and skin symptoms polymorphic: urticarial, papular and annular lesions appear first and blistering usually follows in a few weeks (Huilaja et al., 2014; Lipozenčić et al., 2012). The main autoantigen in PG is known to be BP180 and the diagnosis is made by clinical appearance, DIF and serological studies (Huilaja et al., 2014; Lipozenčić et al., 2012; Schmidt & Zillikens, 2013). PG is mainly treated with topical and oral corticosteroids combined with antihistamines (Huilaja et al., 2014; Lipozenčić et al., 2012; Schmidt & Zillikens, 2013). PG is associated with fetal complications (miscarriage, fetal growth restriction and preterm labor), and its presence should be taken into account in a follow-up of PG pregnancy (Huilaja et al., 2013).

2.7.2 Linear IgA disease

Linear IgA disease (LAD) presents with blisters forming annular shapes, also known as the string-of-pearls sign, urticarial lesions and mucosal involvement are also often seen (Schmidt & Zillikens, 2013). LAD is the most common pemphigoid disease in children but also affects adults, most often those aged around 60 years (Gottlieb et al., 2017; Lings & Bygum, 2015; Schmidt & Zillikens, 2013). A 97 kDA extracellular part of BP180 is the major target antigen in linear IgA disease, and only a minority of patients’ sera react with NC16A domain of BP180 (Schmidt & Zillikens, 2013). Diagnosis of LAD is based on linear IgA deposits at the BMZ,
seen in DIF (Guide & Marinkovich, 2001; Schmidt & Zillikens, 2013). LAD can be drug-induced, vancomycin being the most common reported cause. Other antibiotics, lithium, cyclosporine, captopril, phenytoin, diclofenac, amiodarone, somatostatin, cefamandole, interleukin-2 and interferon-γ have also been implicated in case reports (Guide & Marinkovich, 2001). Drug-induced LAD usually resolves after the culprit drug has been discontinued whereas disease without drug-induced etiology is most often treated with dapsone (Guide & Marinkovich, 2001; Schmidt & Zillikens, 2013).

### 2.7.3 Mucous membrane pemphigoid

Mucous membrane pemphigoid (MMP) is a chronic, autoimmune disease that affects the mucous membranes, but skin involvement can also be seen. Scarring of mucosae is typical and it can be even life-threatening when it affects and obstructs the airways (Amber et al., 2018a; Schmidt & Zillikens, 2013). The oral mucosa are most often affected, but the ocular, nasopharyngeal, nasal, anogenital, laryngeal and oesophageal mucosa can be affected as well. Several target antigens in the BMZ have been established: BP230, BP180, laminin 332, laminin 311, type VII collagen and α6β4 integrin. Diagnosis requires a typical clinical picture and characteristic DIF findings of the perilesional mucosa and/or skin showing linear deposits of IgG and/or IgA and/or C3 in the BMZ. Other autoimmune blistering diseases can have shared findings in DIF, therefore the clinical appearance of the disease is important in differential diagnosis. In IIF of salt-split skin, the antibodies against laminin 332 and collagen VII bind along the floor of the artificial split and antibodies against BP180, BP230 and α6β4 integrin bind to the roof (Amber et al., 2018a; Schmidt & Zillikens, 2013).

Severe and rapidly progressive MMP is usually treated with prednisolone and cyclophosphamide, whereas in mild disease, topical corticosteroids, in combination with dapsone or tetracycline might be sufficient; then if response is not achieved, oral prednisolone, azathioprine or mycophenolic acid can be added to the therapy (Chan et al., 2002; Schmidt & Zillikens, 2013; Xu, Werth, Parisi, & Sollecito, 2013). Since laminin-332 MMP is associated with a significant cancer morbidity, patients with laminin 332 autoantibodies should be carefully screened for potential underlying malignancy (Egan et al., 2001; Schmidt & Zillikens, 2013).

Recently, the role of DPP-4is in MMP onset was evaluated in a cohort of 313 MMP patients of whom 64 (21%) were diabetics (Gaudin et al., 2018). In 17 of these, a DPP-4i had been used before the onset of MMP, with vildagliptin being the
most common. Among those patients of whose DPP-4i therapy was discontinued upon MMP diagnosis, complete remission during first year of follow-up was more frequent than among MMP control patients without preceding DPP-4 inhibitor therapy. The authors concluded that DPP-4 inhibitors may be responsible for some MMP cases (Gaudin et al., 2018).

2.7.4 Anti-laminin γ1/p200 pemphigoid

In anti-laminin γ1/p200 pemphigoid, autoantibodies bind to a 200kDa protein, laminin γ1 at the BMZ (Dainichi et al., 2009). Its clinical manifestation often includes erythema and tense bullae or blisters, making it difficult to distinguish from BP. Mucosal involvement can also be seen (Amber et al., 2018a; Commin et al., 2016; Schmidt & Zillikens, 2013). Psoriasis is reported to be associated with anti-laminin γ1/p200 pemphigoid (Ohata et al., 2015). As in other autoimmune bullous diseases, DIF is important in diagnosis of anti-laminin γ1/p200 pemphigoid and it shows linear deposits of IgG and/or C3 at the BMZ as in some other pemphigoid diseases (Amber et al., 2018a). Therefore, a diagnosis of anti-laminin γ1/p200 pemphigoid can be made by the detection of p200 autoantibodies by immunoblotting of dermal extracts or by IIF of salt-split-skin where autoantibody staining is seen at the floor of the artificial split. Treatment options and practices are the same as in BP (Schmidt & Zillikens, 2013).

2.7.5 Epidermolysis bullosa acquisita

In epidermolysis bullosa acquisita (EBA), autoantibodies are produced against type VII collagen (Amber et al., 2018a; Kasperkiewicz et al., 2016). The disease presents as fragility of the skin to mechanical trauma and blister formation, which can leave significant scars and hyperpigmentation. Another, inflammatory form of the disease often has the same clinical appearance as other autoimmune bullous diseases like BP, linear IgA dermatosis and MMP (Amber et al., 2018a; Kasperkiewicz et al., 2016). Since DIF in EBA shows linear deposits of IgG, C3 and IgG in BMZ, EBA can be distinguished from other pemphigoid diseases by the u-serrated pattern seen in DIF, which appears as an n-serrated pattern in other pemphigoid diseases (Amber et al., 2018a). In IIF of salt-split-skin, autoantibodies label the floor of the artificial split. Systemic corticosteroid and other immunosuppressive agents are used in the treatment of EBA (Amber et al., 2018a; Schmidt & Zillikens, 2013).
2.8 Other subepidermal autoimmune bullous diseases

2.8.1 Dermatitis herpetiformis

Dermatitis herpetiformis (DH) is an autoimmune disease in which celiac disease (CD) is manifested as a pruritic papulovesicular dermatosis (Collin, Salmi, Hervonen, Kaukinen, & Reunala, 2017). The incidence of DH varies between 0.4 and 3.5 per 100,000 per year in different populations; the incidence in Finland is among the highest (Salmi, Hervonen, Kautiainen, Collin, & Reunala, 2011). The incidence of DH is reported to have decreased in Finland and in the UK, while the incidence of CD has increased (Salmi et al., 2011; West, Fleming, Tata, Card, & Crooks, 2014). The mean age at the diagnosis varies between 33 and 53 years in Europe according to different studies (Salmi et al., 2011). DH is associated with autoimmune disorders: type I diabetes and thyroid diseases are more common in patients with DH. Alopecia areata, vitiligo, rheumatoid arthritis, Sjögren syndrome and lupus erythematosus are also more common in the DH population (Bolotin & Petronic-Rosic, 2011a). Furthermore, an elevated risk of developing lymphomas has been reported in patients with DH who do not adhere to a gluten-free diet (Hervonen, Vornanen, Kautiainen, Collin, & Reunala, 2005; Lewis et al., 1996).

The autoantigen in DH is epidermal transglutaminase 3 (TG3), while tissue transglutaminase 2 is the autoantigen in CD (Bolotin & Petronic-Rosic, 2011a; Collin et al., 2017; Sárdy, Kárpáti, Merkl, Paulsson, & Smyth, 2002). Rash in DH presents as papules and vesicles, but as DH is usually extremely pruritic, only secondary signs of scratching, excoriations, scars and erosions, may be seen (Fig. 4a and b). Skin symptoms and findings are typically located symmetrically on the buttocks, knees and elbows and may also be seen on the scalp (Bolotin & Petronic-Rosic, 2011a). Diagnosis is based on combination of clinical presentation, histopathological examination, DIF microscopy and serologic testing (Bolotin & Petronic-Rosic, 2011b; Caproni, Antiga, Melani, Fabbri, & Italian Group for Cutaneous Immunopathology, 2009). DIF of unaffected skin close to an active lesion is pivotal in diagnosis and shows granular deposits of IgA in the dermal papillae or along the BMZ (Bolotin & Petronic-Rosic, 2011b; Caproni et al., 2009; Reunala, Salmi, & Hervonen, 2015). Histopathological examination shows subepidermal blistering and the presence of neutrophils and some eosinophils at the dermal papillae. Serological testing involves ELISA for IgA-class anti-tissue transglutaminase antibodies and IIF for IgA antibodies to endomysium tissue. However, DH cannot be ruled out through negative serology findings alone.
Most DH patients show findings of CD in bowel biopsies, even though the symptoms of CD may be only mild or absent (Bolotin & Petronic-Rosic, 2011a).

A lifelong gluten-free diet is the basis of the treatment of DH. However, while dapsone relieves the rash within a few days, it can take months for the gluten-free diet to alleviate the skin symptoms (Bolotin & Petronic-Rosic, 2011b; Reunala et al., 2015). Therefore, dapsone is often used in the beginning of treatment or when a gluten-free diet alone is not sufficient to keep patient symptom free. (Bolotin & Petronic-Rosic, 2011b; Reunala et al., 2015).

Fig. 4. a and b. Erythema and excoriations in dermatitis herpetiformis (Department of Dermatology, Oulu University Hospital).
3 Objectives of the study

The incidence of BP has increased in several countries but the reasons for this tendency are only partially understood. Recently drugs, especially those used to treat diabetes, have been of particular interest, but large population-based studies of drugs as risk factors for BP are scarce. Although case reports of the association of BP and DH have been published, no epidemiological studies have been conducted so far. These facts lent inspiration to the present thesis.

The objectives of this study were to investigate drugs, DH and CD as risk factors for BP utilizing data from national Finnish registries. The studies were conducted in a matched case-control study setting. The specific objectives of the studies I-IV were:

I  To investigate if DPP-4is and metformin are associated with BP and which of these drugs are most strongly associated with BP.
II To determine whether other conventional diabetes drugs are associated with an increased risk for BP.
III To investigate whether drugs used to treat neurological and psychiatric conditions are associated with the risk for BP.
IV To study the association between preceding DH and CD and BP.
4 Materials and methods

4.1 Populations and databases

Study populations and diagnosis codes (I, II, III, IV)

This was a retrospective database study of patients diagnosed with BP in Finland between 1st January 1987 and 31st December 2013. Patients in the BP group were selected by their BP diagnoses, defined by the International Classification of Diseases (ICD)-9 codes 6945A and 6945B, and ICD-10 code L12.0. The control population consisted of patients diagnosed with basal cell carcinoma (BCC) as defined by the ICD-9 codes 1730A-1739A and ICD-10 codes C44.01, C44.11, C44.21, C44.31, C44.41, C44.51, C44.61, C44.71, C44.81 and C44.91 over the same time period as that described above. ICD-9 codes were used in Finland from 1987 to 1995 and ICD-10 codes have been used since 1996. BCC was selected as a control population because, like BP, it affects elderly people, but it is not an inflammatory skin disease (Diepgen & Mahler, 2002; Wong, Strange, & Lear, 2003). BCC control patients were randomly matched to BP cases by sex, age and year of the diagnosis (within a margin of two years) in a 4:1 ratio.

In publication IV diagnoses of DH and CD in the study populations were identified by searching the ICD-9 and ICD-10 codes 6940A and L13.0 for DH and 5790A and K90.0 for CD.

For both BP and BCC, the first diagnosis that was found in the database was used. The same principle was applied for all other diagnoses received by our selected populations, including for comorbidities. Patients aged under 40 years were excluded from the study.

Databases used (I, II, III, IV)

All the following data were obtained from the database of the Care Register for Health Care (CRHC): Details of diagnosis, date of diagnosis, age, sex, hospital admissions and treatment periods. The register was formerly called the Hospital Discharge Register. It contains data on the diagnosis codes of hospitalized inpatients from the year 1969 onwards and on outpatient visits to hospitals from 1998 onwards. The CRHC includes basic information e.g. sex, date of birth, admission and discharge days, diagnoses of the patient’s diseases, area of residence
and the hospital the patient was treated in. In addition to that, other variables concerning e.g. surgical procedures, traumas have been added to the register later (Sund, 2012). The CRHC does not include detailed clinical information like data on histological or blood test samples or clinical presentation or information on socioeconomic status. It also does not include data collected in the primary health care setting.

Data on drugs used by patients and controls were obtained from the Social Insurance Institution of Finland. The registry includes data on all drugs reimbursed from the year 1995 onwards. In order to ensure the availability of complete medication data for the two years preceding the first BP diagnosis, only patients diagnosed between the years 1997 and 2013 were included in the studies. Information on the reimbursed drugs including their dates of purchase was obtained from the registry. Drugs were classified according to the Anatomical Therapeutic Chemical (ATC) classification system and data were collected using ATC codes. All reimbursed drugs purchased during the two years prior to the diagnosis of BP were included.

Diabetes drugs (I, II) were first classified by pharmacological subgroups, every drug in each subgroup was then examined individually. Since several new drugs were approved in Finland during the study period, data were collected for each drug from the date of its first entry to the Finnish market. Where data were examined in pharmacological subgroups, the observation period started from the date of the approval of the first available drug in the subgroup. The dates of approval were obtained from the website of the Finnish Medicines Agency Fimea (www.fimea.fi). The mean age of patients treated with or without DPP-4is was calculated starting from 2007 when the first DPP-4i was approved in Finland. Drugs used for neurological and psychiatric diseases were also examined in pharmacological subgroups and then individually (III). Drugs in pharmacological main group N (Nervous system) were analyzed, excluding subgroups N01 and N02 (anesthetics, analgesics).

The unique personal identification number given to every Finnish citizen was used to combine the data from the two databases, this was done by the Social Insurance Institution of Finland. After that, data were analyzed anonymously and without any identification details.
4.2 Statistical analyses (I, II, III, IV)

Statistical methods (I, II, III, IV)

All statistical analyses were performed using the SAS software package. Analyses were performed in a matched case-controlled manner. Possible associations between the examined drugs and diagnoses and BP were evaluated using a conditional logistic regression model and presented with ORs and 95% CIs. A P-value less than 0.05 was considered significant. Following the finding in study I of an association between DPP-4i use and BP, a sensitivity analysis was performed to confirm the association.

Adjustments (I, II, III, IV)

In studies I and II, ORs were adjusted for diabetes, Alzheimer’s disease, vascular
dementia, other/unspecified dementia, Parkinson’s disease, multiple sclerosis,
subarachnoid hemorrhage, intracerebral hemorrhage, cerebral infarction and
epilepsy. In study IV, in addition to the neurological conditions mentioned above,
psychiatric conditions (schizotypal and delusional disorder, schizophrenia, bipolar
affective disorder, major depressive disorder, neurotic, stress-related and
somatoform disorders and personality disorder) were also adjusted for in the
analysis. In study III, adjustments were made for Alzheimer’s disease, vascular
dementia, other/unspecified dementia, Parkinson’s disease, multiple sclerosis,
subarachnoid hemorrhage, intracerebral hemorrhage, cerebral infarction, epilepsy,
schizotypal and delusional disorder, schizophrenia, bipolar affective disorder,
major depressive disorder, neurotic, stress-related and somatoform disorders,
personality disorders, delirium due to known physiological condition, other mental
disorders due to known physiological condition, personality and behavioral
disorders due to known physiological condition, unspecified mental disorder due
to known physiological condition.

4.3 Ethical issues and study permissions (I, II, III, IV)

Being a register-based study, patients were not contacted at any point and no
interventions or examinations were made. Therefore, a statement was not required
from the Ethics committee, but permission for the study was obtained from the
Oulu University Hospital district. Permission to use data from the CRHC and the
Social Insurance Institution of Finland registries was obtained from the respective register administrators.
5 Results

5.1 Subject characteristics (I, II, III, IV)

The CRHC database search returned records for 4524 patients with BP who were treated in Finnish hospitals between the years 1987 and 2013. The present studies included a subgroup of 3397 BP cases diagnosed between the years 1997 and 2013. A total of 66138 cases of BCC were identified. Of these 12941 were randomly selected to comprise the control group and were matched by age, sex and year of diagnosis to the BP group in a 1:4 ratio. Because matching was done prior to obtaining drug reimbursement data, 579 BP patients had fewer than four controls because of missing drug data in some control subjects. The characteristics of the cases and controls are shown in table 2.

Table 2. Baseline characteristics of the study populations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BP cases n = 3397</th>
<th>Controls n = 12941(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (range)</td>
<td>76.6 (40–102)</td>
<td>76.7 (40–101)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>2028 (59.7)</td>
<td>7766 (60.0)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>1369 (40.3)</td>
<td>5175 (40.0)</td>
</tr>
</tbody>
</table>

\(^1\) Age, sex and year of the diagnosis matched in 1:4 ratio. Due to availability of drug reimbursement data, 579 patients had fewer than four basal cell carcinoma controls.

5.2 Diabetes among study populations (I, II)

In the whole study population of 4524 BP patients and 66138 controls, 42 (0.93%) of BP patients and 329 (0.50%) of control individuals with BCC had type I diabetes mellitus (DM I). Type II diabetes mellitus (DM II) was also more common among BP patients (19.6%) than BCC patients (12.3%). In the BP population, 0.35% of individuals were recorded as having DM of unspecified type, as were 0.18% of the BCC population.

5.3 DPP-4 inhibitor use and risk of bullous pemphigoid (I)

The DPP-4i vildagliptin was associated with tenfold increased risk for BP compared with control individuals (adjusted OR 10.4, 95% CI 4.56–23.80) but sitagliptin was not associated with any such increase in risk (adjusted OR 1.37, 95% CI 0.93–2.01). Treatment with regimens combining metformin and either
sitagliptin or vildagliptin increased the risk of BP (OR 2.40 and 6.71, respectively). The mean latency from the first purchase of vildagliptin to BP diagnosis was 449 days (range 36–729 days). There was no significant difference in age at BP diagnosis depending on DPP-4i use: the mean age at the time of diagnosis was 77.7 years among BP patients who had received DPP-4i treatment and 76.7 years in those who had not.

After adjusting for diabetes and several neurological diseases, the risk of having BP diagnosis after DPP-4i monotherapy within the preceding two years was heightened threefold in women (adjusted OR 3.26, 95% CI 1.97–5.39), but no such increased risk was seen in men (adjusted OR 1.47, 95% CI 0.88–2.47). Additionally, the risk for developing BP after vildagliptin use was 13-fold greater in women compared with controls (adjusted OR 13.0, 95% CI 3.85–43.60) and almost ninefold greater in men (adjusted OR 8.85, 95% CI 2.80–28.00), however the difference between sexes was not statistically significant (P=0.54).

5.4 Other diabetes drugs and the risk of bullous pemphigoid (I, II)

Of the study populations diagnosed between 1997 and 2013, 582 (17.1%) of BP patients and 1555 (12.0%) of controls had received at least one diabetes drug other than insulin during the two years preceding diagnosis of either BP or BCC. When studying diabetes drugs other than insulins and DPP-4is, no heightened risk for BP was found (after adjustment for either diabetes or diabetes and certain neurological diseases) concerning sulfonylureas, thiazolidinediones, combinations of metformin and rosiglitazone or pioglitazone, guar gum or repaglinide. Metformin monotherapy was not associated with an elevated risk for BP (adjusted OR 1.05, 95% CI 0.88–1.24). Differences between genders were not found.

5.5 Use of drugs for psychiatric and neurologic diseases and the risk of bullous pemphigoid (III)

After adjustment for several neurological and psychiatric conditions the following drugs affecting the nervous system were found to be associated with an elevated risk for BP: carbamazepine, pregabalin, biperiden, levodopa and decarboxylase inhibitor, levomepromazine, perphenazine, periciazine, haloperidol, melperone, quetiapine, sulpiride, risperidone, hydroxyzine, diazepam, chlordiazepoxide, oxazepam, lorazepam, nitrazepam, temazepam, zopiclone, amitriptyline, doxepin, citalopram, sertraline, escitalopram, mianserin, mirtazapine, venlafaxine,
duloxetine, donepezil, rivastigmine, galantamine, carbachol and memantine. The latency between first purchase of each of these drugs and the first BP diagnosis was over one year except for hydroxyzine (263 days).

5.6 Dermatitis herpetiformis and celiac disease and the risk for bullous pemphigoid (IV)

In the study population of 3397 BP cases, 41 (1.2%) individuals were identified who also had a diagnosis of DH, whereas in the BCC group of 12947 patients there were only 7 (0.1%) individuals with DH. Preceding CD was found in 34 (1.0%) BP cases and 53 (0.4%) controls. Twelve (29%) of the BP patients with DH also had CD. The risk of BP after DH diagnosis was 22-fold greater than the risk of BCC after DH diagnosis (OR 22.30, 95% CI 9.99–49.70). Preceding CD doubled the risk of BP (OR 2.54, 95% CI 1.64–3.92). This elevation remained unchanged after adjustment for neurological disorders. The differences between genders were not statistically significant but there was a trend towards heightened risk among males.

Dermatitis herpetiformis was diagnosed 3.3 years (mean) before BP and 10.0 years (mean) before BCC. Celiac disease preceded BP 4.9 years and BCC 7.2 years. Mean age at DH diagnosis in the BP group was 64.9 years (median 64.0, first quartile 54.0, third quartile 78.0) and 65.6 in the BCC group (median 73.0, first quartile 56.0, third quartile 76.5). Mean age was 76.7 among BP patients with no preceding DH and 68.8 among the BP patients with preceding DH.

5.7 The use of dapsone and DPP-4 inhibitors in study populations (IV)

Eighteen of the 41 BP patients who had preceding DH (43.9%) had purchased dapsone in the two years prior to their BP diagnosis, and 14 (34%) had purchased dapsone in the 6 months before the diagnosis. Only one BP patient with preceding CD had a DPP-4i during the two years before their diagnosis of BP. No subject with DH in either the BP or control population received any DPP-4i.
6 Discussion

6.1 DPP-4 inhibitors increase the risk of bullous pemphigoid (I)

This study was one of the first to report the association between vildagliptin and BP in a case-controlled setting. The findings of the present study are in line with those of a French study of 61 diabetic BP patients, which also found an increased risk for BP after the use of vildagliptin, but not other DPP-4 inhibitors (Benzaquen et al., 2018). Another study of 82 BP patients found an increased risk for BP with linagliptin use, although that study also found the association with vildagliptin to be the strongest of all the DPP-4is (Kridin & Bergman, 2018). A French study also found that DPP4i intake was higher in patients with BP than in general population (Plaquevent et al., 2018). In a EudraVigilance-pharmacovigilance database study, the proportions of BP patients experiencing adverse events with DPP-4is varied between vildagliptin, linagliptin, saxagliptin and sitagliptin, but again with vildagliptin being the most often reported (García et al., 2016). In a Japanese pharmacovigilance database study, a similar disproportionality was noted, particularly with vildagliptin, linagliptin and tenegliptin (Arai, Shirakawa, Konishi, Sagawa, & Terauchi, 2018).

DPP-4i-related BP has been suggested to represent a non-inflammatory type of BP, in that such patients have smaller blisters, milder erythema and limited distribution of skin lesions compared with the clinical picture of BP in patients who have not received a DPP-4i (Izumi et al., 2016; Ujiie et al., 2017). However, since clinically typical BP has also been described in patients who have used DPP-4i prior to disease onset, (Chijiwa et al., 2018; Fania et al., 2017; Kridin & Bergman, 2018; Skandalis et al., 2012) it remains unclear whether or not the clinical presentation of DPP-4i-induced BP actually has some specific features. Kridin et al did not find atypical clinical presentation to be more common among DPP-4i-associated BP than among patients who had not used DPP-4is (Kridin & Bergman, 2018). Mucosal involvement, however, was more common among DPP-4i-associated BP than non-DPP-4i-associated BP (22.2% vs 6.5%, respectively) (Kridin & Bergman, 2018). A Japanese study of 9 DPP-4i-associated BP also found mucosal involvement, assessed using the BPDAI, to be more extensive in DPP-4i-associated BP (Chijiwa et al., 2018). No such differences were seen in other domains of the BPDAI. Other case-controlled studies found the association between DPP-4i and BP to be stronger in males than in females (Benzaquen et al.,
a result that contrasts with our study, which found the association to be stronger among females. In the present study, the use of DPP-4is did not correlate with patient age at the diagnosis. Corresponding results were found also in Japanese study in which the DPP-4 inhibitor use did not have significant effect on the age at the BP diagnosis (Kawaguchi et al., 2018). Benzaquen et al reported the association between DPP-4is and BP to be stronger among patients aged over 80 years than in those aged under 80 years (Benzaquen et al., 2018) whereas in Israeli study, the association was strongest in patients younger than 70 years (Kridin & Bergman, 2018).

In the present study, at 449 days the mean time interval between the first purchase of vildagliptin and diagnosis of BP was rather long. An analysis of the European pharmacovigilance database showed the mean latency from DPP-4i initiation to onset of BP varying between 6 and 19 months (García et al., 2016). In other case-controlled studies, the median time between DPP-4i initiation and BP onset was 8.2 months and 10.4 months (Benzaquen et al., 2018; Kridin & Bergman, 2018). In a French Pharmacovigilance database study, the median time from the initiation of DPP-4i therapy to the onset of BP was 10 months (Bene et al., 2016). The long latency between the initiation of DPP-4i treatment and BP onset suggests that DPP-4i-associated BP is a drug-aggravated disease rather than purely an adverse drug reaction.

There are only few studies of the clinical course of DPP-4i-associated BP. Benzaquen et al reported that 95% of patients whose DPP-4i therapy was discontinued achieved partial or complete remission during the follow-up period of 3-30 months, while only 55% of those who continued DPP-4i therapy achieved remission (Benzaquen et al., 2018). In a case-series of eight DPP-4i-associated BP patients, when DPP-4i treatment was discontinued in all patients and topical corticosteroids or other BP treatments were introduced, the median time to clinical remission was 3 months (range 0.5–10 months) (García-Díez et al., 2018). In contrast, in a report of eight cases of DPP-4i-associated BP, all continued taking DPP-4is after diagnosis of BP and were reported to be in stable condition (which was not defined precisely), however, three still needed systemic corticosteroid therapy (Kawaguchi et al., 2018). A recent study of 108 BP patients compared outcomes in BP patients whose DPP4i medication was discontinued (45.3%) with those whose DPP4i medication was continued (54.7%). No differences were found in the median time to achieve disease control, the time to first relapse, the relapse rate, or the mean initial dose of clobetasol propionate cream used for treatment (Plaquevent et al., 2018). Overall, evidence for the effect of DPP-4i discontinuation
on the disease course remains somewhat contradictory and this issue needs to be studied further.

In BP, IgG autoantibodies mainly target the NC16A domain of BP180 (Amber et al., 2018a; Schmidt & Zillikens, 2010). Cases of DDP-4i-associated BP autoantibodies targeting the NC16A domain have been described (Aouidad et al., 2013; Esposito et al., 2017; Fania et al., 2017; Haber et al., 2016; Yoshiji et al., 2017) but it has been also suggested that autoantibodies in DPP-4i-associated BP preferentially target parts of BP180 other than the NC16A domain (Izumi et al., 2016; Kawaguchi et al., 2018; Mai et al., 2018; Sakai et al., 2017). In a Japanese cohort of 168 BP patients, anti-NC16A antibodies were less often detected in BP patients who had received DPP-4i therapy than in those who had not (65.6% vs 82.3% respectively) (Kawaguchi et al., 2018). Mean antibody titers were also lower in the DPP-4i-treated group. When studying the human leukocyte antigen (HLA) profiles of Japanese patients with DPP-4i-associated BP, a non-inflammatory phenotype was found to be associated with HLA-DQB1*03:01 allele (Ujiie et al., 2017).

Dipeptidyl peptidase 4 is a cell surface aminopeptidase that is expressed in many tissues such as the liver, lung, kidney and intestines as well as in endothelial cells and lymphocytes (Drucker & Nauck, 2006). Additionally, CD26/DPP4 is a surface T cell activation antigen (Klemann, Wagner, Stephan, & von Hörsten, 2016). The expression of CD26/DPP-4 is upregulated in T-cell lymphomas, psoriasis, lichen planus and atopic dermatitis (Novelli et al., 1996; Van Lingen et al., 2008).

In our recent study, the expression of CD26/DPP-4 was significantly elevated in the skin lesions of BP patients compared to healthy controls, but no difference in CD26/DPP-4 expression was noted between DPP-4i-treated BP patients and patients with no preceding DPP-4i therapy (Lindgren et al, unpublished data). Certain specific histological features have, however, been reported in patients with DPP-4 inhibitor associated BP: Chijiwa et al showed that levels of infiltrating eosinophils in the skin biopsies were lower in DPP-4i-associated BP patients than in non-DPP-4i-associated BP, but no significant difference was found in circulating eosinophil counts (Chijiwa et al., 2018). Less infiltrating eosinophils in skin samples were also noted among BP patients with autoantibodies against other parts of BP180 than NC16A domain compared to patients with autoantibodies against NC16A domain in Japanese study (Izumi et al., 2016). Half of these patients (n=7) with autoantibodies against other parts of BP180 than NC16A domain had received DPP-4is prior to the BP diagnosis. Kridin et al reported higher circulating eosinophil count in BP patients who had not been exposed to a DPP-4i than in those
who had (Kridin & Bergman, 2018). On the other hand, in an animal model, the inhibition of CD26/DPP-4 was demonstrated to lead to increased mobilization of eosinophils in the blood (Forssmann et al., 2008).

Vildagliptin and sitagliptin have also been reported to induce polyarthritis (Crickx et al., 2014; Saito et al., 2013). Furthermore, a case-controlled study of 283 diabetic patients reported that psoriasis, Crohn’s disease, and Hashimoto thyroiditis were more frequent in patients treated with DPP-4is than in those who received no DPP-4i treatment (Kridin et al., 2018). In contrast, DPP-4is have also been suggested to decrease the risk of autoimmune diseases: in a cohort study of a U.S. insurance database the use of linagliptin, saxagliptin or sitagliptin slightly reduced the risk of rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, psoriasis and inflammatory bowel disease (Kim et al., 2015). Notably, the risk of autoimmune blistering skin diseases was not analyzed in this study. To summarize, currently only limited data are available concerning the association of DPP-4is with other autoimmune diseases than BP.

6.2 Diabetes drugs other than DPP-4 inhibitors are not associated with an increased risk for bullous pemphigoid (II)

Since the association between DPP-4is and BP has been shown in the present study (I) as well as previous studies (Benzaquen et al., 2018; Kridin & Bergman, 2018) and DM is a common comorbidity of BP (II), this study (II) further investigated whether the other conventional diabetes medications may also carry a heightened risk for BP. The present study (II) did not find association between the conventional diabetes drugs studied and BP. Since DPP-4is are often used in combination with metformin, causative role of metformin could not initially be ruled out in early reports of DPP-4i-associated BP (Skandalis et al., 2012). Later, as it became clear that the onset of BP symptoms was related to the addition of DPP-4is to previous long-standing metformin therapy (Esposito et al., 2017; Skandalis et al., 2012) and that skin symptoms improve after cessation of DPP-4i while the metformin therapy continues (Bene et al., 2015; Mendonça et al., 2016), it seemed likely that metformin was not responsible for causing BP. Supporting this view, to best of my knowledge, there are no reported BP cases due to metformin in English literature. An Israeli study also found that metformin was not associated with an increased risk for BP (Kridin & Bergman, 2018). Insulin, sulfonylureas, metformin and acarbose were studied as a part of a case-controlled study of 105 patients with BP, and no association was found between treatment with any of these drug classes and
BP (Tan et al., 2017). Similarly, no such association was found by other case-controlled studies (Benzaquen et al., 2018; Lloyd-Lavery, Chi, Wojnarowska, & Taghipour, 2013). A Japanese study of a database of drug adverse events, also found no increased risk of BP associated with any class of antihyperglycemia drug other than DPP-4is (Arai et al., 2018).

6.3 Drugs affecting nervous system may contribute to elevated bullous pemphigoid risk (III)

Knowing the association between neurological and psychiatric diseases and BP ( Försti et al., 2017), the association of certain drugs affecting nervous system with BP was studied (III). When analyzing drugs in the ATC classification main group N, nervous system, 34 drugs were associated with an increased risk of BP. Interestingly, the butyrophenone derivates and the anticholinesterases used for dementia were the only subgroups in which all the studied drugs were associated with increased BP risk. In other groups, for example the benzodiazepines, some of the drugs were associated with an increased risk and some were not. Hydroxyzine seemed to be associated with a remarkable increase in the risk of BP, but this probably reflects the use of hydroxyzine to treat pruritus that actually is a pre-diagnosis BP symptom, rather than hydroxyzine truly predisposing patients to BP. When analyzing the of the drugs that were found to be associated with BP risk, no similarities between their pharmacological properties and chemical structures were found that might explain the elevated risk.

Neuroleptics, especially those with an aliphatic side chain, have previously been reported to increase the risk of BP (Bastuji-Garin et al., 1996; Bastuji-Garin et al., 2011). However, since later case-controlled studies of BP risk factors found no effect of neuroleptics or other central nervous system drugs on BP risk (Lloyd-Lavery et al., 2013; Tan et al., 2017), the evidence seems somewhat contradictory. In the present study, use of levomepromazine, which is a neuroleptic with an aliphatic side-chain, almost tripled the risk for BP. However, the butyrophenone derivates was the only subgroup of neuroleptics in which all drugs were associated with BP.

In the present study, the association between several of the studied drugs and BP risk remained following adjustment for the presence of numerous neurological and psychiatric diseases, thus the results cannot be explained solely by the greater neurological and psychiatric morbidity of BP patients. Since, aside from one exception, the mean time interval between the first purchase of these drugs and
diagnosis of BP was more than one year, chronic drug exposure seems to be required for an effect on BP risk. Knowing that BP180 and BP230 are expressed in both the skin and nervous system, the potential effect of chronic intake of nervous system drugs on the expression or pathogenicity of these antigens should be further studied.

6.4 Dermatitis herpetiformis is a risk factor for bullous pemphigoid (IV)

The earliest case reports of overlapping clinical presentation of BP and DH have were published almost 50 years ago (Honeyman, Honeyman, Lobitz, & Storrs, 1972; Jablonska, Chorzelski, Beutner, Maciejowska, & Rzęsa, 1976). To the best of my knowledge, the present study was the first to report an association between DH and BP in a large study population. Taylor et al found no association between BP and autoimmune disorders, but neither DH nor CD were included in the study (Taylor, Venning, Wojnarowska, & Welch, 1993). The relatively high incidence of DH in Finland (Salmi et al., 2011) may have contributed to the present study’s discovery of an association of DH and BP.

In 1972, Honeyman et al described a 61-year-old patient with a clinical presentation of DH whose initial DIF showed IgA and C3. Four months later, the DIF findings had shifted to IgG, IgM and C3, which is a profile more closely resembling that of BP. At this time the patient also tested positive for antibodies against BMZ (Honeyman et al., 1972). Since then, other reports of DH developing into BP have been described: Setterfield described a patient who, for 25 years, had had a pruritic skin eruption on the knees, elbows, buttocks and scalp (Setterfield, Bhogal, Black, & McGibbon, 1997). He developed blistering on the same areas and DIF showed granular IgA deposits and also C3 at the BMZ. Four months later after discontinuing dapsone, the skin symptoms became more widespread and DIF again detected granular deposits of IgA in the dermal papillae but also after salt-split, IgG on the epidermal side of the BMZ. Western immunoblotting found 180kDa antigen supporting a diagnosis of BP (Setterfield et al., 1997).

Ameen et al reported an 84-year-old female who had DH and Crohn’s disease and whose skin symptoms responded well to a gluten-free diet and dapsone (Ameen, Bhogal, & Black, 2000). Eleven years later, she presented with large blisters and had become unresponsive to dapsone treatment. Her DIF findings had changed: initially it had shown IgA and C3 as granular deposits within the papillary dermis but 11 years later the DIF showed IgG and C3 at the BMZ accompanied by bright
IgA staining in the dermal papillae. The authors suggested epitope spreading to explain this disease evolution (Ameen et al., 2000). Epitope spreading has also been hypothesized to be one mechanism that leads to the production of pathognomonic IgA antibody production against TG3 and the development of DH in CD patients (Kárpáti et al., 2018; Reunala et al., 2015). It may also happen in BP patients (Di Zenzo et al., 2011). Epitope spreading is a possible explanation for the association between DH and BP that was found by the present study but due to the study setting, this hypothesis could not be examined further.

Susceptibility for both CD and DH is known to be associated with the HLA-DQ2 and DQ8 haplotypes (Collin et al., 2017; Kaukinen, Partanen, Mäki, & Collin, 2002) whereas HLA-DQB1*0301 is overrepresented among BP patients (Amber, Zikry, & Hertl, 2017; Sun et al., 2018). Vaira et al described a patient with BP who later developed DH and CD, who had an HLA profile predisposing to BP, DH and CD (Vaira, Della Valle, Fanoni, Pontini, & Muratori, 2013). To the best of my knowledge, no other studies of the probable shared predisposing factors have been conducted.

Many physical factors have been reported to induce BP, including thermal burns, radiotherapy, photodynamic therapy, surgical procedures, ultraviolet light exposure, insect bites, ostomies, skin grafts and cupping (Azizpour, Nasimi, Shakoei, Mohammadi, & Azizpour, 2018; Dănescu et al., 2016; Mai et al., 2018). It has been hypothesized that events such as irradiation could directly disturb the BMZ and alter its antigenic properties or that patients who develop BP after irradiation, might have already had circulating anti-BMZ-antibodies, with the tissue damage leading to deposition of antibodies at the BMZ and subsequently BP onset (Mul et al., 2007). In a large Finnish cohort of DH patients, only 8% of patients needed dapsone to control their skin symptoms after adhering to a gluten-free diet (Hervonen et al., 2012) whereas in the present study, 34% of the patients with DH had bought dapsone during the 6 months preceding their BP diagnosis. The high frequency of dapsone therapy among the patients with DH and subsequent BP in the present study may reflect the activity of these patients’ skin symptoms. Active inflammation and scratching the skin cause tissue damage, which could lead to changes in the antigenic properties of BMZ proteins and thus contribute to the onset of BP.

In the present study, DH preceded BP by 3 years and CD 4.9 years (means). In case reports of DH and subsequent BP, the mean time between the diagnoses has varied between 4 months and 25 years (Ameen et al., 2000; Murphy, Bhogal, Banerjee, & Black, 2003; Setterfield et al., 1997). Wide variation in the time
interval between preceding comorbidities and BP has reported in earlier studies: the reported time between diagnoses of psoriasis and BP has varied between 3 and 25 years (Chen et al., 2011; Kridin & Bergman, 2017). In our previous study, multiple sclerosis preceded BP by 12 years and psychiatric comorbidities by 9 to 11 years (Försti et al., 2016b). In the current study, BP occurred at a younger mean age (68.8 years) in patients who also had DH compared with those with no preceding DH (76.7 years), whereas the overall mean age at DH diagnosis (64.9 years) was higher than that seen in the non-BP Finnish DH population during the years 2000–2009 (48.6 years) (Salmi et al., 2011). Other possible distinct clinical features of BP preceded by DH could not be determined due to the study setting.

6.5 Strengths of the study

That this study utilized one of the largest ever BP cohorts described is a major strength (Försti et al., 2017). The study population obtained from the CRHC register is national, covers almost all Finnish BP patients, treated in all hospitals regardless of socioeconomic status or insurance cover, and includes data spanning decades. The comprehensive nature of the drug reimbursement data set is also a major strength of the study: it covers all reimbursed drugs in Finland and therefore can be considered more reliable than other methods, such as interviewing the patients or retrieving drug data from patient records. Since DH has a particularly high incidence in Finland (Salmi et al., 2011), the study population itself can also be considered a strength of the study.

6.6 Limitations of the study

Due to the use of routinely collected registry data there was no certainty that all the BP cases were confirmed using DIF analysis, ELISA or other diagnostic measures. Due to the study setting, detailed clinical information was also absent. Furthermore, no information was available regarding the actual onset of BP symptoms. Additionally, patients without blistering or extensive symptoms may have been misdiagnosed or never presented at hospital dermatology clinics. However, in Finland, DIF studies are mainly conducted in hospitals and therefore BP diagnoses are largely made in the hospital setting. Since the present study utilized the CRHC data set, which covers all the public hospitals in Finland, it is likely that the present study includes the majority of the BP patients truly diagnosed in Finland during the study period.
Data on drug use were obtained from the drug reimbursement registry of the Social Insurance Institution of Finland, which contains information on reimbursed drugs only. Therefore, data on any medications used in the hospital setting, or which lacked reimbursement status were not included in the present study. Although the present study cohort included all patients treated for BP in Finnish hospitals between the years 1997 and 2013, some of the drugs were used too infrequently to allow reliable statistical analysis.

Concerning study III, the adjustments for other diagnoses did not cover all the indications for which e.g. benzodiazepines or neuroleptics are used. This might have affected the calculated ORs. In addition, all diagnoses used in the present study were obtained from the Finnish CRHC database, which includes only diagnoses made in the hospital setting. Therefore, it is possible that some diagnoses, such as temporary insomnia or anxiety, which are often diagnosed in the primary health care setting, may have been under-represented in the present study. Neuroleptics are also used “off-label” i.e. for unapproved indications and this may have affected the results.

The use of BCC patients as control population may have introduced confounding factors in the present study. Since exposure to ultraviolet radiation is a known risk factor for BCC (Wong et al., 2003), it is possible that BCC patients have more active lifestyle and thus they might be less prone to diabetes and/or have less severe diabetes. When the control group is constructed of patients with a certain diagnosis, it is always possible that factors associated with that diagnosis, may act as confounding factors.

Since this was a register-based study, the histological or serological studies of the patients were not available. Therefore, specific features of vildagliptin-associated BP or possible causes of the association of DH and BP could not be studied in more detail. Finally, as an epidemiological study, the present study alone is not able to prove any direct causal relationship between drug use and BP.

6.7 Future prospects

The potential specific clinical and prognostic features of DPP-4i-associated BP need to be studied in the future to clarify the role of DPP-4 inhibition in the pathomechanism of BP. The discovery of the association between DPP-4i treatment and BP arouses interest in other possible triggering factors. Knowing the factors that predispose towards BP may help us better understand the process that leads to the loss of immunotolerance in BP. Since neurological and psychiatric diseases are
known to be associated with BP, and since drugs for these diseases are widely used, the potential role played by these drugs as triggering factors for BP need to be studied in the future.

This study brought novel knowledge of an association between DH and BP. Studies with detailed clinical, serological and immunofluorescence study data are needed to further understand and confirm this association.
7 Conclusions

This thesis studied the risk factors for BP. Based on the findings of the studies described here, the following main conclusions can be drawn:

- Treatment with DPP-4is, especially vildagliptin, markedly increases the risk for BP. DPP-4i use is likely to be a triggering factor in some cases of BP, but further studies are needed to describe the mechanisms behind this, and to characterize probable clinical, immunological and genetic features of patients with DPP-4i-associated BP.
- This study supports the view that oral diabetes medications other than DPP-4is are not associated with an increased risk for BP and therefore can be considered safe for use in BP patients.
- Drugs used for psychiatric and neurologic diseases are associated with an increased risk for BP.
- Previously diagnosed DH and CD are risk factors for subsequent BP and clinicians treating patients with DH should be aware of this association if a patient’s skin symptoms alter or the response for gluten-free diet and/or dapsone is lost.
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67


Original publications


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