INCIDENCE, MORTALITY, COMORBIDITIES, AND TREATMENT OF BULLOUS PEMPHIGOID IN FINLAND

Anna-Kaisa Försti
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INCIDENCE, MORTALITY, COMORBIDITIES, AND TREATMENT OF BULLOUS PEMPHIGOID IN FINLAND

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UNIVERSITY OF OULU, OULU 2017
Bullous pemphigoid (BP) is an autoimmune skin disease predominantly found in elderly people, which causes blistering of the skin and severe itching. The incidence of BP reported by previous studies has varied greatly between 0.05 and 42.8 per 1 million persons per year. Higher incidences have been reported in Western Europe and the USA, while countries around the Mediterranean have reported lower rates. However, the epidemiology of BP has not previously been studied in any Scandinavian country. The one-year mortality of BP is highly variable with estimates between 11% and 41% worldwide. As for comorbidities, the previous studies have shown that BP is associated with neurological disorders.

The aim of this study was to investigate the incidence and mortality of BP in Finland, to assess the treatments used for BP, and the potential contribution of systemic glucocorticoid treatment to the high mortality rate found in BP patients. A further aim was to obtain more specific information about the neurological diseases associated with BP, and to clarify the less studied association with psychiatric disorders. For these purposes, we collected the records of all immunologically confirmed BP patients diagnosed in the Oulu University Hospital between 1985 and 2012, and, for a sub-study III, data for all patients diagnosed with BP in Finnish hospitals between 1987 and 2013.

We found that the incidence of BP in Northern Finland has increased over the past two decades to approximately 27 new BP cases per 1 million persons per year. The one-year mortality of BP patients is 17%, and the standardized mortality ratio (SMR) is 7.6. Common comorbidities found in the sample of BP patients were: cardiovascular diseases (76%), neurodegenerative diseases (41%), skin conditions other than BP (37%) and type 2 diabetes (23%). Many neurodegenerative diseases of the central nervous system were associated with BP, as were many psychiatric disorders. The association was strongest between multiple sclerosis (MS) and BP, with MS patients having almost a 6-fold higher risk of BP than controls.

The present study reports for the first time the incidence and mortality of BP in Finland, and provides new information about the association between BP and neurological and psychiatric disorders.

**Keywords:** autoimmune bullous diseases, BP180, bullous pemphigoid, collagen XVII, epidemiology, Finland, incidence, mortality, neurological comorbidity, psychiatric comorbidity, skin diseases, standardized mortality ratio
Tiivistelmä


Tämän tutkimuksen tarkoituksena oli selvittää pemfigoidin ilmaantuvuus ja kuolleisuus Suomessa, tutkia sen hoitoon käytettyjä lääkkeitä sekä arvioida systemisen glukokortikoidihoidon osuutta korkeaan kuolleisuuteen. Lisäksi tavoitteena oli saada yksityiskohtainen tieto pemfigo- diin liittyvistä neurologisista sairauksista ja selvittää lisää aiemmissa tutkimuksissa ristiriitaisia sairauksia ja yhteyttä pemfigoidiin ja neurologisiin sairauksiin. Tutkimuksen tavoitteena oli selvittää, onko pemfigoidiksi diagnoositettu 17% vuoden kuluessa ja vakioitu kuolleisuusuhde 7,6. Yleisiä oheissairauksia pemfigoidia sairastavilla olivat sydän- ja verisuonisairaudet (76%), neurodegeneratiiviset sairaudet (41%), muut ihosairaudet (37%) sekä tyyppi 2 diabetes (23%). Tutkimuksessa todettiin, että monet neurodegeneratiiviset sairaudet ja monet psykiatriset sairaudet liittyvät pemfigoidiin. Yhteys oli vahvempi pesäkekovettumataudin (MS-tauti) ja pemfigoidin välillä, ja MS-tautia sairastavilla riski sairastaa pemfigoidiin oli lähes 6-kertainen verrattuna kontrollipotilaisiin.

Tämä tutkimus on ensimmäinen, joka raportoi pemfigoidin ilmaantuvuuden ja kuolleisuuden Suomessa. Tutkimus antaa lisää olemuksen tietoa pemfigoidin yhteydestä neurologisiin ja psykiatrisiin sairauksiin.

Asiasanat: autoimmuunirakkulatauti, BP180, epidemiologia, ihosairaus, ilmaantuvuus, kollageeni XVII, kuolleisuus, neurologinen oheissairaus, pemfigoidi, psykiatrinen oheissairaus, rakkulainen pemfigoidi, Suomi, vakioitu kuolleisuusuhde
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My warm thanks and hugs go to my friends and their families. « Good friends help you find important things when you have lost them. Your smile, you hope, and your courage. » Thank you all for being there and sharing this wonderful life with me.

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Oulu, 16th March 2017 Anna-Kaisa Försti
### Abbreviations

<table>
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<tr>
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<th>Definition</th>
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<tbody>
<tr>
<td>ALS</td>
<td>amyotrophic lateral sclerosis</td>
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<tr>
<td>BCC</td>
<td>basocellular carcinoma</td>
</tr>
<tr>
<td>BP</td>
<td>bullous pemphigoid</td>
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<tr>
<td>BPAG</td>
<td>bullous pemphigoid antigen</td>
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<tr>
<td>BP180</td>
<td>bullous pemphigoid antigen 180</td>
</tr>
<tr>
<td>BP230</td>
<td>bullous pemphigoid antigen 230</td>
</tr>
<tr>
<td>BPDAI</td>
<td>Bullous Pemphigoid Disease Area Index</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation, cluster of designation</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>C3</td>
<td>complement 3</td>
</tr>
<tr>
<td>DIF</td>
<td>direct immunofluorescence microscopy</td>
</tr>
<tr>
<td>DPP-4</td>
<td>dipeptidyl peptidase-4</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>HLA</td>
<td>human leukocyte antigen</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>ICD</td>
<td>international classification of diseases</td>
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<tr>
<td>IgA</td>
<td>immunoglobulin A</td>
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<tr>
<td>IgE</td>
<td>immunoglobulin E</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
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<tr>
<td>IgM</td>
<td>immunoglobulin M</td>
</tr>
<tr>
<td>IIF</td>
<td>indirect immunofluorescence microscopy</td>
</tr>
<tr>
<td>IF</td>
<td>immunofluorescence</td>
</tr>
<tr>
<td>IRR</td>
<td>incidence rate ratio</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo Dalton</td>
</tr>
<tr>
<td>LAD</td>
<td>linear immunoglobulin A disease</td>
</tr>
<tr>
<td>NaCl</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>NOHD</td>
<td>the Northern Ostrobothnia Hospital District</td>
</tr>
<tr>
<td>MMP</td>
<td>mucous membrane pemphigoid</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-Mental State Examination</td>
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<tr>
<td>mol</td>
<td>mole</td>
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<tr>
<td>MS</td>
<td>multiple sclerosis</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PASW</td>
<td>Predictive Analytics SoftWare</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SMR</td>
<td>standardized mortality ratio</td>
</tr>
<tr>
<td>STATA</td>
<td>Data Analysis and Statistical Software</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor alpha</td>
</tr>
<tr>
<td>UK</td>
<td>the United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>the United States of America</td>
</tr>
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Original publications

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1 Introduction

Bullous pemphigoid (BP) is an autoimmune skin disease, which belongs to the pemphigoid group. BP usually affects elderly people after the 7th decade of life, and considerably impairs the patient’s quality of life with symptoms like intense pruritus and blistering of the skin. The diagnosis of BP is confirmed by immunological investigations, and an evaluation by a dermatologist is needed. In cases of BP that involve widespread blistering, treatment is generally started in a dermatologic ward (Di Zenzo et al. 2012, Schmidt & Zillikens 2013).

The mainstream treatment of BP consists of systemic and topical glucocorticoids. However, systemic glucocorticoids, especially when used in elderly people, are associated with potentially severe side-effects, like diabetes, osteoporosis, heart failure, and irritation of the gastric mucosa. Moreover, it is suspected that the use of systemic glucocorticoids may contribute to the high mortality rate seen in BP patients (Feliciani et al. 2015, Joly et al. 2002, Venning et al. 2012). However, before the invention of glucocorticoids, the mortality rate of BP was even higher than now with estimates at 40% (Zillikens 2009).

The idea of this study arose in daily practice in the Department of Dermatology at the Oulu University Hospital, where we noticed an increase in the number of new BP patients. By the start of this study in 2010, only one previous study had reported an increasing incidence of BP (Langan et al. 2008), and there were no epidemiological data at all for BP in Finland, or even in other Scandinavian countries. In general, epidemiology is a branch of science that investigates phenomena related to health and diseases, such as the incidence or mortality of a disease in different populations. Epidemiology can, for example, study how common a disease is in a certain population, reveal factors that predispose patients to particular diseases, or determine causal relations between phenomena, such as response to treatment. In this study, we wanted to gather epidemiological data on BP in Finland. The study started with investigations of the incidence of BP in the Northern Ostrobothnia Hospital District (NOHD), continued with mortality assessments, and was augmented with an examination of a large, national data set of more than 4500 BP patients, and their neurological and psychiatric comorbidities.
2 Review of the literature

2.1 General aspects of bullous pemphigoid (BP)

The pemphigoid diseases were first distinguished from another autoimmune skin disease, pemphigus by their clinical and histopathological features in 1953 (Lever 1953). Nowadays, BP is a well-defined autoimmune blistering skin disease, and the most common of the pemphigoids. In BP, autoantibodies target the basement membrane structures between the epidermis and dermis of the skin causing subepidermal blisters. In pemphigus, blister formation occurs in the upper layer of the skin, between keratinocytes (Lever 1953, Schmidt & Zillikens 2013).

2.2 Epidemiology of bullous pemphigoid

2.2.1 Incidence

Based on studies around the world, the yearly incidence of BP varies between 0.05 and 42.8 per 1 million inhabitants per year (Alpsoy et al. 2015, Joly et al. 2012, Langan et al. 2008, Marazza et al. 2009, Uzun et al. 2006). The incidence of BP was 6.1–6.6 per 1 million persons per year in Germany between 1989 and 1997 (Jung et al. 1999, Zillikens et al. 1995), 12.1 in Switzerland between 2001 and 2002 (Marazza et al. 2009) and 21.7 in France between 2000 and 2005 (Joly et al. 2012), and the incidence has been reported to have increased in recent years (Bernard et al. 1995, Joly et al. 2012, Langan et al. 2008). A UK study found that the incidence of BP increased 5-fold over 11 years to 2006 (Langan et al. 2008), whereas in France the increase was 3-fold over 15 years to 2005 (Bernard et al. 1995, Joly et al. 2012). A recent study from Serbia reported that the incidence of BP increased 0.06 cases per 1 million persons per year over a 20-year time period to 2010 (Milinkovic et al. 2016).

Besides true variation of incidence, other reasons behind notably variable numbers may include differences in diagnostic criteria used, different age structures of study populations and differences in the study countries’ healthcare systems (Langan et al. 2008, Marazza et al. 2009, Naldi et al. 2012). Most of the studies have used the databases of dermatology departments/clinics – an approach that may result in underestimation of the incidence of BP through lack of diagnosis of the mildest cases (Langan et al. 2008). Only one study, by Langan and her coworkers,
investigated the incidence of BP based on the general practice database, and reported the incidence as high as 42.8 new BP cases per 1 million persons per year in the UK (Langan et al. 2008). However, it is unknown which of the cases captured by this study were verified by immunological examinations.

While BP is the most common autoimmune bullous disease in Western Europe and in the USA, countries around the Mediterranean have reported higher incidences of the other autoimmune blistering skin disease, pemphigus (Alpsoy et al. 2015, Baican et al. 2010, Bastuji-Garin et al. 1995, Chams-Davatchi et al. 2005). Pemphigus is known to be associated with Jewish origin and certain human leukocyte antigen (HLA) genotypes, namely HLA-DRB1*04, HLA-DRB1*14, and HLA-DQB1*05 (Ahmed et al. 1990, Alpsoy et al. 2015, Simon et al. 1980). In Brazil, there is also an endemic form of pemphigus, called fogo selvagem (Culton et al. 2008). Differing life expectancies may also play a role in the different distributions of pemphigus and BP, since BP typically affects older persons than pemphigus (Alpsoy et al. 2015).

BP is a disease of elderly people occurring mostly after the age of 70 years (Alpsoy et al. 2015, Joly et al. 2012, Jung et al. 1999, Langan et al. 2008, Marazza et al. 2009). According to the study by Joly and coworkers the incidence of BP in the 70-year-old or above population is 162 cases per 1 million persons per year, and among persons aged 85 years or above, as high as 507 per 1 million persons per year (Joly et al. 2012). Slightly more cases are found in females, the female-male ratio being 1.1 in Germany (Zillikens et al. 1995), 1.5 in France (Bernard et al. 1995, Joly et al. 2012) and up to 5.1 in Kuwait (Nanda et al. 2004). However, females are overrepresented in older age groups, which may affect the sex distribution of BP (Alpsoy et al. 2015), and indeed, in the study by Langan and coworkers, the adjusted incidence was equal for both sexes (Langan et al. 2008).

2.2.2 Mortality

BP is associated with a high mortality rate: in most of the studies around 20% of affected individuals die within the first year after the diagnosis, though the range has varied between 10.8% and 41%. BP patients also die more often than their age-adjusted counterparts in the general population, with the standardized mortality ratio (SMR) varying between 1.9 and 9.6. (Brick et al. 2014a, Cai et al. 2013, Colbert et al. 2004, Gual et al. 2014, Joly et al. 2005b, Joly et al. 2012, Langan et al. 2008, Lee & Kim 2014, Parker et al. 2008, Roujeau et al. 1998, Rzany et al. 2002).
Advanced age among BP patients has been associated with increased mortality by many studies (Cortes et al. 2011, Cortes et al. 2012, Gual et al. 2014, Joly et al. 2005b, Parker et al. 2008, Rzany et al. 2002). A prospective multicenter study in France examined prognostic factors of BP in a study group of 170 patients, and found that advanced age and weak general condition were the main factors that correlated with decreased one-year survival (Joly et al. 2005b). By contrast, the severity of clinical symptoms did not correlate with mortality (Joly et al. 2005b). Another multicenter study comprising 369 patients from Germany (Rzany et al. 2002) reported that the main risk factors for death within one year were advanced age, low serum albumin level, high erythrocyte sedimentation rate and systemic glucocorticoid dose more than 37 mg per day at discharge. Altogether, the existing literature indicates that the elevated mortality of BP patients compared with age-adjusted reference population is probably caused by poor general health, comorbid diseases, and possibly also by side-effects of immunosuppressive treatment for BP (Alpsoy et al. 2015, Joly et al. 2002).

### 2.2.3 Associated diseases

In general, BP patients have many comorbidities due to advanced age (Cai et al. 2013, Gual et al. 2014). Among these, the most frequently reported are cardiovascular diseases, neurological diseases and diabetes (Cai et al. 2013, Gual et al. 2014, Marazza et al. 2009, Parker et al. 2008). Although an association between BP and malignancies has been suspected (Chorzelski et al. 1978), recent studies have not established any such connection (Cai et al. 2015, Jedlickova et al. 2010, Lindelof et al. 1990, Ong et al. 2014).

**Neurological comorbidities**

Evidence has accumulated to indicate that there exists an association between BP and neurological disorders (Table 1) (Brick et al. 2014b, Chen et al. 2011b, Langan et al. 2011, Ong et al. 2013, Taghipour et al. 2010). A case-control study of 89 BP patients, which investigated the association between BP and internal diseases including diabetes mellitus, neurological diseases, malignant tumors, benign prostate hyperplasia, hypertension and ischemic heart diseases found that only neurological diseases were statistically significantly associated with BP (Jedlickova et al. 2010). Later studies have showed that BP is particularly associated with stroke, dementia, and Parkinson’s disease (Table 1) (Brick et al. 2014b, Chen et al. 2011b, Langan et al. 2011, Ong et al. 2013, Taghipour et al. 2010).
However, in previous studies, the types of dementia and cerebrovascular stroke have not been specified. With regard to temporal relationships in these studies, neurological disorders mostly preceded BP (Brick et al. 2014b, Chen et al. 2011b, Langan et al. 2011, Taghipour et al. 2010), but only a few studies with conflicting results have indicated whether the risk of developing neurological disorders is elevated following a diagnosis of BP (Brick et al. 2014b, Langan et al. 2011, Ong et al. 2014). These findings demonstrate the need for further investigation into which specific neurological disorders are associated with BP, the temporal relationships, and possible causality.

In Finland, neurological and psychiatric disorders belonged to the same medical specialty until 1960, when they were separated to their own entities. In order to classify disorders, the International Classification of Diseases (ICD) has been used since 1968 in Finland (Lönnqvist et al. 2009), and already in this 8th revision of the ICD, neurological and psychiatric disorders were their own entities. In the currently used ICD 10, the category of neurological diseases includes all diseases of the central and peripheral nervous systems, some muscle diseases, some congenital conditions such as cerebral palsy and some diseases of the spinal cord. The diseases associated with BP by previous studies tend to be disorders that have so-called neuroinflammatory and neurodegenerative mechanisms, such as Alzheimer’s disease and Parkinson’s disease (Heneka et al. 2015, Hirsch & Hunot 2009). Neurodegeneration is a process that happens in the central nervous system (CNS) with a progressive loss of structure or function of neurons, including death of neurons. Recent studies have found that many inflammatory mechanisms are linked with neurodegeneration (Chen et al. 2016).

**Psychiatric comorbidities**

Psychiatry investigates disorders that affect aspects of mental health such as mood, cognition and behavior. In the ICD 10, psychiatric disorders are named ‘mental and behavioural disorders’, and the category includes diseases with well-known organic origins such as alcohol abuse, and diseases with much more heterogeneous pathogenesis such as schizophrenia, mood disorders, and personality disorders (Lönnqvist et al. 2009). Neuroinflammatory mechanisms have also been recognized in these disorders (Bhattacharya et al. 2016, Fanning et al. 2015, Muller et al. 2015)
Table 1. The association between bullous pemphigoid and neurological and psychiatric disorders in previous studies.

<table>
<thead>
<tr>
<th>Author, year, country</th>
<th>Database/population</th>
<th>n(BP)</th>
<th>Years</th>
<th>Disorders with a statistically significant association with bullous pemphigoid (BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ong, 2013, the UK</td>
<td>National Health Service Information Center</td>
<td>9317</td>
<td>1999-2011</td>
<td>Motor neuron disease</td>
</tr>
<tr>
<td>Chen, 2011, Taiwan</td>
<td>National Health Insurance Research Database</td>
<td>3485</td>
<td>1997-2008</td>
<td>Stroke, dementia, Parkinson’s disease, epilepsy, schizophrenia, psoriasis</td>
</tr>
<tr>
<td>Langan, 2011, the UK</td>
<td>National, General practice database hospitals in North-East Italy</td>
<td>868</td>
<td>1996-2006</td>
<td>Stroke, dementia, Parkinson’s disease, multiple sclerosis, epilepsy</td>
</tr>
<tr>
<td>Stinco, 2005, Italy</td>
<td>11 hospitals</td>
<td>238</td>
<td>1995-2000</td>
<td>Neurological disease (Parkinson’s disease or multiple sclerosis)</td>
</tr>
<tr>
<td>Bastuji-Garin, 2011, France</td>
<td>11 hospitals</td>
<td>201</td>
<td>2003-2007</td>
<td>Major cognitive impairment, bedridden condition, Parkinson’s disease, unipolar or bipolar disorder</td>
</tr>
<tr>
<td>Li, 2013, China</td>
<td>1 hospital</td>
<td>190</td>
<td>1992-2012</td>
<td>Neurological diseases</td>
</tr>
<tr>
<td>Taghipour, 2010, the UK</td>
<td>1 hospital</td>
<td>90</td>
<td>2004-2008</td>
<td>Cerebrovascular disease, dementia</td>
</tr>
<tr>
<td>Jedlickova, 2010, the Czech Republic</td>
<td>2 hospitals</td>
<td>89</td>
<td>1991-2006</td>
<td>Neurological diseases</td>
</tr>
<tr>
<td>Brick, 2014b, the USA</td>
<td>Olmsted County, Rochester Epidemiology Project</td>
<td>87</td>
<td>1960-2009</td>
<td>Dementia, Parkinson’s disease</td>
</tr>
<tr>
<td>Teixeira, 2014, Portugal</td>
<td>1 hospital</td>
<td>77</td>
<td>1998-2010</td>
<td>Dementia, stroke, bedridden condition</td>
</tr>
<tr>
<td>Casas-de-la-Asunción, 2014, Spain</td>
<td>1 hospital</td>
<td>56</td>
<td>2002-2012</td>
<td>Dementia, Parkinson’s disease</td>
</tr>
<tr>
<td>Kwan, 2015, Malaysia</td>
<td>1 hospital</td>
<td>43</td>
<td>2004-2013</td>
<td>Dementia</td>
</tr>
</tbody>
</table>

Some studies have reported an association between BP and psychiatric diseases (Table 1), but their results remain controversial (Bastuji-Garin et al. 2011, Chen et al. 2011b, Teixeira et al. 2014). Chen and coworkers showed in a large Taiwanese study population, that patients with schizophrenia are more likely to develop BP (Chen et al. 2011b), and in the French study by Bastuji-Garin and coworkers, unipolar and bipolar disorders were independent risk factors for BP (Bastuji-Garin et al.)
On the contrary, no association was seen between BP and depression in the study by Teixeira and coworkers (Teixeira et al. 2014).

### 2.2.4 Predisposing factors

Previous studies have shown that the age-adjusted incidence of BP is increasing (Brick et al. 2014a, Joly et al. 2012, Langan et al. 2008), but the factors behind this trend are not known. The statistical association between BP and neurodegenerative diseases is well recognised, with neurodegenerative diseases mostly preceding BP. The temporal relationship raises the question as to whether nervous system damage can trigger an immunological process that leads to the development of BP. However, any molecular mechanisms behind such a process are unknown (Brick et al. 2014b, Chen et al. 2011b, Langan et al. 2011, Seppänen 2013).

Medications, such as diuretics, neuroleptics, antidiabetics and tumor necrosis factor alpha (TNFα) inhibitors as well as vaccines have been suggested to predispose patients to BP (Oh et al. 2016, Stavropoulos et al. 2014). A French study, which comprised 201 BP patients, found an association between BP and the use of spironolactone or phenothiazine psycholeptics (Bastuji-Garin et al. 2011). Another study from the UK reported an association between BP and loop diuretics (Lloyd-Lavery et al. 2013). Increasing numbers of case reports have linked BP with the use of dipeptidyl peptidase-4 (DPP-4) inhibitors (“gliptins”), which are common in the treatment of diabetes, but this association needs to be further investigated (Stavropoulos et al. 2014). Interestingly, a Japanese study group reported that BP patients using DPP-4 inhibitors were more likely to have atypical BP, characterized by a non-inflammatory appearance of BP, and autoantibodies that target the mid-portion of the bullous pemphigoid antigen 180 (BP180, also known as collagen XVII or BP antigen 2 [BPAG2]), but not the NC16A domain as is found in typical BP (Izumi et al. 2016).

In some cases, physical triggers such as ultraviolet light, radiation therapy, and trauma have been thought to induce BP (Lo Schiavo et al. 2013). Genetic susceptibility seems to be of less importance in BP than in pemphigus. Furthermore, there is not such variation in the incidence of BP according to geographic area or ethnic origin, as in pemphigus (Alpsoy et al. 2015). Regardless, an association between BP and certain HLA genotypes has been reported. HLA genotypes, in general, play a key role in genetic predisposing factors in autoimmunity because of their role in antigen presentation (Lo Schiavo et al. 2013). The HLA-DQB1*0301 allele is associated with enhanced susceptibility to BP among Caucasians (Delgado
et al. 1996), as are HLA-DRB1*04 and DRB1*1101 in Japanese people (Okazaki et al. 2000). Interestingly, HLA-DRB1*04 has also been associated with pemphigus, especially in Ashkenazi Jewish patients (Ahmed et al. 1990).

2.3 Clinical presentation and prognosis of bullous pemphigoid

2.3.1 Clinical features

BP is characterized by subepidermal, tense blisters and erythema which turn into erosions and crusts within days (Fig. 1).

Fig. 1. Widespread blistering and erythema on the limbs of a bullous pemphigoid patient (Department of Dermatology, Oulu University Hospital).

Blisters usually appear on the trunk and proximal flexural sites of the limbs. Intense pruritus is typical of BP, and it can precede blisters by several weeks or even months, sometimes occurring with atypical skin lesions like excoriations, eczema, papular or urticarial lesions (Di Zenzo et al. 2012, Schmidt & Zillikens 2013). In up to 20% of cases, the only lesions seen at the time of BP diagnosis are atypical and without
blistering (Di Zenzo et al. 2012). Approximately 10–20% of BP patients have mucous membrane involvement, usually in the oral cavity (Nishie 2014, Schmidt & Zillikens 2013). A clinical scoring system, the BP Disease Area Index (BPDAI) has been developed by an international panel of experts as a tool for the objective evaluation of BP symptom severity (Murrell et al. 2012).

### 2.3.2 Clinical course and prognosis

BP appears to be a chronic disease with the possibility of relapse after successful treatment (Di Zenzo et al. 2012), and, as described above, it carries a high mortality rate. Clinical symptoms include severe pruritus and in many cases widespread skin erosions, which can cause pain, disturb sleep and remarkably decrease the patient’s quality of life. Few studies have characterized the clinical course of BP. In a UK study conducted among 82 BP patients between 1975 and 1988, the disease lasted from nine weeks to 17 years with median treatment time of approximately two years (Venning & Wojnarowska 1992). Another study comprising 118 BP patients from France investigated the risk of relapse within one year of treatment cessation, and found, that high titers of BP180 identified by enzyme-linked immunosorbent assay (ELISA) examination in patients in clinical remission predicted relapse with a high positive predictive value (91%) (Bernard et al. 2009). However, at the time of diagnosis, the prognosis of an individual patient is difficult to predict.

### 2.4 Other pemphigoid subtypes

BP is the most common subtype of the pemphigoid diseases, the other major subtypes being mucous membrane pemphigoid (MMP), pemphigoid gestationis, linear immunoglobulin A (IgA) disease (LAD), and epidermolysis bullosa acquisita (EBA). Anti-p200 (anti-laminin γ1) pemphigoid and lichen planus pemphigoides are rare types with fewer than 100 reports of each worldwide. In BP, pemphigoid gestationis and in linear IgA disease, the main autoantigen is BP180 while other proteins can also serve as autoantigens in other pemphigoid types. The different types cannot be diagnosed by clinical appearance alone, hence, diagnostic investigations are needed (Schmidt & Zillikens 2013).
**2.4.1 Mucous membrane pemphigoid**

MMP is rarer than BP with an incidence between 1.3 and 2.0 per 1 million person-years (Bernard et al. 1995, Bertram et al. 2009). Earlier, the term cicatricial pemphigoid was used as a synonym for MMP, but nowadays, it refers to a rare variant in which mucosal symptoms are scarce, and skin lesions heal by scarring (Schmidt & Zillikens 2013). In MMP, symptoms predominantly localize to mucosal surfaces, most often to the oral cavity or conjunctiva, and the severity of the disease is highly variable. The autoantigens for MMP are heterogeneous and include BP180, bullous pemphigoid antigen 230 (BP230 or BP antigen 1, BPAG1), laminin 332, \( \alpha 6\beta 4 \) integrin, and collagen VII (Rashid et al. 2006, Schmidt & Zillikens 2013).

**2.4.2 Pemphigoid gestationis**

Pemphigoid gestationis occurs mostly in the 2nd or 3rd trimester of pregnancy, and typically starts with intense pruritus around the navel. Skin symptoms vary, and include erythema, papules, urticarial plaques or erythema multiforme-like target lesions, followed by blisters after some weeks (Ambros-Rudolph 2011, Huilaja et al. 2014, Sadik et al. 2016). Most (approximately 75%) patients experience worsening of symptoms during delivery, but in the majority of cases, the disease is limited to six months thereafter. In pemphigoid gestationis, as in BP, the major autoantigen is the NC16A domain of BP180 (Huilaja et al. 2014, Sadik et al. 2016, Schmidt & Zillikens 2013).

**2.4.3 Linear IgA disease**

The reported incidence of LAD varies between 0.26 and 1.0 per 1 million persons per year (Bernard et al. 1995, Bertram et al. 2009, Wong & Chua 2002). LAD has two peaks of onset: it is the most common pemphigoid disease in children, mostly occurring before the age of five years. Its other peak in incidence is seen in the population aged over 60 years (Schmidt & Zillikens 2013). Clinical features typical of LAD include annular lesions with blisters, and consistently with other pemphigoid types, urticarial or eczematous lesions can also appear. Mucosal involvement is seen in about 70% of cases (Schmidt & Zillikens 2013). LAD autoantibodies are mainly of the IgA type, and target BP180 (Marinkovich et al. 1996, Zillikens et al. 1999).
2.4.4 Epidermolysis bullosa acquisita

In contrast to the other major pemphigoid types, the autoantibodies in EBA do not target BP180, but collagen VII, locating in lower basement membrane zone (Fig. 2) (Woodley et al. 1984, Woodley et al. 1988). Its clinical appearance can vary, and based on its manifestation, it can be classified into two subtypes. The classical mechanobullous EBA subtype manifests as blisters and erosions following friction of the skin, whereas the inflammatory type more closely resembles BP or MMP. In the mechanobullous type, skin lesions heal with scarring and milia formation. Mucosal lesions occur in approximately half of the cases in each subtype (Chen et al. 2012, Schmidt & Zillikens 2013). The incidence of EBA in Europe is approximately 0.2–0.5 per 1 million person-years (Bernard et al. 1995, Bertram et al. 2009).

2.5 Pathophysiology of bullous pemphigoid

2.5.1 Hemidesmosomes

Hemidesmosomes are adhesion structures in the skin, which bind epidermal basal cells to the underlying extracellular matrix. The extracellular matrix beneath the layer of basal keratinocytes forms a structure called the basement membrane, which binds the epidermis to the dermis. The basement membrane consists of two layers: the lamina lucida and the lamina densa (Fig. 2). Hemidesmosomes are comprised of many molecules including BP180, BP230, plectin and α6β4 integrin, and they interact with several other molecules in the basement membrane zone including laminin 111 (which includes the laminin γ1 chain), laminin 332 and collagens IV and VII (Fig. 2) (Goletz et al. 2014, Nishie 2014, Schmidt & Zillikens 2013). In all pemphigoid diseases, autoantibodies are directed at the structural molecules of hemidesmosomes or adjacent proteins causing breakage of the adhesion between basal cells and the extracellular matrix and, consequently, subepidermal blisters (Nishie 2014, Schmidt & Zillikens 2013).
2.5.2 Bullous pemphigoid antigens in the skin and pathomechanisms in bullous pemphigoid

In BP, the major autoantigen is BP180, more specifically the extracellular 16th non-collagenous (NC16A) domain of BP180 (Fig. 2). Autoantibodies against another protein, BP230 and autoantibodies against other parts of BP180 aside from the NC16A domain are also often involved, but their pathogenic importance in BP is somewhat unclear (Di Zenzo et al. 2008, Diaz et al. 1990, Jordon et al. 1967, Labib et al. 1986, Nishie 2014, Schmidt & Zillikens 2013).

Typically, the autoantibodies to NC16A are of the immunoglobulin G (IgG) type, but immunoglobulin E (IgE) and IgA antibodies are also sometimes found (Nishie 2014, Schmidt & Zillikens 2013, Yayli et al. 2011). The pathogenic role of IgG antibodies has been demonstrated in mouse models (Liu et al. 1993), and titers correlate with clinical disease activity (Hofmann et al. 2002). The presence of IgE autoantibodies has been associated with more severe phenotypes and extensive skin lesions (Dopp et al. 2000, Hofmann et al. 2002). Blister formation is finally
mediated by complement activation and the inflammatory mechanisms that follow. However, a complement-independent pathway can also be involved (Fig. 3) (Nishie 2014). Recent studies have also shown that NC16A-reactive CD4+ T-cells may have a role in blister formation, probably in certain genetically susceptible individuals. Nevertheless, the reasons behind the onset of autoimmune process and the formation of autoantibodies are not known (Nishie 2014, Schmidt & Zillikens 2013).

Fig. 3. Potential mechanisms of blister formation in bullous pemphigoid (modified from Lo Schiavo et al. 2013, Nishie 2014)

2.5.3 Bullous pemphigoid antigens in other tissues

As well as in the skin, BP180 is also expressed in the CNS, gastrointestinal tract, urinary bladder, heart, eye, placenta, and amniotic membranes (Aho & Uitto 1999, Claudepierre et al. 2005, Huilaja et al. 2008, Seppänen et al. 2006, Van den Bergh & Giudice 2003) In the CNS, it is predominantly located in the soma and proximal axons of neurons. The areas of strong expression of BP180 are the deepest, ganglionic layer of the cerebral cortex, the hippocampus, and many nuclei (hypoglossal nucleus, oculomotor nucleus, nucleus basalis of Meynert, supraoptic
nucleus and subthalamic nuclei) (Seppänen et al. 2006, Seppänen 2013). As epidemiological studies have affirmed statistical association between BP and neurological disorders (Brick et al. 2014b, Chen et al. 2011b, Langan et al. 2011, Ong et al. 2013), an interesting question has been posed, as to whether autoimmunization against BP180 would be involved in the pathogenesis of neurological disorders (Seppänen 2013).

BP230 has also been detected in tissues other than skin including central and peripheral nervous systems, and cardiac and skeletal muscle. This protein exists in many isoforms, and the epithelial (BPAG1-e) and neuronal (BPAG1-a) isoforms, and other variants have differences in their localization and molecular structure (Chen et al. 2011a, Kunzli et al. 2016, Laffitte et al. 2005). Regardless of these differences, BP230 is a potential shared target of antigen response in the skin and CNS (Seppänen 2013).

2.6 Diagnostics of bullous pemphigoid

2.6.1 Clinical criteria

In clinically typical cases, when widespread tense blisters are seen on eczematous skin of an elderly person who suffers from intensive itching, the diagnosis of BP is easy, and can be confirmed by direct immunofluorescence microscopy (DIF) examination of a perilesional skin biopsy (Di Zenzo et al. 2012, Vaillant et al. 1998). A study by the French Bullous Study Group showed that, in cases with positive DIF findings, i.e. linear deposits of IgG or complement 3 (C3) along the basement membrane zone, three of the following four clinical features are enough to make a diagnosis of BP with high sensitivity (90%) and specificity (83%): 1) age higher than 70 years 2) absence of atrophic scars 3) absence of mucosal involvement 4) absence of head and neck skin involvement (Di Zenzo et al. 2012, Vaillant et al. 1998). In atypical cases, in which blisters are not seen, and the appearance of the skin is e.g. urticarial or eczematous, or when just intense itching is present, laboratory investigations are of greater importance (Di Zenzo et al. 2012).

2.6.2 Direct immunofluorescence microscopy (DIF)

When clinical features suggest BP, DIF is the gold standard and the most sensitive (91%) and a very specific (98%) diagnostic test (Sardy et al. 2013). In this
investigation, a skin biopsy is taken from healthy looking skin near blisters. The sample must be snap frozen or placed in a suitable transport medium, otherwise immunoreactants may rapidly degrade (Mihai & Sitaru 2007). Typically, DIF reveals linear deposits of IgG or C3, or both, along the basement membrane zone, but weaker linear IgA or IgE staining can also be involved. In BP, linear fluorescence seems n-serrated while in EBA the pattern is u-serrated (Di Zenzo et al. 2012, Schmidt & Zillikens 2013). For more accurate diagnostics, DIF can also be performed using the so-called salt-split skin technique (Sardy et al. 2013). In this method, after incubating the skin biopsy with 1 mol/l sodium chloride (NaCl) solution, the epidermis and dermis are separated, and antibodies are seen either in the epidermal (roof of the blister) or dermal (base of the blister) side of the artificial blister. In BP, autoantibodies attach to the epidermal side, while in some other pemphigoids, like EBA, antibodies are seen in the base (Di Zenzo et al. 2012).

2.6.3 Indirect immunofluorescence microscopy (IIF)

In an IIF examination, IgG autoantibodies from a BP patient’s serum are seen to bind to the basement membrane zone of a substrate, for example monkey or rabbit esophagus tissue. The sensitivity of this investigation varies between 60–80%, although greater sensitivity can be achieved by using salt-split skin as a substrate (Di Zenzo et al. 2012, Sardy et al. 2013). Other circulating antibodies, like IgA, IgE and IgM, can also be searched for using this technique (Di Zenzo et al. 2012).

2.6.4 Enzyme linked immunosorbent assay (ELISA)

An ELISA can detect different parts of BP180, such as the most important BP autoantigen, the NC16A domain, using recombinant protein techniques. The NC16A ELISA investigation is commercially available, and provides a highly sensitive (70–98%) and specific (98%) test for BP diagnostics (Di Zenzo et al. 2012, Schmidt & Zillikens 2013, Tampoia et al. 2012). Combining this with another commercial ELISA for BP230 raises sensitivity, but the BP230 assay alone is not recommended in the diagnostics of BP, since its sensitivity when used alone is 60–70% (Di Zenzo et al. 2012, Schmidt & Zillikens 2013). A high concentration of anti-BP180 NC16A antibodies in serum also correlates with a high level of disease activity (Hofmann et al. 2002), and an elevated risk of relapse after discontinuation of treatment (Bernard et al. 2009, Schmidt & Zillikens 2013, Tampoia et al. 2012). All the same, the NC16A ELISA is not able to detect BP in patients who express
autoantibodies to regions of BP180 other than the NC16A domain (Nishie 2014, Schmidt & Zillikens 2013).

### 2.6.5 Histopathology

Light microscopy of a BP affected skin biopsy cannot differentiate BP from other types of pemphigoid, and is thus an additional investigation. However, characteristic findings are subepidermal splitting and the presence of dermal inflammatory cells, particularly eosinophils. Also other inflammatory cells including lymphocytes and neutrophils can be involved (Di Zenzo et al. 2012, Schmidt & Zillikens 2013).

### 2.6.6 Other diagnostic techniques

As well as ELISA, immunoblotting and immunoprecipitation techniques are also used to investigate the exact molecular target of autoantibodies. This allows the detection of different antigens in different types of pemphigoids, for example laminin \( \gamma \)1, a 200 kilodalton (kDa) glycoprotein, in anti-laminin \( \gamma \)1 pemphigoid (Dainichi et al. 2009). However, as ELISA is much easier to carry out, it has mostly replaced immunoblotting (Di Zenzo et al. 2012).

### 2.7 Treatment of bullous pemphigoid

#### 2.7.1 Drugs used to treat bullous pemphigoid

There have been few prospective, controlled studies of the treatment of BP (Kirtschig et al. 2010). However, both the British Association of Dermatologists and the European Dermatology Forum (EDF) have published guidelines for its management (Feliciani et al. 2015, Venning et al. 2012). There are no official guidelines in Finland, but clinical practices broadly follow the aforementioned guidelines.

According to the British Association of Dermatologists and the EDF guidelines, systemic corticosteroids are the best established treatment for BP, and starting doses between 0.5 and 0.75 mg/kg/day are recommended (Feliciani et al. 2015, Kirtschig et al. 2010, Venning et al. 2012). Doses higher than 0.75 mg/kg do not usually give additional benefit, and the adverse effects of high doses of systemic
corticosteroids are well recognized (Kirtschig et al. 2010). Very potent topical corticosteroids are also effective and safe in extensive BP (Joly et al. 2002), but their use may be limited by practical factors such as cost and, in elderly patients, lack of mobility limiting the body sites to which cream may be self-applied (Feliciani et al. 2015, Kirtschig et al. 2010, Venning et al. 2012). Topical corticosteroids milder than clobetasol propionate 0.05% are also recommended for the treatment of cases with mild and localized symptoms (Joly et al. 2002, Kirtschig et al. 2010, Terra et al. 2014, Venning et al. 2012). Prospective, controlled studies of treatment options are needed, and currently a randomized, controlled trial is underway in the UK and Germany, comparing the safety and efficacy of doxycycline and systemic prednisolone as first-line treatment of BP (Chalmers et al. 2015).

In severe BP that does not respond to oral corticosteroids, many anti-inflammatory and immunosuppressive drugs have been used in order to achieve remission, and to reduce corticosteroid doses. In a French study, 100 patients with extensive BP were randomized to receive prednisolone 1 mg/kg/day, prednisolone 1 mg/kg/day together with azathioprine from 100 to 150 mg/day, or prednisolone 1 mg/kg/day together with four rounds of large-volume plasmapheresis during the first 2 weeks (Guillaume et al. 1993). No differences were seen between groups in terms of the proportion of patients achieving complete remission at day 28 or at 6 months. However, severe complications were more common in the azathioprine group (Guillaume et al. 1993). Another randomized, controlled study comprised 25 BP patients who were treated with prednisone, either alone (30–80 mg/day), or in combination with azathioprine 2.5 mg/kg/day (Burton et al. 1978). This three-year study reported a 45% reduction in the cumulative dose of prednisone in the group which received azathioprine, with minimal additional side effects (Burton et al. 1978). A more recent study compared the efficacy and safety of methylprednisolone 0.5 mg/kg/day combined with either azathioprine 2 mg/kg/day or mycophenolate mofetil 2 g/day (Beissert et al. 2007). The primary outcome was complete remission, which was achieved by all patients in both groups. However, remission was induced sooner in the azathioprine group. Hepatotoxicity was more common in the azathioprine group, but more infections occurred in the mycophenolate mofetil group (Beissert et al. 2007, Bystryn 2008).

One small randomized trial compared the effects of prednisone 40–80 mg/day with those of tetracycline 2 g/day plus nicotinamide 1500 mg/day (Fivenson et al. 1994). In this study, there was no statistically significant difference in the treatment response between these two treatment protocols, but adverse reactions were fewer
in the tetracycline-nicotinamide group. However, the small number of patients in this study limits the conclusions that may be drawn (Fivenson et al. 1994).

Overall, there is little evidence for the benefits of adjuvant therapies. Studies have reported no additional benefit in disease control of adjuvant azathioprine, mycophenolate mofetil or plasma exchange when compared with prednisolone monotherapy. Neither was tetracycline together with nicotinamide more effective than prednisone (Beissert et al. 2007, Burton et al. 1978, Fivenson et al. 1994, Guillaume et al. 1993, Kirtschig et al. 2010, Venning et al. 2012). However, small populations or methodological issues limit the conclusions that can be drawn from many of these studies (Kirtschig et al. 2010). Methotrexate, dapsone, cyclofosfamide, cyclosporin, rituximab, chlorambucil and intravenous immunoglobulin are other examples of therapies that have been used for the treatment of BP, but without supporting evidence from clinical trials (Venning et al. 2012).

The use of therapies for BP varies widely in Europe. In Denmark, azathioprine is frequently used in combination with oral glucocorticoids (Kibsgaard et al. 2014), while in Sweden, methotrexate monotherapy is common (Kjellman et al. 2008). In France, topical treatment with very potent corticosteroid cream (0.05% clobetasol propionate) is preferred to systemic treatment (Joly et al. 2002). However, each patient should be treated individually taking into account factors such as comorbidities, other medications, the patient’s own opinion, and practical factors.

2.7.2 Mechanism of action of glucocorticoid treatment

Glucocorticoids, administered both topically and systemically, are widely used in BP and in other skin diseases (Jackson et al. 2007). In cells, the effects of glucocorticoids are mediated via glucocorticoid receptors, which are expressed in practically all human cell types. After binding to the intracytoplasmic glucocorticoid receptor, the resulting complex is transferred to the nucleus, where it binds to steroid-responsive genes (Jackson et al. 2007, Rhen & Cidlowski 2005). By various mechanisms, glucocorticoids suppress the production of many inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukins (e.g. IL-1, IL-2, IL-6 and IL-8). Glucocorticoids also broadly affect the distribution and activity of all the body’s inflammatory cells. The proliferation of T-lymphocytes is inhibited, while B-lymphocytes are affected only by higher doses of glucocorticoids. Glucocorticoids also lower the numbers of circulating eosinophils and monocyte-macrophage-line cells. In turn, circulating neutrophil
numbers increase because of accelerated release from the bone marrow (Jackson et al. 2007).

2.7.3 Side-effects of glucocorticoid treatment

Short-term glucocorticoid therapy is usually quite well tolerated, but prolonged or high-dose use is associated with many side-effects, especially in elderly people (Table 2).

<table>
<thead>
<tr>
<th>Tissue/system</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal gland</td>
<td>Adrenal atrophy, withdrawal syndrome</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>Hypertension, edema, thrombosis, vasculitis</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Hyperglycemia, dyslipidemia, obesity, hypocalcemia, hypokalemia</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Changes in behavior, cognition, memory, and mood. Peripheral neuropathy</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Gastrointestinal bleeding, pancreatitis, peptic ulcer, esophagitis</td>
</tr>
<tr>
<td>Immune system</td>
<td>Broad immunosuppression, activation of latent viruses</td>
</tr>
<tr>
<td>Skin</td>
<td>Atrophy, purpura, hirsutism, hyperpigmentation, acne, acneiform eruptions, infections, delayed wound healing</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
<td>Osteoporosis, bone necrosis, muscle atrophy, growth retardation</td>
</tr>
<tr>
<td>Eyes</td>
<td>Cataracts, glaucoma</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>Delayed puberty, fetal growth retardation, hypogonadism, amenorrhea</td>
</tr>
</tbody>
</table>

The most prevalent and obvious side-effects are osteoporosis and hyperglycemia. In bone, glucocorticoids induce apoptosis of osteoblasts and increase the activity of osteoclasts resulting in significant demineralization, and consequently, increased risk of bone fractures. Osteonecrosis and myopathy are also known to be associated with glucocorticoid treatment (Jackson et al. 2007).

The development of hyperglycemia is common during prolonged glucocorticoid treatment, especially, when there is pre-existing glucose intolerance.
Glucocorticoids elevate blood glucose by enhancing hepatic gluconeogenesis, and decreasing peripheral glucose uptake in muscles (Jackson et al. 2007). They also cause hyperlipidemia, most commonly the elevation of triglycerides, and hypertension which may result from renal sodium retention (Jackson et al. 2007, Rhen & Cidlowski 2005).

Since glucocorticoids act on BP via various immunosuppressive mechanisms, they carry an elevated risk for many bacterial, viral, fungal, and parasite infections (Jackson et al. 2007). Glucocorticoids also suppress the symptoms of infections, like fever, making them more difficult to recognize. Fungal and cutaneous staphylococcal infections are commonly seen in patients receiving glucocorticoid therapy. A potentially life-threatening infection related generally to immunosuppression and also to glucocorticoid treatment is Pneumocystis jiroveci (formerly known as Pneumocystis carinii) pneumonia. Coexisting hyperglycemia, for its part, makes patients even more susceptible to infections (Jackson et al. 2007).

Other relatively common side-effects of glucocorticoid therapy include hypothalamus-pituitary gland-adrenal gland (HPA axis) suppression, gastrointestinal irritation, acne, cataracts and changes in mood, behavior or cognition (Jackson et al. 2007, Rhen & Cidlowski 2005). Individual patient characteristics affect the risk of side-effects: postmenopausal women are at great risk of osteoporosis, as are patients with rheumatoid arthritis. Any condition that causes hypoalbuminemia leads to an increased free fraction of glucocorticoids, i.e. an increase in the amount the drug biologically active, and thereby, increased toxicity. Smoking, alcohol intake and the consumption of many drugs, for their part, increase the risk for peptic ulcer disease (Jackson et al. 2007). All things considered, BP patients, who are usually elderly people, are prone to the side-effects of glucocorticoids, and it has been suggested that the heightened mortality seen in BP patients may be partly due to the medications used to treat the condition (Feliciani et al. 2015).
3 Aims of the study

Given the lack of information previously reported in Finland or other Scandinavian countries, this study was designed to obtain information about the epidemiology, incidence and mortality of BP in Finland, and its comorbidities. The specific aims of the publications I–III were as follows:

I  To determine the incidence of BP in the NOHD and to document the changes in incidence over time.

II To investigate the mortality and comorbidities of BP patients in the NOHD, medications used to treat BP, and possible associations between treatment and mortality.

III To investigate the association between BP and neurological and psychiatric disorders, temporal causality between these diseases, and the incidence of BP in the whole of Finland.
4 Materials and methods

4.1 Study populations and databases

4.1.1 Northern Ostrobothnia Hospital District study population (I, II)

The study population of the first and the second publications consists of BP patients living in the NOHD, which locates in Northern Finland. The centre of the NOHD is the Oulu University Hospital, which has the only department of dermatology in the area, and the only department of pathology that performs immunofluorescence diagnostics of skin biopsies.

The study population of the first publication covers all BP patients diagnosed in the outpatient clinic or ward of the Oulu University Hospital between 1st Jan 1985 and 31st Dec 2009. The second publication extended this study period to include the years 2010–2012. The study populations in both publications comprised of records retrieved by a search of the Oulu University Hospital database using codes from the ICD versions 8–10 (code 694 in ICD 8, 694.5 and 694.6 in ICD 9, and L12 in ICD 10). The initial BP diagnoses in each case retrieved were further evaluated against the diagnostic criteria used in this study. ICD 8, 9, and 10 were used in Finland between the years 1968 and 1986, 1987 and 1995, and from the year 1996 onwards, respectively (Statistics Finland, http://www.tilastokeskus.fi) (Lönnqvist et al. 2009).

Variables in the Northern Ostrobothnia Hospital District study population

The evaluation of the patient records was done by one researcher (A-K.F.), and a structured form for every patient was used to collect data about diagnostic tests, duration of symptoms, mucosal involvement, treatments used for BP, other medications, comorbidities before and after the diagnosis of BP, and date of death (Fig. 4). If the date of death was not available in a patient’s hospital records, it was obtained from the Local Office of Northern Finland or from Statistics Finland.

Every treatment used for BP was recorded with the data on the duration of each medication, and the order in which they were used. The starting dose of prednisolone was registered as milligrams (mg).

Diagnoses preceding BP and comorbidities were recorded as of interest if they could be classified as one of the following categories: autoimmune disease,
Fig. 4. Data collection form used in the NOHD population (translated into English).

<table>
<thead>
<tr>
<th>Social security number:</th>
<th>Municipality of residence:</th>
<th>Gender: (1=male, 2=female)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis: ________________________
1) pemphigus vulgaris 2) pemphigus foliaceus 3) bullous pemphigoid 4) mucous membrane pemphigoid 5) linear IgA disease

Diagnosis date: __________ dd mm yyyy

Diagnostic methods: ________________________
(0=not known, if the examination has been done or not, 1=positive 2=negative 3=the examination has not been done)

Clinical features:
- Histological examination of the
- Lesional skin biopsy:
- Direct immunofluorescence of the perilesional skin biopsy:
- Indirect immunofluorescence:
  - BP180 ELISA:

How long before the diagnosis symptoms have begun? (select one):
1) < 1 month 2) 1-6 m 3) 6-12 m 4) > 12 m 5) not known

Blisters/erosions (select one):
- 1) skin 2) mucous membranes 3) skin and mucous membranes

Treatment: the order of drugs and the duration of each treatment:
1) < 6 months 2) 6-12 m 3) 1-2 years 4) > 2 y 5) not known

<table>
<thead>
<tr>
<th>Prednisolone, systemic mg (starting/highest dose)</th>
<th>Drug 1, Duration</th>
<th>Drug 2, Duration</th>
<th>Drug 3, Duration</th>
<th>Drug 4, Duration</th>
<th>Drug 5, Duration</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Corticosteroid, topical</td>
<td>__________</td>
<td>__________</td>
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<tr>
<td>Azathioprine</td>
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<tr>
<td>Methotrexate</td>
<td>__________</td>
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<tr>
<td>Dapsone</td>
<td>__________</td>
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<tr>
<td>Tetacycline</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
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<tr>
<td>Other, what</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
</tr>
</tbody>
</table>

Other diseases at the time of the BP diagnosis:
1) yes 2) no

- Myocardial infarction:
- Diabetes:
- Cardiovascular diseases:
- Neurodegenerative diseases:
- Other skin disease(s) besides BP: __________

Other diseases following the BP diagnosis:
1) yes 2) no

- Severe infections (treated in Oulu University Hospital):
- Bone fractures:
- Diabetes:
- Peptic ulcer:
- Psychiatric symptoms:
- Cataracts:

Date of death: __________ dd mm yyyy

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malignancy, type 2 diabetes, cardiovascular disease, neurodegenerative disease or skin condition other than BP. Autoimmune diseases included diseases defined in a study conducted in Denmark (Eaton et al. 2010), except for autoimmune skin diseases, which were registered as ‘other skin condition’. Multiple sclerosis, was recorded as a neurodegenerative disease, although being also of autoimmune origin. Neurodegenerative diseases included e.g. cerebrovascular strokes, dementias and Parkinson’s disease, whereas epilepsy and congenital conditions were excluded. The cardiovascular disease category included all kinds of cardiac and blood vessel diseases. The comorbidities registered following the diagnosis of BP included severe infections, osteoporosis, type 2 diabetes, peptic ulcer, psychiatric symptoms, cataracts, and glaucoma. Diagnoses preceding BP and comorbidities were included, if the disease was mentioned in patient’s record. The verification of diagnoses was not possible.

**Groupings used in the Northern Ostrobothnia Hospital District study population**

In the second publication, the risk of death in BP patients was analyzed in three different treatment groups. The groups were formed according to the expected prognosis for each group. Group one consisted of BP patients treated with oral prednisolone together with topical corticosteroids. Group two was formed of patients treated with topical corticosteroids, with or without oral tetracycline. Group three comprised patients who received both oral prednisolone and an adjuvant immunosuppressant (azathioprine or methotrexate). Patients were not excluded from group three if they had used topical corticosteroids and/or tetracycline and/or dapsone, before prednisolone was initiated.

**4.1.2 National study population (III)**

The study population of the third publication was obtained from the Finnish Care Register for Health Care (formerly named the Finnish Hospital Discharge Register) maintained by the National Institute of Health and Welfare (https://www.thl.fi/en/web/thlfi-en). The register covers all Finnish hospitals managed by local authorities, municipal federations and central government as well as the largest private hospitals, and contains, for example, the identification number of the patient and hospital, primary diagnosis together with three subsidiary diagnoses, and duration of hospital stay. Outpatient visits are included from 1998.
onwards. Studies evaluating the validity of Finnish registries and comparing registry data with patient records, have confirmed that the coverage and accuracy of the Care Register are well-suited for epidemiological research (Sund 2012). However, most of the validation studies have investigated vascular diseases, mental disorders or injuries, and the number of skin diseases in validation studies is small (Aro et al. 1990, Sund 2012).

The third publication covered the study period between 1st Jan 1987 and 31st Dec 2013, and thus, ICD 9 and 10 codes for BP were used (6945A and 6945B in ICD 9, and L12.0 in ICD 10). The control population consisted of patients with basocellular carcinoma (BCC; 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, C44.91), because it is a common diagnosis, not inflammatory of origin, and BCC patients tend to be of a similar age to the BP population. Primary and subsidiary diagnoses were gathered for BP patients and for controls from the Care Register for Health Care. A large range of central and peripheral nervous system diseases, and psychiatric disorders were included in the study (please see III, Table 1). Dementias were classified as CNS diseases, although they belong to the category of mental and behavioural disorders in the ICD 10. Diseases with known non-neuroinflammatory origins, such as congenital conditions, epilepsy, and organic disorders resulting from the use of drugs or psychoactive substances, were excluded.

4.2 Diagnostic criteria

4.2.1 Diagnostic criteria in the Northern Ostrobothnia Hospital District study population (I, II)

The diagnosis of suspected BP patients identified from the Oulu University Hospital patient records was evaluated with the following criteria: clinical features, histopathological and immunopathological examination of skin biopsies, and serological assays including IIF and BP180 ELISA. In publications I and II, the diagnosis of BP was considered to be verified if clinical features were appropriate for BP and either the DIF or IIF was positive. Patients with only clinical features characteristic of BP were excluded, as were patients with only positive histology and/or positive BP180 ELISA.

For a positive BP diagnosis, these studies followed the guidelines of the British Association of Dermatologists for the management of bullous pemphigoid,
(Venning et al. 2012). Clinical features: non-scarring blisters and/or erosions of the skin with or without mucous membrane lesions. In atypical BP cases: eczematous or urticarial, and itchy lesions of the skin without blistering, evaluated by a dermatologist. Histopathologic examination: subepidermal blister. DIF: linear deposits of IgG and/or C3 along the basement membrane zone. IIF: circulating autoantibodies in the serum against the basement membrane zone detected by IIF performed on frozen sections of monkey or rabbit esophagus tissue. ELISA: circulating autoantibodies to recombinant human BP180 protein’s NC16A domain. Histopathological examinations and DIFs were performed at the Department of Pathology, the Oulu University Hospital; IIFs and ELISAs were performed in the HUSLAB, Helsinki (the manufacturer of the BP180 ELISA kit: Mbl, Medical & Biological laboratories Co., LTD, Japan). ELISA assays have been taken in the Oulu University Hospital since 2002 and are analysed in the HUSLAB. The BP180 ELISA kit did not change over the study period.

4.2.2 Diagnostic criteria in the national Care Register for Health Care study population (III)

The third publication was a register based study, and immunological validation of BP diagnoses was not possible. As well as a search hit based on the relevant ICD codes, an age of 40 years or above was required, since BP rarely affects younger individuals, and the possibility of false diagnoses was considered to be high in younger age groups. When analysing comorbid diseases, only primary psychiatric diagnoses made in a specialized care setting were included in order to ensure the validity of psychiatric diagnoses.

4.3 Statistical analyses (I, II, III)

Statistical analyses were carried out using STATA (Data Analysis and Statistical Software, MP 11.2 StataCorp LP, College Station, TX 77845, USA) and PASW (Predictive Analytics SoftWare, Versions 18 and 20, Chicago, IL, USA). The crude incidence of BP was calculated by dividing the number of new BP cases in one year by the number of individuals who were at risk (the mean number of the 40-year-old or above NOHD population within each year). The result was given as the number of BP cases per 1 million persons. The age-adjusted incidences were calculated by the direct standardization method using the general NOHD population and the general European population as references. Incidence rate ratios
(IRR) of BP were estimated by the Poisson regression model, and the results were shown as adjusted and unadjusted IRRs. In adjusted IRRs, age at BP diagnosis (recorded in categories 0–59, 60–69, 70–79, 80–89 and ≥90) and sex were used as potential confounding factors. The change in incidence over time was analyzed in the five-year periods 1985–1989, 1990–1994, 1995–1999, 2000–2004, and 2005–2009. The incidence did not change significantly between the years 1985 and 2004, thus, these years were combined into one time period (1985–2004), and the mean incidence level of this combined time period was used as a reference for the later time period 2005–2009 in the analysis.

When analyzing the mortality of BP patients in different subgroups of treatment, a Kaplan-Meier survival analysis was applied. Standardized mortality ratios (SMR) were calculated by an indirect method, and mortality in the BP study population was compared with mortality in the general population. The impact of polypharmacy on mortality was calculated in the categories 0, 1–5, 6–10, and 11 or more drugs in regular use, and hazard ratios (HR) for different categories were computed by Cox regression analysis. The results were adjusted for age at the time of the diagnosis and for sex. A negative binomial model was used to calculate the risk ratio for the categories of numbers of concomitant medications, and statistical significance was tested by the log-rank test for equality of survival functions. Data on the age structure and mortality in the general Finnish population were provided by Statistics Finland (http://www.tilastokeskus.fi), and data on the general European population by Eurostat (http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search_database).

In the study that investigated the association between BP and neurological and psychiatric disorders, the association between a preceding neurological or psychiatric disorder and BP was analyzed using a logistic regression model, and results were presented as odds ratios (OR). When calculating an association between BP and a subsequent neurological or psychiatric disorder, Cox regression analysis was applied, and results were given as hazard ratios (HR). Both of these statistical models were adjusted for age at the time of the diagnosis and for sex. A latency period was defined as the period between the date of diagnosis of the BP or BCC diagnosis to the date of diagnosis of the neurologic or psychiatric disorder of interest. If the diagnosis of a specified neurological or psychiatric disorder did not emerge, the follow-up stopped at the date of death or at the date of the last recorded visit. In the national population, the age-standardized incidence was calculated using an indirect method.
In all three publications, characteristics of the study populations were expressed as proportions, means, (with standard deviation [SD]) and medians, when appropriate. All rates and ratios were given with a 95% confidence interval (95% CI). A two sided P-values less than 0.05 was considered to be statistically significant.

4.4 Permissions and ethical aspects (I, II, III)

Since this was a register based study, and patients were not contacted, a statement of the ethical committee was not required. The permission to use data from the patient records of the Oulu University Hospital was obtained from the Medical Director of the Oulu University Hospital, and permission to use the national data was granted by the National Institute of Health and Welfare. A description of the scientific research data file concerning e.g. data privacy protection was provided to the Office of the Data Protection Ombudsman, Finland (www.tietosuoja.fi), as requested.
5 Results

5.1 Characteristics of the study populations

5.1.1 Characteristics of the Northern Ostrobothnia Hospital District study population (I, II)

The initial query of the Oulu University Hospital patient records database returned 172 cases of BP living in the NOHD area. Thirteen records were excluded for not meeting the specified diagnostic criteria and the remaining 159 cases were included in the final analysis of publication I. There were 80 females and 79 males, the female-male ratio being almost 1:1, and the mean age at the time of the diagnosis was 77.0 for females and 76.5 years for males. The youngest BP patient was 40 years old and the oldest was 93. The mean age at diagnosis was 73.4 years between 1985 and 1989, 74.0 between 1990 and 1999, and 79.0 between 2000 and 2009. The total population living in the NOHD area increased from circa 340 000 to 400 000 between 1985 and 2009, and the proportion of elderly (70–90-year old) persons increased from 6.6% to 10.0%.

In publication II, the study population of BP patients living in the NOHD area included 198 patients. The cases diagnosed between 1985 and 2009 were the same 159 patients as in article I. In article II, the study period was extended to include the years 2010–2013, and 39 additional patients were included. In this study population, there were 102 females (51.5%) and 96 males (48.5%), the mean±SD age was 77.5 years ±10.4, and range 40–96 years.

5.1.2 Characteristics of the national study population (III)

The national study population included 4524 BP patients diagnosed in Finnish Hospitals between 1987 and 2013. The control population consisted of 66138 BCC patients. All were aged over 40 years. There were 2719 (60.1%) women in the BP population, and 36240 (54.8%) women in the control population. The mean±SD age at the time of diagnosis was 77±11 years for BP and 73±12 years for BCC.
5.2 Clinical features of bullous pemphigoid (I)

Clinical features were analyzed among the NOHD population according to information supplied by the patient records of the Oulu University Hospital. In 148/159 (93.1%) of cases BP manifested as typical blisters or erosion of the skin, and in 11/159 (6.9%) both the skin and oral mucous membranes were affected. There was no apparent difference between females and males regarding the affected sites. Diagnosis was made within one month of initial symptom onset in 27.0% of patients; 72.3% were diagnosed within six months and in 7.6% diagnosis was made more than 12 months after the initial symptoms were reported. The data on the onset of symptoms were missing in 5.0% of cases.

5.3 Incidence of bullous pemphigoid (I, III)

In the NOHD area, the crude incidence was 17 new BP cases per 1 million person-years (95% CI 15 to 20) between 1985 and 2009. When the European standard population was used as a reference, the age-standardized incidence was 14 BP cases per 1 million person-years (95% CI 12 to 17). The crude incidence rates in the periods 1985–1989, 1990–1994, 1995–1999, 2000–2004, and 2005–2009 were 15, 12, 16, 16, and 27, respectively. The incidence of BP was 1.8-fold (IRR=1.8, 95% CI 1.3 to 2.6, p<0.001) higher in the 2005–2009 period than the mean incidence level in the 1985–2004 period. The age- and sex-adjusted IRR was 1.4 (95% CI 1.0 to 2.0, p=0.043).

The age-adjusted incidence was 24 (95% CI 19 to 30) for males and 14 (95% CI 11 to 17) for females. The incidence of BP increased with age in both genders and was highest among elderly men: 451 new cases per 1 million person-years among men between 80–89 years, and 1068 new cases per 1 million person-years among 90–100-year old men.

In the national BP data, the crude incidence of BP was 25 per 1 million per year between 1987 and 2013. Using the European standard population as a reference, the age-adjusted incidence was 18 per 1 million per year. The increasing trend in the incidence of BP over time was also seen in national population (data not shown), but it is partly explained by the involvement of outpatient visits from 1998 onwards.
5.4 Mortality of bullous pemphigoid (II)

In the study population of 198 BP patients from the NOHD, 33 (16.7%) died within the first year of diagnosis, and 60 (30.3%) within two years. When 1-year mortality was compared with that of the age-matched general population, BP patients had 7.6-fold greater risk of death (SMR=7.56, 95% CI 4.98 to 10.14).

5.4.1 Comorbidities and mortality

In the NOHD population (n=198), we found the following comorbidities prior to the diagnosis of BP: cardiovascular diseases in 76.3% of patients, neurodegenerative diseases in 40.9%, other skin conditions (non-melanoma skin cancers included) in 37.4%, type 2 diabetes in 23.2%, malignant disease (excluding non-melanoma skin cancers) in 8.6% and autoimmune diseases other than BP in 3.5%. Of these, only malignant disease predicted increased 1-year mortality, the age- and sex-adjusted HR being 2.4 (95% CI 1.1 to 5.5, p=0.047).

5.4.2 Concomitant medications and mortality

Polypharmacy was common among BP patients (n=197): the number of regularly used concomitant medications (excluding medications for BP) ranged from 0 to 19. While 14 patients (7.1%) did not have any medication, 85 (43.1%) had 1–5 drugs, 74 (37.6%) 6–10 drugs, and 24 (12.2%) 11 or more drugs.

Polypharmacy had a statistically significant association with mortality, and the higher the number of drugs was, the greater the 1-year and 2-year mortality. For example, the patients who had 11 or more drugs in regular use had a 2-fold higher risk of death within two years than those who had 1–5 drugs (age- and sex-adjusted HR=2.15, 95% CI 0.99 to 4.67). The number of regularly used drugs increased over time, since the mean (median) number of drugs was 4.6 (4), 5.1 (5), 6.3 (6) and 7.4 (7) in 1985–1989, 1990–1999, 2000–2009 and 2010–2012, respectively, and a linear 1.17-fold (95% CI 1.07 to 1.31) increasing trend was seen between time periods.

5.4.3 Treatment for bullous pemphigoid and mortality

In 124/198 (62.6%) of cases, the first-line treatment for BP in the Oulu University Hospital was oral prednisolone together with topical corticosteroids (usually 0.1%
The second most common first-line treatment was topical corticosteroid alone in 59/198 (29.8%) of cases, usually for patients with milder symptoms. Tetracycline (with topical corticosteroids) was the initial treatment in 15/198 (7.6%) of cases. In half of the cases (100/198; 50.5%) only prednisolone and topical corticosteroids were used, whereas azathioprine was added to prednisolone in 25 patients, and methotrexate in two patients. Adjuvant therapy has become more common in recent years, since of those patients treated with azathioprine 12%, 16%, 28% and 44% were diagnosed in the 1980s, 1990s, 2000s and 2010s, respectively.

The mortality of BP patients was analyzed according to three different treatment groups (see Materials and methods: Groupings used in the Northern Ostrobothnia Hospital District study population). There were 100 patients in group 1 (prednisolone alone), 40 patients in group 2 (topical corticosteroid with or without tetracycline), and 26 patients in group 3 (prednisolone together with an adjuvant immunosuppressant). The mean age at diagnosis was 77.8 years in group 1, 82.0 in group 2, and 72.5 in group 3.

The patients in group 1 seemed to have the lowest 1- and 2-year survival, but because of limited numbers of cases, especially in group 3, statistical comparison was not reliable. There were no statistically significant differences between groups 1 and 3 in the mean starting dose of prednisolone (45.6 mg and 46.5 mg in groups 1 and 3, respectively, p=0.79) or in the number of concomitant drugs (6.1, 6.3 and 5.0 in groups 1, 2 and 3, respectively, p=0.41). The mortality rate of the entire population remained unchanged throughout the study period.

5.5 Association with neurological diseases (III)

Based on the national data, the most prevalent neurological diseases among BP patients (n=4524) were cerebral infarction (n=859), ‘other or unspecified dementia’ (n=607), and Alzheimer’s disease (n=617). The strongest association was found between MS and BP: MS patients had 5.9-fold greater risk of incident BP compared with controls. Dementias were also significantly associated with subsequent BP with 3.8-fold greater risk in ‘other or unspecified dementia’, 3.6-fold greater risk in vascular dementia, and 2.6-fold greater risk in Alzheimer’s disease. Several other central nervous system diseases preceded BP as well: subarachnoid hemorrhage, intracerebral hemorrhage, and cerebral infarction with 2.1-fold, 2.6-fold, and 1.8-fold greater risks, respectively, compared with controls. The mean time between
dementia and BP was three years whereas MS developed approximately 12 years before BP.

The analysis of risk for BP patients to develop neurological diseases later in life, showed a greater risk with e.g. epilepsy (2.0-fold), dementias (1.2–1.8-fold), viral meningitis (2.3-fold) and viral encephalitis (2.0-fold).

5.6 Association with psychiatric diseases (III)

The most common (primary) psychiatric diagnoses in patients with BP were depression (n=259), ‘neurotic, stress-related and somatoform disorders’ (n=132), and schizotypal and delusional disorders (including schizoaffective disorders) (n=114). The risk for BP was especially heightened in schizophrenia (2.7-fold), in schizotypal and delusional disorders (2.1-fold) as well as in personality disorders (2.2-fold). These disorders preceded BP by approximately 7 to 11 years. In schizotypal and delusional disorders the risk was bidirectional, and the risk for these disorders was 1.8-fold in BP patients compared with BCC controls.
6 Discussion

6.1 Increasing incidence of bullous pemphigoid in Finland (I, III)

This study (I) was among the first to report an increasing incidence of immunologically confirmed BP in an age- and sex-adjusted study population, and the first to report the incidence of BP in Nordic countries. The present study showed, that the incidence of BP in the NOHD increased 1.4-fold between 2005 and 2009 compared with the preceding 20 years. The same kind of increasing trend with the greatest rise in the 2000s was seen in the national Finnish data (III). The increase in the incidence of BP in publication I was, however, smaller than that found in previous studies. A five-fold increase over 11 years has previously been reported in the UK (Langan et al. 2008), and a three-fold increase over 15 years in France (Joly et al. 2012).

It has been discussed whether improvements in the diagnostics and awareness of BP could explain the increase in BP incidence (Bertram et al. 2009, Naldi et al. 2012). In publication I, BP diagnoses were verified by direct or indirect IF, which had been in use over the entire study period. BP180 NC16A ELISA examinations have been taken in the Oulu University Hospital since 2002, but in the present study, BP patients diagnosed based only on clinical features and positive ELISA were excluded from analyses. Moreover, the incidence of BP increased most notably towards the end of the 2000s, by which time the awareness of BP was widespread in the primary health care setting in the area from which this study population was drawn. The Oulu University Hospital has the only dermatology department in the NOHD area, and the only pathology department capable of performing IF examinations. Therefore, all suspected BP patients in the area are referred to the Oulu University Hospital.

The overall incidence of BP in Northern Finland in the 2000s was between 16 and 27 new cases per 1 million person-years. In the 1980s and 1990s, the incidence varied between 12 and 16 per 1 million person-years. These numbers are close to the incidences reported in France between 2000 and 2005 (21.7 per 1 million person-years) (Joly et al. 2012), and in Scotland between 1991 and 2001 (14 per 1 million person-years) (Gudi et al. 2005). Lower incidences were reported in Germany in the 1980s and 1990s: 6.6 and 6.1 new cases per 1 million person-years (Jung et al. 1999, Zillikens et al. 1995), and in France during the same era: approximately 7 BP cases per 1 million person-years (Bernard et al. 1995).
The mean age of BP patients in this study did not differ greatly from that found by previous studies, with the exception of the study by Bernard and his coworkers, in which the mean age, at 82.4 years, was notably higher (Bernard et al. 1995). Generally speaking, the Finnish population is somewhat older than the general European population; the crude incidence over the entire study period in our study was 17 BP cases per 1 million person-years, and the age-standardized incidence (using the standard European population as a reference) 14.

When looking at the incidence of BP in Southern Europe and around the Mediterranean, much lower numbers are seen. The incidences of BP in Tunisia, Kuwait, and Romania have been reported as 3.84, 2.6, and 2.5 per 1 million person-years, respectively (Baican et al. 2010, Nanda et al. 2004, Zaraa et al. 2011). In all of these countries, the reported incidence of pemphigus, another autoimmune skin disease, is higher than that of BP, with genetic factors, different life expectancies, and possibly environmental factors, likely contributing to the differences (Alpsoy et al. 2015).

6.2 High mortality among bullous pemphigoid patients (II)

In the present study, the 1- and 2-year mortality rates of BP patients were high, at 16.7% and 30.3%, respectively. In recent European studies, reports of the one-year mortality of BP patients have ranged between 13% in Spain (Gual et al. 2014) and approximately 40% in France (Joly et al. 2012, Roujeau et al. 1998). Outside Europe, reported mortality rates include 11–23% in the USA (Brick et al. 2014a, Colbert et al. 2004, Parker et al. 2008), 13–23% in China (Li et al. 2013, Zhang et al. 2013), 27% in Singapore (Cai et al. 2013), and 19% in Korea (Lee & Kim 2014). The somewhat higher mortality rates in France compared with other countries could be explained by the slightly older age of French study populations, and higher drop-out rates in some of the studies conducted in other countries (Joly et al. 2012).

Though BP patients are elderly people, the SMR of 7.56 reported in this study indicates that the risk of death within one year of diagnosis in BP patients is 7.6-fold greater than that of the reference population of the same age. The SMRs (calculated for all BP age-groups together) have varied from 1.9 in the USA to 7.6 in our study (Brick et al. 2014a). In Finland, the one-year mortality was quite low, 17%, whereas SMR was among highest. The high SMR might reflect the relatively older structure of the Finnish population compared to the standard European population.
6.3 Factors increasing the mortality rate in bullous pemphigoid (II)

This study showed that factors that increase the 1-year mortality of BP patients include polypharmacy and comorbid malignancies. The higher the number of drugs in regular use, the greater the 1- and 2-year mortality. Polypharmacy can indicate poor general health, which previous studies have shown to increase mortality among BP patients (Joly et al. 2005a). Polypharmacy itself was independently associated with increased mortality in a large population of elderly individuals in a study by Gomez and coworkers (Gomez et al. 2014). In this study, despite adjustment for various confounding factors such as age, gender, comorbidities and smoking status, the risk of death remained 1.8-fold higher in persons receiving six or more drugs daily compared with those who did not use any medication. Another population-based study from Kuopio, Finland reported that excessive polypharmacy (≥10 drugs) was associated with 2.2-fold elevated risk of 5-year mortality in a population aged 80 years and over (Jyrkka et al. 2009). The main limitation of the Finnish study was that comorbidities were not adjusted for (Jyrkka et al. 2009).

It can be argued that polypharmacy is a cause rather than a marker of a heightened risk of death. Nevertheless, there are several theoretical possibilities for the true causality of this association. Because of altered physiology, elderly people are prone to the side-effects of drugs, and multiple medications can also result in unexpected drug-drug or drug-disease interactions. Moreover, drugs that affect the CNS can potentially cause dizziness and cognitive impairment, both of which increase the risk of accidents (Gomez et al. 2014, Jyrkka et al. 2009).

Previously, an association between BP and malignancies has been suspected (Chorzelski et al. 1978), but several recent studies have dispelled such a connection (Cai et al. 2015, Jedlickova et al. 2010, Lindelof et al. 1990, Ong et al. 2014). However, as might be expected, those BP patients who also had a diagnosis of a malignant disease had lower 1-year survival in the present study. Although this study recorded relatively few malignancies – present in approximately 17 (8.6%) BP patients, we found a statistically significant 2.4-fold greater risk of death in those patients compared with BP patients with no accompanying malignancy. The malignancies detected were heterogeneous including for example tumors of the gastrointestinal tract, prostate cancer, lung cancer, breast cancer, ovarian cancer, melanoma, lymphoma and leukemia.
6.4 Systemic glucocorticoids and mortality (II)

Systemic glucocorticoids have been the mainstay of BP therapy for decades (Feliciani et al. 2015, Kirtschig et al. 2010, Venning et al. 2012). However, their use carries a risk for side-effects, particularly among elderly patients. A prospective study randomized 341 BP patients to receive either oral prednisone (1mg/kg for extensive disease, 0.5mg/kg for moderate disease), or topical clobetasol propionate cream 40g/day (for both extensive and moderate BP) (Joly et al. 2002). In this study, the overall survival in extensive BP was significantly better in those treated with topical clobetasol propionate compared with those treated with oral prednisone 1mg/kg. However, there was no difference in survival between topical treatment group and the prednisone 0.5mg/kg group. Disease control in both extensive and moderate BP was even better with the topical treatment (Joly et al. 2002).

In the present study, three treatment groups with different expected prognoses were compared, and a trend towards lower survival of the patients treated solely with oral prednisolone was seen. Unfortunately, the number of cases, especially in group 3 (treated with oral prednisolone together with an adjuvant immunosuppressant) was so small (n=26), that statistical procedures were not reliable.

Nowadays, the international consensus on glucocorticoid treatment of BP is, that starting doses of more than 0.75mg/kg of prednisolone should usually be avoided, but in some treatment resistant cases, doses up to 1 mg/kg may be used (Feliciani et al. 2015, Kirtschig et al. 2010, Venning et al. 2012). In milder disease, lower doses can be sufficient to achieve remission (Feliciani et al. 2015, Kirtschig et al. 2010, Venning et al. 2012). More prospective, controlled studies are needed to investigate glucocorticoid sparing adjuvant therapies and other glucocorticoid-free potential therapies. A well-designed, prospective, randomized study comparing doxycycline and oral prednisolone is currently ongoing in the UK and Germany (Chalmers et al. 2015).

It is not currently possible to predict which patients will experience serious side-effects when starting systemic glucocorticoid therapy for BP. However, attention should be paid to factors that increase the toxicity of glucocorticoids, like low serum albumin, simultaneous use of other drugs that impair gastric mucosa, and comorbidities like diabetes, osteoporosis and heart failure (Jackson et al. 2007).
6.5 Increased risk of bullous pemphigoid in patients with neurological and psychiatric disorders (III)

To be able to investigate neurological and psychiatric comorbidities with adequate statistical power, the study population of the third publication was obtained from the national Finnish Care Register. In publication III, both the CNS and the peripheral nervous system diseases among BP patients were comprehensively examined with the very interesting discovery that many neurodegenerative diseases of the CNS, but none of the peripheral nervous system diseases, were associated with BP. This finding is in line with the hypothesis that BP180, which is expressed both in the skin and the CNS, could be an autoantigen that BP shares with many neurodegenerative diseases (Seppänen 2013). In fact, previous studies have concentrated mainly on the CNS diseases (Brick et al. 2014b, Chen et al. 2011b, Langan et al. 2011, Taghipour et al. 2010) and the present study was the first that also examined the peripheral nervous system diseases on a large scale.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects both the CNS and the peripheral nervous system (Soinila et al. 2006). However, in a large, national study population of more than 9000 BP patients, a bidirectional association was found between BP and ALS (Ong et al. 2013). In the present study, the number of ALS patients was quite small (n=7), which is probably why no statistically significant association emerged.

Another new insight made in this study was the association of BP with psychiatric disorders. Previous studies have not established an association between BP and schizotypal and delusional disorders, personality disorders, or ‘neurotic, stress-related and somatoform disorders’ (Bastuji-Garin et al. 2011, Chen et al. 2011b, Teixeira et al. 2014), as did the present study. The results are in line with those of Chen and coworkers, who reported a 2.6-fold heightened risk of developing BP in patients with schizophrenia (2.7-fold risk in this study), and a 1.8-fold heightened risk of BP in patients with bipolar or unipolar disorders (1.5-fold risk for unipolar, and 1.7-fold risk for bipolar disorder in this study) (Chen et al. 2011b).

However, the strongest association we found was between BP and MS. In patients with pre-existing MS the risk of BP diagnosis was even 5.9-fold greater than in non-MS patients. Similarly, another large, population-based study of more than 5000 MS patients found a 6.7-fold heightened risk of developing BP following an MS diagnosis (Langer-Gould et al. 2010). In the present study, a significantly increased risk of BP was found after a diagnosis of dementia. In contrast to other
studies, the current study also classified dementias into specific diagnostic categories, and found, that the risk for BP was 2.6-fold greater in Alzheimer’s disease, 3.6-fold greater in vascular dementia, and 3.8-fold greater in ‘other or unspecified dementia’ when compared with non-dementia patients. Despite a well-recognized epidemiological association, and the identification of shared autoantigens, the exact molecular mechanisms behind the link between BP and neurodegenerative diseases are still poorly understood.

It is important for clinicians to be mindful of the risk of BP in neurologically ill patients, whose ability to report symptoms of BP, such as intense pruritus, may be impaired. In these cases, anxiety or restlessness may be indicators of pruritus. Pruritus can also disturb sleep, thereby further impairing memory and cognition.

6.5.1 Pathomechanisms behind the epidemiological association between bullous pemphigoid and neurological disorders

Some studies have investigated the association between neurological diseases and BP at molecular level (Chen et al. 2011a, Foureur et al. 2006, Kokkonen et al. 2017, Recke et al. 2016). Interestingly, serum samples of patients with both BP and various neurological disorders have been shown to recognize both BP180 and BP230 antigens in human brain extract (Chen et al. 2011a). A few studies have also shown IgG autoantibodies to BP180 to be present in the sera of patients with dementia or Parkinson’s disease in the absence of BP (Foureur et al. 2006, Kokkonen et al. 2017, Messingham et al. 2016).

A French study comprising 69 dementia patients and 69 controls, all aged over 69 years, measured serum BP180 antibody levels in both groups, and found that the presence of BP180 antibodies was significantly associated with a diagnosis of dementia (Foureur et al. 2006). None of the 138 patients had clinical signs of BP (Foureur et al. 2006).

Another recent study investigated patients with Parkinson’s disease (n=24) and dementia (n=26), both groups without evidence of an autoimmune skin disease (Messingham et al. 2016). The control group comprised of 23 dermatological patients without autoimmune or neurological diseases. In this study, only one patient with unspecified dementia had autoantibodies to the NC16A region of BP180 detectable by commercial ELISA. Nevertheless, when reactivity to BP180 outside the NC16A domain, was also investigated by immunoblotting, nine out of 24 (37.5%) Parkinson’s patients and six out of 26 (23.1%) dementia patients had positive results (Messingham et al. 2016). The authors further investigated the
ability of BP180 reactive Parkinson’s patients’ sera to recognize tyrosine-
hydroxylase positive dopaminergic neurons in the rat substantia nigra, a brain
structure that degenerates in humans with Parkinson’s disease. Very interestingly,
all tested serum samples (n=4) colocalized with tyrosine-hydroxylase positive
neurons. Moreover, when BP180 was absorbed from the sera, the neurons of the
substantia nigra were no longer recognized (Messingham et al. 2016).

A study performed by our own department (Kokkonen et al. 2017) investigated
a population of well-characterized patients with Alzheimer’s disease (n=115) and
40 neurologically healthy controls. This study reported that 18% of Alzheimer’s
patients versus 3% of controls had IgG autoantibodies against the NC16A domain
of BP180 in their sera, detected by both ELISA and immunoblotting. NC16A
autoantibody levels also inversely correlated with scores on the Mini-Mental State
Examination (MMSE) i.e. the higher the antibody levels, the lower the MMSE
score, and the more severe the cognitive impairment. However, the sera of 18
Alzheimer’s patients who had autoantibodies to NC16A did not show binding to
the skin basement membrane in an indirect immunofluorescence (IIF) examination,
and none of the patients had any of the skin symptoms characteristic of BP
(Kokkonen et al. 2017).

Results of some other studies contradict these findings. A study that searched
for autoantibodies to BP180 and BP230 in patients with multiple sclerosis (MS)
and Parkinson’s disease, found fewer positive results in neurological patients than
in healthy controls (Recke et al. 2016). One possible explanation to this result is
that patients in this study were relatively young, with the mean age of Parkinson
patients being approximately 63 years and that of MS patients only 33 years (Recke
et al. 2016). It was not indicated whether the serum samples of this study were, for
example, taken at the time when the neurological disease was diagnosed. This has
relevance, as epidemiological studies have established that at least clinical
symptoms of BP develop several years after a neurological diagnosis (Cordel et al.

The current hypothesis proposed by several research groups (Foureur et al.
2006, Kokkonen et al. 2017, Messingham et al. 2016) is, that BP180 is an antigen
that BP shares with several neurological disorders, which most probably explains
the epidemiological association between these diseases. Still, many details remain
unclear, such as the role of BP180 autoantibodies in progression of neurodegenerative diseases, and what other factors must accompany the presence of BP180 NC16A autoantibodies to precipitate an outbreak of BP.
6.6 Strengths and limitations of the study

The first and second publications (I, II) each included a well-characterized study population, selected according to strict inclusion criteria, with every BP diagnosis confirmed by immunological verification. These studies also had the very long, 25-year study period. A single researcher (A-K.F) examined all patient records and collected extensive data on clinical symptoms, treatments, and comorbidities. However, in the second publication (II), which examined the mortality rate in BP, the small number of deaths prevented firm conclusions from being drawn. Since all the studies were based on hospital records, they may have missed any mild cases of BP that were treated in the primary health care setting.

The third publication (III) captured the second largest BP study population in the world to date (Chen et al. 2011b, Ong et al. 2013) by using national data from the Finnish Care Register for Health Care over a long study period. The Finnish registers are unique worldwide, because they cover practically the entire population of the country and have been maintained for decades. However, in this register-based study, it was not possible to verify the diagnoses of BP. One more limitation was that outpatient visits were only recorded from 1998 onwards in the Care Register.

6.7 Future prospects

New studies are required to clarify the reasons behind the increase of the incidence of BP, which is not explained solely by the aging population. As the greatest increase in its incidence happened in the late 2000s, factors with a rapid effect on the incidence, such as the availability of new drugs, may be involved. For example, recent studies have proposed an association between DPP-4 inhibitors and BP (Bene et al. 2016, Garcia et al. 2016, Stavropoulos et al. 2014). Well-designed epidemiological studies and studies investigating potential molecular mechanisms in drug-induced pemphigoid are needed. As discussed above, large, prospective studies of current treatments for BP are required, as are investigations into alternatives to glucocorticoid therapy, so that elderly BP patients could be treated effectively, but without the harmful, in worst case fatal side-effects. Future challenges include that of describing the molecular mechanisms behind the association between BP and neurological and psychiatric disorders.
7 Conclusions

Based on studies I-III, the following conclusions can be drawn:

- The present incidence of BP in Finland is approximately 27 new cases per 1 million persons per year, and the incidence has increased over time.
- Based on the Northern Finland study population, the mortality rate within one year of a BP diagnosis is approximately 17%, and the SMR is 7.6.
- Common comorbidities in BP patients are: cardiovascular diseases (76%), neurodegenerative diseases (41%), skin conditions other than BP (37%), and type 2 diabetes (23%).
- A diagnosis of malignant disease before BP was present in 8.6% of cases, and a malignant disease predicted a 2.4-fold increased risk of one-year mortality.
- Polypharmacy is common in BP patients, and it is associated with increased mortality.
- Many diseases of the CNS that cause neurodegeneration or neuroinflammation, and many psychiatric disorders are associated with BP. The association is stronger when the neurological or psychiatric disorder precedes BP than vice versa.
- The strongest association between BP and a neurodegenerative disease is that with MS.
- Diseases of the peripheral nervous system are not associated with BP.
List of references


Original publications


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Original publications are not included in the electronic version of the dissertation.
1392. Korhonen, Vesa (2016) Integrating near-infrared spectroscopy to synchronous multimodal neuroimaging : applications and novel findings
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INCIDENCE, MORTALITY, COMORBIDITIES, AND TREATMENT OF BULLOUS PEMPHIGOID IN FINLAND

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