Tampere University

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"It always seems impossible until it is done."

Nelson Mandela

To the memory of my loving grandparents
Ida and Viljam who always believed in me.

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Pirkkala, August 2019

Eriika Mansikka

ABSTRACT

Dermatitis herpetiformis (DH) is an extraintestinal manifestation of coeliac disease presenting with an intensely itchy and blistering rash mainly on the elbows, knees, and buttocks. Diagnosis is based on the demonstration of granular IgA deposits in the papillary dermis by examination with direct immunofluorescence (IF). The disease is caused by gluten, a protein found in wheat, rye and barley, which initiates an autoimmune response in genetically predisposed individuals. This leads to small bowel mucosal damage typical of coeliac disease and, in some individuals, to a blistering rash typical of DH. At present, 13 % of adults with coeliac disease have DH in Finland. The incidence of DH is decreasing, whereas the reverse is true for coeliac disease. The mainstay of treatment for DH and coeliac disease is a life-long gluten-free diet (GFD), which in DH also heals the rash.

In the research presented in the present dissertation, a cohort of patients with DH diagnosed between 1970 and 2014 at Tampere University Hospital, Finland were investigated. The first aim was to evaluate diagnostic delay in DH. The second aim was to study whether small bowel histological findings have changed over the 45-year period and to determine how mucosal damage correlates to serum transglutaminase 2 (TG2) antibody levels. The third aim was to examine if the presence or absence of small bowel villous atrophy at diagnosis affects the long-term prognosis of DH patients on a GFD. The fourth aim was to examine, by gluten challenge, whether DH patients on a long-term GFD treatment could have developed gluten tolerance, as suggested by a few earlier studies.

The dissertation consists of four separate studies. In Study **I**, the duration of the rash before diagnosis was examined from hospital records in 446 DH patients. The diagnosis was considered delayed when the duration of the rash before diagnosis was two years or longer. Factors associated with the delayed diagnosis were analysed in more detail using follow-up questionnaires obtained from 217 patients. Over the study period, the median duration of the rash before diagnosis decreased and the number of patients with delayed diagnosis decreased. Female gender and the presence of villous atrophy correlated with the delayed diagnosis, whereas age at diagnosis and the activity of the rash did not. According to the follow-up questionnaire, bone fractures or malignancies were shown not to occur more often

in those patients with a delayed diagnosis compared to those with a non-delayed diagnosis.

In Study **II**, the severity of small bowel villous atrophy was examined in 393 DH patients over the 45-year study period. The prevalence of severe (subtotal/total) villous atrophy (SVA) was shown to decrease over time. At the same time, an increase was seen in both partial villous atrophy (PVA) and normal villous architecture. Patients with villous atrophy had higher TG2 antibody levels than those with normal villous architecture. However, several patients with villous atrophy had normal TG2 antibody levels, indicating that a negative test result does not always exclude villous damage in DH.

In Study **III,** long-term prognoses were compared between DH patients with and without small bowel villous atrophy at diagnosis (n=352) and 128 coeliac disease controls. Initial data was gathered from the patient records and follow-up data was collected via questionnaires from 181 DH patients on a GFD. At the DH diagnosis, 98 (28%) patients had normal villous architecture and 254 (72%) had villous atrophy. Clinical recovery did not differ significantly between the DH groups, nor did the presence of long-term illnesses, coeliac disease-related complications or quality of life (QoL). By contrast, the coeliac disease controls had osteopenia/osteoporosis, thyroid diseases and malignancies more often compared to the DH patients.

In Study **IV**, 19 asymptomatic DH patients who had adhered to a GFD for a mean of 23 years were challenged with gluten for up to 12 months. Before the challenge skin biopsies showed negative IgA and transglutaminase 3 (TG3) deposits in 84% of the patients and normal villous mucosa in all of them. The gluten challenge caused a relapse of the rash and/or villous atrophy in 18 (95%) DH patients; 15 (79%) patients showed a rash within a mean of 5.6 months and three (16%) had only small bowel villous atrophy.

The results of the present dissertation show that diagnostic delay in DH has decreased over time. Further, the prevalence of SVA decreased during the 45 years study period and high serum TG2 antibody levels reveals rather well whether the patients have villous atrophy. However, the presence of villous atrophy at the time of diagnosis was shown not to effect GFD treatment response or long-term morbidity and QoL and hence has no effect on the prognosis of DH. Importantly, the gluten challenge showed that a life-long GFD treatment remains justified in all DH patients.

TIIVISTELMÄ

Ihokeliakia on keliakian suoliston ulkopuolinen manifestaatio, joka aiheuttaa kutisevan ja pienirakkulaisen ihottuman. Ihottumaa esiintyy tyypillisesti kyynärpäissä, polvissa ja pakara-alueella. Ihokeliakian diagnoosi perustuu terveeltä iholta otettavaan koepalaan ja siitä tehtävään immunofluoresenssitutkimukseen, jossa havaitaan rakeiset IgA kertymät verinahassa. Sairauden aiheuttaa gluteeni eli vehnän, rukiin tai ohran proteiini, joka käynnistää immuunireaktion geneettisesti alttiilla henkilöllä. Immuunivälitteisen reaktion seurauksena suolistoon tulee keliakialle tyypillinen nukkavaurio tai tulehdus ja osalla ihmisistä iholle kehittyy lisäksi ihokeliakialle tyypillisiä rakkuloita. Nykyisin Suomessa 13%:lla aikuisista keliakiapotilaista on ihokeliakia. Ihokeliakian ilmaantuvuus on laskussa, kun taas keliakian ilmaantuvuus päinvastoin lisääntyy. Ihokeliakian ja keliakian hoitona on elinikäinen gluteeniton ruokavalio, joka parantaa suoliston vaurion sekä ihokeliakiassa myös ihottuman.

Tämän väitöskirjatyön tutkimuskohorttina olivat vuosina 1970-2014 Tampereen Yliopistollisessa sairaalassa diagnosoidut ihokeliakiapotilaat. Ensimmäisenä tavoitteena oli tutkia ihokeliakian diagnostista viivettä. Toisena tavoitteena oli selvittää ihokeliakiapotilaiden suolivaurion vaikeusasteessa tapahtuneita muutoksia ja sitä, kuinka seerumin transglutaminaasi (TG) 2 vasta-aineet liittyvät suolivaurioasteeseen. Kolmantena tavoitteena oli selvittää, vaikuttaako diagnoosivaiheen suolivaurion olemassaolo hoidettujen ihokeliakiapotilaiden pitkäaikaisennusteeseen. Neljäntenä osatyönä oli gluteenialtistus, jossa tutkittiin, pitkään gluteenittomalla ruokavaliohoidolla olleiden ihokeliakiapotilaiden gluteenin sietokyvyn palautumisen mahdollisuutta, kuten muutamissa aiemmissa tutkimuksissa oli esitetty.

Väitöskirja koostuu neljästä erillisestä osatyöstä. Osatyössä **I** tutkittiin 446 ihokeliakiapotilaalla diagnostista viivettä eli iho-oireiden alkamisesta diagnoosiin kestänyttä aikaa sairaskertomuksista kerätyllä aineistolla. Diagnoosia pidettiin viivästyneenä, jos oireiden alkamisesta diagnoosiin kesti kaksi vuotta tai enemmän. Viivästyneeseen diagnoosiin liittyviä tietoja täydennettiin kyselytutkimustiedolla (n=217). Diagnostisen viiveen mediaaniaika lyhentyi sekä viivästynyt diagnoosi muuttui harvinaisemmaksi seurantajakson aikana. Viivästyneeseen diagnoosiin

yhdistyi naissukupuoli sekä vaikeampiasteinen suolen nukkavaurio, mutta ei diagnoosi-ikä tai iho-oireiden vaikeusaste. Potilailla joiden diagnoosi oli viivästynyt, ei kuitenkaan todettu useammin luunmurtumia tai lisääntynyttä syöpäriskiä verrattuna potilaisiin, joilla diagnoosiin päästiin nopeammin.

Osatyössä **II** tutkittiin suolen nukkavaurion olemassaoloa 393 ihokeliakiapotilaalla 45 vuoden tarkastelujakson aikana. Vaikea suolivaurio harvinaistui tarkastelujakson aikana, kun taas lievempi suolivaurio ja normaali suolirakenne yleistyivät. Seerumista tutkittavat TG2 vasta-aineet olivat useammin koholla silloin, kun suolistossa todettiin vaurio, verrattuna tilanteeseen, jossa nukkavauriota ei todettu. Osalla tutkittavista vasta-aineet olivat kuitenkin negatiiviset ohutsuolen nukkavauriosta huolimatta. Vasta-aineita tutkimalla ei täten löydetä kaikkia ihokeliakiapotilaita, joilla on suolivaurio.

Osatyössä **III** tutkittiin, vaikuttaako suolivaurion olemassaolo ihokeliakiapotilaan pitkäaikaisennusteeseen. Sairaskertomustieto kerättiin 352 ihokeliakiapotilaalta, joista diagnoosivaiheessa 98:lla (28%) oli todettu normaali suolirakenne ja 254 (72%) potilasta, joilla oli todettu suolivaurio sekä kontrollina olleilta 128 keliakiapotilaalta. Tietoja täydennettiin kyselytutkimuksen avulla 181 ihokeliakiapotilaalta. Ihokeliakiapotilaiden välillä ei havaittu eroa iho-oireiden keston, keliakiaan yhdistettyjen komplikaatioiden, pitkäaikaissairauksien tai elämänlaadun välillä. Keliakiakontrolleilla todettiin kuitenkin useammin osteopeniaa tai osteoporoosia, kilpirauhassairauksia ja syöpiä verrattuna ihokeliakiapotilaisiin.

Osatyössä **IV** toteutettiin 12 kuukauden gluteenialtistus oireettomille, keskimäärin 23 vuotta gluteenittomalla dieetillä olleille, ihokeliakiapotilaille. Ennen altistusta potilailla ei todettu nukkavauriota suolistossa ja 84%:lla ei todettu ihokoepalassa IgA/TG3 kertymiä. Altistus johti 18 (95%) potilaalla iho-oireiden ja/tai suolivaurion uusiutumiseen eli relapsiin. Iho-oireita tuli keskimäärin 5.6 kuukauden kuluttua 15 (79%) potilaalle ja kolmella (16%) todettiin vain suolivaurio relapsin yhteydessä.

Väitöskirjatyön tulokset osoittavat, että ihokeliakian diagnostinen viive on lyhentynyt. Ohutsuolivaurioaste on ihokeliakiassa lieventynyt 45 vuoden aikana, ja seerumin korkeat TG2 vasta-aineet liittyvät usein ohutsuolivaurion olemassaoloon. Diagnoosivaiheen suolivaurion olemassaolo ei kuitenkaan vaikuta gluteenittoman ruokavaliohoidon vasteeseen eikä sairastumisriskiin tai elämänlaatuun ihokeliakiassa. Gluteenialtistus tuki aiempaa tietoa siitä, että tiukka gluteeniton ruokavalio on edelleen välttämätön kaikilla ihokeliakiapotilailla.

CONTENTS

ABBREVIATIONS

AGA	antigliadin antibodies
ARA	antireticulin antibodies
BMD	bone mineral density
CI	confidence interval
DGP	deamidated gliadin peptides
DH	dermatitis herpetiformis
DLQI	The Dermatology Life Quality Index
ELISA	enzyme-linked immunosorbent assay
EmA	endomysium antibodies
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
GFD	gluten-free diet
GSRS	The Gastrointestinal Syptom Rating Scale
HLA	human leukocyte antigen
i.e.	id est, in other words
IEL	intraepithelial lymphocyte
IF	immunofluorescence
IFN-γ	interferon-γ
IgA	immunoglobulin A
IL	interleukin
PGWB	The Psychological General Well-Being questionnaire
PVA	partial villous atrophy
Q_1	lower quartile
Q_3	upper quartile
QoL	quality of life
SVA	subtotal/total villous atrophy
TCR	T-cell receptor
TG2	transglutaminase 2 or tissue transglutaminase
TG3	transglutaminase 3 or epidermal transglutaminase
TG6	transglutaminase 6

Vh/CrD	villous height, crypt depth ratio

ORIGINAL PUBLICATIONS

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

I Mansikka E, Salmi T, Kaukinen K, Collin P, Huhtala H, Reunala T, Hervonen K. (2018) Diagnostic delay in dermatitis herpetiformis in a high-prevalence area. Acta Derm Venereol 98: 195-199

II Mansikka E, Hervonen K, Salmi TT, Kautiainen H, Kaukinen K, Collin P, Reunala T. (2017) The decreasing prevalence of severe villous atrophy in dermatitis herpetiformis: a 45-year experience in 393 patients. J Clin Gastroenterol 51: 235-239

III Mansikka E, Hervonen K, Kaukinen K, Collin P, Huhtala H, Reunala T, Salmi T. (2018) Prognosis of dermatitis herpetiformis patients with and without villous atrophy at diagnosis. Nutrients 10: 641

IV Mansikka E, Hervonen K, Kaukinen K, Ilus T, Oksanen P, Lindfors K, Laurila K, Hietikko M, Taavela J, Jernman J, Saavalainen P, Reunala T, Salmi T. (2019) Gluten challenge induces skin and small bowel relapse in long-term gluten-free diet treated dermatitis herpetiformis. J Invest Dermatol. Epub ahead of print 15.4.2019 doi: 10.1016/j.jid.2019.03.1150

INTRODUCTION

Dermatitis herpetiformis (DH) is an autoimmune disease characterized by a highly itchy and symmetrical rash typically on elbows, knees, and buttocks (Bolotin and Petronic-Rosic, 2011a). The majority of DH patients have small bowel villous atrophy, but only a minority have overt gastrointestinal symptoms (Gawkrodger et al., 1984, Reunala et al., 1984). In 2011, the prevalence of DH in Finland was 75 per 100, 000, which is the highest reported rate to date (Salmi et al., 2011).

Similarly to coeliac disease, DH patients have immunoglobulin A (IgA) autoantibodies in the serum and small bowel mucosa against the transglutaminase 2 (TG2) enzyme, which occurs in the small intestine and other tissues (Dieterich et al., 1998, Korponay-Szabó et al., 2004). Diagnosis of DH is based on the clinical picture and pathognomonic IgA deposits in the papillary dermis detected in an uninvolved skin biopsy with a direct immunofluorescence (IF) examination (Zone et al., 1996).

The treatment for DH and coeliac disease is a life-long gluten-free diet (GFD). In DH, a GFD heals both the enteropathy and the rash. As alleviation of the rash is often slow, additional treatment with dapsone medication is frequently needed upon starting a GFD. The long-term prognosis of DH patients on a GFD seems to be good, but there is an increased risk of lymphoma during the first five years following diagnosis (Lewis et al., 1996). However, it is not known whether a delay in DH diagnosis and the presence or absence of small bowel villous atrophy affect the prognosis. Surprisingly, there have been studies suggesting that some DH patients adhering to a GFD or normal gluten-containing diet could re-develop gluten tolerance and go into remission (Bardella et al., 2003, Paek et al., 2011).

The aim of the present study was to investigate diagnostic delay in a large DH cohort collected prospectively at the Tampere University Hospital, Finland. As small bowel biopsies have been taken routinely from this DH cohort, additional aims were to examine whether mucosal findings have changed over the past 45 years and whether the presence villous atrophy affects the prognosis. A further aim was to examine how TG2 antibodies associate with the villous atrophy in DH. Finally, a gluten challenge study was performed with long-term GFD-treated DH patients to investigate whether gluten tolerance had re-developed to some of the patients.

REVIEW OF THE LITERATURE

1 DERMATITIS HERPETIFORMIS

1.1 History

Dermatitis herpetiformis (DH) was initially delineated and named by the American dermatologist Louis Duhring at the University of Pennsylvania in 1884 (Duhring, 1884). In 1940, it was discovered that sulphones relieved the intensively itchy rash in DH and thereafter response to this medication was used as a diagnostic tool for DH (Costello, 1940). Histological skin biopsy findings for DH were documented in the early 1960s (Pierard and Whimster, 1961). A breakthrough in the diagnosis of DH was made in 1969, when granular immunoglobulin A (IgA) deposits were found at the dermal papillae of DH patients by direct IF examination (Van der Meer, 1969). Subsequently, this finding became the diagnostic hallmark of the disease (Seah and Fry, 1975, Zone et al., 1996). Moreover, it was demonstrated in the 1960s that the majority of DH patients have enteropathy, which was usually asymptomatic (Marks et al., 1966). Thereafter, it was documented that both enteropathy (Fry et al., 1969) and the rash responded to a strict gluten-free diet (GFD) (Fry et al., 1973, Reunala et al., 1977). Later, it was shown that a gluten challenge induced a relapse of the DH rash and small bowel villous atrophy, indicating that adherence to a GFD should be life-long in DH (Leonard et al., 1983a). The genetic association between DH and coeliac disease became evident when the same human leukocyte antigen (HLA) alleles were found to predispose to both conditions, and DH and coeliac disease were shown to occur even in the same families (Katz et al., 1972, Reunala et al., 1976). Based on all of the observations above, it became obvious that DH is a specific cutaneous manifestation of coeliac disease, and not only an associated condition as was previously often thought (Reunala, 1998).

1.2 Clinical features

DH is characterized as a highly itchy and symmetrical rash with small papulovesicular eruption favouring the extensor surfaces of the elbows and knees (Bolotin and Petronic-Rosic, 2011a, Figure 1.). Additionally, the buttocks and scalp can be

affected, and more rarely also the upper back, abdomen, or face. Oral blisters are rare in DH (Patinen et al., 2002). In addition to representative herpetiform vesicles, the clinical spectrum can comprise erythematous macules and urticarial plaques and papules. Intense pruritus and easily excoriated vesicles often result in the presentation of crusted papules, erosions, and erythaema (Bolotin and Petronic-Rosic, 2011a, Junkins-Hopkins, 2010). The severity of the DH rash can range from mild to severe in different individuals, and even in the same individual the rash can show a fluctuating course of disease activity. Palmar purpura is one – albeit more infrequent – presentation of DH, and it can be the sole presentation or occur coincidentally with the typical blistering DH rash (Karpati et al., 1986).

Linear IgA disease resembles DH closely (Chanal et al., 2013), and pruritic skin diseases, such as scabies or atopic dermatitis can be difficult to differentiate from DH (Bolotin and Petronic-Rosic, 2011a). Regardless of the coexisting enteropathy, obvious gastrointestinal symptoms are rare at the DH diagnosis. The prevalence of gastrointestinal symptoms in DH has varied from 10% to 38% in previous Finnish studies, with the prevalence being highest in children with DH (Alakoski et al., 2012, Pasternack et al., 2017, Reunala et al., 1984). When present, the gastrointestinal symptoms are generally mild and typically include abdominal pain or loose stools (Collin et al., 2017, Kárpáti, 2004).

Similarly to coeliac disease, DH has also been associated with other autoimmune diseases (Gaspari et al., 1990, Reijonen et al., 1991). Studies featuring patients with DH showed that 1–2 % have type 1 diabetes and the frequency of autoimmune thyroid disease is as high as 4% (Gaspari et al., 1990, Hervonen et al., 2004, Reijonen et al., 1991, Reunala and Collin, 1997). Sjögren syndrome, vitiligo, and alopecia areata are diseases which have associated at a low frequency with DH (Reunala and Collin, 1997). Most of the associated autoimmune diseases seem to develop prior to the DH diagnosis (Reunala and Collin, 1997). However, a recent register study from Finland found that patients with previously diagnosed DH have a 22-fold risk for the later development of bullous pemphigoid (Varpuluoma et al. 2019).

1.3 Diagnosis by skin biopsy

DH diagnosis is based on the typical clinical picture and pathognomonic granular IgA deposits in the papillary dermis detected with direct IF (Zone et al., 1996, Figure 2). IF test is known to be highly sensitive (95%) for DH (Bresler and Scott, 2015), but in order to avoid false-negative results, the skin biopsy should be obtained from

normal appearing skin near the rash, i.e. the perilesional area, since deposition is less intense in the inflamed and never involved skin areas (Zone et al., 1996).

Granular IgA in DH skin is known to target the epidermal transglutaminase (TG3) enzyme (Sárdy et al., 2002). This is closely related, but not identical to tissue transglutaminase (TG2) (Lorand and Graham, 2003), which is a major autoantigen in coeliac disease (Dieterich et al., 1998). IgA and TG3 have been shown to disappear from the dermis of DH patients on a GFD at the same time (Hietikko et al., 2018a), but surprisingly, IgA deposits can persist in the skin for many years after the skin symptoms are controlled with a GFD (Garioch et al., 1994, Reunala et al., 2015b). Consequently, IgA deposits are probably present in the skin even if a patient has initiated GFD a few weeks or months before the IF examination. However, it is recommended that the skin biopsy is taken when the patient is still on a gluten-containing diet. Only after the confirmed DH diagnosis, the patients should be advised to adhere to a strict GFD.

Histopathological findings in a lesional skin biopsy are not required for a DH diagnosis (Bolotin and Petronic-Rosic, 2011b). If taken, ideal areas are an intact vesicle or erythematous skin, and the typical findings include non-specific subepidermal blister and papillary microabscesses together with neutrophil and a few eosinophil infiltrates (Pierard and Whimster, 1961).

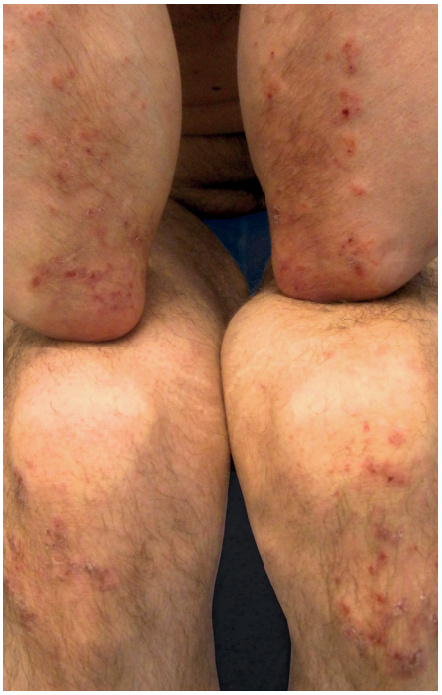

Figure 1. Symmetrical dermatitis herpetiformis rash with excoriated blisters and crusts on the elbows and knees.

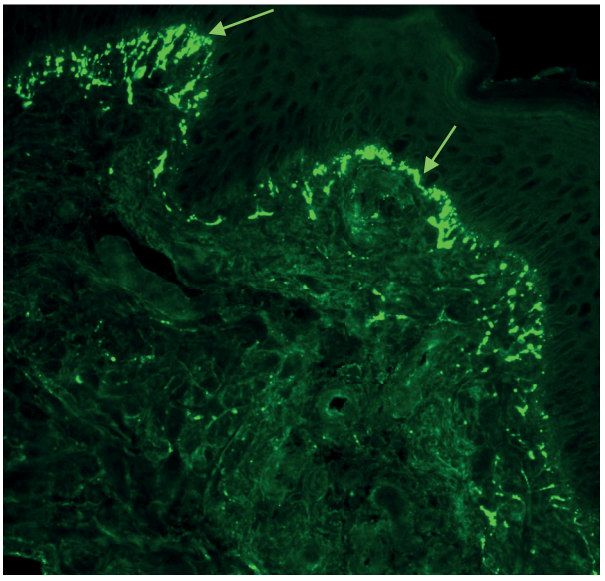

Figure 2. Granular immunoglobulin A (IgA) deposits at the papillary dermis (arrows) of dermatitis herpetiformis patient detected with direct immunofluorescence examination.

1.4 Small bowel findings and serum antibodies

At diagnosis, approximately 75% of DH patients have small bowel mucosal villous atrophy and crypt hyperplasia identical to coeliac disease (Fry et al., 1969, Marks et al., 1966, Reunala et al., 1977), and the remainder have normal villous architecture but coeliac-type inflammation with increased densities of intraepithelial lymphocytes (IELs). Similarly to coeliac disease, an increased density of γδ+ IELs studied from frozen small bowel mucosal samples is relatively representative finding in DH (Järvinen et al., 2003, Savilahti et al., 1997). Furthermore, it was shown already in 1988 that the small bowel mucosa of children with DH contain deposited IgA (Kárpáti et al., 1988). In 2004, Korponay-Szabó and colleagues discovered that IgA deposits detected in frozen small bowel mucosal samples of both coeliac disease and DH patients target the TG2 enzyme (Korponay-Szabó et al., 2003). Thereafter, it has been shown that the majority of DH patients have gluten-dependent TG2-specific IgA deposits in the small bowel mucosa, but the prevalence of these deposits seems to be lower than in coeliac disease (Koskinen et al., 2008, Salmi et al., 2014). At present, examinations of γδ+ IELs and TG2-specific IgA deposits from frozen small bowel mucosal samples are special investigations used only for study purposes in DH.

At diagnosis, DH patients have a similar autoantibody response in the serum as coeliac disease patients, and these antibodies are described in detail in Chapter 2.2. In both DH and coeliac disease, the circulating IgA autoantibodies described first were against reticulin (ARA) (Seach et al., 1971, Vainio et al., 1986), then against endomysium (EmA), and finally against TG2, which is considered the autoantigen of coeliac disease (Dieterich et al., 1998, Dieterich et al., 1999). EmA and TG2-antibody tests both measure antibodies directed against TG2 (Korponay-Szabó et al., 2003), but since the indirect IF EmA test is more laborious and subjective in the interpretation, the TG2 antibody ELISA test is now used as the first-line serological screening test in coeliac disease (Giersiepen et al., 2012).

In DH studies, the IgA-class TG2 antibody and EmA test have shown a specificity of 98% but slightly lower sensitivity figures (89-93%) than for coeliac disease (Dieterich et al., 1997, Kumar et al., 2001, Reunala et al., 1987). There is preliminary evidence suggesting that the occurrence of TG2-targeted antibodies in the serum reflects the severity of small intestinal damage in DH (Dahlbom et al., 2010, Hällström, 1989, Reunala et al., 1987). TG2-targeted coeliac autoantibody levels can be utilized in the follow-up of GFD treatment (Reunala et al., 2015a).

In addition to TG2 antibodies, circulating IgA-class autoantibodies against TG3 exist in the majority of DH patients at diagnosis (Hull et al., 2008, Sárdyet al., 2002). Even though TG3 is considered the autoantigen of DH, circulating antibodies against TG3 are occasionally detected also in the serum of coeliac disease patients without DH (Hull et al., 2008, Reunala et al., 2015a, Sárdy et al., 2002). However, these antibodies are less prevalent, the titres are lower, and the antibodies express a lower affinity for TG3 in coeliac disease compared to DH (Reunala et al., 2015a, Salmi et al., 2016, Sárdy et al., 2002). In addition, gluten-dependency is not as evident as it is in DH (Salmi et al., 2016) where TG3 antibody levels have shown to decrease on GFD in parallel to the levels of TG2 antibodies (Reunala et al., 2015a). Currently serum TG3 antibody measurements are solely used in research setting as their specificity for DH and exact role is yet to be fully elucidated.

Moreover, measurement of IgA-class antibodies against gliadin (AGA) are no longer used in DH diagnostics, and the clinical validity of deamidated gliadin peptide (DGP) antibodies is currently obscure (Jaskowski et al., 2010, Reunala et al., 1987).

2 COELIAC DISEASE

2.1 Clinical features

In the past, coeliac disease was considered a rare malabsorptive disease causing severe steatorrhoea, cachexia, and the failure to thrive mostly in children (Visakorpi and Immonen, 1967). However, presently coeliac disease is widely recognized as the most common food-related disorder, and it can be diagnosed at any age. Furthermore, the clinical spectrum of the disease is highly versatile. According to a recent classification, coeliac disease can be divided into classical, non-classical and asymptomatic coeliac disease (Ludvigsson et al., 2013).

The classical presentation of coeliac disease includes gastrointestinal and malabsorptive symptoms such as diarrhoea, abdominal pain, weight loss, nutritional deficiencies, and growth retardation in children (Ludvigssonet al., 2013, Visakorpi and Mäki, 1994). These symptoms are not specific to coeliac disease, and further, it must be recognized that the symptoms can be mild or vague, for example moderate gastrointestinal discomfort, loose stools, or flatulence without malabsorption.

Non-classical symptoms are nowadays increasingly common in coeliac disease (Fasano and Catassi, 2001, Green and Cellier, 2007) and symptoms can derive from various organs other than the gastrointestinal tract. Besides DH, the cutaneous manifestation of coeliac disease, the best described extraintestinal symptoms of coeliac disease include neurological conditions such as gluten ataxia, polyneuropathy, and epilepsy (Hadjivassiliou et al., 2006, Jericho et al., 2017), joint pain or arthritis (Nurminen et al., 2018, Savilahti et al., 2010) or gynaecological problems, such as delayed puberty, infertility, and recurrent miscarriages (Tersigni et al., 2014). Dental enamel defects found in adult patients' permanent teeth are due to subclinical childhood coeliac disease, and they can also be present in DH (Aine et al., 1992, Ballinger et al., 1994, Collin and Reunala, 2003). Extraintestinal symptoms further include liver problems, and the severity can vary from mild reversible dysfunction and hypertransaminasemia to liver failure (Bardella et al., 1995, Kaukinen et al., 2002).

Coeliac disease can also be diagnosed in totally asymptomatic individuals, and these patients are typically diagnosed through screening (Fasano and Catassi, 2001).

Serological mass screening of the general population is not recommended at present; instead, active case findings with screenings among patients with an increased risk for coeliac disease is currently highly advised (Husby et al., 2012). The most important patient groups with an increased risk of coeliac disease – and thus also the target groups for coeliac disease screening – are listed in Table 1. The highest prevalence of coeliac disease is in first-degree relatives and especially in monozygotic twins and siblings with the same HLA risk alleles (Fasano et al., 2003, Greco et al., 2002, Singh et al., 2015). Moreover, coeliac disease is more common in patients with autoimmune thyroid disorders (Reunala and Collin, 1997, Roy et al., 2016) as well as patients with multiple endocrine diseases (Collin et al., 2002). In addition, an increased risk for coeliac disease is found in patients with a selective IgA deficiency, and IgG class coeliac antibodies should be used for screening in these patients (Collin et al., 1992).

Table 1. The most important patient and disease groups with an increased risk of coeliac disease.

Risk groups for coeliac Disease	Prevalence of coeliac disease (%)
First degree relatives	8–10 %
Type 1 diabetes	5–9 %
Hyper- or hypothyreoiditis	2–6 %
Sjögren's syndrome	2–4%
Addison disease	6–8%
Selective IgA deficiency	7–10 %
Down syndrome	4–14 %

Data collected from Collin, 1992; Collin and Mäki, 1994; Gale et al., 1997; Bonamico et al., 1998; Collin et al., 2002; Biagi et al., 2006; Fasano et al., 2003; Mårild et al., 2013a; Mårild et al., 2016; Singh et al., 2015; and Kurppa et al., 2018.

2.2 Diagnosis by small bowel biopsy and autoantibodies

The gold standard in the diagnosis of coeliac disease is the small bowel biopsy, obtained during gastroscopy, revealing villous atrophy and crypt hyperplasia. Biopsies should be taken while the patient is adhering to a normal gluten-containing diet, and multiple biopsy specimens should be taken to detect patchy villous atrophy (Lebwohl et al., 2011, Pais et al., 2008). Proper specimen orientation is essential as it reduces the risk of both false-negative and -positive results (Arguelles-Grande et al., 2012, Taavela et al., 2013).

In coeliac disease, the small bowel mucosal damage progresses gradually from inflammatory changes to overt villous atrophy and crypt hyperplasia when the patient is consuming gluten. The Marsh classification has been used to describe the stepwise development of mucosal lesions (Marsh, 1992). To assess the mucosal damage in more detail, villous height/crypt depth ratios (Vh/CrD) should be measured from at least three different sites, and generally mean values of Vh/CrD ≥2 are considered normal (Kuitunen et al., 1982, Taavela et al., 2013). However, in clinical practice, small bowel biopsy findings are often divided more roughly into three subgroups: normal villous architecture, partial villous atrophy and severe villous atrophy.

An increase in small bowel mucosal IELs is typical for coeliac disease and considered the first detectable abnormal histological feature (Marsh, 1992). The lymphocytes in the lamina propria are mainly CD3+ IELs, and these cells express either T-cell receptor (TCR) αβ+ or γδ+. In untreated coeliac disease, the densities of αβ+ and γδ+ cells are elevated, and an especially increased density of γδ+ T-cells is considered relatively specific for coeliac disease (Holm et al., 1992; Järvinen et al., 2003). Furthermore, densities of CD3+ and γδ+ IELs are typically increased already in early-stage coeliac disease, and the density of γδ+ IELs often remains elevated even when the patients adhere to a GFD (Järvinen et al., 2003). However, determination of γδ+ IELs requires frozen small bowel mucosal specimens, and therefore, it should be used only in problematic cases with diagnostic difficulties. Further, when frozen small bowel mucosal specimens are available, the presence of intestinal TG2-specific autoantibody deposits can also be examined. In coeliac disease, TG2-targeted autoantibodies are produced mainly by B-lymphocytes in the lamina propria (Marzari et al., 2001), and with direct IF examination intestinal TG2-specific IgA deposits can be detected with high sensitivity and specificity in coeliac disease (Koskinen et al., 2010). Moreover, these deposits appear even before the development of villous atrophy and occur also in seronegative coeliac disease patients (Koskinen et al., 2008, Salmi et al., 2006).

Serological tests were developed to screen patients suspected of suffering from coeliac disease. The first test developed was the ARA, which seemed reliable in experienced hands (Hällström, 1989) but showed highly variable sensitivity and specificity in different studies (Lock et al., 1999, Mäki et al., 1984, Sulkanen et al., 1998). The IgA-class AGA test was also used contemporarily with the ARA until more accurate serological methods became available (Leffler and Schuppan, 2010, Savilahti et al., 1983). The EmA test started to replace these two tests in 1984 (Chorzelski et al., 1984) and when TG2 was identified as the autoantigen in coeliac

disease, an enzyme-linked immunosorbent assay (ELISA) method for detecting IgA-class TG2 antibodies was developed (Dieterich et al., 1997, Sulkanen et al., 1998). Subsequently, it was shown that TG2 is also the antigen of the ARA and EmA tests (Korponay-Szabóet al., 2003). The TG2 ELISA antibody test is cheaper and considered more sensitive but slightly less specific than the EmA test, which has a specificity of 97–100% (Rostom et al., 2005). In the EmA test, human umbilical cord is needed as the substrate, the indirect IF microscopic evaluation is subjective, and the test requires skilful laboratory personnel (Schyum and Rumessen, 2013). Therefore, the IgA-class TG2-antibody ELISA test should primarily be used when coeliac disease is screened. The measurement of DGP antibodies has shown relatively high specificity (90–98%) in coeliac disease but this test method is currently lacking extensive use in clinical practice (Adriaanse and Leffler, 2015). Moreover, circulating antibodies against transglutaminase 6 (TG6) have been proposed to be valuable in identifying patients with coeliac disease-related neurological symptoms like cerebellar ataxia (Hadjivassiliou et al., 2006).

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommended the serology-based diagnosis of coeliac disease in children already in 2012. According to the criteria, diagnosis without small bowel biopsies can be made when the TG2 antibody level is more than ten times the upper limit of normal and the EmA test is positive in a different blood sample. In addition, the HLA DQ2 or DQ8 haplotype must be present (Husby et al., 2012). Recently, the National Guidelines for coeliac disease (Celiac Disease, Current Care Guidelines, 2018) in Finland recommended that coeliac disease in adults can also be diagnosed without small bowel biopsies when the TG2 antibody level is at least ten times the normal limit and the subsequent EmA test is also positive. It has been estimated that serology-based criteria could be sufficient to make the diagnosis in at least one third of adult patients with coeliac disease (Fuchs et al., 2019).

3 EPIDEMIOLOGY IN DERMATITIS HERPETIFORMIS AND COELIAC DISEASE

DH is most common among individuals of northern European origin. In the early 1970s, the prevalence was 10.4 per 100,000 in Finland (Reunala and Lokki, 1978). In the 1980s, similar or somewhat higher figures were found in Sweden, Scotland, and Ireland (Buckley et al., 1983, Gawkrodger et al., 1984, Mobacken et al., 1984). In 2011, the prevalence of DH in Finland was 75,3 per 100,000 – the highest figure reported to date (Salmi et al., 2011). At the same time, the prevalence in the United Kingdom (UK) was lower, at 30.3 per 100,000 (West et al., 2014). Importantly, in both Finland and the UK, the prevalence of DH was eight times lower than the prevalence of coeliac disease (Salmi et al., 2011, West et al., 2014).

A rather high prevalence of DH – 10.2 per 100,000 – has been reported in the United States (US) state of Utah (Smith et al., 1992, Westet al., 2014). DH is rare in African-Americans (Hall et al., 1990). A few small case series have been published on Asia populations but none have been published on Africa populations (Zhang et al., 2012). A study from Japan showed the occurrence of DH type disease with mostly fibrillar IgA deposits in the papillary dermis (Ohata et al., 2012). Despite the similarities, the disease is likely to differ from the classical DH occurring in the Caucasian population. Overall, the geographical differences in the prevalence of DH and coeliac disease are mainly explained by HLA genetics and wheat consumption habits (Kang et al., 2013).

In the 1970s, the annual incidence of DH was 1.3 per 100,000 in Finland (Reunala and Lokki, 1978) and by the early 1980s, the incidence of DH and coeliac disease was almost the same (Collin et al., 2007). Later studies from Finland and the UK have shown that the incidence of DH has significantly decreased in recent decades, from 5.2 to 2.7 per 100,000 in the Finnish study and from 1.8 to 0.8 per 100,000 in the UK study (Salmi et al., 2011, West et al., 2014). At the same time the incidence of coeliac disease has increased fourfold in the UK and Finland (West et al., 2014, Virta et al. 2017). Seroepidemiological studies suggest that in addition to the better recognition of coeliac disease, there has also been a true increase in the incidence in recent decades (Lohi et al., 2007). Importantly, as only 0.7% of the Finnish adult

population has a coeliac disease diagnosis (Ilus et al., 2014), the disease is still greatly underdiagnosed in Finland, like in many other countries (Fasano et al., 2003).

Age at onset and gender differ markedly between DH and coeliac disease. In contrast to coeliac disease, DH is rare in childhood in Finland, where approximately 4% of the DH patients are diagnosed under the age of 16 (Hervonen et al., 2014, Kivelä et al., 2015) but seems more common among children in Hungary and Italy (Antiga et al., 2013, Dahlbom et al., 2010). The mean age at DH diagnosis has increased significantly from 35 to 51 years in men and from 36 to 46 years in women during the last 30 years. Currently, the mean age at DH diagnosis is 49 years in Finland (Salmi et al., 2011). In coeliac disease, there are two peak ages of onset: early childhood and the fourth or fifth decades of life (Tack et al., 2010). Similarly, to DH, the age at diagnosis seems to be increasing also in adults with coeliac disease in Finland (Collin et al., 2018). There is a slight or moderate male preponderance in DH studies (Salmi et al., 2011), whereas in adults with coeliac disease, females markedly outnumber males (Green and Cellier, 2007).

4 PATHOGENESIS OF DERMATITIS HERPETIFORMIS AND COELIAC DISEASE

4.1 Genetic background and familial occurrence

Practically all patients with DH and coeliac disease carry the HLA alleles DQ2 or DQ8 (Spurkland et al., 1997). It is important to note that 25–35% of the Caucasoid population carries the HLA DQ2 and DQ8 haplotypes, and only a minority of these will develop DH or coeliac disease (Monsuur et al., 2008). On the other hand, the absence of the HLA DQ2 or DQ8 in patients suspected for the disease excludes DH and coeliac disease with a negative predictive value of more than 99% (Caproni et al., 2009).

Due to the common genetic background, the prevalence of DH and coeliac disease are increased in first-degree relatives (Fasano et al., 2003, Hervonen et al., 2002, Rubio–Tapia et al., 2008). A recent meta-analysis showed that the prevalence of coeliac disease was 7.5% among first-degree relatives (Singh et al., 2015). In a Finnish family study, 18% of patients with DH and 19% of patients with coeliac disease had one or several affected first-degree relatives, most of whom had coeliac disease (Hervonen et al., 2002). Monozygotic twins are known to have a high concordance of coeliac disease (Nisticò et al., 2006). Interestingly, monozygotic twins can also be affected by different gluten-sensitive phenotypes; one twin pair can have DH and the other coeliac disease (Hervonen et al., 2000).

4.2 Gluten and other environmental factors

DH and coeliac disease have unique pathogenesis among autoimmune diseases. In genetically predisposed individuals, the exogenous factor responsible is dietary gluten. Gluten is the storage protein for alcohol-insoluble prolamine glycoproteins: gliadin in wheat, secalin in barley, and hordein in rye. The major amino acid constituents of gluten are proline, glutamine, and hydrophobic amino acids, which make gluten resistant to complete degradation by gastric, pancreatic, and brush-border enzymes. When the partly digested gluten proteins and peptides reach the

small intestinal lumen in affected individuals, they start a cascade of adaptive and innate immunological reactions leading to gluten-sensitive enteropathy typical of coeliac disease and DH (Shan et al., 2002).

Other environmental factors than gluten have been proposed for the complete explanation of the development of coeliac disease. Changes in infant feeding practices were considered to be important in the Swedish epidemic of coeliac disease in the 1980s (Ivarsson et al., 2000). However, a recent controlled study on breast-feeding and early introduced gluten did not show any increased risk for coeliac disease development (Vriezinga et al., 2014). Gastrointestinal infections and especially the rotavirus in children have been proposed as additional risk factors (Stene et al., 2006). In addition, the augmented use of antibiotics can disturb normal gut microbiota, which may increase the risk for coeliac disease (Mårild et al., 2013b).

4.3 Immunopathology in gluten-sensitive enteropathy

The partly digested gluten peptides enter via transcellular or paracellular routes into the lamina propria of small intestinal mucosa (Shan et al., 2002). In affected individuals, an adaptive immune reaction occurs that is dependent on deamidation of gliadin peptides by the TG2-enzyme (Qiao et al., 2009). Deamidation increases the immunogenicity, facilitating binding to the HLA-DQ2 or -DQ8 molecules on antigen presenting cells (Molberg et al., 1998). These antigen-presenting cells interact with gliadin-specific CD4+T cells, which produce pro-inflammatory cytokines, such as interfreron γ (IFN-γ) and interleukin (IL) 21, which leads to tissue damage (Bodd et al., 2010, Lundin et al., 1993). These CD4+ cells are also able to bind to gluten-specific B-cells which then differentiate into plasma cells releasing TG2 and gliadin antibodies to circulation (Rauhavirta et al. 2016). See Figure 3.

The innate immune response to gliadin occurs in the epithelial component of the intestinal mucosa and involves the increased production of cytokines, in particular IL-15, by enterocytes and macrophages (Mention et al., 2003). The result is the differentiation of IELs into cytotoxic CD8+T cells. The cumulative resulting damage to the intestinal mucosa from this cascade of inflammatory mediators is the villous atrophy and crypt hyperplasia in coeliac disease and DH.

4.4 Rash and skin IgA deposits in dermatitis herpetiformis

Granular IgA deposits within the papillary dermis are pathognomonic for DH (Zone et al., 1996). Biopsies taken routinely from perilesional skin and stained with the direct IF method show typical IgA deposits, which are also present in skin areas not affected by the DH rash (Zone et al., 1996). The autoantigen for deposited cutaneous IgA is TG3 (Sárdyet al., 2002), an enzyme normally present only in the epidermis. Thus, the TG3-bound IgA aggregates in the dermis are thought to derive from the blood vessels (Preisz et al., 2005, Taylor et al., 2015). Supporting this hypothesis, patients with DH have frequently circulating high-avidity IgA-class TG3 antibodies, and one study has also found TG3-IgA immune complexes in the blood (Collin et al., 2017, Görög et al., 2016, Sárdy et al., 2002).

A valid hypothesis suggests that immune pathogenesis in DH begins in the small bowel from latent or manifest gluten-sensitive enteropathy, i.e. coeliac disease (Collin et al., 2017, Reunala et al., 1998). See Figure 3. The gluten-induced immune response in the small bowel releases circulating IgA-class TG2 autoantibodies in both coeliac disease and DH, whereas TG3 autoantibodies are found mainly in DH (Dieterich et al., 1998, Korponay-Szabó et al., 2004, Reunala et al., 2015a). Importantly, the TG3 protein has not been found in the small bowel like the TG2 enzyme, and therefore epitope spreading could be a reason for the production of TG3 antibodies (Sárdy et al., 2002). In line with this, a recent study showed that patients with untreated DH secreted high levels of TG3 antibodies into the small bowel organ culture medium, and TG3-secreting plasma cells were also seen in the small bowel mucosa (Hietikko et al., 2018b).

The initial events of blister formation in DH are still unclear. Recently, Taylor and colleagues (2015) showed that TG3 present in IgA aggregates in DH skin is enzymatically active and binds soluble fibrinogen. This fits well with earlier studies showing marked fibrin deposition and the upregulation of a urokinase-type plasminogen activator in evolving DH blisters (Airola et al., 1995, Reitamo et al., 1981). An influx of neutrophils and macrophages into developing DH lesions is a rather late phenomenon, which may be initiated by the activation of fibrinogen to fibrin, resulting in fibrinolysis (Davalos and Akassoglou, 2012, Görög et al., 2016, Reitamo et al., 1981). A second open question is why the blistering DH lesions occur on the knees, elbows, and buttocks even though IgA-TG3 aggregates are also deposited at sites never involved in blister formation (Zone et al., 1996). The most likely explanation for this unique distribution involves the influence of local factors, such as pressure and stretching, which may directly activate TG3 in dermal

aggregates in the same way that mechanical force activates TG2 in blood vessel walls (Huelsz-Prince et al., 2013).

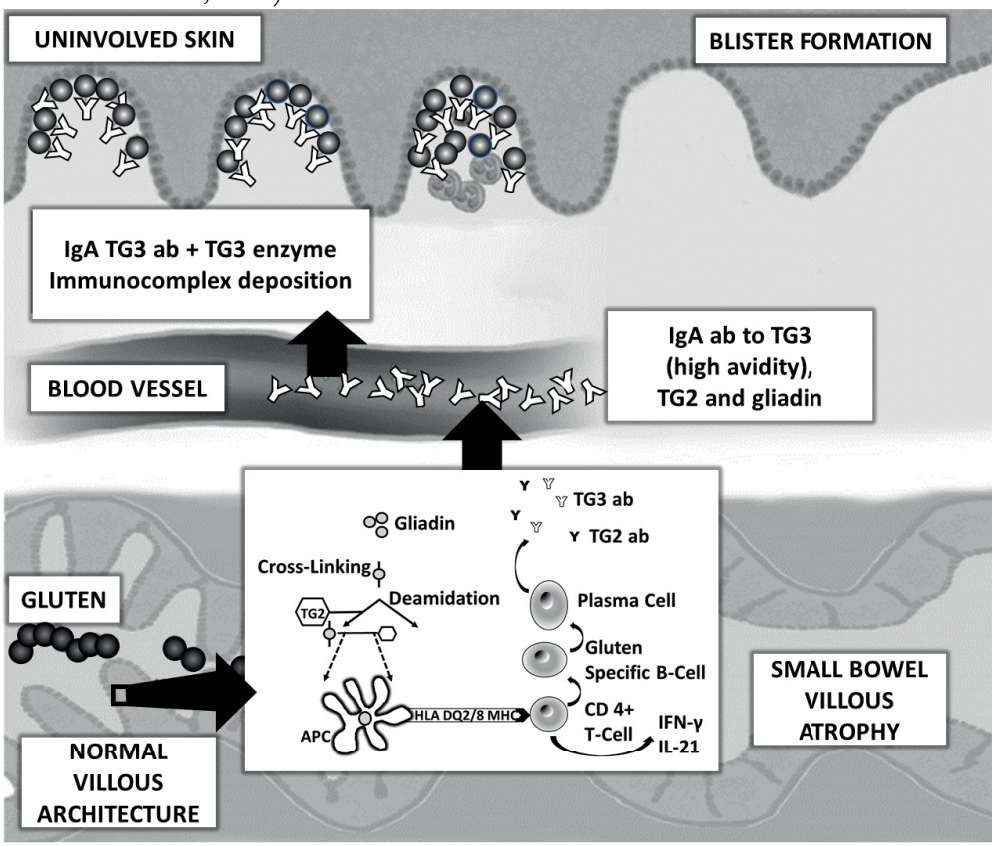

Figure 3. Proposed pathogenesis of dermatitis herpetiformis and coeliac disease adapted from Reunala et al. 2015b.

Abbreviations: APC=antigen presenting cells, TG= transglutaminase, ab= antibodies, IFN= interferon, IL=interleukin.

After dietary (gluten-containing) wheat, rye or barley is digested in the small bowel lumen, it is absorbed through the lamina propria. Gliadin is deamidated close to the lamina propria by TG2. Thereafter, CD4+ cells recognize the gliadin peptides presented by HLA DQ2/DQ8 antigen presenting cells (APC). This results in cytokine production leading to mucosal damage and the activation of gluten-specific B-cells. B-cell activation leads differentiated plasma cells to produce TG2 antibodies and cytokine production then leads to mucosal damage

Epitope spreading is one explanation for the development of TG3 antibodies. TG2 and TG3 antibodies circulate in the blood vessels, and thereafter TG3 antibodies leave the circulation and enter the dermis. TG3 antibodies make immunocomplex depositions with TG3 enzymes. IgA TG3 antibody +TG3 enzyme immunocomplexes are deposited near the papillary dermis and lead to neutrophilic abscesses and subepidermal blister production in the skin.

5 TREATMENT OF DERMATITIS HERPETIFORMIS AND COELIAC DISEASE

5.1 Gluten-free diet

The most important treatment for both DH and coeliac disease is a strict life-long GFD, which is initiated by patients after the diagnosis is confirmed. A GFD means the avoidance of wheat, rye, and barley and foods made from these cereals. Gluten-free oats, i.e. oats not contaminated by gluten traces from other cereals, have been shown to be safe both in DH (Reunala et al., 1998) and coeliac disease (Janatuinen et al., 1995, Pinto-Sánchez et al., 2017). Even though the role of oats as a part of a GFD is somewhat controversial worldwide, gluten-free oats are available for coeliac disease and DH patients for example in Finland, Canada, the UK, and the US.

In an ideal GFD diet, gluten intake would be zero, but this is almost impossible to achieve. There is no evidence of a single definitive threshold for a safe gluten intake in DH and coeliac disease. Tolerance to gluten traces also seems to vary between individuals (Lähdeaho et al., 2011). For most coeliac disease patients, a daily intake of 10 milligrams of gluten is unlikely to cause small bowel damage (Akobeng and Thomas, 2008). The European Commission has issued a regulation for foods that are labelled "gluten-free". These foods can contain less than 20 parts per million (ppm) of gluten. This is equal to 20 mg/kg of food, which is also a widely accepted threshold for a GFD. Studies have shown that this amount of gluten is safe for patients with coeliac disease (See et al., 2015). In the Western diet, people eat on average 13 g of gluten per day, but individual variation is large (Antvorskov et al., 2018, Köhler-Brands et al., 1997).

In DH, GFD heals both the rash and the small bowel villous atrophy. Regardless of adhering strictly to the GFD, skin symptoms can take several months to a few years to clear (Fry et al., 1973, Reunala et al., 1977). In coeliac disease, the alleviation of gastrointestinal symptoms usually takes a few weeks after starting a GFD. Small bowel mucosal recovery seems to be slow in adult patients with coeliac disease, and normalization of the villous architecture may take even 2–5 years (Lanzini et al., 2009, Rubio-Tapia et al., 2010, Wahab et al., 2002).

As a GFD can be personally and socially problematic (Zarkadas et al., 2013), all patients should receive dietary advice by dietitian when starting the diet (See et al., 2015). While it is important to avoid gluten by replacing it with alternative products, it is equally essential to ensure an adequate amount of nutrients, vitamins, fibre, and calcium in the diet (Ciacci et al., 2015). A GFD is also more expensive when compared to a normal gluten-containing diet, and the availability of gluten-free products can be limited. Travelling and eating outside the home can be especially problematic (Whitaker et al., 2009; Zarkadas et al., 2013). Due to these discomforts, some coeliac disease patients may often have dietary lapses or even non-adherence to a GFD (See et al., 2015).

A long-term follow-up study conducted in Finland showed that 98% of DH patients adhered to a GFD and 72% of them adhered strictly without any lapses (Hervonen et al., 2012). In another Finnish study, adherence to a strict GFD was 88% among patients with coeliac disease, and dietary lapses were associated with a younger age at diagnosis, being a teenager, and existing symptoms (Kurppa et al., 2012). However, dietary adherence rates have been shown to vary, and based on self-reported questionnaires in several European countries, strict dietary adherence was only seen in 40–62% of patients with coeliac disease (See et al., 2015).

The prevailing view is that GFD treatment should be life-long both in DH and coeliac disease. There are nevertheless a few reports suggesting that a small proportion of DH patients could go into remission and be able to re-introduce gluten to their diet without developing any symptoms or signs of DH (Garioch et al., 1994, Gawkrodger et al., 1984, Paek et al., 2011). Furthermore, in a recent Italian study (Bardella et al., 2003) in which DH patients were gluten-challenged, seven (18%) did not show clinical or small bowel histological relapse. There are studies also suggesting that patients with coeliac disease could re-develop gluten tolerance and enter clinical and histological remission (Hopman et al., 2008, Matysiak-Budnik et al., 2007). However, further studies are needed to verify what the possible mechanisms for gluten tolerance could be and how frequently this is achieved in patients with DH and coeliac disease adhering to a GFD treatment.

5.2 Dapsone medication in dermatitis herpetiformis

After a DH diagnosis, all patients are advised to start the GFD treatment. Even when adhering strictly to a GFD, the rash and intensive pruritus usually subsides within a few weeks to several months (Fry et al., 1973, Reunala et al., 1977). Patients with a

widespread rash and intense itch need additional treatment with dapsone, and in our cohort this drug was initiated in 65% of newly diagnosed DH patients (Hervonen et al., 2012).

Dapsone has antibacterial and anti-inflammatory effects, and in the skin it inhibits neutrophil- and eosinophil-mediated tissue damage. Dapsone relieves the rash in a couple of days, but it has no effect on the small bowel enteropathy or IgA deposits in the skin (Bolotin and Petronic-Rosic, 2011b, Zone et al., 1996).

For children, the starting dose of dapsone is usually 0.5 mg/kg/day. For adults, a dosage of 25–50 mg/day is usually enough to control the pruritus and the development of new skin lesions in most patients (Gawkrodger et al., 1984, Reunala et al., 1977). The dapsone dose is slowly reduced, and the drug treatment can be stopped after an average of two years on a strict GFD (Bolotin and Petronic-Rosic, 2011b, Garioch et al., 1994).

Dapsone treatment rarely causes agranulocytosis, but it can have a dose-dependent risk for haematological adverse effects, such as haemolysis and methaemoglobinaemia (Bolotin and Petronic-Rosic, 2011b). Therefore, patients need to be followed-up until the dapsone medication has been discontinued (Garioch et al., 1994, Leonard and Fry, 1991).

Topical treatment with 5% dapsone gel available in the US has been reported to have beneficial effects on the DH rash (Burbidge and Haber 2016). In contrast, even potent or superpotent topical steroids have shown only mild or no impact on pruritus when used as a monotherapy (Caproni et al., 2009, Bolotin and Petronic-Rosic, 2011b).

6 PROGNOSIS OF DERMATITIS HERPETIFORMIS AND COELIAC DISEASE

6.1 Long-term prognosis on a gluten-free diet

In DH, the GFD treatment offers a good long-term prognosis in regard to mortality in addition to the healing of the rash and small bowel mucosa. In a recent Finnish DH study, the standardized mortality rate (0.70) was significantly lower than that for the general population (Hervonen et al., 2012). In contrast to DH, most studies on coeliac disease have shown an increased risk for all-cause mortality (Tio et al., 2012). See Table 2. The overall risk in 17 studies was 1.24, and the most common causes of death were lymphoproliferative and cardiovascular diseases (Tio et al., 2012). Compared to the DH mortality study (Hervonen et al., 2012), knowledge about adherence to a GFD has been scant in coeliac disease studies. A previous DH mortality study from UK reported normal (hazard ratio 0.93) all-cause mortality (Lewis et al., 2008).

The results for bone mineral density (BMD) in DH patients are somewhat contradictory. In two studies, BMD was shown not to differ from that of the healthy controls (Abuzakouk et al., 2007, Lheure et al., 2017). However, in one study DH patients were found to have a lower BMD compared to non-coeliac controls, but a higher BMD compared to coeliac disease patients at diagnosis (Di Stefano et al., 1999). Long-term studies suggest that DH patients on a GFD do not have lowered BMD, unlike patients with coeliac disease (Lorinczy et al., 2013). The risk for bone fractures in GFD-treated DH patients seems not to be increased according one study (Lewis et al., 2008, Pasternack et al., 2018). This is in contrast to the results of a meta-analysis study performed for coeliac disease, where the risk of hip fractures, and fractures in general was increased (Heikkilä et al., 2015).

The most serious complication of DH and coeliac disease is the development of a lymphoma or other malignancy. The association of DH and lymphoma was first reported in the 1980s, when a very high, 100-fold relative risk was found (Leonard et al., 1983b). After that, the reported risk rates of lymphoma in DH have been markedly lower – 2–10-fold compared to general population (Collin et al., 1996, Sigurgeisson et al., 1994, Viljamaa et al., 2006). Importantly, the risk of lymphoma

seems to be elevated only during the first five years after DH diagnosis, but not thereafter, whereas the risk persists with coeliac disease for over 15 years (Grainge et al., 2012, Lewis et al., 1996). Furthermore, DH patients developing a lymphoma have been reported not to follow a GFD or adhere to it strictly (Hervonen et al., 2005). The risk for any cancer or gastrointestinal carcinoma is not increased in GFD-treated DH patients (Collin et al., 1996, Hervonen et al., 2012, Lewis et al., 2008, Sigurgeisson et al., 1994, Swerdlow et al., 1993) which again is in contrast to coeliac disease (Askling et al., 2002, Green et al., 2003, West et al., 2004, Table 2).

Table 2. Comparison of the long-term prognosis of gluten-free diet-treated patients with dermatitis herpetiformis and coeliac disease

	Dermatitits Herpetiformis	Coeliac disease
Bone mineral density	Normal	Lowered in most studies
Risk of bone fractures	Not increased	Increased
Risk of Non-Hodgkin's lymphoma	Increased for the first five years after diagnosis	Increased
Risk of gastrointestinal malignancies	Not increased	Increased
Mortality	Reduced	Slightly increased
Quality of life	Not decreased	Decreased in most studies

6.2 Refractory disease

In DH, there are a few earlier reports describing patients, whose rash does not response to a strict GFD (Gariochet al., 1994, Hervonen et al., 2016, Leonard and Fry, 1991). One reason for the non-response is intentional or inadvertent ongoing gluten consumption. However, this seems not always to be the case. A recent Finnish study of 403 DH patients, found seven (1.7%) non-responsive patients, who had been on a strict GFD for a mean of 16 years (Hervonen et al., 2016). These patients still needed dapsone for the active rash, although their GFD was considered strict, the serum coeliac autoantibodies were negative, and the small bowel mucosa had healed. This condition, named as refractory DH, seems to be different from refractory coeliac disease since there were no signs of lymphoma or other complications for which refractory coeliac disease is known (Hervonen et al., 2016).

In coeliac disease, there are patients whose symptoms and small bowel villous atrophy do not response to a strict GFD. In these cases, ongoing gluten ingestion

must always be ruled out. This condition has been termed refractory coeliac disease, and it has overt malabsorptive symptoms together with persistent villous atrophy for a minimum of 6–12 months despite a strict GFD (Rubio-Tapia and Murray, 2010). Refractory coeliac disease is classified as subtype I or II based on the detection of a normal or aberrant IEL T-cell population in small bowel biopsy specimens. In type I, there is a normal IEL immunophenotype, whereas in type II, the immunophenotype is abnormal. The prognosis in these subtypes is different; in type I nutritional support together with pharmacologic therapy usually improves the disease, whereas in type II, the prognosis is poor. In the latter subtype patients are at risk for severe complications, such as intestinal T-cell lymphoma and premature death (Rubio-Tapia and Murray, 2010). The prevalence of refractory coeliac disease is thought to be very rare. In Finland, refractory coeliac disease was diagnosed in 44 (0.36%) of 12,243 coeliac disease patients (Ilus et al., 2014). Of these, 68% had type I, 23% had type II, and 9% remained uncharacterized.

6.3 Quality of life

Quality of life (QoL) has been described as psychological, physical, and social well-being and the awareness of one's position in life compared to others (Calman, 1984, Deepak et al., 2018). When QoL is monitored, it enables a more comprehensive evaluation of the disease and the benefits of the treatment.

There is little data about QoL in DH. A recent study featuring 52 newly diagnosed DH patients showed that QoL was decreased and the burden of untreated DH was even greater among patients with gastrointestinal symptoms (Pasternack et al., 2017). After adherence to a GFD for one year, QoL was at the same level as that of the normal controls (Pasternack et al., 2017), a result found also earlier in long-term GFD-treated DH patients (Pasternack et al., 2015).

More extensive QoL research has been performed for coeliac disease. At diagnosis, the patients with coeliac disease have shown to have reduced QoL when compared to healthy controls (Green et al., 2001, Ukkola et al., 2011). Moreover, diagnostic delay has been shown to associate with reduced QoL (Fuchs et al., 2018, Paarlahti et al., 2013). After one year on a GFD, the majority of coeliac disease patients reported an improvement in their health-related QoL (Greenet al., 2001, Mustalahti et al., 2002). However, 25 % of coeliac disease patients undergoing long-term GFD-treatment reported having persistent gastrointestinal symptoms and reduced QoL (Hallert et al., 2002, Häuser et al., 2006, Paarlahti et al., 2013).

Gender seems to affect QoL in DH and coeliac disease. Compared to males, female DH patients have shown to have a lower QoL at diagnosis but not on GFD treatment (Pasternack et al., 2017). Furthermore, on GFD DH females have shown to suffer more commonly from abdominal symptoms compared to males (Pasternack et al., 2015). Similarly, females with coeliac disease have shown to have lower QoL both at diagnosis (Nordström et al. 2011) and on GFD when compared to male patients (Hallert et al., 2002, Pulido et al., 2013, Roos et al., 2006, Zarkadas et al., 2006).

7 AIMS OF THE PRESENT STUDY

The main aims of the present DH study were to investigate the diagnostic delay and the severity and long-term prognosis of villous atrophy in DH utilizing a prospectively collected patient series at the Tampere University Hospital. The possibility of remission from DH after long-term GFD treatment was investigated with a 12-month gluten challenge.

The specific aims were:

1. To investigate the diagnostic delay in DH and factors associated with delayed diagnosis (**I**).

2. To study whether small bowel histological findings at DH diagnosis have changed over the last 45 years, and to investigate the correlations of serum TG 2 antibodies and the degree of mucosal damage (**II**).

3. To examine whether the presence or absence of villous atrophy at diagnosis affects the long-term prognosis of DH (**III**).

4. To determine whether the development of gluten-tolerance is possible in long-term GFD-treated DH patients using a gluten challenge (**IV**).

8 DERMATITIS HERPETIFORMIS PATIENTS AND COELIAC DISEASE CONTROLS

8.1 Dermatitis herpetiformis patients

Table 3. Patients with dermatitis herpetiformis (DH) and the coeliac disease controls.

Study	Study design	Number of DH patients	Men (%)	Mean age at DH diagnosis: years (range)	Mean age at the time of the study: years (range)	Control patients
I. Diagnostic delay	Retrospective cohort study	446	229 (51)	43 (3–83)	-	-
	Questionnaire	217	118 (54)	45 (5–78)	63 (18–96)	-
II. Prevalence of small bowel villous atrophy	Retrospective cohort study	393	214 (54)	45 (18–84)	-	-
III. Prognosis in patients with and without small bowel villous atrophy	Retrospective cohort study	352	177 (50)	42 (3–84)	63 (18–96)	248 coeliac controls
	Questionnaire	181	96 (53)	45 (5–78)	62 (18–96)	128 coeliac controls
IV. Gluten challenge	Prospective study	19	13 (68)	35 (19–57)	58 (37–72)	-

The DH study cohorts in Studies **I–IV** (Table 3) derived from our prospectively collected series of DH patients at the Department of Dermatology, Tampere University Hospital. This single-centre DH cohort consists a total of 502 patients who had been diagnosed and treated at a special DH outpatient clinic between 1970 and 2014. All patients suspected of having DH by general practitioners in health care centres and private dermatologists were referred to the Department of Dermatology, Tampere University Hospital for the confirmation of a DH diagnosis. This was

based on the typical clinical picture and the detection of granular IgA deposits in the papillary dermis by direct IF examination. All patients were asked to undergo gastroscopy to obtain small bowel biopsies at the Department of Gastroenterology and Alimentary Tract Surgery (or previously at the Department of Internal Medicine) at Tampere University Hospital. After the diagnosis, all patients were advised to adhere to a GFD routinely. About 65% of the patients received dapsone, from 12.5 to 50mg/day, to control the skin symptoms (Hervonenet al., 2012). The patients on a GFD were followed up routinely by an experienced dermatologist in the outpatient clinic for at least 1–2 years or until the rash had cleared up and the need for dapsone medication stopped.

Study **I** included 446 DH patients. Of the 502 patients, 56 were excluded from further analysis: 21 had died more than 20 years previously and adequate data was lacking, and 35 had a prior (≥2 years earlier) diagnosis of coeliac disease. In Studies **II** and **III**, 53 patients had to be excluded as a small bowel biopsy result was not available, and in Study **III**, and additional 41 patients were excluded due to inadequate follow-up data. Long-term follow-up data for Studies **I** and **III** was obtained by questionnaires (see Chapter 9.5) collected between December 2015 and May 2016. The questionnaires were mailed to all 420 DH patients living in the Pirkanmaa Hospital District, and the overall response rate was 62%. In Studies **I** and **III**, 217 and 181 patients had adequate data for further analysis, respectively (Table 3).

Gluten challenge Study **IV** was a prospective study that included 19 GFD-treated DH patients from the cohort described above who volunteered to participate. They had adhered to a GFD for a mean of 23 (range 5–40) years and were all in clinical remission, i.e. they did not use dapsone and had been rash-free for at least three years.

8.2 Coeliac disease controls (III)

The controls included 248 coeliac disease patients suffering from abdominal symptoms at the time of the diagnosis (Study **III**, Table 3). The diagnosis of all control patients was based on histological analysis of small bowel biopsy specimens at the Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital.

The follow-up data from coeliac disease controls were collected on May 2016 by specific questionnaires (see Chapter 9.5) mailed to the 222 adult coeliac disease patients who were still alive. A total of 128 (58%) responded.

9 METHODS

9.1 Duration and activity of rash and skin immune deposit examinations

In Studies **I–IV**, data on demographic characteristics, the duration and severity of the rash, and the results of coeliac autoantibodies and haemoglobin values at DH diagnosis were gathered from the patient records of Tampere University Hospital. In Study **I**, the duration of the rash before diagnosis was collected from all patients. Diagnosis was defined as delayed when the duration of the rash before the diagnosis was two years or longer. The severity of the rash at DH diagnosis was scored as mild, moderate, or severe according to presence of a few, several, or many blisters, macular eruptions, and erosions on the knees, elbows, buttocks, and scalp or elsewhere on the body based on descriptions in the medical records (Studies **I**, **III**, and **IV**). In Study **IV**, the severity of the rash was based on clinical examination.

In Study **IV**, a punch biopsy was taken in the absence of skin symptoms from normal appearing forearm skin or from perilesional skin. The specimens were stored at -70°C before the examinations. To investigate IgA deposits, sections cut from the specimens were stained with TRITC-conjugated goat antihuman IgA (1:50) (A18786, Life Technologies, Frederick, Maryland, USA) at the coeliac disease research laboratory. Skin IF-IgA intensity was graded negative, positive, or strong positive. TG3 deposits were stained with FITC-conjugated rabbit polyclonal TG3 antibody (1:100) (A030, Zedira, Darmstadt, Germany). All sections were further double-stained for IgA and TG3 with TRITC goat antihuman IgA (1:50) (A18786, Thermo Fisher Scientific, Waltham, MA, USA) as previously described (Hietikko et al., 2018c).

9.2 Small bowel biopsy examinations

After the DH diagnosis, a gastroscopy and a small bowel biopsy was performed in about 75% of the patients. In the 1970s, biopsies were obtained with a Crosby capsule under x-ray control and thereafter upon upper gastrointestinal endoscopy.

Small bowel mucosal samples were scored morphologically as SVA, PVA, or normal villous architecture. Pathologists with a special knowledge of intestinal pathology made the interpretations. Small bowel biopsy results at DH diagnosis were collected from the medical records (Studies **I–IV**).

In Study **IV** at pre- and post-challenge, 6–8 forceps biopsy specimens were taken from the distal part of the duodenum during gastroscopy. At least two specimens were stained with haematoxylin and eosin and investigated with light microscopy. At least three well-oriented villous crypt units were examined by measuring the villi lengths and crypt depths; the mean was calculated and a ratio >2.0 was considered normal (Kuitunenet al., 1982, Taavelaet al., 2013). The remaining specimens were freshly embedded in OCT (Tissue-Tec.), snap-frozen in liquid nitrogen, and stored at -70°C before examinations. Stainings of CD3+ and γδ+ IELs were carried out on 5 μm-thick frozen sections, and the normal values were <37 cells/mm for CD3+ and <4.3 cells/mm for γδ+ IELs (Järvinenet al., 2003).

Small bowel TG2-targeted IgA deposits (Study **IV**) were studied from unfixed, frozen, 5 μm-thick small bowel mucosal sections by direct IF. These were stained with mouse monoclonal anti-TG2 antibody (CUB7402; NeoMarkers, Fremont, California, USA), followed by detection with fluorescein isothiocyanate (FITC) -labelled rabbit anti-human IgA antibody (Dako A/S, Glostrup, Denmark; (Korponay-Szabóet al., 2004). Based on their presence along the basement membrane in the villous-crypt area, the deposits were graded as positive or negative as in our previous study (Salmi et al., 2014).

9.3 Serum antibody measurements

The investigated serum IgA-class TG2-targeted antibodies in Studies **I–IV** were ARA, EmA, and/or TG2 antibodies depending on the time of testing. Over time, EmA has replaced ARA in clinical practice, but in our laboratory these tests have proved to be highly identical (Mäki, 1995). In Study **IV**, TG3 antibodies were also analysed.

In Study **I**, the serum of 250 patients with DH was investigated by IgA-class ARA and/or EmA. In Study **II**, 96 patients with DH were examined for IgA-class TG2 antibody levels. In Study **III**, 163 DH patients and 148 controls were examined with ARA, EmA, or TG2 antibodies. In Study **IV**, the investigated IgA-class antibodies were TG2 antibodies, TG3 antibodies, and EmA.

Serum IgA-class ARA and EmA were measured with an indirect IF method. In ARA, a typical R1 pattern was required in rat kidney and liver sections (Hällström, 1989), and in EmA, human umbilical cord was used as a substrate; a titre of 1:≥5 was regarded as positive in both tests. TG2 antibodies were determined with a commercially available ELISA kit (TG2; Celikey, Phadia GmbH, Freiburg, Germany). In Study **I**, values ≥5.0 absorbance units (AU)/mL were considered positive, as were values ≥3.0 AU/ml in Study **IV**. TG3 antibodies were determined with a commercially available ELISA kit (TG3; Immunodiagnostik, Bensheim, Germany), and values >30 AU/ml were considered positive.

9.4 Gluten challenge study protocol (IV)

The inclusion criteria were a diagnosis of DH based on the typical clinical picture, the presence of granular IgA deposits in the papillary dermis with direct IF examination, adherence to a GFD for at least five years, and the absence of skin symptoms for at least three years. Exclusion criteria were an age >80 years, severe cardiovascular disease, previous cancer, immunosuppressive or anti-coagulation medication other than acetylsalicylic acid, and the use of dapsone during the past three years.

Of the 19 DH study patients, a small bowel biopsy result at diagnosis was available for 14 subjects: 12 (86%) had villous atrophy and two (14%) had normal villous architecture. Before the gluten challenge, the patients had been on a GFD for a mean of 23 (range 5–40) years. Sixteen patients had adhered to the diet strictly and three reported having 1–5 dietary lapses per month. HLA DQ2 and DQ8 genotypes were determined using the Olerup SSP DQB1 low-resolution kit (Olerup AB, Saltsjöbaden, Sweden/Qiagen Vetriebs GmbH, Vienna, Austria).

A detailed flowchart of the study protocol is shown in Figure 4. The gluten challenge was initiated by giving the patients 200 g of commercially available wheat bread to be consumed daily for three days (Anderson et al., 2000). The first follow-up visit was conducted at day 6, after which the patients started to adhere to a normal gluten-containing diet. The minimum daily amount of gluten was set at 1 gram, which was verified with a follow-up telephone call after the first three weeks and thereafter with a three-day dietary diary that was filled by the patients before every follow-up visit. Follow-up visits were conducted every three months until the final examination at 12 months. The patients had been informed to contact the researchers when the DH rash or severe gastrointestinal symptoms appeared. The

researchers then decided if the challenge had to be discontinued. In addition, the challenge was discontinued if the serum EmA and TG2 antibodies were positive.

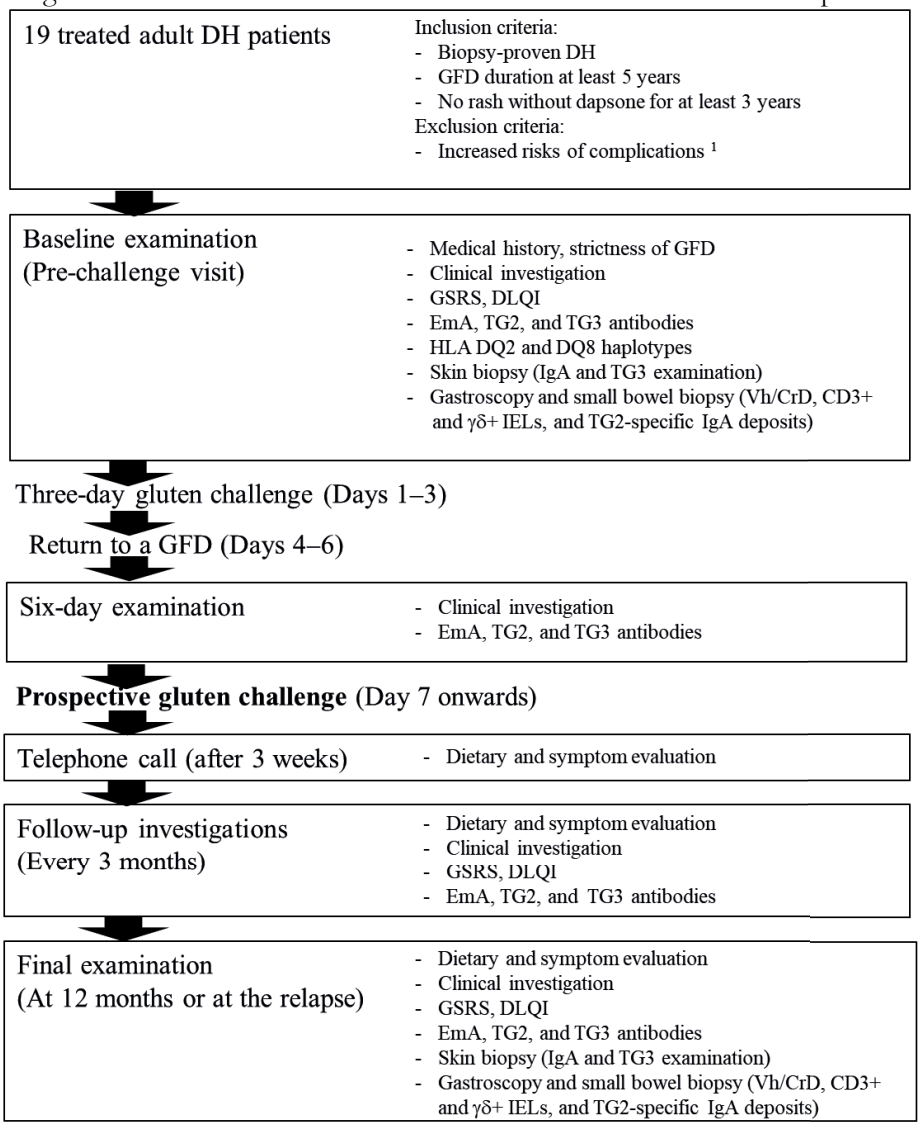

Figure 4. Flowchart of the gluten challenge study protocol including 19 gluten-free diet-treated dermatitis herpetiformis (DH) patients.

Abbreviations: GFD = gluten free diet, GSRS = Gastrointestinal Symptom Rating Scale, DLQI = Dermatology Life Quality Index, EmA = endomysium antibodies, TG = transglutaminase, Vh/CrD = villous height, crypt depth ratio, IEL = intraepithelial lymphocytes

[1] An age over 80 years, severe cardiovascular disease, previous malignancies, and the use of dapsone, immunosuppressive or anti-coagulation medication other than acetylsalicylic acid

Questionnaires (III, IV)

The disease-specific study questionnaires for the DH patients and coeliac disease controls were designed for study purposes (Studies **I** and **III)** and are available online as supplementary material for the publication of Study **III** (www.mdpi.com/2072-6643/10/5/641). The questionnaires included questions about the presence and duration of DH and coeliac disease-related symptoms before and after the diagnosis, the occurrence of malignancies and fractures, strictness of the GFD, smoking and other lifestyle characteristics, and the family history of coeliac disease or DH. Compliance with a GFD was reported as a strict diet without dietary lapses, dietary lapses once per month, dietary lapses one to five times per month, or dietary lapses once per week. In addition, the questionnaire included questions about the presence of coeliac disease complications and associated diseases, malignancies, other long-term illnesses, and regularly used medications.

The Psychological General Well-Being (PGWB) questionnaire used in Study **III** is a validated 22-item questionnaire that evaluates self-perceived health-related well-being and distress, and it includes six dimensions: anxiety, depressed mood, positive well-being, self-control, vitality, and general health (Dimenäs et al., 1996). The total score ranges from 22 to 132, with a higher score indicating better quality of life.

The Gastrointestinal Symptom Rating Scale (GSRS) questionnaire was used in Studies **III** and **IV**. The GSRS includes 15 items and assesses the severity and existence of gastrointestinal symptoms in five categories: diarrhoea, indigestion, constipation, abdominal pain, and reflux (Svedlund et al., 1988). It uses a seven-point Likert scale for each question: one indicates an absence of symptoms and seven indicates severe symptoms.

The Dermatology Life Quality Index (DLQI) (Studies **III** and **IV**) is a 10-item dermatology-specific quality of life instrument (Finlay and Khan, 1994). The questionnaire includes six different sections: symptoms and feelings, daily activities, leisure, work and school, personal relationships, and treatment unit. The scores for all ten questions are calculated together, and the total score varies from a minimum of 0 to a maximum of 30, with a higher score indicating a more impaired life quality.

9.5 Statistical analysis

For the descriptive statistics and further analysis, patients in Studies **I** and **II** were divided into three groups according to the year of DH diagnosis: 1970−1984,

1985−1999, and 2000−2014. In Study **III**, DH patients were divided into two groups according to presence or absence of villous atrophy at diagnosis.

In all studies, categorical variables are expressed as percentages and numbers. Numeric data are described as the means with ranges or the 95% confidence interval (CI), or as the medians with ranges or lower and upper quartiles (Q_1−Q_3), as appropriate.

In Studies **I**, **II**, and **III**, the two-sided χ^2 test, Fisher's exact test, and/or Kruskal–Wallis test were used as appropriate. In Study **I**, binary logistic regression analysis was used when identifying factors associated with delayed diagnosis. Univariate analysis was used initially for each associating factor, and then multivariable analysis was used for detecting the independence of delay-associated parameters found in the univariate analysis. In Study **II**, the linearity of small-bowel histology findings across the three 15-year periods was tested by using the Cochran–Armitage test, and the analysis of variance. Differences in TG2 IgA antibody levels between patients with PVA or SVA and normal villous architecture were determined using the Kruskall-Wallis test followed by pairwise multiple comparisons with the Dunn test. In Study **III**, logistic regression analysis was used to adjust the study groups according to age at the time of the study, and sex, age at diagnosis, calendar period of diagnosis, severity of rash, presence of gastrointestinal symptoms, and small bowel biopsy findings at diagnosis were all taken into account in the multivariable analysis. In Study **IV**, the statistical comparisons within-subjects were performed by a permutation test or Wilcoxon matched-pairs signed-rank test with exact *p*-values, and correlations were assessed by Spearman's correlation coefficient method. The normality of the variables was tested by using the Shapiro–Wilk W-test.

In all studies, statistical significance was set at a *p*-value below 0.05. Analyses were performed using IBM SPSS Statistics for Windows (Version 23.0., IBM Corp., Armonk, NY, USA) or the Stata 15.0 (StataCorp LP; College Station, Texas USA) statistical package.

9.6 Ethical aspects

The Declaration of Helsinki (1975) was followed in each study. The study protocol and usage of the register-based data was approved by the Regional Ethics Committee of Tampere University Hospital. Informed consent was obtained from each patient participating in the questionnaire studies and the gluten challenge study.

10 RESULTS

10.1 Diagnostic delay in dermatitis herpetiformis (I)

Of the 446 DH patients diagnosed between 1970 and 2014 with an available small bowel biopsy result, 151 were diagnosed between 1970 and 1984, 161 between 1985 and 1999, and 134 between 2000 and 2014. The median duration of the rash before DH diagnosis was 10 months, and the mean was 3.1 years for the entire study period. In the three 15-year periods, the median duration decreased significantly, from 12.0 to 11.0 months and then to 8.0 months ($p = 0.002$). Statistical significance was found when the first and third period were compared.

In 378 patients with available data, the rash was considered mild in 72 (19%), moderate in 193 (51%), and severe in 113 (30%) at the time of diagnosis. In the follow-up questionnaire, 41% reported having suffered from gastrointestinal symptoms at diagnosis. There was no significant change in the prevalence of severe rash or the presence of gastrointestinal symptoms between the three study periods.

Of the study patients, 389 (87%) had sufficient information for the analysis of the factors associated with delayed diagnosis, i.e. the presence of the rash before diagnosis for two years or more. Altogether, 142 (37%) patients had a delayed diagnosis. The frequency of delayed diagnosis decreased significantly from 47% to 38% and then to 25% from the first to the third study period. The diagnosis of DH between 1970 and 1984 was significantly associated with delayed diagnosis in both the univariate ($p = 0.001$) and multivariable ($p = 0.017$) analyses. Similarly, a significant association was detected between villous atrophy (SVA or PVA) at diagnosis and delayed diagnosis in both the univariate ($p = 0.001$) and multivariable ($p = 0.003$) analyses. Female sex at diagnosis associated significantly with delayed diagnosis in the univariate analysis ($p = 0.043$; Table 2 in the publication for Study **I**). In the 250 DH patients with available data, ARA and/or EmA positivity associated significantly with diagnostic delay in the univariate analysis ($p = 0.001$). DH patients with a delayed diagnosis did not report more bone fractures or malignancies in the follow-up questionnaires compared to those with a non-delayed diagnosis.

10.2 The prevalence of villous atrophy and serum TG2 antibody levels in dermatitis herpetiformis (II)

Of the 393 DH patients in Study **II**, DH was diagnosed in 144 patients between 1970 and 1984, in 144 patients between 1985 and 1999, and in 105 patients between 2000 and 2014. Out of all the patients, small bowel mucosa was considered normal in 108 (28%), while 145 (37%) had PVA and 140 (36%) had SVA in the small bowel mucosa at diagnosis. In the three 15-year periods, a significant decrease ($p = 0.032$) occurred in the prevalence of SVA, from 42% to 35% and then to 29%. At the same time, an 8% increase from 33% to 37% and then to 41% occurred in the prevalence of PVA ($p = 0.22$), albeit statistically insignificantly, and there was a 5% increase in normal villous architecture (from 25% to 28% and then to 30%, $p = 0.34$).

Serum TG2 IgA antibody levels were collected from DH patients in the last 15-year period. In 28 patients with normal villous architecture, median TG2 levels were significantly lower compared to the 28 patients with SVA (2.8 vs 41.5 AU/ml, $p<0.001$) and 40 patients with PVA (2.8 vs 16.5 AU/ml, $p = 0.046$). Normal TG2 antibody levels were detected in 15 (54%) patients with normal villous architecture, 12 (30%) with PVA, and 3 (11%) patients with SVA.

10.3 Prognosis of dermatitis herpetiformis patients with and without villous atrophy (III)

Of the 352 DH patients analysed in Study **III**, 98 (28%) had normal small bowel villous architecture and 254 (72%) had villous atrophy (PVA or SVA) at diagnosis. Parallel to the results in Study **II**, 28 patients with normal villous architecture in Study **III** had ARA, EmA or TG2 antibodies significantly less often compared to the 139 patients with villous atrophy (39% vs 73%, $p<0.001$; Table 1 in the publication for Study **III**). The median age at diagnosis was significantly higher in the DH patients with normal villous architecture compared to the DH patients with villous atrophy (52 vs 37 years, $p<0.001$). After the DH diagnosis, 80% of the DH patients started dapsone treatment. The median duration of dapsone treatment was slightly – but not significantly – longer in DH patients with normal villous architecture compared to patients with villous atrophy at diagnosis (36 vs 24 months, $p = 0.097$). The severity of the rash and haemoglobin levels at diagnosis did not differ significantly between the DH groups.

Follow-up data collected with the study questionnaires was available for 181 DH patients, of whom 39 (22%) had normal villous architecture and 142 (78%) had villous atrophy at diagnosis. The median follow-up time of all DH patients was 22 years. At the follow-up, DH patients with normal villous architecture were significantly older compared to the DH patients with villous atrophy (68 vs 61 years, $p<0.001$). After age adjustment at the follow-up, no significant differences between the DH study groups were detected in the presence of coronary heart disease, hypertension, type 1 or 2 diabetes, thyroid diseases, cerebrovascular diseases, osteopenia/osteoporosis, or malignancies (Figure 1 in the publication for Study **III**). Correspondingly, no significant differences were detected in the use of physician-prescribed regular medications between the DH study groups after age adjustment. Only the usage of vitamin D was more frequent among DH patients with villous atrophy compared to those without villous atrophy (40% vs 23%, $p = 0.050$).

The long-term QoL and severity of gastrointestinal symptoms measured with the PGWB, DLQI, and GSRS questionnaires did not differ between the DH groups. Likewise, no differences were detected between the DH groups in the strictness of the GFD, BMI, smoking habits, or physical activity.

Compared to the DH patients, the coeliac disease controls were more often female (47% vs 81%, $p<0.001$). The strictness of the GFD at the time of the study did not differ between the DH patients and coeliac disease controls. As the DH patients with normal villous architecture were slightly younger (61 vs 65 years) compared to the coeliac disease controls, adjustment for current age was made in all analyses between the study groups. The total number of long-term illnesses was significantly lower (median 1 vs 2, $p<0.001$) in DH patients compared to the coeliac disease controls. The DH patients also had significantly less thyroid disease ($p = 0.019$) and osteopenia/osteoporosis ($p = 0.012$) compared to the coeliac disease controls. However, the presence of bone fractures did not differ significantly between the DH patients and the coeliac disease controls even though an increased trend towards more bone fractures was seen in the coeliac disease controls. The DH patients reported significantly fewer malignancies compared to the coeliac disease controls (7% vs 19%, $p = 0.016$). When the DH patients were compared to the coeliac disease controls, it was shown that the DH patients used significantly less calcium ($p = 0.011$) and vitamin D ($p<0.001$) medication than the controls did. Moreover, the DH patients with normal villous architecture used significantly more statin medication compared to the coeliac disease controls ($p = 0.010$).

The coeliac disease controls were shown to have significantly lower PGWB general health scores compared to the DH patients with villous atrophy ($p = 0.022$),

but otherwise QoL did not differ between the DH patients and the controls when measured with the PGWB. According to the GSRS questionnaire, the coeliac disease controls had significantly higher scores in total symptoms, gastrointestinal pain, and diarrhoea compared to the DH patients (Table 3 in the publication for Study **III**).

10.4 Gluten challenge in dermatitis herpetiformis patients on a gluten-free diet (IV)

By definition, none of the 19 GFD-treated patients participating in the gluten challenge study had the rash at pre-challenge, but three (16%) had skin IgA and TG3 deposits and two had slightly elevated serum TG3 antibody levels. Serum TG2 antibodies and EmA were negative in all patients. Small bowel histology was normal in all 19 patients but 11 (58%) had increased densities of CD3+ IELS and 17 (89%) had increased densities of γδ+ IELs (Table 4). Sixteen patients carried the HLA-DQ2 haplotype and three carried the HLA-DQ8 haplotype.

Gluten challenge caused a relapse of the rash and/or small bowel villous atrophy in 18 (95%) DH patients after a mean of six months. The first relapse occurred after one month and the last was discovered when a small bowel biopsy was performed at the final examination after 12 months of the gluten challenge (Figure 5). Fifteen (79%) patients relapsed with the rash (Figure 6) and altogether 15 (79%) developed small bowel villous atrophy (Table 1 in the publication for Study **IV**). Twelve (63%) patients had elevated levels of serum TG2 antibodies post-challenge. Two patients without the rash developed high levels of serum TG2 antibodies (100 and 54 AU/ml), resulting in the challenge being discontinued; a small bowel biopsy showed villous atrophy in both patients. In addition, one patient did not show any rash or gastrointestinal symptoms and the serum EmA and TG2 antibodies were negative, but a small bowel biopsy at 12 months evinced villous atrophy (Figure 6).

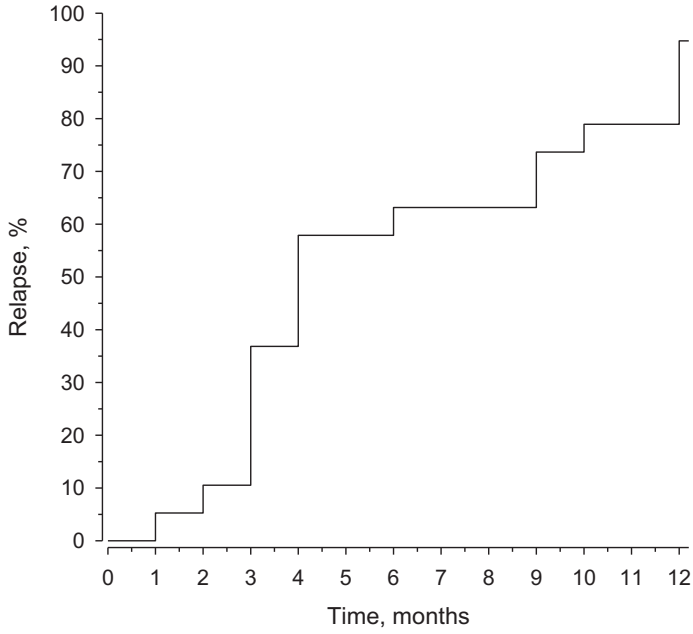

Figure 5. The Kaplan-Meier estimator measuring the relapse % in time (months) after the initiation of the gluten challenge in the 19 patients with dermatitis herpetiformis.

During the gluten challenge, skin IgA became positive in ten DH patients, and all ten also experienced the rash (Figure 6). In double stainings, TG3 co-localized with IgA in all skin biopsy specimens. Two patients relapsing with typical but mild DH rash remained negative for skin IgA and TG3 deposits. In both patients, the rash had appeared less than two weeks before the skin biopsy was taken. Both patients had markedly elevated levels of serum TG3 antibodies and also villous atrophy.

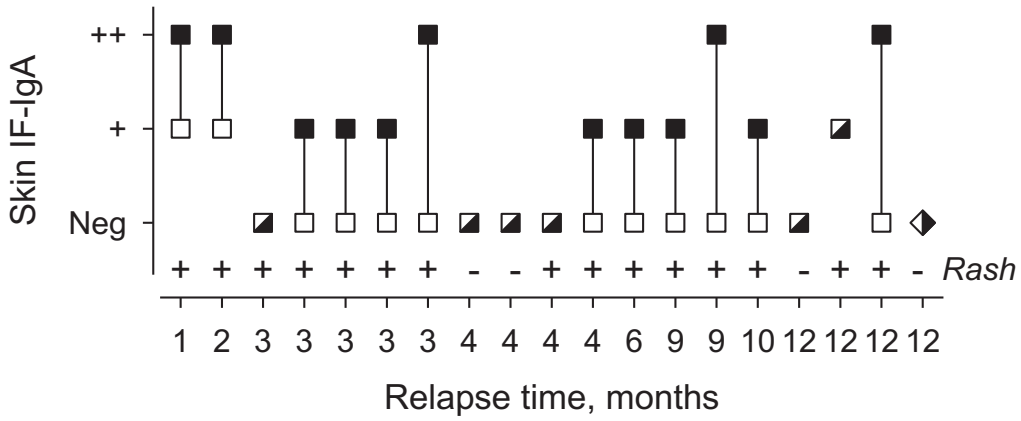

Figure 6. Skin immunofluorescence (IF) biopsy results in 19 dermatitis herpetiformis patients at the pre-challenge □ and post-challenge ■ visit. The symbol ◪ depicts a situation when IF result remained unchanged. The skin IF-IgA result was graded negative (-), positive (+) or strong positive (++). The presence or absence of a rash at the post-challenge is shown with + or -. The symbol ◆ marks the one patient who did not relapse during the study.

The duration of the GFD before the challenge correlated significantly with the relapse time (r = 0.62, CI: 0.24–0.84; Figure 2 in the publication for Study **IV**). One patient did not relapse during the 12-month gluten challenge. At the final examination, he was asymptomatic, his skin IgA and TG3 deposits and serum TG2 and TG3 antibodies were negative, and the small bowel mucosa was normal. The dietary diary showed that the patient's gluten consumption had been at least eight grams of gluten per day. After a two-year follow-up on a gluten-containing diet, the patient was still asymptomatic and the skin IgA and serum TG2 and TG3 antibodies were negative.

When the pre-challenge data were compared to the post-challenge data, a significant increase was seen in the median levels of serum TG3 and TG2 antibodies and EmA (Table 4). In the small bowel specimens, the mean Vh/CrD decreased significantly and the mean densities of CD3+ and γδ+ IELs increased significantly. The GSRS score showed a mild but not significant increase. However, the mean DLQI score increased significantly (Table 4).

Table 4. Pre- and post-challenge data comparisons in 19 gluten-challenged patients with dermatitis herpetiformis.

	Pre-challenge	Post-challenge	p-value
Skin IgA positive, n (%)	3 (16)	13 (68)	0.003
Skin TG3 positive, n (%)	3 (16)	13 (68)	0.003
TG3-ab, median (range)	4 (0–41)	89 (5–89)	<0.001
TG2-ab, median (range)	0 (0–0)	12 (0–100)	<0.001
EmA, median (range)	0 (0–0)	200 (0–4000)	<0.001
Vh/CrD, mean (SD)	2.8 (0.59)	1.2 (0.87)	<0.001
CD3+IELs, mean (SD)	40 (15)	74 (29)	<0.001
γδ+IELs, mean (SD)	10.8 (8.0)	16.5 (11.3)	0.018
TG2-specific IgA deposits in the small bowel mucosa, n (%)	0 (0)	17 (100)	0.002
GSRS total, mean	1.66 (0.55)	1.86 (0.73)	0.22
DLQI mean	0.11 (0.32)	1.58 (2.04)	<0.001

Abbreviations: IgA = immunoglobulin A, TG = transglutaminase, ab = antibodies, EmA = endomysium antibodies, Vh/CrD = villous height, crypt depth ratio, SD = standard deviation, IELs = intraepithelial lymphocytes, GSRS = Gastrointestinal Symptom Rating Scale, DLQI = Dermatology Life Quality Index

11 DISCUSSION

11.1 Delayed diagnosis in dermatitis herpetiformis

The present study examining DH patients diagnosed in 1970–2014 disclosed that the median duration of the rash before the diagnosis has decreased over the period from 12 months to 8 months. However, considering the troublesome and severely itchy skin symptoms, which reduces the patient's QoL (Pasternack et al., 2017), the time from the onset of the rash to the diagnosis of DH is still far too long. Importantly, in the last 15 years, as many as one quarter of the DH patients experienced the rash for two years or more before being diagnosed, i.e. they had a delayed diagnosis. Females and patients with small bowel villous atrophy were shown to be especially at risk for a delayed diagnosis.

Compared to the median duration, the mean duration was longer, at 3.1 years from the onset of the rash to the diagnosis in this study. This is in line with earlier DH studies with far smaller numbers of patients; a study from Ireland (Egan et al., 1997) reported a somewhat shorter (1.6 years) duration of the rash to diagnosis, while a study from Germany (Rose et al., 2010) reported a similar (3.2 years) duration. By contrast, the duration from the onset of gastro-intestinal symptoms to the diagnosis has been notably longer in coeliac disease studies. In hospital cohorts and questionnaire studies, the duration of symptoms before diagnosis has ranged from three to 11 years (Green et al., 2001, Häuser et al., 2006, Rampertab et al., 2006, Ukkola et al., 2011). Moreover, in a recent Finnish study 32% of coeliac disease patients reported their symptoms presenting for over 10 years before the diagnosis (Fuchs et al., 2018).

In the present study, DH patients with villous atrophy at diagnosis were shown to have a delayed diagnosis more often compared to those with normal villous architecture. It is probable that the higher prevalence of villous atrophy is the result rather than the cause of the delayed diagnosis. In line with this, the DH patients evincing normal villous architecture and only coeliac-type inflammation in the small bowel seem to be at an early stage of gluten-induced autoimmune activity. Moreover, a higher frequency of positive ARA/EmA antibodies in DH patients with a delayed

diagnosis is likely a reflection of the progression of small bowel mucosal changes towards villous atrophy.

In Finland, the decrease in diagnostic delay may be a result of better awareness of DH due to nationwide guidelines for the diagnosis and treatment of coeliac disease (Coeliac disease, Current Care Guidelines, 2018). Physicians can access these guidelines online, which also include DH with clinical pictures of the rash and recommendations on how and where to diagnose patients. The present study showed that diagnosis before the year 2000 was significantly associated with a longer diagnostic delay and this result can be partially explained by these guidelines, which became available from 1997 onwards. The diagnostic delay in coeliac disease likewise became shorter in Finland after the publication of these guidelines (Fuchs et al., 2014).

In this study, the female gender was associated with a delayed DH diagnosis. Fuchs et al. (2014) have also reported a similar finding in Finnish coeliac disease patients. Furthermore, parallel results have been detected in other coeliac disease studies (Rampertab et al., 2006, Vavricka et al., 2016). We did not seek to determine whether the diagnostic delays in the present study were due to the inability of doctors to recognise DH or due to the patients. However, it could be expected that the females with an obvious rash and intense itch would seek help from a doctor just as rapidly as males would. In a recent coeliac disease study, the diagnostic delay was caused especially by doctors, and it was even more pronounced in female patients (Vavricka et al., 2016). Nevertheless, the fundamental reasons why female patients seem to have a longer diagnostic delay in DH and coeliac disease remain obscure.

11.2 Villous atrophy in dermatitis herpetiformis

11.2.1 Prevalence of severe villous atrophy is decreasing

During the present DH study covering a period of 45 years, a significant decrease occurred in the prevalence of SVA, and there was a concomitant – albeit not significant – increase in PVA and normal villous architecture. This tendency towards milder small bowel villous findings in DH patients is in line with coeliac disease studies, where the small bowel mucosal damage has become milder over time (Volta et al., 2014; Brar et al., 2007). At present, active risk-group screening detects many new cases of coeliac disease (Husby et al., 2014; Leffler et al., 2010). Therefore,

patients with asymptomatic coeliac disease are also diagnosed earlier (Volta et al., 2014). The improved coeliac disease diagnostics obviously lead to a smaller cohort of undiagnosed coeliac disease patients continuing to consume gluten and developing severe villous damage and DH. It seems, however, that a rather long period of time is needed for the phenotype change from classical coeliac disease to DH (Gawkrodger et al., 1993, Salmi et al., 2015). Patients with normal small bowel mucosal histology but positive EmA or TG2 IgA antibodies could especially be at risk for developing DH when gluten exposure continues. Supporting this, one quarter of the newly diagnosed DH patients in the present study showed normal villous architecture, and half of them had serum TG2 IgA antibodies.

In the last 15-year period of this DH study, serum TG2 IgA antibody levels were shown to associate significantly with SVA and PVA. This finding agrees well with previous DH and coeliac disease studies (Dahlbom et al., 2010, Leffler and Schuppan, 2010, Singh et al., 2015). However, some of the DH patients with villous atrophy had normal TG2 antibody levels, and a few patients with normal villous architecture had increased antibody levels. This shows that the sensitivity of the TG2 antibody test for detecting villous atrophy is not 100% in DH, but overall, a positive TG2 antibody test is a relatively good predictor of the presence of villous atrophy in child and adult patients with DH (Dahlbom et al., 2010).

It is important to acknowledge, however, that a negative TG2 antibody test does not exclude DH. In this study, as much as 30% of the patients had a negative TG2 antibody test at DH diagnosis, whereas in earlier studies the percentage was 20 (Kumar et al. 2001, Rose et al. 2009). As fewer DH patients currently evince villous atrophy at DH diagnosis, the negative TG2 antibody result is even more probable. In the present study, 54% of the patients with normal villous architecture were seronegative.

11.2.2 Similar prognosis in patients with and without villous atrophy

The current study established that the prognosis of DH patients with and without villous atrophy at diagnosis does not differ in regard to GFD treatment response, nor in the long-term development of chronic illnesses and coeliac disease-associated complications or QoL.

After the DH diagnosis, 80% of DH patients in this study started dapsone medication. The duration of dapsone treatment was considered the active disease time period, which lasted until the patients were symptom-free with a GFD alone.

The median duration of dapsone treatment (30 months) was in line with earlier GFD treatment studies (Fry et al., 1982, Gawkrodger et al., 1984). Importantly, even though in the present study the DH patients with normal small bowel mucosa used dapsone slightly longer compared to those with villous atrophy, the difference was not statistically significant. Therefore, the responsiveness of the DH rash to a GFD does not seem to depend on the absence or presence of small bowel mucosal damage at diagnosis. In addition, this study also showed that after long-term adherence to a GFD, the presence of chronic illnesses, complications, and QoL are equal in DH patients with and without villous atrophy at diagnosis. Moreover, an earlier DH study from our group showed that the mortality of DH patients with villous atrophy at diagnosis does not differ from that of DH patients with normal villous architecture at diagnosis (Hervonen et al., 2012). Therefore, it is obvious that GFD-treated DH patients have a similar short- and long-term prognosis that is independent of the presence or absence of villous atrophy at diagnosis. Due to this, routine gastroscopy with small bowel biopsies for DH patients is no longer recommended in the Finnish Coeliac Disease Guidelines (2018). However, the need for small bowel biopsy is obvious when the patient has obscure gastrointestinal symptoms or a suspicion of malignancy, as recommended earlier in an Italian DH guideline (Caproni et al., 2009).

In this study, the median age at DH diagnosis was significantly higher in patients without villous atrophy compared to those with villous atrophy (52 vs 37 years), but the diagnostic time period did not differ between the study groups. Therefore, the earlier reported finding by our group (Salmi et al., 2011) that age at diagnosis in DH has increased continuously from 1970 does not explain the difference between the two study groups. However, it is possible that elderly people might have a less prominent immune response resulting in milder small bowel mucosal alterations. In line with this, older patients with coeliac disease have shown to be more commonly seronegative, and a trend towards less severe histopathology has also been observed with increasing age (Salmi et al., 2006, Vivas et al., 2008).

In agreement with a previous study showing a slightly higher frequency of autoimmune diseases in patients with coeliac disease compared to patients with DH (Reunala and Collin, 1997), thyroid disease associated more often with coeliac disease than with DH in this study. Compared to the DH patients, the GFD-treated coeliac disease controls in the present study had significantly more malignancies and long-term illnesses, and osteopenia or osteoporosis were more common in particular. Earlier studies have shown that even in GFD-treated coeliac disease patients, the risk of osteoporotic fractures, malignancies, and mortality is increased (Asklinget al., 2002, Kamycheva et al., 2017, West et al., 2004). Additionally, the

GFD-treated coeliac disease controls in the present study reported poorer general health and gastrointestinal pain and diarrhoea more often compared to the DH patients. These results are in line with a recent long-term study by our group, which showed DH patients on a GFD had a better QoL and fewer gastrointestinal symptoms when compared to coeliac disease patients (Pasternack et al., 2015).

11.3 Gluten challenge causes relapse in long-term gluten-free diet treated dermatitis herpetiformis patients

In this study, 18 (95%) of the GFD treated DH patients participating in the 12-month gluten challenge study showed disease relapse in the skin and/or in the small bowel mucosa. In 15 patients, the challenge induced a DH rash and 80% of these patients also showed a progression towards small bowel mucosal villous atrophy. Interestingly, however, three relapsed patients did not develop the rash or skin IgA and TG3 deposits but evinced the development of villous atrophy. One challenged patient did not develop any signs of DH or coeliac disease during a two-year follow-up on a normal gluten-containing diet.

IgA and TG3 deposits are considered pathognomonic for untreated DH (Donaldson et al., 2007, Sárdyet al., 2002). At pre-challenge, three patients in this study had IgA and TG3 deposits in the skin, but 16 were negative. This fits well with their long (mean 23 years) adherence to a GFD. During the GFD treatment, these deposits are known to disappear, but they can persist for many years after the rash and small bowel villous atrophy have healed (Hietikko et al., 2018a). In the present study, we observed a rather fast (mean 6.2 months, range 3–12 months) re-appearance both IgA and TG3 in 10 challenged patients, which is an unreported finding. Surprisingly, two patients with a mild DH rash and markedly elevated serum TG3 antibody levels remained negative for skin IgA and TG3 deposits. As their skin biopsies were taken correctly from the perilesional skin area (Donaldson et al., 2007, Zoneet al., 1996), one possibility could be that IgA and TG3 were not yet detectable by the conventional IF technology used in the study. The results of this study suggest that if a patient has adhered to a GFD for several years and skin IgA is negative, at least three-month gluten challenge is necessary before reappearance of IgA can be expected and skin biopsy should be taken. However, if the duration of GFD is shorter, weeks to months, there is no need to discontinue GFD before the diagnostic skin biopsy, since IgA deposits persist in the skin for a long time after patient has initiated GFD (Garioch et al., 1994, Reunala et al., 2015b).

It is possible that the three relapsed patients with no rash or skin IgA or TG3 deposits had a phenotype change to coeliac disease. A change of phenotype from coeliac disease to DH has been reported earlier mostly in patients not adhering strictly to a GFD (Gawkrodger et al., 1993, Salmi et al., 2015). However, it is also possible that these DH patients without the rash at the relapse could have developed skin symptoms later if gluten consumption had continued. Importantly, most DH patients relapsing with the rash also had villous atrophy, strongly indicating that the small bowel mucosa is the primary target of the gluten-induced autoimmune reaction in DH (Collinet al., 2017, Sárdy et al., 2002).

In this study, the duration of the GFD before the gluten challenge correlated with the relapse time, and a similar result was documented previously by Leonard et al. (1983a). In their gluten challenge study of 12 DH patients, 92% relapsed with a rash, and 64% of these also developed small bowel villous atrophy. Later, Bardella et al. (2003) reported a lower relapse rate of 82% in 38 challenged patients. However, all seven non-relapsed patients were children, and their compliance with a GFD was only moderate or poor before the challenge. Due to this, the authors suggested that diagnosis in childhood and dietary lapses could induce the redevelopment of a tolerance to gluten. On the contrary, another well performed study showed that all gluten-challenged DH children relapsed (Kosnai et al., 1986, Table 5). In the current study, none of the patients had been diagnosed in childhood, but three patients reported dietary lapses when adhering to a GFD. One of these was the non-relapsed patient who had adhered for 36 years to a GFD. This may be one reason for the non-relapse, and a follow-up longer than two years on a normal gluten-containing diet is required before a persistent tolerance to gluten can be confirmed. However, until further knowledge becomes available, life-long adherence to a GFD is justified in all DH patients.

Table 5. Gluten challenge and remission studies in dermatitis herpetiformis.

Challenge studies	**Patients**	**GFD duration before challenge, mean years (range)**	**Non-relapsed patients on gluten challenge**	**Challenge duration, mean months (range)**	**Comment**
Mansikka et al., 2019 Finland	19 adults	23 (5–40)	1 [1] (5%)	6 (1–12)	Duration of GFD before challenge correlated with the relapse time. Ten skin IgA-negative patients tested positive after the challenge.
Bardella et al., 2003 Italy	38 adults and children	7.3	7 [2] (18%)	2 (1–6) [4]	All 7 patients in remission were children who did not follow a strict GFD before the challenge. At pre-challenge, all had lost skin IgA deposits and had normal small bowel mucosa.
Kosnai et al., 1986 Hungary and Finland	16 children	1.5 (0.9–3.7)	0	5 (3–29)	All children had the rash and villous atrophy after the challenge.
Leonard et al., 1983 England	12 adults	7.6 (2.6–11.7)	1[3] (8%)	3 (0.5–9)	Duration of GFD before the challenge associated with the relapse time. Three skin IgA-negative patients tested positive after the challenge.
Remission studies	**Patients**	**Study years**	**Patients in remission**		
Paek et al., 2011 USA	86 34 adhered to a GFD	1972–2010	10 (12%)	-	No skin IgA or small bowel data during remission.
Garioch et al., 1994 UK	133 on a GFD 77 on a normal diet	1967–1992	2 (5%) 8 (10%)	-	All except one patient in remission had IgA deposits in the skin. No small bowel data during remission.
Gawkrodger et al., 1984 Scotland	51 on a GFD 25 on a normal diet	1972–1983	0 2 (8%)	-	

[1] Two-year follow-up time on a gluten-containing diet; [2] median follow-up time 12 (range 3–21) years on a gluten-containing diet; [3] one-year follow-up time on a gluten-containing diet; [4] in relapsed patients; GFD = gluten free diet

11.4 Strengths and limitations of the study

The main strength in Studies **I–III** is the large patient cohort collected from 1970 onwards at a special DH out-patient clinic at Tampere University Hospital (Hervonen et al., 2012). The diagnosis was invariably based on the presence of granular IgA deposits in the papillary dermis (Zone et al., 1996). Two experienced dermatologists examined and treated almost all the present patients with DH. In addition, small bowel biopsy results were available for the majority of the patients, since endoscopy with small bowel biopsy has been performed routinely for most newly diagnosed patients with DH. Moreover, a GFD has been recommended to all DH patients. Most of them have started GFD treatment and have had excellent dietary adherence rates (Hervonen et al., 2012). In Study **I**, data on the duration of the rash before the diagnosis was retrieved primarily from the medical records, which is more reliable than questionnaire-based data obtained several years or even decades after the diagnosis. The major strengths of Study **IV** are the well-defined long-term GFD-treated study group and the thoroughly conducted gluten challenge with regular follow-up visits and clinical, serological, and small bowel mucosal biopsy endpoints. At the relapse, we could also examine the re-appearance of IgA and TG3 deposits in the skin and TG2 deposits in the small bowel mucosa, which are rather new research findings that have not been previously examined during a gluten challenge (Donaldson et al., 2007, Salmi et al., 2014, Sárdy et al., 2002).

The main limitation in Studies **I–III** is the retrospective study design. In addition, the definition of delayed diagnosis in Study **I**, i.e. two years or more, was based only on personal clinical experience. The definition was adapted from a previous study in which the median duration of rash before diagnosis was 9 months in patients with refractory DH and 12 months in control DH patients (Hervonen et al., 2016). Furthermore, as the follow-up data for Studies **I** and **III** were collected from questionnaires, selection and recall bias is possible. The disease-specific questionnaires were designed for this study and have not been validated. In addition, even though widely used in coeliac disease studies, the GSRS and PGWB questionnaires have not been validated specifically for coeliac disease. Additionally, in Study **II** and **III**, there was no opportunity for the more accurate examination of mucosal samples other than the routine histological evaluation performed by the pathologists. A limitation of Study **IV** is the relatively small number of patients, as is the rather short follow-up time of the non-relapsed DH patient.

12 CONCLUSIONS AND CLINICAL IMPLICATIONS

DH is the best known extraintestinal manifestation of coeliac disease, and Finland has the highest prevalence of this autoimmune skin disease. Diagnosis is based on pathognomonic granular IgA deposits in the papillary dermis. The mainstay of treatment is a life-long GFD. The mean age at diagnosis is increasing in Finland and mortality in GFD-treated DH patients is lower than that in the general population. However, there has been a lack of insight on the diagnostic delay and prognostic value of small bowel histological changes. Finally, earlier studies have suggested that some GFD-treated DH patients may go into remission by re-developing gluten tolerance.

The principal conclusions of the present study are as follows:

1. The time from the onset of rash to diagnosis in DH has decreased over the past 45 years. Even at present, one quarter of DH patients have a delayed diagnosis, i.e. they have the rash for two years or more before diagnosis. Delayed diagnosis is more common in female patients and those with villous atrophy.
2. Small bowel histology in DH has altered over the past 45 years towards milder small bowel damage. Serum TG2 IgA antibody levels are more reliable in detecting DH patients with villous atrophy than those without atrophy.
3. DH patients with or without small bowel villous atrophy at diagnosis have a similar and excellent long-term prognosis on GFD treatment.
4. A gluten challenge of up to one year induces DH rash and/or villous atrophy in the vast majority of long-term GFD-treated DH patients. DH rash relapse results in the rather rapid appearance of skin IgA and TG3 deposits in most – but not all – patients.

As is the case with coeliac disease, it is important to increase the awareness of DH, and both are treatable with a GFD. At present, the duration of the rash before diagnosis in DH is still excessively long in Finland, and, similarly to coeliac disease,

there is an unexplained diagnostic delay particularly in female patients. The awareness of DH and coeliac disease among general practitioners in Finland seems to have improved, which is probably the result of the online available Current Care Guidelines (2018). A trend towards milder small bowel mucosal findings was observed in the present study, and importantly, the DH patients with and without villous atrophy had a similar short- and long-term prognosis on a GFD treatment. These results revealed that there is no need to perform routine gastroscopies at DH diagnosis to obtain small bowel biopsies. This has now been updated in the Finnish Coeliac Disease Guidelines (2018). Furthermore, the present DH study showed that elevated serum TG2 antibodies indicate rather well the presence of villous atrophy in DH. As the TG2 IgA antibody ELISA test is widely available, it can be easily used to detect the majority of DH patients with villous atrophy, but it does not detect all individuals with villous atrophy. In clinical work however, it is imperative to recognize that a negative serum TG2 antibody result does not exclude DH, and if the clinical picture is compatible with DH, further investigations with skin IF biopsy are necessary.

The present gluten challenge study showed that even after long-term GFD treatment, all except one DH patient relapsed. The reason for the non-relapse could be the rather short follow-up time on the gluten-containing diet. Therefore, a strict life-long GFD still seems necessary for all adult DH patients. Whether the redevelopment of a tolerance to gluten may be achieved in some DH children not adhering strictly to a GFD remains a possibility, although it was not examined in the present dissertation. The results of the present gluten challenge study showed a relapse in both the skin and the small bowel mucosa. In addition, a few patients without any skin symptoms evinced small bowel villous damage in the relapse, indicating that the primary target in gluten intolerance is in small bowel mucosa also in DH. In the present DH study, both the small bowel mucosal TG2 deposits and skin IgA and TG3 deposits were shown to reappear at the relapse. However, the exact immune reaction initiating from the small bowel mucosal TG2-targeted IgA deposits and leading to skin IgA/TG3 deposits and the rash requires is not known. Further studies to investigate this would be of value.

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13 ORIGINAL PUBLICATIONS

PUBLICATION

I

Diagnostic delay in dermatitis herpetiformis in a high-prevalence area

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Diagnostic Delay in Dermatitis Herpetiformis in a High-prevalence Area

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Dermatitis herpetiformis (DH) is an extra-intestinal manifestation of coeliac disease. The highest currently reported prevalence of DH is in Finland, but knowledge of diagnostic delay is limited. This study investigated the duration of rash prior to diagnosis in 446 patients with DH, analysing the results in 3 periods of 15 years. The diagnosis was considered delayed when the duration of rash before diagnosis was 2 years or longer. Factors associated with delayed diagnosis were analysed. Within the 45 years, the median duration of rash before diagnosis decreased significantly, from 12.0 to 8.0 months ($p = 0.002$) and the occurrence of a delayed diagnosis decreased from 47% to 25% ($p = 0.002$). Female sex, the presence of villous atrophy, and a diagnosis of DH before the year 2000 were significantly associated with delayed diagnosis. In conclusion, the present study showed that one-quarter of patients currently have a diagnostic delay of 2 years or more, which is far from ideal.

Key words: dermatitis herpetiformis; coeliac disease; diagnostic delay; gluten-free diet; dapsone.

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Dermatitis herpetiformis (DH) is a cutaneous manifestation of coeliac disease presenting as an itchy polymorphic blistering rash on the elbows, knees, buttocks and scalp (1). Diagnosis of DH is based on the presence of typical skin symptoms and the demonstration of IgA in the papillary dermis during direct immunofluorescence examination (2). Although 75% of patients with DH have small bowel mucosal villous atrophy at diagnosis, only a minority have marked gastrointestinal symptoms (3, 4). The treatment of DH is a lifelong gluten-free diet (GFD), similar to treatment of coeliac disease (4, 5). A GFD results in healing of the enteropathy and the rash, but the rash alleviates slowly and additional treatment with dapsone (4,4'-diaminodiphenylsulfone) is frequently needed at the start of dietary treatment (6, 7).

DH is considered relatively uncommon, having the highest reported prevalence of 75.3 per 100,000 people in Finland and a lower prevalence in UK and the USA (8–10). In contrast to the established increase in the incidence of coeliac disease, the incidence of DH decreased in both Finland and UK during the 1990s (8, 9). DH constitutes a diagnostic challenge to general practitioners and other non-dermatologists, and can easily be misdiagnosed as other itchy or blistering skin diseases (11, 12). Early diagnosis is warranted in DH, since ongoing symptoms reduce quality of life, and undiagnosed DH predisposes to complications, such as lymphoma and low bone mineral density (13–15).

For coeliac disease, the median time from onset of gastrointestinal symptoms to diagnosis in Finland is currently 3 years (16). Fortunately, diagnostic delay has decreased over the past decades in Finland, other European countries, and the USA (17–20). However, up-to-date knowledge about diagnostic delay in DH is lacking. The aim of this study was to investigate the changes in the diagnostic delay in DH and to analyse possible factors associated with delayed diagnosis. Our prospectively collected large DH cohort enabled us to perform delay analyses for 3 periods of 15 years.

PATIENTS AND METHODS

All patients suspected of having DH in the Tampere region, Finland, are referred by private dermatologists and general practitioners working in healthcare centres to the Department of Dermatology, Tampere University Hospital, for confirmation of DH diagnosis. Clinical suspicion of DH is an adequate reason for referral regardless of coeliac autoantibody result, and the referral policy for DH has remained the same for the years of the present study. In Tampere University Hospital, diagnosis of DH is based on the detection of typical clinical symptoms and the presence of granular IgA deposits in the papillary dermis (2), and this diagnostic procedure has remained unchanged during the study period. All patients are treated at a special DH clinic, where they are advised to adhere to a lifelong GFD.

Our DH cohort consists of 512 patients who had been diagnosed and treated at the DH clinic between 1970 and 2014. A total of 446 patients were included for further analysis. Of the 66 patients excluded, 10 did not have IgA deposits in the skin, 21 had died over 20 years previously and their data was not available, and 35 had a previous diagnosis of coeliac disease. Data on the duration of the rash before diagnosis were collected from medical records held at Tampere University Hospital. The diagnosis was considered delayed when the duration of the rash before diagnosis was 2 years or more. The definition for delayed diagnosis was based on a previous study performed in our hospital district (21) and

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clinical experience. In December 2015, a special questionnaire that included questions about the duration of the rash, presence of gastrointestinal symptoms before the diagnosis and the occurrence of malignancies and fractures was sent to all 413 surviving patients with DH. A total of 237 (56%) patients responded, and 217 were included in the analysis of gastrointestinal symptoms. Self-reported bone fractures and malignancies were recorded from questionnaires. Excessive-trauma fractures (the trauma causing the fracture considered sufficient to cause a bone fracture for any person) and stress fractures were excluded from further bone fracture analyses, and non-melanoma skin cancers were excluded from further malignancy analyses.

The severity of the rash at DH diagnosis was collected from medical records and scored as mild, moderate, or severe according to the presence of a few, several, or many blisters; macular eruptions; and erosions on the knees, elbows, buttocks, scalp, or elsewhere on the body. Small bowel biopsy results at diagnosis collected from medical records were graded by experienced pathologists as subtotal villous atrophy (SVA), partial villous atrophy (PVA), or normal mucosa (22).

The results of reticulin and/or endomysium autoantibodies at diagnosis were collected from medical records and categorized as positive or negative.

The study protocol was approved by the Ethics Committee of Pirkanmaa Hospital District (R15143). Informed consent was obtained from each study participant responding to the questionnaire.

For the descriptive statistics and further analysis, the patients were grouped into three 15-year periods according to the year of their DH diagnosis: 1970−1984, 1985−1999, and 2000−2014. Diagnostic delay was expressed as the median with lower and upper quartiles (Q_1–Q_3), and also as mean, in order to be able to compare it with previous studies. The 2-sided χ^2 test, Fisher's exact test, and the Kruskal–Wallis test were used for verifying the relationship between the year of diagnosis of DH, diagnostic delay, severity of rash, and gastro-intestinal symptoms. The significance level was set at ≤ 0.05.

To identify factors associated with delayed diagnosis, binary logistic regression analysis was used. Univariate analysis was used at first for each associating factor, then multivariable analysis was used for detecting the independence of delay-associated parameters found in the univariate analysis. In the multivariable analysis, sex, age at diagnosis, calendar period of diagnosis, severity of rash, presence of gastrointestinal symptoms, and small bowel biopsy findings at diagnosis were all taken into consideration. Associations are expressed in terms of odds ratios (OR) with 95% confidence intervals. To determine whether the patients with delayed diagnosis developed more bone fractures or malignancies, Pearson χ^2 test and Fisher's 2-sided exact test were used. A p-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 23.0. Armonk, NY: IBM Corp.).

RESULTS

Patients, duration of rash before diagnosis, and small bowel biopsy findings

Of the 446 patients with DH, 229 (51%) were males. Mean age at diagnosis was 43 (range 3–83) years. The number of patients with DH diagnosed within the three 15-year periods was 151 in 1970−1984, 161 in 1985−1999, and 134 in 2000−2014 (**Table I**). As already reported in our previous study (8), the median age at diagnosis increased significantly ($p < 0.001$), from 33 years in the first study period to 54 years in the third study period.

During the entire study period, the median duration of the rash before diagnosis was 10 months and the mean was 3.1 years. The median duration of the rash decreased significantly from 12.0 to 11.0 and then to 8.0 months ($p = 0.002$) during the three 15-year periods, respectively (**Fig. 1**); statistical significance was found when the first and third period were compared (Table I). The corresponding mean values were 37 (range 0.1–240), 43 (0.1–528), and 34 (1–480) months, respectively.

The rash was severe at diagnosis in 112 (29%) of the 386 patients, and 89 out of 217 (41%) reported having had gastrointestinal symptoms at diagnosis. There was no difference in the occurrence of severe rash or gastrointestinal symptoms at diagnosis during the 3 study periods (Table I). The small bowel biopsy result was available for 347 (78%) patients, of whom 131 (38%) had SVA, 116 (33%) PVA, and 100 (29%) normal mucosal morphology (Table I). Overall, there was no statistically significant change in the three 15-year periods; however, a decreasing trend was seen in the occurrence of SVA and there was a concomitant increasing trend in normal villous architecture.

Factors associated with delayed diagnosis

Of the 446 patients in the study, 389 (87%) had sufficient information for the analysis of factors associated with the delayed diagnosis (**Table II**). Altogether, 142 (37%) patients had a delayed diagnosis, i.e. time from onset of rash to diagnosis was 2 years or more. The percentages in the 3 study periods were 47%, 38% and 25%, respectively (Table I).

Table I. Demography, duration of rash, delayed diagnosis, and small bowel biopsy findings in the three 15-year periods in 446 patients with dermatitis herpetiformis (DH)

Number of patients with DH	1970–1984 $n = 151$	1985–1999 $n = 161$	2000–2014 $n = 134$	p-value
Males, *n* (%)	74 (49)	83 (52)	72 (54)	0.727
Age at diagnosis; median (Q_1–Q_3)	33 (23–47)	42 (30–54)	54 (35–65)	< 0.001
Duration of rash before diagnosis, months, median (Q_1–Q_3)	12.0 (7–48)	11.0 (6–36)	8.0 (4–24)	0.002[a]
Delayed diagnosis[b], *n* (%)	55 (47)	56 (38)	31 (25)	0.002
Severe rash at diagnosis, *n* (%)	36 (32)	33 (24)	43 (35)	0.134
Presence of gastrointestinal symptoms at diagnosis, *n* (%)	43 (48)	56 (49)	56 (48)	0.990
Small bowel histology at diagnosis, *n* (%)				
Subtotal villous atrophy	51 (46)	48 (37)	32 (30)	0.214[c]
Partial villous atrophy	35 (31)	45 (34)	36 (35)	
Normal mucosa	26 (23)	38 (29)	36 (35)	

[a]Third period compared with the first period. [b]Duration of 2 years or more. [c]All biopsy results analysed together.

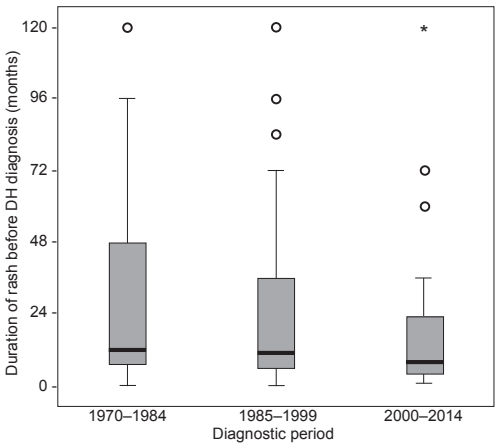

Fig. 1. Duration of rash before diagnosis in patients with dermatitis herpetiformis (DH) in the three 15-year diagnostic periods. The lower and upper limits of the boxes indicate the 25% and 75% confidence intervals and the black bars indicate median values. The whiskers extend to 1.5 times the height of the box and the circles are outliers that exceeded the interquartile range ± 1.5 times, The asterisk is an extreme outlier that exceeded the interquartile range +3 times.

Female sex (OR 1.5) was significantly ($p=0.043$) associated with delayed diagnosis in the univariate analysis, but not in multivariable analysis ($p=0.061$, Table II). Age at diagnosis was not associated with the delay. DH diagnosis performed during the first study period was significantly associated with delayed diagnosis, both in the univariate (OR 2.62, $p=0.001$) and multivariable (OR 2.32, $p=0.017$) analyses (Table II). The severity of rash or presence of gastrointestinal symptoms at diagnosis was not connected with delayed diagnosis. By contrast, villous atrophy (subtotal or partial) at diagnosis was significantly associated with diagnostic delay, both in the univariate (OR 2.70, $p=0.001$) and multivariable (OR 2.52, $p=0.003$) analyses. Furthermore, the positivity of coeliac autoantibodies (reticulin and/or endomysium) was significantly associated with diagnostic delay in the univariate analysis (OR 2.63, $p=0.001$) in the 250 patients with available autoantibody results.

The patients with delayed diagnosis did not develop more bone fractures (17.6%) than those without long delay in diagnosis (14.3%) ($p=0.523$), nor did they develop more malignancies (7.4% vs. 6.0% respectively; $p=0.765$).

DISCUSSION

This long-term study of DH patients diagnosed between 1970 and 2014 analysed time tendencies from the onset of the rash to diagnosis. In the three 15-year study periods, the median durations of rash before diagnosis were 12, 11 and 8 months, respectively. Importantly, the rash duration in the third and latest period was significantly shorter than in the first period. One reason for this seems to be better awareness of the disease due to nationwide guidelines for the diagnosis and treatment of coeliac disease in Finland, which were published in 1997 and are updated regularly (23). General practitioners and other physicians can access these guidelines online, and they also include DH, with clinical pictures of the rash and recommendations on how and where to diagnose patients.

Similarly to DH, the diagnostic delay for coeliac disease became shorter in Finland after the publication of the Current Care Guidelines (17). However, it is noteworthy that the diagnostic delay in coeliac disease is still much longer than in DH; in approximately one-third of Finnish patients with coeliac disease (17) and DH, there are delays of over 10 and 2 years, respectively.

The present DH cohort of 446 patients showed that the mean time from the first symptoms of rash to diagnosis was 3.1 years. Two previous DH studies with a smaller number of patients, one from Ireland (24) and another from

Table II. Associations between clinical characteristics, time of diagnosis, and delayed diagnosis[a] in 389 patients with dermatitis herpetiformis

	n	Delayed diagnosis %	Univariate OR (95% CI)	*p*-value	Multivariable[c] OR (95% CI)	*p*-value
Sex						
Male	199	32	1		1	
Female	190	42	1.5 (1.02–2.33)	0.043	1.64 (0.98–2.74)	0.061
Age at diagnosis						
> 50 years	146	30	1		1	
30–50 years	141	40	1.68 (0.99–2.85)	0.056	1.15 (0.80–2.74)	0.214
< 30 years	102	41	1.63 (0.996–2.65)	0.052	0.88 (0.60–2.50)	0.581
Calendar period of diagnosis						
2000 to 2014	124	47	1		1	
1985 to 1999	147	38	1.85 (1.09–3.12)	0.022	1.55 (0.81–2.97)	0.184
1970 to 1984	118	25	2.62 (1.52–4.51)	0.001	2.32 (1.17–4.62)	0.017
Severity of rash at diagnosis						
Severe	104	33	1		1	
Moderate	181	39	1.30 (0.63–2.31)	0.573	1.64 (0.39–1.90)	0.858
Mild	65	37	1.21 (0.78–2.16)	0.313	1.30 (0.63–2.66)	0.184
Small bowel biopsy finding at diagnosis						
Normal	87	23	1		1	
Villous atrophy[b]	222	45	2.70 (1.53–4.75)	0.001	2.52 (1.36–4.68)	0.003

[a]Rash lasting ≥2 years before diagnosis of dermatitis herpetiformis. [b]Partial or subtotal villous atrophy. [c]280 patients in this analysis.
CI: confidence interval; OR: odds ratio.

Germany (25), documented a shorter (1.6 years) or similar (3.2 years) duration to diagnosis, respectively (**Table III**). By comparison, a recent Swiss study showed that, in bullous pemphigoid, the mean time from the first symptoms to diagnosis was much shorter (6.1 months) (26). It is clear that the blistering rash with accompanying intense itch in DH means that there is usually little delay before the patient contacts a physician. When DH is suspected, the patient is sent directly to our University Hospital for a final diagnosis. It is, however, noteworthy that the time needed for diagnosis can vary greatly among patients with DH. In the present study, the range was from 0.1 to 44 years, and in the 2 previous DH studies, the longest durations were 4 and 20 years, respectively (24, 25).

In contrast to DH, the delay in diagnosis of coeliac disease has received much attention in recent years. The diagnostic delay for coeliac disease in hospital cohorts and questionnaire studies has been reported to be very long, from 3 to 11 years (Table III) (16, 19, 20, 27–29). Consuming a gluten-containing diet for such a long time may increase the risk of developing DH, which is the most common extra-intestinal manifestation of coeliac disease (1, 30). An important issue is how long the patients with DH have had preceding undiagnosed coeliac disease in the small bowel. The time period can be very long (up to 30 years), as shown by studies reporting a phenotype change from partially GFD-treated coeliac disease to DH (31, 32). Moreover, coeliac-type dental enamel defects observed in adults with DH suggest that undiagnosed coeliac disease had been present in childhood in many of the patients (33).

Small bowel mucosal damage in patients with DH ranges from severe, to partial villous atrophy, to normal villous structure with coeliac-type inflammatory changes (22). The present study analysed whether the small bowel damage could be linked to the delay in diagnosis of DH. It was found that the patients with villous atrophy significantly more often had a long diagnostic delay than those with normal villous mucosa. Villous atrophy can be a result rather than a cause of delayed diagnosis; after initial onset of gluten intolerance, it can take a long time before villous atrophy develops in coeliac disease (34, 35). Therefore, patients with DH who have undamaged villous morphology could be in the early stages of this process. Similarly, we found that the patients with positive coeliac autoantibodies significantly more often had a delayed diagnosis than those with negative autoantibodies, which probably reflects the same thing; since the diagnosis becomes delayed the autoantibody response has time to progress.

Several studies of coeliac disease have shown that females have a longer diagnostic delay than males (17, 20, 29). Similarly, in the univariate statistical analysis of the present DH study, females significantly more often had a delayed diagnosis than did males. Irritable bowel syndrome is common in females, and may mask the diagnosis of coeliac disease (20). Patients with DH rarely have obvious gastrointestinal symptoms, and the rash with intense itch is the reason for seeking medical advice. Therefore, the reasons why females seem to have delayed diagnosis in both coeliac disease and DH compared with males remain unknown.

Untreated DH has shown to decrease patients' quality of life, which improves along with GFD treatment after diagnosis (13). The current study did not detect association with delayed diagnosis and development of bone fractures or malignancies, but the burden and complications related to delayed diagnosis of DH are yet to be fully elucidated.

The main strengths of the present study were the large patient cohort, collected from 1970 onwards at the single DH clinic. The diagnosis was invariably based on the presence of granular IgA deposits in the papillary dermis (2). Two experienced dermatologists have treated almost all of the present patients with DH, making notes on the presence of the rash at the time of diagnosis. In addition, small bowel biopsy results were available for the majority of patients, since endoscopy with small bowel biopsy has been performed routinely for all newly diagnosed patients with DH (22). The main limitations were the retrospective study design and a rather low response rate to the questionnaire. However, the data on the duration of the rash before the diagnosis was retrieved primarily from the patients' medical records, and only the data on the presence of gastrointestinal symptoms was derived from the questionnaires. A further limitation is that the threshold of 2 years or more for delayed diagnosis is

Table III. Time from the onset of symptoms to diagnosis in dermatitis herpetiformis (DH) and coeliac disease (CD) in the present and previous studies

Studies	Patients, *n*, (M/F ratio) Years diagnosed	Age at diagnosis, years, mean (range)	Duration of symptoms before diagnosis, years, mean (range)	Comment on diagnostic delay
DH studies				
Present study, 2017, Finland	446 (1.1)[a], 1970–2014	43.0 (3–83)	3.1 (0.1–44)	Decreased over time. Longer in females
Rose et al. 2010, Germany	32 (1.5)[a], 1996–2008	43.0 (10–84)	3.2 (0.1–20)	
Egan et al. 1997, Ireland	54 (1.8)[a], 1984–1993	42.0 (18–79)	1.6 (0.25–4)	
CD studies				
Vavricka et al. 2016, Switzerland	1,689 (0.29)[b], not given	31.1 (0–83)	7.3 (0–65)	Decreased from 1990 to 2010. Longer in females
Ukkola et al. 2011, Finland	490 (0.30)[b], 2007–2008	49.0 (16–84)[c]	3.0 (0–59)[c]	
Hurley et al. 2012, Wales	347 (0.50)[a], 1996–2005	49.9 (16–88)	6.1 (0.1–15)	
Rampertab et al. 2006, USA	590 (0.47)[a], 1952–2004	43.4 (16–83)	4.6 (0–60)	Decreased over time. Longer in females
Häuser et al. 2006, Germany	446 (0.41)[b], not given	37.3 (0–85)	4.4 (0–62)	
Green et al. 2001, USA	1,138 (0.34)[b], not given	45.2 (18–88)	11.0 (0–70)	

[a]Hospital cohort. [b]Questionnaire study to Coeliac Disease Society members. [c]Median.

somewhat arbitrary; however, it is partially based on our previous study in which the median duration of rash before diagnosis was 9 months in patients with refractory DH and 12 months in control patients with DH (21). In addition, from a clinical point of view, we consider a diagnostic delay of 2 years or more with itchy rash to be far too long.

In conclusion, the present long-term study showed that one-third of patients had a diagnostic delay of at least 2 years. Female sex, villous atrophy at diagnosis, and a DH diagnosis before the year 2000 were significantly associated with delayed diagnosis. Even though the situation has improved over the past 45 years, the diagnostic delay is still unacceptably high for a country with such a high prevalence of the disorder.

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PUBLICATION

II

The decreasing prevalence of severe villous atrophy in dermatitis herpetiformis

A 45-year experience in 393 patients

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The Decreasing Prevalence of Severe Villous Atrophy in Dermatitis Herpetiformis

A 45-Year Experience in 393 Patients

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Goals: We analyzed from our prospectively collected series of patients with dermatitis herpetiformis (DH) whether small-bowel histologic findings are changing and how serum tissue transglutaminase (TG2) IgA antibodies correlate to mucosal damage.

Background: DH is an extraintestinal manifestation of celiac disease presenting with itchy blistering rash and pathognomonic IgA deposits in the skin. Prominent gastrointestinal symptoms are rare, and small-bowel findings range from severe villous atrophy (SVA) and partial villous atrophy (PVA) to normal mucosa with inflammatory changes.

Methods: The cohort included 393 patients (214 male and 179 female) with DH having small-bowel biopsies performed at Tampere University Hospital since 1970. The small-bowel findings were calculated in the three 15-year periods, and in the last period they were correlated with serum IgA class TG2 antibody levels measured by enzyme-linked immunosorbent assay.

Results: The prevalence of SVA decreased significantly ($P = 0.032$), from 42% in the first study period to 29% in the last study period. A concomitant increase was seen in PVA, from 33% to 41%, and normal villous architecture, from 25% to 30%. The patients with SVA ($P < 0.001$) and PVA ($P = 0.046$) had significantly higher TG2 antibody levels than those with normal villous architecture.

Conclusions: This long-term study in patients with DH disclosed a significant decrease in the occurrence of SVA. Serum IgA TG2 antibody levels correlated to damage in the small bowel. The trend toward milder small-bowel histology in DH suggests that a similar pattern could occur in the pool of undiagnosed celiac disease from which DH develops.

Key Words: dermatitis herpetiformis, celiac disease, small-bowel histology, severe villous atrophy, transglutaminase antibodies

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Dermatitis herpetiformis (DH) is an extraintestinal manifestation of celiac disease presenting with itchy, blistering rash mainly on the elbows, knees, and buttocks.[1–3] Diagnosis is confirmed by direct immunofluorescence examination of uninvolved skin showing granular IgA deposits in the upper dermis.[4] Small-bowel biopsies performed on patients with DH in the 1960s detected celiac enteropathy.[5,6] Thereafter, the rash was also shown to respond to a strict gluten-free diet (GFD) and relapse on gluten challenge.[7–9] Further evidence that DH is an extraintestinal manifestation of celiac disease came from immunogenetic and family studies. The patients of both diseases had the same strong association with HLA DQ2.[10] Moreover, DH and celiac disease were shown to cluster in the same families, and monozygotic twins, one with DH and the other with celiac disease, were also reported.[11]

Gender differences are obvious between adults with DH and celiac disease; male patients dominate in DH and female patients dominate in celiac disease.[2] Moreover, only a minority of patients with DH present with prominent gastrointestinal symptoms or signs of malabsorption, and, interestingly, about one fourth of the patients have no villous atrophy in the small bowel.[8,12] Nevertheless, these patients with DH also have mucosal inflammation compatible with early developing celiac disease,[13,14] and their rash responds to a GFD treatment similarly to the patients with villous atrophy.[8]

The diagnostic capability of celiac disease has increased within the past decades, resulting in the detection of numerous new cases.[15,16] In our area in Finland, the ratio of diagnosed celiac disease to DH was 1 to 1 in the early 1980s, whereas at present it is 8 to 1, and 0.7% of the adult population has the disorder.[17,18] In contrast to celiac disease, the incidence of DH is now decreasing both in Finland and the United Kingdom.[19,20] This fits to the hypothesis that DH develops on the basis of undiagnosed celiac disease by formation of IgA-class epidermal transglutaminase antibodies, which are deposited into the skin.[21,22] Whether the increased detection of celiac disease could have an impact on the severity of enteropathy in DH is of interest. The aim of the present study covering the years from 1970 to 2014 was to analyze whether any changes have occurred in the degree of small-bowel mucosal damage. A further aim was to compare small-bowel findings and serum IgA TG2 antibodies to elucidate whether the antibody levels also in patients with DH correlate to the damage in the small-bowel mucosa.

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PATIENTS AND METHODS

The study group comprised all adult patients with DH (214 male and 179 female) who were admitted and diagnosed at the Department of Dermatology in 1970 to 2014. Thereafter, a small-bowel biopsy was performed at the Department of Gastroenterology and Alimentary Tract Surgery or previously at the Department of Internal Medicine in Tampere University Hospital. The diagnosis of DH was based on the blistering, itchy rash mainly on the elbows, knees, and buttocks, and the demonstration of granular IgA deposits in the papillary dermis by direct immunofluorescence examination.[4]

Small-bowel biopsy was offered to all patients before starting a GFD treatment, and 75% of all 524 patients accepted the examination. In the 70s, biopsies were obtained with Crosby capsule under x-ray control and thereafter upon upper gastrointestinal endoscopy. Small-bowel mucosal samples were stained by hematoxylin-eosin and scored morphologically as severe villous atrophy (SVA; ie, subtotal or total villous atrophy compatible with Marsh-Oberhuber types III-b and III-c), partial villous atrophy (PVA, type III-a), or normal villous architecture.[15] Pathologists with a special knowledge of intestinal pathology made the interpretations.

Serum samples taken from 96 patients with DH diagnosed in 2000 to 2014 were examined for TG2 antibodies with IgA anti-TG2 enzyme-linked immunosorbent assay (ELISA) kits (Celikey; Phadia, GmbH, Freiburg, Germany), as previously described.[23] Levels below 5 absorbance units (AU)/mL were considered negative, and the highest measured level was 120 AU/mL.

The linearity of small-bowel histology findings across the three 15-year periods was tested by using Cochran-Armitage test and analysis of variance. Differences in TG2 IgA antibody levels between patients with SVA or PVA and normal villous architecture were determined using the Kruskall-Wallis test followed by pairwise multiple comparisons with the Dunn test.

RESULTS

Patients in the Three 15-Year Periods

The number of the patients with DH was 144 in 1970 to 1984, 144 in 1985 to 1999, and 105 in 2000 to 2014 (Table 1). Male patients (n = 214) outnumbered female patients (n = 179), and no significant change occurred in the three 15-year periods (Table 1). The mean age of the patients at diagnosis increased significantly ($P < 0.001$), from 37.7 to 52.1 years, during the study, and this occurred both in male and female patients (Table 1).

Small-Bowel Histology in the Three 15-Year Periods

Of all the 393 patients with DH, 140 (35.6%) showed SVA, 145 (36.9%) showed PVA, and 108 (27.5%) showed normal villous architecture in small-bowel histology. In the three 15-year periods, prevalence of SVA decreased from 42% to 35% and then to 29% ($P = 0.032$, Table 1 and Fig. 1). A concomitant increase, although not significant ($P = 0.22$ and 0.34), occurred in the prevalence of PVA, from 33% to 41%, and normal villous architecture, from 25% to 30% (Table 1 and Fig. 1).

TG2 IgA Antibodies and Small-Bowel Histology in the Last 15-Year Period

Serum TG2 IgA antibody levels in DH patients with SVA, PVA, and normal villous architecture diagnosed in 2000 to 2014 are shown in Figure 2. These were significantly elevated in the 28 patients with SVA (median, 41.5 AU/mL; $P < 0.001$) and in the 40 patients with PVA (median, 16.5 AU/mL; $P = 0.046$) compared with the levels in 28 patients with normal villous architecture (median, 2.75 AU/mL). Three (11%) patients with SVA, 12 (30%) with PVA, and 15 (54%) with normal villous architecture had normal TG2 IgA antibody levels.

DISCUSSION

The main finding in the present study in 393 patients with DH was that the occurrence of SVA has become less common within the observation period of 45 years. The prevalence of SVA decreased from 42% to 35% and then to 29% in the 3 study periods, and a concomitant increase, although not significant, occurred in the percentage of PVA and normal villous architecture. At the same time with these changes the mean age at diagnosis increased significantly, from 38 to 52 years, which agrees with our previous report including also small-bowel nonbiopsied patients with DH.[19] In our previous register study, we found no basic differences in the mean ages of adult patients with DH and celiac disease; the mean age in DH was 39 years and that of celiac disease was 44 years.[17] The age at the diagnosis of celiac disease is highly dependent of the diagnostic accuracy, because the symptoms of patients are often vague. By contrast, we believe that in DH the intensive itching brings the patient to the physician very soon. We do not see any alteration or flaws in the diagnostic procedure in DH over the past decades. Thus, the explanation for the increased age at diagnosis in DH may be improved diagnostic accuracy resulting in markedly increased incidence of celiac disease in our country.[17,18] High index of suspicion and screening in at-risk groups

TABLE 1. Sex, Mean Age, and Small-Bowel Histology at Diagnosis in 393 Patients With Dermatitis Herpetiformis

	1970-1984 (N = 144)	1985-1999 (N = 144)	2000-2014 (N = 105)	P*
No. male patients (%)	77 (53)	76 (53)	61 (68)	0.50
Mean age (range)	37.7 (18-69)	43.7 (20-84)	52.1 (19-75)	< 0.001†
Small-bowel biopsy [n (%)]				
Severe villous atrophy	60 (42)	50 (35)	30 (29)	0.032
Partial villous atrophy	48 (33)	54 (37)	43 (41)	0.22
Normal villous architecture	36 (25)	40 (28)	32 (30)	0.34

The cohort was prospectively collected from 1970, and the results are expressed in three 15-year periods.
*For linearity.
†Similar increase in male and female patients.

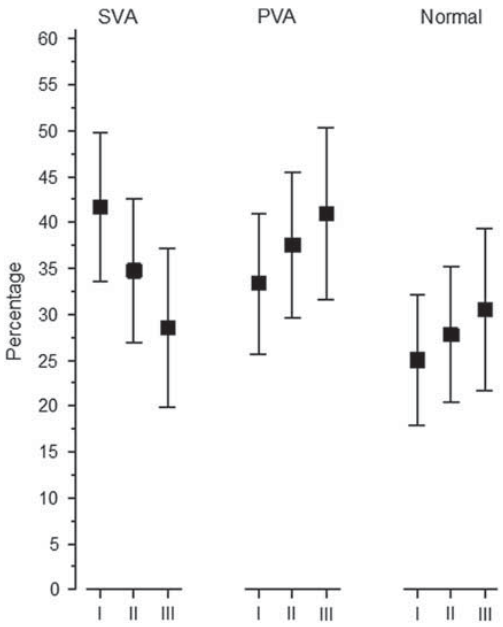

FIGURE 1. Severe villous atrophy (SVA), partial villous atrophy (PVA), and normal small-bowel villous architecture in 393 patients with dermatitis herpetiformis in three 15-year periods: I = 1970 to 1984, II = 1985 to 1999, III = 2000 to 2014. SVA shows a significant linear decrease ($P = 0.032$), whereas the increases in PVA and normal villous architecture are nonsignificant ($P = 0.22$ and 0.34).

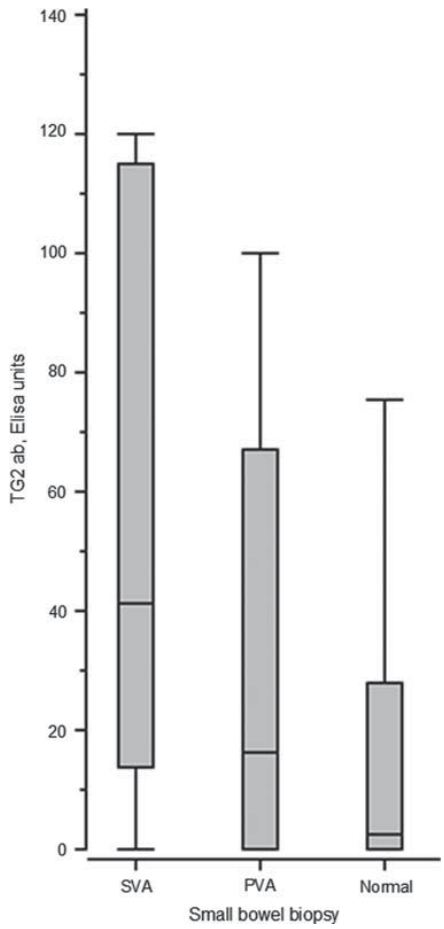

FIGURE 2. Serum tissue transglutaminase (TG2) IgA antibody levels (median with interquartile range whiskers showing 5 and 95 percentiles) measured at diagnosis by ELISA test in 96 patients with dermatitis herpetiformis. Patients with severe villous atrophy (SVA; $P < 0.001$) and partial villous atrophy (PVA; $P = 0.046$) have significantly higher TG2 antibody levels than the patients with normal villous architecture.

reveal many new cases of celiac disease.[16,24] Patients particularly with asymptomatic celiac disease will be diagnosed earlier,[25] and also these patients are placed on a GFD. Because of this, a smaller potential cohort of undetected celiac disease will nowadays continue on gluten-containing diet and have time to develop SVA and skin manifestation—that is, DH. The long time period needed for phenotype change from celiac disease to DH supports this finding.[26,27]

Serologic screening of celiac disease by measuring TG2 IgA antibodies in ELISA test has become a widely accepted practice.[16,24] The sensitivity and specificity for TG2 IgA antibody ELISA tests have been shown to be high, over 90% in various study populations.[28] This test has been used also in DH, and diagnostic sensitivities lower than those in celiac disease have been found.[23,29–31] In the present study, we correlated TG2 IgA antibody levels to small-bowel findings in a much larger patient population than in previous studies in adults with DH.[30–32] As expected from celiac disease studies,[24,33,34] the levels were significantly elevated and highest in the patients with SVA and lower but still significantly elevated in the patients with PVA. These results agree with a large study of children with DH in which high IgA TG2 antibody levels were associated with the degree of mucosal villous atrophy.[35] Importantly, 11% of the present patients with SVA and 30% with PVA had normal TG2 IgA antibody levels, obviously indicating that normal antibody levels cannot exclude that the patient with DH has damaged mucosa in the small bowel. Although TG2 IgA antibody ELISA test does not detect all DH patients with small-bowel villous atrophy, a need for small-bowel biopsy is obvious only when the patient has prominent gastrointestinal symptoms or a suspicion of malignancy. We agree with this opinion presented in an Italian guideline[36] when we now know that the DH patients with and without small-bowel villous atrophy have similar excellent prognosis after adherence to a strict GFD.[37]

Strengths of the present study were the long-term prospective survey in a specialized center covering the same catchment area and using the best diagnostic criterion for DH—that is, demonstration of granular IgA deposits in the uninvolved skin.[2,4] Moreover, we have had a close cooperation with dermatologists and gastroenterologist offering routinely small-bowel biopsies to every of our newly diagnosed patients with DH. Three-fourths of these—that is, 393 patients—had a successful biopsy, whereas the number of patients and percentage of biopsies have been much lower in earlier studies in adults with DH.[32,38,39]

We excluded children with DH from the present study—the percentage of whom has been only 4% of all of our patients[40] but markedly higher in Hungarian and Italian series.[35,41] The mean age of our 18 children with DH was 10 years at diagnosis, and a half of them had SVA.[40] Limitations in the present study were different methods (capsule, endoscopy) to obtain small-bowel biopsy samples and no opportunity to more accurately examine the mucosal samples than routine histologic evaluation. However, we have previously shown that also the DH patients with normal mucosal histology have inflammatory changes such as increased numbers of gamma/delta T cells.[13,14] Moreover, the rash in DH patients with normal small-bowel villous architecture responds to a GFD similarly to the patients with villous atrophy,[8] confirming that also these patients belong to the same pool of skin manifestation of celiac disease.[3] In accordance, several celiac disease studies have focused attention to the patients presenting with normal mucosal histology together with endomysium or TG2 IgA antibodies, and they have shown that on a gluten-containing diet many of them later develop villous atrophy.[42–44] This condition has been termed as early developing[43] or potential celiac disease.[45] It is of interest that DH seems to be able also to arise from this condition, as one fourth of the present patients had normal villous architecture and a half of them presented with TG2 IgA antibodies. The trend toward milder small-bowel histology in DH suggests that a similar pattern could occur in the pool of undiagnosed celiac disease from which DH develops possibly as an autoimmune reaction against epidermal transglutaminase.[21,22] In agreement with this, recent studies in celiac disease have shown that the severity of symptoms and small-bowel mucosal damage have become milder.[25,46] Brar et al[46] examined 499 patients with adult celiac disease in 1981 to 2004 and could show that there was a significant trend over time for a greater proportion of patients presenting as atypical/silent celiac disease and having PVA. Whether environmental factors such as changes in gluten consumption,[47] intestinal microbiota,[48] and smoking[49] might have had an effect on the decreased severity of small bowel villous atrophy in celiac disease and DH needs to be addressed in future studies.

In conclusion, the present long-term prospective study in DH showed that severe enteropathy has significantly decreased in this common extraintestinal manifestation of celiac disease. TG2 IgA antibody levels in ELISA were highest in patients with SVA, but negative test results occurred as well. The trend toward milder small-bowel histologic damage in DH suggests that a similar pattern could occur in the pool of undiagnosed celiac disease from which DH develops.

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PUBLICATION

III

Prognosis of dermatitis herpetiformis patients with and without villous atrophy at diagnosis

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Prognosis of Dermatitis Herpetiformis Patients with and without Villous Atrophy at Diagnosis

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Abstract: Dermatitis herpetiformis (DH) is a cutaneous manifestation of coeliac disease. At diagnosis, the majority of patients have villous atrophy in the small bowel mucosa. The objective of this study was to investigate whether the presence or absence of villous atrophy at diagnosis affects the long-term prognosis of DH. Data were gathered from the patient records of 352 DH and 248 coeliac disease patients, and follow-up data via questionnaires from 181 DH and 128 coeliac disease patients on a gluten-free diet (GFD). Of the DH patients, 72% had villous atrophy when DH was diagnosed, and these patients were significantly younger at diagnosis compared to those with normal small bowel mucosa (37 vs. 54 years, $p < 0.001$). Clinical recovery on a GFD did not differ significantly between the DH groups, nor did current adherence to a GFD, the presence of long-term illnesses, coeliac disease-related complications or gastrointestinal symptoms, or quality of life. By contrast, the coeliac disease controls had more often osteopenia/osteoporosis, thyroid diseases, malignancies and current gastrointestinal symptoms compared to the DH patients. In conclusion, villous atrophy at the time of DH diagnosis does not have an impact on the clinical recovery or long-term general health of DH patients.

Keywords: dermatitis herpetiformis; coeliac disease; gluten-free diet; small bowel; villous atrophy; prognosis

1. Introduction

Dermatitis herpetiformis (DH) is an extraintestinal manifestation of coeliac disease currently affecting approximately 13% of coeliac disease patients [1,2]. DH induces intense pruritus and a symmetrical papulovesicular rash typically on the elbows, knees, and buttocks [3]. Coeliac disease and DH are genetically predisposed by the human leukocyte antigen (HLA) *DQ2* or *DQ8* haplotypes, and exogenous gluten causes an immune response and small bowel mucosal injury in both [4,5]. Furthermore, autoantibodies against endogenous enzyme tissue transglutaminase (TG2) are characteristically present in the serum and the intestine in both conditions [6–9].

Diagnosis of DH is verified with the detection of pathognomonic granular immunoglobulin A (IgA) deposits in the uninvolved skin by direct immunofluorescence (IF) examination [10]. This IgA is known to target epidermal transglutaminase (TG3) [11], which is considered the autoantigen in DH,

while in coeliac disease it is TG2 [6]. In addition to the skin, TG3 antibody response is often present in the sera of DH patients, although TG3 antibodies are occasionally also found in the serum of some coeliac disease patients without DH [12–14].

At the time of the DH diagnosis, some degree of small bowel mucosal villous atrophy is known to exist in approximately 75% of patients, but the remainder have normal villous architecture with only coeliac-type inflammation [15,16]. Regardless of the small bowel mucosal alterations, DH patients only rarely present with obvious gastrointestinal symptoms [17,18].

A strict life-long gluten-free diet (GFD) is the mainstay of treatment in both DH and coeliac disease. However, resolution of DH rash can take months or even longer on the dietary treatment, and therefore, DH patients with severe skin symptoms are additionally treated with dapsone medication to control the rash more quickly [3,19]. Coeliac disease and DH both carry an increased risk of concomitant autoimmune conditions such as thyroid diseases and type 1-diabetes; furthermore, the risk of developing non-Hodgkin lymphoma is increased [20–22]. Mortality in coeliac disease, but not in DH, has shown to be increased [23]. A GFD is known to have a preventive effect against the development of lymphoma in DH [24], but other than that, previous research about the factors influencing the prognosis of DH is lacking. Currently, it is not known whether DH patients with small bowel villous atrophy at diagnosis have a worse outcome compared to those with normal small bowel mucosa, and furthermore, whether the prognosis of DH patients with villous atrophy is corresponding to that of classical coeliac disease patients. This issue is of importance when necessary investigations, at the time of DH diagnosis, are assessed.

The aim of the current study was to assess whether the presence of villous atrophy at DH diagnosis would affect clinical recovery on a GFD or the long-term prognosis of DH. In addition, DH patients were compared to classical coeliac disease controls with abdominal symptoms at diagnosis and a histologically confirmed diagnosis. The hypothesis of this study was that the presence or absence of villous atrophy at diagnosis would not be an influential factor in the prognosis of DH.

2. Materials and Methods

Between 1970 and 2014, a total of 526 DH patients were diagnosed at the Department of Dermatology, Tampere University Hospital. During the study period, all patients with DH living in a defined area around Tampere were diagnosed at this dermatology unit since IF biopsies required for the diagnosis were not performed elsewhere. Each DH patient's diagnosis was based on the typical clinical picture and the demonstration of granular IgA deposits in skin biopsies [10]. In addition, all diagnosed patients were routinely suggested to undergo gastroscopy and small bowel biopsy obtainment at the time of the diagnosis while on a gluten-containing diet. After diagnosis, a strict GFD was advised to all patients and dapsone was instituted in those with severe skin symptoms. According to routine treatment policies, all patients were followed up at a DH outpatient clinic until the rash had cleared and the dapsone medication could be discontinued. In this study, all DH patients without prior coeliac disease diagnosis (made $\geq$2 years earlier) diagnosed between 1970 and 2014 and having an available small bowel biopsy result and commencing on a GFD after diagnosis, were included as study patients. Altogether, 352 DH patients fulfilled the inclusion criteria and were included as DH study patients. Further, 248 classical coeliac disease patients with abdominal symptoms at diagnosis and a histologically confirmed diagnosis at Tampere University Hospital during the same time period served as controls.

Data on demographic characteristics, the severity of clinical symptoms and small bowel mucosal histology, and the results of coeliac autoantibodies and hemoglobin values at the time of DH or coeliac disease diagnosis were gathered from the patient records of Tampere University Hospital between March and October 2016. The small bowel biopsy results were graded as subtotal villous atrophy (SVA), partial villous atrophy (PVA), or normal mucosa according to the analysis of the routine pathologist as previously described [16]. In DH patients, the skin symptoms at the time of the diagnosis were graded as mild, moderate, or severe according to the presence of a few, several or many blisters,

macular eruptions and erosions. The grading was performed by one dermatologist. In addition, the commencement and duration of dapsone medication after diagnosis was recorded.

Follow-up data were collected using questionnaires (see below for more detail) mailed to all 294 living DH patients fulfilling the inclusion criteria of this study (on December 2015) and the 222 living coeliac disease controls (on May 2016). The final response rate was 62% for the DH patients and 58% for the coeliac disease patients; hence, the follow-up study included 181 DH and 128 coeliac disease patients.

The study protocol and usage of the register-based data were approved by the Regional Ethics Committee of Tampere University Hospital (R15143), and furthermore, informed consent was obtained from each patient participating in the follow-up study.

2.1. Questionnaires

The disease-specific questionnaire designed for this study, the Psychological General Well-Being (PGWB) [25] and Gastrointestinal Symptom Rating Scale (GSRS) [26] questionnaires were mailed to the DH and coeliac disease study patients. PGWB and GSRS questionnaires are validated questionnaires, which have been widely applied in previous coeliac disease studies [27–31]. In addition, the DH patients received the Dermatology Life Quality Index (DLQI) questionnaire [32].

The disease-specific questionnaire included both open and multiple-choice questions. The patients were asked about the presence and duration of DH and coeliac disease-related symptoms before and after the diagnosis, the strictness of the GFD, smoking and other lifestyle characteristics, the number of children born, the family history of coeliac disease or DH, and the patient's current height and weight. Compliance with a GFD was reported as strict diet without dietary lapses, dietary lapses once per month, dietary lapses one to five times per month, or dietary lapses once per week. In addition, the questionnaire included questions about the presence of coeliac disease complications and associated diseases, malignancies, other long-term illnesses, and the regular usage of physician-prescribed medications and over-the-counter (OTC) medications. In the malignancy analysis, non-melanoma skin cancers were excluded, as were excessive trauma fractures in bone fracture analyses.

As previously described, the validated 22-item PGWB questionnaire evaluates self-perceived health-related well-being and distress and includes six dimensions: Anxiety, depressed mood, positive well-being, self-control, vitality, and general health [25]. The total score ranges from 22 to 132, with a higher score indicating better quality of life. The 15-item GSRS questionnaire assesses the severity and existence of gastrointestinal symptoms in five categories: Diarrhea, indigestion, constipation, abdominal pain, and reflux [26]. It uses a seven-point Likert scale for each question: One indicates an absence of symptoms and seven indicates severe symptoms. The DLQI is a 10-item dermatology-specific quality of life instrument. The questionnaire includes six different sections: Symptoms and feelings, daily activities, leisure, work and school, personal relationships, and treatment unit. The scores of all ten questions are calculated together, and the total score varies from a minimum of 0 to a maximum of 30, with a higher score indicating a more impaired life quality [32].

2.2. Statistical Analysis

A Two-sided chi-squared test was used to compare the categorical variables and a Kruskall–Wallis test was performed to assess differences between the continuous variables. Logistic regression analysis was used to standardize the study groups according to age at the time of the study. Statistical significance was set at $p < 0.05$. The analyses were performed using IBM SPSS Statistics for Windows (Version 23.0., IBM Corp., Armonk, NY, USA).

3. Results

3.1. DH Patients with Normal Villous Architecture Compared to DH Patients with Villous Atrophy at Diagnosis

Of the 352 DH patients, 98 (28%) had normal villous architecture, and 254 (72%) had small bowel mucosal villous atrophy (PVA or SVA) at the time of the DH diagnosis (Table 1). GFD was not initiated

before the diagnosis in study participants. Mean time since the year of DH diagnosis, was 20 years in the DH patients with normal villous architecture and 23 years in the DH patients with villous atrophy, and the difference was not statistically significant. The median age at diagnosis was significantly higher in the DH patients with normal villous architecture compared to the DH patients with villous atrophy ($p < 0.001$, Table 1). At diagnosis, the DH patients with villous atrophy were significantly more often serum coeliac autoantibody-positive compared to the DH patients with normal villous architecture (73% vs. 39%, $p < 0.001$, Table 1). The severity of the DH rash at diagnosis did not differ significantly between the DH groups ($p = 0.862$). Eighty percent of all DH patients used dapsone after the diagnosis. The duration of dapsone usage was longer in the DH patients with normal villous architecture compared to the DH patients with villous atrophy at diagnosis (median 36 vs. 24 months), but the difference was not statistically significant ($p = 0.097$, Table 1).

Table 1. Demographic data and disease-related characteristics of 98 dermatitis herpetiformis (DH) patients with normal small bowel villous architecture and 254 DH patients with villous atrophy at diagnosis, and 248 coeliac disease (CD) control patients.

	DH Patients		CD Controls (*n* = 248)	*p*-Value *
	With Normal Villous Architecture (*n* = 98)	With Villous Atrophy (*n* = 254)		
Females; *n* (%)	50 (51)	125 (49)	193 (78)	<0.001
Age at diagnosis; median (range)	52 (3–84)	37 (4–78)	42 (7–75)	<0.001 [a]
Coeliac autoantibodies [1] present in the serum at diagnosis; *n* (%)	28/72 (39)	139/191 (73)	124/148 (84)	<0.001 [a]
Haemoglobin level at diagnosis [2], g/L; median (Q_1–Q_3) [3]	138 (128–148)	136 (129–146)	130 (121–140)	0.057
Dapsone treatment used; *n* (%)	75/93 (81)	191/243 (79)	-	-
Duration of dapsone treatment, months; median (range)	36 (5–324)	24 (2–384)	-	-

* *p*-value measured across the three study groups; [1] Transglutaminase 2-, endomysium-, or antireticulin IgA antibodies; [2] Statistical analysis was further performed for patients ≥16 years of age and for females and males separately—there were no statistically significant differences between the three groups; [3] Interquartile range; [a] Statistically significant difference ($p < 0.001$) between DH patients with normal villous architecture and DH patients with villous atrophy.

Of the 181 DH patients with available follow-up data, 39 (22%) had normal villous architecture, and 142 (78%) had villous atrophy at the time of DH diagnosis. The median follow-up time was 20 years in patients with normal villous architecture and 23 years in the DH patients with villous atrophy at diagnosis (Table 2). The presence of gastrointestinal symptoms at diagnosis did not differ between the DH study groups according to the follow-up study questionnaire ($p = 0.170$). At the time of the follow-up study, DH patients with normal villous architecture were significantly older compared to those DH patients who had villous atrophy at diagnosis (Table 2).

The strictness of the GFD and BMI did not differ between the DH study groups at the time of the study (Table 2). Similarly, no significant differences were detected in smoking habits or physical activity: 3% of DH patients without villous atrophy and 13% of patients with villous atrophy at diagnosis were current smokers, and 49% and 69% exercised at least three times a week, respectively.

Table 2. Follow-up data of 39 dermatitis herpetiformis (DH) patients with normal villous architecture and 142 DH patients with small bowel mucosal villous atrophy at diagnosis, and 128 coeliac disease (CD) control patients.

	DH Patients		CD Controls (*n* = 128)	*p*-Value *
	With Normal Villous Architecture (*n* = 39)	With Villous Atrophy (*n* = 142)		
Females; *n* (%)	18 (46)	67 (47)	104 (81)	<0.001
Follow-up time, years; median (range)	20 (1–44)	23 (1–42)	18 (6–43)	0.003
Age; median (range)	68 (52–85)	61 (18–96)	65 (34–85)	<0.001 [a]
BMI, kg/m^2; median (range)	25 (19–37)	25 (16–38)	26 (15–46)	0.772
Strict adherence to GFD, no dietary lapses; *n* (%)	30 (77)	101 (71)	107 (84)	0.170 [b]
Number of long-term illnesses; median (range)	1 (0–7)	1 (0–14)	2 (0–9)	<0.001
Number of prescription medications used; median (range)	2 (0–11)	1 (0–18)	3 (0–16)	0.078

Table 2. *Cont.*

	DH Patients		CD Controls (n = 128)	p-Value *
	With Normal Villous Architecture (n = 39)	With Villous Atrophy (n = 142)		
Uses statin medication; n (%)	14 (36)	21 (15)	15 (12)	0.001 [c]
Uses antihypertensive medication; n (%)	20 (51)	50 (35)	49 (38)	0.188
Uses proton pump inhibitor medication; n (%)	5 (13)	16 (11)	16 (13)	0.938
Number of over-the-counter medications used; median (range)	0 (0–5)	1 (0–7)	2 (0–7)	<0.001
Number of children born; median (range)	2 (0–5)	2 (0–6)	2 (0–5)	0.497
First-degree relatives with DH or CD; n (%)	13 (33)	53 (37)	55 (43)	0.464

BMI: Body mass index; GFD: Gluten-free diet. * p-value measured across the three study groups; [a] Statistically significant difference ($p < 0.001$) between DH patients with normal villous architecture and DH patients with villous atrophy at diagnosis; [b] p-value was tested for categorical variables including categories: strict diet, dietary lapses once per month, dietary lapses 1–5 times/month, dietary lapses once per week; [c] Statistically significant difference ($p = 0.003$) between DH patients with normal villous architecture and DH patients with villous atrophy at diagnosis.

At the time of the follow-up study, coronary heart disease and hypertension were significantly more common among the DH patients with normal villous architecture compared to the DH patients with villous atrophy at diagnosis (Figure 1); however, after adjustment for the current age, significant differences disappeared ($p = 0.198$, OR = 0.482 and $p = 0.273$, OR = 0.653, respectively). Significant differences were not detected in the presence of type 1- or 2-diabetes, thyroid diseases, cerebrovascular diseases, osteopenia or osteoporosis, or malignancies between the DH study groups (Figure 1). Patients with self-reported bone fractures were slightly more numerous among the DH patients with villous atrophy than among those with normal villous architecture at diagnosis, but the difference was not statistically significant ($p = 0.321$, Figure 1).

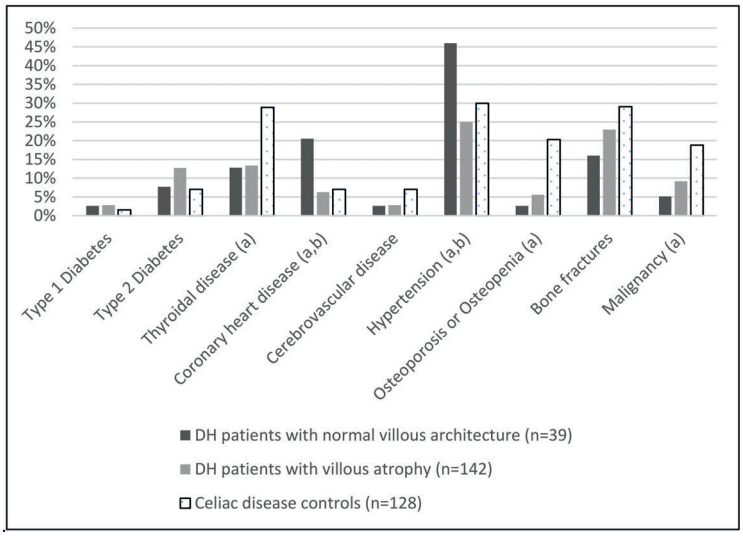

Figure 1. Percentages of dermatitis herpetiformis (DH) patients with normal small bowel mucosal villous architecture and with villous atrophy at diagnosis, and coeliac disease control patients with long-term illnesses or complications at the time of the follow-up study. (a) Statistically significant difference ($p < 0.05$) between the three study groups; (b) statistically significant difference ($p < 0.05$) between DH patients with normal villous architecture and DH patients with villous atrophy at diagnosis.

Statistically significant differences were not detected in the use of physician-prescribed regular medications between the DH study groups; even the significant difference in the use of statin

medication disappeared after adjustment for the current age (OR = 0.479, $p = 0.88$, Table 2). Furthermore, the total amount of used OTC medications was similar in the DH groups; only the usage of vitamin D was more frequent among DH patients with villous atrophy at diagnosis compared to those without villous atrophy (40% vs. 23%, $p = 0.050$).

The presence of gastrointestinal symptoms or the quality of life according to the total or the subscores of the GSRS, PGWB (Table 3) and DLQI questionnaires at the time of the study did not differ between the DH study groups

Table 3. The Psychological General Well-Being (PGWB) and Gastrointestinal Symptom Rating Scale (GSRS) questionnaires' median and interquartile range (Q1–Q3) results for the gluten-free diet-treated dermatitis herpetiformis (DH) patients with normal villous architecture and with villous atrophy at diagnosis, and the coeliac disease (CD) controls at the time of the follow-up study.

		DH Patients				CD Controls (n = 128)		p-Value *
		With Normal Villous Architecture (n = 39)		With Villous Atrophy (n = 142)				
PGWB		median	(Q_1–Q_3)	median	(Q_1–Q_3)	median	(Q_1–Q_3)	
	Total	110	(99–116)	110	(101–117)	106	(96–117)	0.200
	Anxiety	26	(23–27)	26	(23–27)	25	(23–28)	0.891
	Depression	17	(16–18)	17	(16–18)	17	(15–18)	0.587
	Well-being	18	(16–20)	18	(16–20)	18	(16–20)	0.279
	Self-control	16	(15–17)	16	(15–17)	16	(14–17)	0.295
	General health	13	(12–15)	14	(12–16)	13	(11–15)	0.022
	Vitality	20	(17–21)	19	(17–21)	18	(16–20)	0.104
GSRS		median	(Q_1–Q_3)	median	(Q_1–Q_3)	median	(Q_1–Q_3)	
	Total	1.6	(1.3–2.0)	1.7	(1.3–2.3)	2.1	(1.5–4.2)	<0.001
	Diarrhoea	1.0	(1.0–1.7)	1.3	(1.0–2.3)	1.7	(1.0–2.7)	0.006
	Indigestion	1.8	(1.5–2.5)	2.0	(1.5–2.5)	2.0	(1.5–3.0)	0.227
	Constipation	1.7	(1.0–2.3)	1.7	(1.0–2.3)	1.7	(1.0–2.4)	0.482
	Pain	1.3	(1.0–1.7)	1.7	(1.0–2.0)	1.7	(1.3–2.3)	0.007
	Reflux	1.0	(1.0–1.5)	1.0	(1.0–2.0)	1.5	(1.0–2.0)	0.084

* p-value measured across the three study groups.

3.2. Comparisons between the DH Patients and the Classical Coeliac Disease Controls

Compared to the DH patients, the coeliac disease controls were more often female (Tables 1 and 2), and their median diagnostic age was significantly lower compared to DH patients with normal villous architecture at diagnosis (Table 1).

In the long-term follow-up data, there were no observed differences in current smoking habits or physical activity between the DH patients and the coeliac disease controls. By contrast, the total number of long-term illnesses was found to be higher among the coeliac disease controls compared to the DH patients (Table 2). More specifically, after adjustment for the current age, thyroid diseases (OR = 3.443, $p = 0.019$) and osteopenia or osteoporosis (OR = 14.132, $p = 0.012$) were more common among the coeliac disease controls than among the DH patients (Figure 1). However, the presence of self-reported bone fractures did not differ significantly between the DH study groups and the coeliac disease controls. In the malignancy analysis, the coeliac disease controls outnumbered the DH patients after adjustment for the current age (OR = 6.527, $p = 0.016$) (Figure 1).

In the analysis of regularly used physician-prescribed medications and after adjustment for the current age, the coeliac disease controls were found to use less statin medication compared to the DH patients with normal villous architecture (OR = 0.319, $p = 0.01$). In turn, the total number of regularly used OTCs was higher among the coeliac disease patients compared to the DH patients ($p = 0.003$, Table 2), and specifically the use of calcium ($p = 0.011$) and vitamin D ($p < 0.001$) was more common.

Quality of life measured with the PGWB did not differ between the coeliac disease controls and the DH patients with normal villous architecture at diagnosis, but the coeliac disease controls had significantly lower PGWB general health scores compared to the DH patients with villous atrophy at

diagnosis (Table 3). In the GSRS questionnaire, the coeliac disease controls had significantly higher total symptoms gastrointestinal pain and diarrhea scores compared to both DH groups.

4. Discussion

This study demonstrated that the disease severity and the clinical response to a GFD does not differ between DH patients with normal villous architecture and those with villous atrophy at diagnosis. Furthermore, the long-term general health and well-being of DH patients are not influenced by the severity of small bowel mucosal damage at the time of DH diagnosis. The outcomes of the current study were obtained from a large, prospectively collected series of DH patients, all of whom adhered to a GFD treatment. Furthermore, in the present study, the proportions of DH patients with villous atrophy and normal villous architecture were consistent with the findings in earlier DH studies [15,33].

In our previous study, the presence of villous atrophy at DH diagnosis was found to be associated with a delayed diagnosis, i.e., the presence of the rash for two years or more before the diagnosis, suggesting that prolonged diagnosis might enable the small bowel mucosal damage to progress [34]. In the current study, the occurrence of villous atrophy did not associate with the severity of the rash or with the presence of gastrointestinal symptoms at diagnosis. The duration of dapsone medication was considered the most reliable method of determining the active period of rash after adherence to a GFD since the majority of patients used dapsone medication, and the medicine was discontinued as early as possible without a relapse in skin symptoms. The median duration of dapsone usage in DH study groups corresponded well with previous GFD treatment studies [35,36], and even though the duration was longer in DH patients with normal small bowel mucosa than in those with villous atrophy at diagnosis, the difference was not statistically significant. Therefore, presence or absence of small bowel villous atrophy at diagnosis seems not to influence the clinical recovery of the DH rash.

The long-term follow-up performed in the present DH patients further demonstrated that small bowel villous atrophy at diagnosis did not have any impact on the presence of long-term illnesses and complications, or long-term quality of life or the presence of persistent gastrointestinal symptoms. Additionally, our previous study showed that the mortality of DH patients with villous atrophy at diagnosis does not differ from that of DH patients with normal villous architecture, and in fact, the mortality of DH patients was shown to be lower than in the general population [37]. Therefore, all these results show that DH patients with and without villous atrophy at diagnosis have a similar good long-term prognosis when they adhere to a GFD.

In contrast to DH, the mortality rate of the patients with coeliac disease are known to be increased compared to the general population [23,38]. Moreover, when the GFD-treated coeliac disease patients in the current study were compared to the DH patient groups, they had significantly more malignancies and long-term illnesses, especially thyroid diseases and osteopenia or osteoporosis. A previous comparison between DH and coeliac disease also showed a higher frequency of diseases of autoimmune origin in patients with coeliac disease [39], but then another study demonstrated that autoimmune diseases were as common among DH patients without classical coeliac disease symptoms than in those DH patients with preceding coeliac disease diagnosis [40]. In the present study, the coeliac disease controls were further found to have worse self-reported general health, and they had more gastrointestinal symptoms at the time of the study compared to the DH patients. These results fit with our recent study that likewise found a better quality of life and fewer gastrointestinal symptoms among long-term treated DH patients compared to treated coeliac disease patients [41].

The results of the present study thus suggest that the prognosis of different phenotypes of coeliac disease diverge and villous atrophy is not the determinative factor in the outcome of DH. Different adherence rates to GFD or varying lifestyle habits did not explain the outcome differences between coeliac disease and DH study patients in this study. One explanation for the different prognosis between coeliac disease and DH might be slightly diverse autoimmune reactions, but this remains to be elucidated in future studies.

In the current study, the median age at diagnosis in DH patients with normal villous architecture was significantly higher compared to DH patients with villous atrophy. We were aware from our earlier long-term DH studies that the age at diagnosis had increased significantly from 1970 onwards [1], and further, that there was a significant trend towards milder villous atrophy [16]. However, in the present study, the time period of DH diagnosis did not differ significantly between DH study groups. Therefore, the divergence in diagnostic periods does not explain the difference in the diagnostic age between DH patients with and without villous atrophy at diagnosis. One explanation might be, however, that older patients are more prone to develop milder small bowel mucosal alterations, e.g., due to divergent immune responses. Previous research shows that older coeliac disease patients are more likely to remain seronegative and further, a trend toward less severe histopathology has been observed with increasing age at the time of coeliac disease diagnosis [42,43]. Nonetheless, these age-related findings detected in coeliac disease and DH should be examined in more detail in further studies.

As a possible limitation of the current study, it must be recognized that the follow-up data were obtained from questionnaires, which might cause selection bias. Recall bias is always a possibility when requiring data from several decades ago. The disease-specific questionnaire used in the study was designed for this particular study and for comparing the results of the study groups, and it has not been used in other disease studies, and it has not been validated. GSRS is not optimized for coeliac disease, but it has been the most commonly used generic questionnaire in coeliac disease studies [28]. PGWB is not a disease-specific instrument, and therefore, it is possible that it might not assess all of the issues that are having an impact on life in DH and coeliac disease patients. GSRS and PGWB questionnaires have not been validated specifically for coeliac disease. Furthermore, all study patients were recruited from the same hospital, and in the future, results from a more comprehensive geographical distribution would be of value. In turn, the major strengths of the study are: A well-defined, prospectively collected DH cohort from a high prevalence area with excellent dietary adherence rates, and the long follow-up time [1]. Moreover, similar large DH studies with knowledge about the diagnostic small bowel mucosal findings and long-term follow-up data consisting of GFD adherence rates have not been performed previously to our knowledge.

5. Conclusions

The major outcome of this study is that skin IgA-IF proven DH patients evincing coeliac-type small bowel mucosal villous atrophy at diagnosis does not differ from DH patients with non-atrophic small bowel mucosa with regard to GFD treatment response, long-term quality of life, or the presence of chronic illnesses or coeliac disease-associated complications.

Author Contributions: E.M., T.S., K.H., T.R., K.K., and P.C. conceived and designed the experiments and wrote the paper; E.M., T.S., and K.H. performed the experiments; and H.H. analyzed the data.

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PUBLICATION

IV

Gluten challenge induces skin and small bowel relapse in long-term gluten-free diet treated dermatitis herpetiformis

Mansikka E, Hervonen K, Kaukinen K, Ilus T, Oksanen P, Lindfors K, Laurila K, Hietikko M, Taavela J, Jernman J, Saavalainen P, Reunala T and Salmi T.

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ORIGINAL ARTICLE

Gluten Challenge Induces Skin and Small Bowel Relapse in Long-Term Gluten-Free Diet–Treated Dermatitis Herpetiformis

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Dermatitis herpetiformis (DH) is an extraintestinal manifestation of celiac disease causing an itchy, blistering rash. Granular IgA deposits in the skin are pathognomonic for DH, and the treatment of choice is a lifelong gluten-free diet (GFD). Preliminary evidence suggests that there are patients with DH who redevelop gluten tolerance after adherence to a GFD treatment. To evaluate this, we performed a 12-month gluten challenge with skin and small-bowel mucosal biopsy samples in 19 patients with DH who had adhered to a GFD for a mean of 23 years. Prechallenge biopsy was negative for skin IgA and transglutaminase 3 deposits in 16 patients (84%) and indicated normal villous height-to-crypt depth ratios in the small bowel mucosa in all 19 patients. The gluten challenge caused a relapse of the rash in 15 patients (79%) in a mean of 5.6 months; of these 15 patients, 13 had skin IgA and transglutaminase 3 deposits, and 12 had small-bowel villous atrophy. In addition, three patients without rash or immune deposits in the skin developed villous atrophy, whereas one patient persisted without any signs of relapse. In conclusion, 95% of the patients with DH were unable to tolerate gluten even after long-term adherence to a GFD. Therefore, lifelong GFD treatment remains justified in all patients with DH.

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INTRODUCTION

Dermatitis herpetiformis (DH) is an extraintestinal manifestation of celiac disease presenting with an itchy and blistering rash, mainly on the elbows, knees, and buttocks (Bolotin and Petronic-Rosic, 2011; Collin et al., 2017). The diagnosis of DH is confirmed with direct immunofluorescence examination showing granular IgA deposits in the papillary dermis of perilesional skin (Zone et al., 1996). Marked gastrointestinal symptoms are rare in DH, although approximately 75% of patients develop villous atrophy in the small bowel mucosa (Mansikka et al., 2017). Even in patients without obvious changes in the villous structures, intestinal celiac-type inflammation with increased densities of intraepithelial lymphocytes (IELs)—in particular $\gamma\delta^+$ IELs (Savilahti et al., 1992)—is evident. Parallel to celiac disease, transglutaminase 2 (TG2)–targeted autoantibodies are frequently observed in the serum and small-bowel mucosa in untreated DH and are known to respond to a gluten-free diet (GFD) (Dieterich et al., 1999; Salmi et al., 2014). However, in DH, the antigen for deposited cutaneous IgA is epidermal transglutaminase (e.g., transglutaminase 3 [TG3]), another member of the transglutaminase family along with TG2, and IgA-class TG3 antibodies (Abs) are often observed in the serum of patients with DH (Hull et al., 2008; Sárdy et al., 2002).

In DH, the treatment of choice is a GFD, which treats both the rash and small-bowel villous atrophy (Fry et al., 1973). However, adherence to the diet must be strict; and it has been observed that the rash typically disappears after a mean duration of 2 years (Garioch et al., 1994; Reunala et al., 1977), but IgA and TG3 deposits in the skin are known to persist much longer (Hietikko et al., 2018). It is generally accepted that in DH and celiac disease, adherence to GFD treatment should be lifelong (Caproni et al., 2009; Ludvigsson et al., 2014). There are, however, a few studies that show that up to 18% of patients with DH seem to acquire a tolerance to gluten during the GFD and do not relapse upon the reintroduction of gluten (Bardella et al., 2003; Garioch et al., 1994; Leonard et al., 1983; Paek et al., 2011). Likewise, there are sporadic reports demonstrating the development of gluten tolerance during a GFD in patients with celiac disease (Hopman et al., 2008; Matysiak-Budnik et al., 2007).

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Abbreviations: Ab, antibody; DH, dermatitis herpetiformis; EmA, endomysial antibody; GFD, gluten-free diet; IEL, intraepithelial lymphocyte; SD, standard deviation; TG2, transglutaminase 2; TG3, transglutaminase 3

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Table 1. Gluten Challenge Outcome in 19 Patients with Dermatitis Herpetiformis

		Challenge Outcome					
Patient/Sex/Age	Duration of GFD Before Challenge, Y	Rash	Villous Atrophy[2]	Serum TG2-Targeted Abs[4]	Challenge Duration, Mo	Skin IgA and TG3 Deposits Pre/ Postchallenge	Serum TG3 Abs[7] Pre/ Postchallenge
1/M/49	5	+	+	−	1	+/+[6]	−/−
2/M/61	5	+	−	−	2	+/+[6]	−/−
3/M/60	24	+	+[3]	+	3	−/+	−/+
4/M/41	20	+	+	+	3	−/+	−/+
5/M/65	24	+	+[3]	+	3	−/+	−/+
6/F/56	31	+	+[3]	+	3	−/−	−/+
7/M/47	14	+	+[3]	+	3	−/+	−/+
8/M/72	15	+	+[3]	+	4	−/+	+/+[6]
9/M/51	8	+	+[3]	+	4	−/−	−/+
10/F/55	40	+	+	−	6	−/+	−/+
11/F/68	33	+	+[3]	+	9	−/+	−/+
12/F/59	34	+	−[3]	+	9	−/+	−/−
13/M/58	18	+	+	+	10	−/+	−/+
14/M/66	34	+	+	−	12	−/+	−/−
15/F/71	34[1]	+	−	−	12	+/+	−/+
16/M/56	22	−	+[3]	+[5]	4	−/−	+/+[6]
17/F/37	9	−	+[3]	+[5]	4	−/−	−/−
18/M/68	22[1]	−	+	−	12	−/−	−/−
19/M/58	36[1]	−	−	−	12	−/−	−/−
Mean Age at Prechallenge, Y (Range)	**Mean Duration of GFD, Y (Range)**	**Rash, n (%)**	**Villous Atrophy, n (%)**	**Serum TG2-Targeted Abs, n (%)**	**Mean Duration (Range)**	**Negative to Positive, n (%)**	**Negative to Positive, n (%)**
58 (37−72)	23 (5−40)	15 (79)	15 (79)	12 (63)	6.1 (1−12)	10 (53)	10 (53)

Abbreviations: Ab, antibody; F, female; GFD, gluten-free diet; M, male; Mo, month; TG, transglutaminase; Y, year.
[1]Dietary lapses 1−5 times per month.
[2]Villous height-to-crypt depth ratio < 2.0.
[3]TG2-specific IgA deposits in the small bowel mucosa.
[4]Serum TG2 antibodies ≥ 3.0 AU/ml and endomysial antibody titer 1:≥5.
[5]Challenge discontinued because of appearance of serum TG2-targeted antibodies.
[6]At postchallenge, deposits more intense or antibody levels more increased.
[7]TG3 antibodies > 30 AU/ml.

The aim of this study was to investigate in detail whether gluten tolerance may exist in patients with DH after long-term adherence to a GFD. The study specifically focused on examining the skin TG3 and IgA immune response during gluten reintroduction in treated subjects with DH. To address these issues, a gluten challenge of up to 12 months was performed in volunteering patients with DH in remission, and the reappearance of the rash, skin IgA and TG3 deposits, serum TG2- and TG3-targeted Abs, and small bowel mucosal deterioration were examined.

RESULTS

Of the 19 patients with DH who volunteered for this study, 13 were male and 6 were female, and their mean age was 58 years (see Table 1). At the prechallenge examination, none of the 19 patients with DH exhibited a rash, and 16 patients (84%) did not have IgA or TG3 deposits in the skin. Serum TG2-targeted Abs (TG2 and endomysial Abs [EmAs]) were negative in all patients, and two had slightly elevated TG3 Ab levels (40 and 41 AU/ml). The small-bowel villous height-to-crypt depth ratio was normal in all 19 patients (see Figure 1), and none had TG2-specific IgA deposits in the small bowel mucosa. Sixteen patients carried HLA-DQ2 (three homozygous) haplotypes, and three carried HLA-DQ8 haplotypes.

The gluten challenge led to the reappearance of the DH rash in 15 patients (79%) in a mean of 5.6 (range, 1−12) months (see Table 1). At postchallenge, 12 of these patients evinced small-bowel mucosal villous atrophy, and 10 patients with a rash had elevated levels of serum TG2-targeted Abs. However, one patient (patient 12, see Table 1) presenting with a rash but a normal villous height-to-crypt depth ratio at relapse showed a marked increase in the densities of $CD3^+$ (from 31 cells/mm at prechallenge to 91 cells/mm at postchallenge) and $\gamma\delta^+$ (from 10.5 cells/mm at prechallenge to 38.3 cells/mm at postchallenge) IELs. Two patients who did not develop a rash during the challenge (patients 16 and 17, see Table 1) developed high levels of serum IgA-class TG2-targeted Abs (TG2 Ab levels 100 and 54 AU/ml, EmA titers 1:1000 and 1:500, respectively), because of which the challenge was discontinued at 4 months. The small bowel biopsy showed villous atrophy in both patients. In addition, one patient (patient 18, see Table 1) had no rash or serum TG2-targeted Abs, but the small bowel biopsy performed at

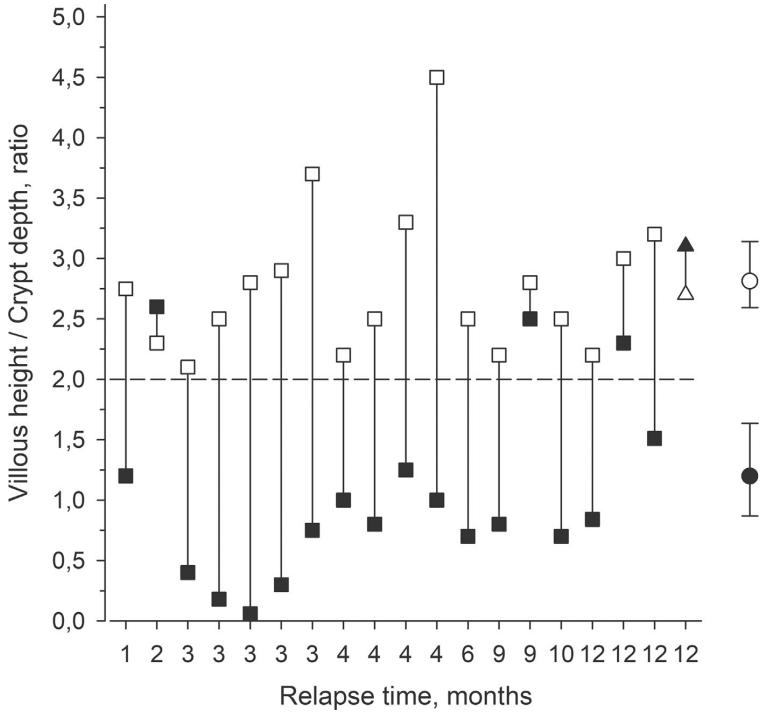

Figure 1. Villous height-to-crypt depth ratios in 19 gluten-challenged patients with dermatitis herpetiformis at pre- and at postchallenge. Eighteen patients experienced relapse during the challenge (pre- □ and postchallenge ■). One patient (pre △ and postchallenge ▲) did not relapse during the challenge. Values above the dashed line are considered normal. The two bars on the right-hand side show mean villous height-to-crypt depth ratios with 95% CIs at pre- and postchallenge depicted with ○ and ●, respectively. CI, confidence interval.

12 months according to the study protocol disclosed villous atrophy. Thus, a relapse was demonstrated in 18 gluten-challenged patients (95%) with DH after a mean of 5.8 months (see Table 1). The remaining patient (patient 19, see Table 1) did not exhibit any skin or gastrointestinal symptoms during the challenge, and at the final examination at 12 months, the small-bowel villous structures were normal, as were the densities of $CD3^+$ and $\gamma\delta^+$ IELs. According to the dietary diary, the patient's gluten consumption had been at least 8 g of gluten per day. After 2 years on a gluten-containing diet, the patient was still asymptomatic, and the skin IgA and serum TG2- and TG3-targeted Abs were negative.

During the gluten challenge, skin IgA became positive in 10 patients with DH, who also developed a rash (see Table 1). In addition, two of the three patients with the positive skin IgA findings at prechallenge (patients 1 and 2, see Table 1) showed more intense fluorescence after the challenge. In double staining, TG3 was found to colocalize with IgA in all skin biopsy samples. Two patients (patients 6 and 9, see Table 1) with the reappearance of the typical DH rash remained negative for skin IgA and TG3 deposits. In both patients, the rash was mild and had appeared a few days to 2 weeks before the skin biopsy samples were taken. However, both had markedly elevated levels (189 AU/ml) of serum TG3 Abs at this time.

When the prechallenge data were compared to the postchallenge data, significant increase in the median levels of serum Abs was noted: TG3 Abs from 4 (range, 0–41) to 89 (range, 5–189) AU/ml; TG2 Abs from 0 to 12 (range, 0–100) AU/ml; and EmA titers from 0 to 1:200 (range, 0–1:4000) ($P < 0.001$, in all analyses). In the small-bowel biopsy samples, the mean villous height-to-crypt depth ratio (see Figure 1) decreased significantly from 2.8 (standard deviation [SD] 0.59) to 1.2 (SD 0.87) ($P < 0.001$), whereas the mean densities of $CD3^+$ and $\gamma\delta^+$ IELs increased significantly from 40 (SD 15) to 74 (SD 29) cells/mm ($P < 0.001$) and from 10.8 (SD 8.0) to 16.5 (SD 11.3) cells/mm ($P = 0.018$), respectively. The Dermatology Life Quality Index mean score increased significantly ($P < 0.001$) from 0.11 (SD 0.32) at prechallenge to 1.58 (SD 2.04) at postchallenge. The total Gastrointestinal Symptom Rating Scale score showed no significant change ($P = 0.22$) with mean scores of 1.66 (SD 0.55) and 1.86 (SD 0.73).

The duration of the GFD before the challenge was shown to correlate significantly with the relapse time ($r = 0.62$, confidence interval = 0.24–0.84; see Figure 2), but age at the time of the DH diagnosis was found not to correlate with the relapse time ($r = -0.23$, confidence interval: −0.62 to 0.25).

DISCUSSION

In this study featuring 19 GFD-treated patients with DH in remission, a gluten challenge was shown to induce relapse in 95% of the patients. The vast majority (15 of 19 patients, 79%) of the relapsed patients experienced a DH rash, and most also developed small-bowel mucosal villous atrophy. In addition, three relapsed patients with no rash exhibited progression to villous atrophy. The remaining patients, however, did not manifest any signs of DH or celiac disease during 2 years on a normal gluten-containing diet.

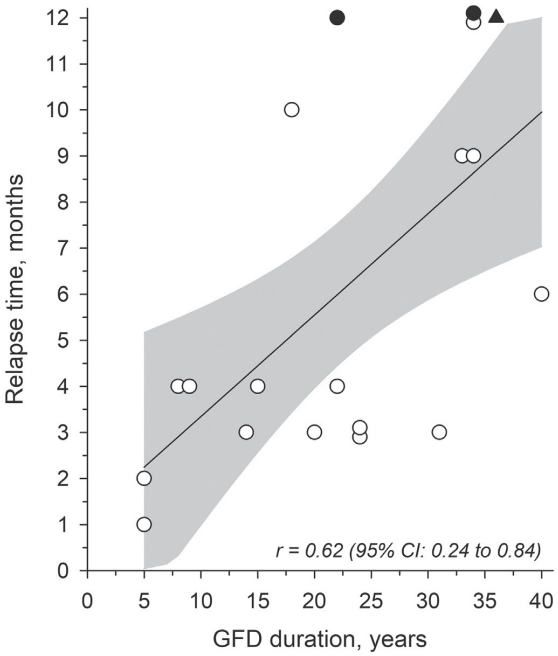

Figure 2. Correlation between the duration of a GFD before the study (years) and relapse time (months) in 19 gluten-challenged patients with dermatitis herpetiformis. The 95% CI of the correlation curve is shown in gray. Patients following a strict GFD before the challenge are marked with ○, and those with a history of dietary lapses are marked with ●. One patient not relapsing during the challenge and with a history of dietary lapses in GFD before the challenge is marked with ▲. CI, confidence interval; GFD, gluten-free diet.

We are aware of two previously conducted gluten challenge studies in adults with DH. Parallel results to ours were found in a study by Leonard et al. (1983): 11 out of 12 patients (92%) with DH relapsed with a rash, and of these, 7 patients (64%) also developed villous atrophy. Bardella et al. (2003) reported 31 patients with DH in whom the rash relapsed within 6 months on a gluten challenge. However, they also observed seven patients (18% of the study group) who did not manifest any type of relapse in the skin or small bowel during the prolonged gluten challenge. It must be noted that these seven patients had been diagnosed in childhood, and compliance with a GFD in these nonrelapsed patients with DH had been only moderate or poor before the challenge. Therefore, the authors suggested that the ingestion of small doses of gluten from childhood may induce immune tolerance (Bardella et al., 2003). Supporting this, the development of tolerance to gluten has also been reported in patients with celiac disease, especially when they have been diagnosed in childhood and continued to eat a normal gluten-containing diet (Hopman et al., 2008; Matysiak-Budnik et al., 2007). In this study, none of the patients had been diagnosed in childhood, and moreover, our previous study on GFD-treated children with DH did not find any patients achieving tolerance to gluten (Hervonen et al., 2014). Furthermore, three of the patients with DH from this series reported dietary lapses when adhering to a GFD, and two of these patients relapsed. The only patient not relapsing during the challenge was documented to consume enough gluten to cause villous atrophy (Lähdeaho et al., 2011). However, this patient had been on a GFD for 36 years, which might cause a delayed DH relapse. Hence, a longer follow-up than 2 years on a normal gluten-containing diet is mandatory before a final conclusion of redeveloped gluten tolerance can be drawn.

In this study, 15 of the gluten-challenged patients with DH developed villous atrophy, and of these, three patients had no rash or skin IgA or TG3 deposits. It has been previously shown that the phenotype of celiac disease can change, especially from the classical disease to DH (Salmi et al., 2015), but it is also probable that the patients with DH without the rash at postchallenge would have developed skin symptoms if gluten exposure had been prolonged. Nonetheless, the results of this study suggest that the gut could be the initial site of the gluten-induced autoimmune reaction, that is, celiac disease, and it is only thereafter that the skin become affected, that is, DH develops (Collin et al., 2017; Sárdy et al., 2002). Nevertheless, we also observed that in three patients with DH who relapsed with the rash, villous atrophy had not developed. It is widely recognized that in celiac disease, small-bowel mucosal changes develop gradually, and villous atrophy is only the end stage of the disease process and not evident in all affected individuals (Kurppa et al., 2009). For instance, the presence of small-bowel mucosal TG2-specific IgA deposits can precede villous atrophy in celiac disease (Koskinen et al., 2008), and the deposits have been shown to occur also in patients with DH with normal small bowel mucosa (Salmi et al., 2014). In agreement with this, one gluten-challenged patient with DH with no villous atrophy had intestinal TG2-specific IgA deposits and increased densities of $CD3^+$ and $\gamma\delta^+$ IELs. In addition, this patient further showed significant levels of TG2-targeted Abs in the serum, also suggesting an ongoing gluten-induced inflammatory response in the small bowel (Katz et al., 2011; Kumar et al., 2001).

At prechallenge, 16 patients were found not to have IgA and TG3 deposits in the skin. These deposits are considered pathognomonic for untreated DH (Donaldson et al., 2007; Sárdy et al., 2002) and are known to resolve slowly during the GFD treatment (Hietikko et al., 2018). In the previous challenge studies by Leonard et al. (1983) and Bardella et al. (2003), 24% and 42% of the patients, respectively, were found not to have IgA deposits in the skin. In these two studies, the patients had adhered to GFD treatment for a mean of 8 years, whereas in this study, the mean duration was 23 years, that is, almost three times longer. This shows that the likelihood of testing negative for skin IgA deposits increases parallel to the duration of the GFD treatment. It is, however, intriguing why skin IgA and TG3 deposits persist for several years after the rash and small-bowel villous atrophy have resolved in patients on a GFD (Hietikko et al., 2018). The explanation might be that IgA and TG3 are deposited in the papillary dermis as immune aggregates in which the TG3 enzyme is active, resulting in covalent cross-linking of the complex to dermal structures (Taylor et al., 2015). In contrast to the very slow disappearance from the skin of a patient on a GFD, this

study documented a rather rapid reappearance (after 3–12 months) of both IgA and TG3 in ten challenged patients, which is to our knowledge a previously unreported finding.

This study also examined whether skin IgA and TG3 aggregates reappear simultaneously with the rash. Coincidental appearance was shown to occur in ten challenged patients. Unexpectedly, two patients developed the DH rash but remained skin IgA- and TG3-negative, though they had markedly elevated TG3 Ab levels in the serum. Skin biopsy samples were taken from the perilesional skin of both patients (Donaldson et al., 2007; Zone et al., 1996), making it unlikely that the results are false negatives. However, there is a possibility that with a short-term rash, the quantity of IgA and TG3 in these two patients was so minute that they were not detectable by the conventional technology that was used. Skin lesions in DH have been previously produced experimentally by the application of potassium iodide, and these studies have shown the activation of elastase and urokinase plasminogen activator enzymes but no alteration in the intensity of IgA deposits (Airola et al., 1997; Reitamo et al., 1981). Furthermore, Taylor and Zone (2018) showed that potassium iodine directly activates IgA-bound TG3 in DH skin and suggested that lesion development is likely dependent on the aberrant activity of the TG3 enzyme.

The major strengths of this study were a well-defined long-term GFD-treated study group from our prospectively collected large DH series (Salmi et al., 2011) and the thoroughly conducted gluten challenge with regular follow-up visits and clinical, serological, and small-bowel mucosal biopsy end points. A limitation, however, was the relatively small number of patients, as was the comparatively short follow-up time for the nonrelapsed patient with DH.

In conclusion, this 12-month gluten challenge study in adult patients with DH showed that 95% of the patients had not achieved tolerance to gluten even after long-term GFD treatment. The gluten challenge was shown to induce a DH rash in the majority of the patients, but intriguingly in a few challenged patients, only small-bowel mucosal deterioration was documented. Moreover, IgA and TG3 aggregates in the skin were not disclosed in every patient with a DH rash. At present, a lifelong adherence to GFD seems justified in all patients with DH.

MATERIALS AND METHODS

Patients and gluten challenge

A total of 19 patients with DH on a GFD from our prospectively collected DH series at Tampere University Hospital participated in the study (see Table 1) (Salmi et al., 2011).

The inclusion criteria were as follows: a diagnosis of DH based on the typical clinical picture, the presence of granular IgA deposits in the papillary dermis with direct immunofluorescence examination, adherence to a GFD for at least 5 years, and absence of skin symptoms for at least 3 years. The exclusion criteria were as follows: age $>$ 80 years, severe cardiovascular disease, previous malignancies, and the use of dapsone or immunosuppressive or anticoagulation medication other than acetylsalicylic acid. At the time of the diagnosis of DH, the mean age of the study participants was 35 (range, 19–57) years, and twelve patients (86%) with available data had small-bowel villous atrophy, and two (14%) had normal villous architecture in the small bowel mucosa.

Before the gluten challenge, the patients had been on a GFD for a mean of 23 (range, 5–40) years. Sixteen patients adhered to the diet strictly, and three reported having 1–5 dietary lapses per month.

The study protocol was approved by the Regional Ethics Committee of Tampere University Hospital (R16039), and all study participants gave their written informed consent.

After the prechallenge investigations, the gluten challenge was initiated by giving the patients 200 g of commercially available wheat bread to be consumed daily for 3 days (Anderson et al., 2000). A follow-up visit was conducted at day 6, and subsequently, the patients commenced a normal gluten-containing diet with a minimum of 10 g of wheat (i.e., about 1 g of gluten) per day. A follow-up telephone call was conducted after 3 weeks, and regular follow-up visits were made every 3 months until the final examination at 12 months. The patients were advised to contact the researchers if they noticed skin or gastrointestinal symptoms, or if they experienced any medical problems during the challenge. In such cases, an extra visit was arranged at the outpatient clinic to consider whether the challenge should be discontinued.

Clinical and dietary evaluation and questionnaires

At each study visit, patients were examined for the presence of skin, gastrointestinal, and other celiac disease–related signs. To ensure the adequate consumption of gluten, a 3-day dietary diary was filled out by the study participants before every follow-up visit and analyzed by a dermatologist experienced in GFD treatment.

Dermatology Life Quality Index and Gastrointestinal Symptom Rating Scale questionnaires assessing the quality of life and the presence of gastrointestinal symptoms were filled out by the patients during the study visits. The Dermatology Life Quality Index is used in dermatological diseases, and it includes six sections: symptoms and feelings, daily activities, leisure, work and school, personal relationships, and treatment unit. Higher scores indicate decreased quality of life (Finlay and Khan, 1994). The Gastrointestinal Symptom Rating Scale has been widely used in previous celiac disease studies (Ludvigsson et al., 2018; Viljamaa et al., 2005), and it includes five categories: diarrhea, indigestion, constipation, abdominal pain, and reflux. Higher scores indicate more severe symptoms (Svedlund et al., 1988).

Skin and small-bowel biopsy samples

Skin and small-bowel biopsy samples were obtained at prechallenge and the end of the gluten challenge to detect findings compatible with DH and/or celiac disease. The skin biopsy sample was taken from uninvolved elbow skin or perilesional skin when the rash had appeared. The samples were fixed in optimal cutting temperature compound (Tissue-Tek O.C.T. Compound, Sakura Finetek USA, Torrance, CA), snap-frozen in liquid nitrogen, and stored at −70 °C until examined. To investigate IgA deposits, sections cut from the samples were stained with TRITC-conjugated goat anti-human IgA (1:50) (A18786, Life Technologies, Frederick, MD). For the examination of TG3 deposits, sections were stained with FITC-conjugated rabbit polyclonal TG3 antibody (1:100) (A030, ZEDIRA GmbH, Darmstadt, Germany). All sections were further double stained for IgA and TG3 as previously described (Hietikko et al., 2018).

During gastroscopy, 6–8 forceps biopsy samples were obtained from the distal part of the duodenum, and at least two samples were stained with hematoxylin and eosin and investigated with light microscopy. At least three well-oriented villous crypt units were measured, and the mean was given as a result. A ratio over 2.0 was considered normal. The remaining samples were freshly embedded

in OCT, snap-frozen in liquid nitrogen, and stored at −70 °C. Stainings of $CD3^+$ and $\gamma\delta^+$ IELs was carried out on 5-μm-thick frozen sections, and the normal values were <37 cells/mm for $CD3^+$ and <4.3 cells/mm for $\gamma\delta^+$ IELs (Järvinen et al., 2003). Small-bowel TG2-targeted IgA deposits were studied from unfixed, frozen, 5-μm-thick small-bowel mucosal sections by direct immunofluorescence as described by Korponay-Szabó et al. (2004).

Serological investigations

Serological investigations were performed at each study visit. The investigated TG2-targeted Abs were TG2 Abs and EmAs (Korponay-Szabó et al., 2003). TG2 Abs were determined with a commercially available ELISA kit (Celikey, Phadia, GmbH, Freiburg, Germany) as previously described (Dieterich et al., 1999), and values ≥ 3.0 AU/ml were considered positive. Serum EmA was measured with an indirect immunofluorescence method with human umbilical cord as a substrate, and a titer of 1:≥5 was regarded as positive (Ladinser et al., 1994). In cases where TG2-targeted Abs were found to convert to positive, the challenge was discontinued, and post-challenge investigations were carried out. TG3 Abs were determined with a commercially available ELISA kit (Immundiagnostik, Bensheim, Germany) as previously described (Reunala et al., 2015), and values >30 AU/ml were considered positive. Elevation of serum TG3 Abs alone was not considered a reliable marker of DH or celiac disease relapse and thus was not considered a sufficient reason for challenge discontinuation. HLA DQ2 and DQ8 genotypes were determined using the Olerup SSP DQB1 low-resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden/Qiagen Vertriebs GmbH, Vienna, Austria).

Statistical analysis

Statistical comparisons within-subjects were performed by permutation test or Wilcoxon matched-pairs signed rank test with exact *P*-values. Correlations were estimated by Spearman correlation coefficient method. The normality of the variables was tested by using the Shapiro-Wilk W test. Stata 15.0 (StataCorp LP, College Station, TX) statistical package was used for the analysis.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of Finnish legislation concerning patient-related data.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: KH, KK, TR, TS; Data Curation: EM, KH, TR, TS; Formal Analysis: EM, KH, TR, TS; Funding Acquisition: TS, KK, KLi; Investigation: EM, KH, TI, PO, TS; Methodology: EM, KK, KLi, KLa, MH, JT, JJ, PS, TR, TS; Supervision: KH, TR, TS; Writing - Original Draft Preparation: EM, KH, KK, TI, PO, KLi, KLa, MH, JT, JJ, PS, TR, TS; Writing - Review and Editing: EM, TR, TS

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