AP&T Alimentary Pharmacology and Therapeutics

Mucosal healing and mortality in coeliac disease

B. Lebwohl*,†, F. Granath†, A. Ekbom†, S. M. Montgomery†,‡, J. A. Murray§, A. Rubio-Tapia§, P. H. R. Green* & J. F. Ludvigsson†,¶

*Celiac Disease Center, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA.
†Clinical Epidemiology Unit, Department of Medicine, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden.
‡Clinical Epidemiology and Biostatistics Unit, Örebro University Hospital, Örebro University, Örebro, Sweden.
§Division of Gastroenterology and Hepatology, Department of Medicine, Mayo Clinic College of Medicine, Rochester, NY, USA.
¶Department of Pediatrics, Örebro University Hospital, Sweden.

Correspondence to:
Dr J. F. Ludvigsson, Department of Pedicatrics, Örebro University Hospital, Sweden.
E-mail: jonasludvigsson@yahoo.com

Publication data
Submitted 10 June 2012
First decision 28 June 2012
Resubmitted 22 October 2012
Accepted 5 November 2012
EV Pub Online 28 November 2012

SUMMARY

Background
Coeliac disease (CD), characterised by the presence of villous atrophy (VA) in the small intestine, is associated with increased mortality, but it is unknown if mortality is influenced by mucosal recovery.

Aims
To determine whether persistent VA is associated with mortality in CD.

Methods
Through biopsy reports from all pathology departments (n = 28) in Sweden, we identified 7648 individuals with CD (defined as VA) who had undergone a follow-up biopsy within 5 years following diagnosis. We used Cox regression to examine mortality according to follow-up biopsy.

Results
The mean age of CD diagnosis was 28.4; 63% were female; and the median follow-up after diagnosis was 11.5 years. The overall mortality rate of patients who underwent follow-up biopsy was lower than that of those who did not undergo follow-up biopsy (Hazard Ratio 0.88, 95% CI: 0.80–0.96). Of the 7648 patients who underwent follow-up biopsy, persistent VA was present in 3317 (43%). There were 606 (8%) deaths. Patients with persistent VA were not at increased risk of death compared with those with mucosal healing (HR: 1.01; 95% CI: 0.86–1.19). Mortality was not increased in children with persistent VA (HR: 1.09 95% CI: 0.37–3.16) or adults (HR 1.00 95% CI: 0.85–1.18), including adults older than age 50 years (HR: 0.96 95% CI: 0.80–1.14).

Conclusions
Persistent villous atrophy is not associated with increased mortality in coeliac disease. While a follow-up biopsy will allow detection of refractory disease in symptomatic patients, in the select population of patients who undergo repeat biopsy, persistent villous atrophy is not useful in predicting future mortality.

Aliment Pharmacol Ther 2013; **37:** 332–339

doi:10.1111/apt.12164

INTRODUCTION

Coeliac disease (CD) is an immune-mediated disorder that occurs in approximately 1% of the Western world.[1, 2] In patients with CD, ingestion of gluten causes small intestinal inflammation and villous atrophy (VA). Typically, patients are recommended a gluten-free diet for life, and with dietary adherence, most symptoms will usually resolve.

Patients with CD are at an increased risk of earlier death.[3, 4] The gluten-free diet may be responsible for the fact that the excess mortality observed in CD decreases towards that of the general population in the years following diagnosis.[4]

The diagnosis of CD is based on characteristic histopathological abnormalities on small intestinal biopsy in the appropriate clinical context. A follow-up biopsy had earlier been considered standard-of-care to confirm healing after adoption of a gluten-free diet.[5] However, subsequently, this practice has declined due to the availability of serology data that normalise after elimination of dietary gluten.[6] As a result, more recent guidelines no longer explicitly mandate a follow-up biopsy.[7] Nevertheless, multiple studies of patients undergoing follow-up small intestinal biopsy have shown that persistent VA is observed in a large proportion of patients, as high as 62–79% in patients in the United States.[8, 9] Persistent VA is often present even in those patients with normalised serologies,[8–12] but is more commonly present in those whose adherence to the gluten-free diet is poor.[9, 11, 12]

It is unknown if survival in CD is related to mucosal recovery. An earlier study found that mucosal healing was associated with a decreased risk of death that did not achieve statistical significance, possibly due to inadequate power (241 patients, with 11 deaths).[9] A second study of apparently asymptomatic individuals with persistent VA (n = 13) reported high rates of malignancy and other morbidity associated with failure to heal.[13] Both of these studies were limited by small size and potential for referral bias.

We therefore examined mortality in a Swedish nationwide cohort study of more than 7000 patients with biopsy-verified CD and a follow-up biopsy.

METHODS

Patients with CD were identified through biopsy reports from all (n = 28) Swedish pathology departments. Using the unique personal identity number (PIN[14]), we matched biopsy data to the Total Population Register and the Swedish Cause of Death Register.

Collection of biopsy data

Collection of biopsy data has been described previously.[4, 15] Between October 2006 and February 2008, we identified patients with CD through computerised biopsy reports from all of the 28 Swedish pathology departments.[15] Of the 29,096 CD patients diagnosed between 1969 and 2008, 7648 had undergone a follow-up biopsy between 0.5 and 5 years after the first diagnostic biopsy with VA (Marsh class 3).[16] For patients with more than one follow-up biopsy, the earliest follow-up biopsy was included.

Classification of follow-up biopsies

Follow-up biopsies were *a priori* dichotomised into persistent VA (Marsh class 3: partial or subtotal-total VA) or improvement (Marsh class 0–2: normal mucosa or inflammation equalling intraepithelial lymphocytosis but without VA).[16] Follow-up histology was determined by local pathologists. Earlier validation has shown that Swedish pathologists correctly classify 90% of samples with VA; further validation showed that patients with VA in this database accurately correlated with a clinical diagnosis of CD in 95% of cases.[15]

MORTALITY DATA

Through the Total Population Register,[17] we were able to ascertain date of death among study participants. Cause-specific mortality was estimated through linkage with the Swedish Cause of Death Register,[18–20] a nationwide register since 1961. Cause-specific mortality was divided into the following four groups based on earlier research in CD: malignancy, cardiovascular disease, respiratory disease and other disease.[21]

Statistical analyses

We compared the survival of patients with persistent atrophy with those with mucosal healing using Cox proportional hazards. Follow-up began on the date of the follow-up biopsy. Follow-up ended on December 31, 2010, or earlier if the patient was lost to follow-up, emigrated or died. We adjusted for the following covariates: age at follow-up biopsy, gender, time period, duration of disease (i.e. time elapsed between diagnosis of CD and follow-up biopsy) and educational attainment.

In a secondary analysis, we compared those who underwent follow-up biopsy with those who did not undergo follow-up biopsy, adjusting for the same covariates as above; follow-up time began on the date of diagnosis.

In preplanned sensitivity analyses, we redefined the window period of the follow-up biopsy. We repeated the Cox model restricting the analysis to three time windows, depending on the time elapsing between the diagnosis of CD and the follow-up biopsy: <1 year; ≥1 year to <3 years and ≥3 years. As multiple prior studies have demonstrated that the mortality risk associated with CD varies over time,[4, 22] we tested the proportional hazards assumption by employing pseudo-time-dependent variables, and derived hazard ratios as a function of time since the follow-up biopsy. We tested for effect modification by introducing interaction terms in the Cox model, and tested their statistical significance via the Wald Chi-squared test.

For cause-specific mortality, follow-up ended on December 31, 2009 since this information was only available to this date.

Serological data

For a subset of patients, the results of antigliadin (AGA), antitissue transglutaminase (TTG), and antiendomysial antibodies (EMA) were available. We obtained computerised data from serology reports and age-specific reference values in individuals undergoing a small intestinal biopsy at one of ten Swedish university hospitals (covering 49% of the Swedish population).[15]

In this subgroup analysis, we identified all patients who had a positive serological result (IgA or IgG) prior to, or within 6 months following, the histological diagnosis of CD. Among these patients, we further identified those who had additional serological testing within 6 months (before or after) their follow-up biopsy. We compared those who converted to seronegative status, with those who remained seropositive with regard to rates of persistent villous atrophy.

Statistical and Power analysis

We used the chi-squared test when comparing proportions of categorical variables, and the *t*-test or Mann–Whitney test when comparing continuous variables. We used a *P*-value cut-off of <0.05 for statistical significance. All statistical calculations were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA).

In a post-hoc power analysis using a two-sided level of significance 0.05 and 80% power, we determined that our sample size would yield a minimally detectable relative risk of 1.27.

Ethics

The study was approved by the Research Ethics Committee of Karolinska Institutet, Stockholm, Sweden.

RESULTS

There were 29 096 individuals in Sweden with biopsy-confirmed evidence of CD. Of these 29 096 patients, 9725 (33%) had a follow-up biopsy. When defining the period of follow-up biopsy as those occurring at least 6 months and no more than 5 years after the initial diagnosis of CD, 7648 (79% of all patients with >1 biopsy) were included for further analysis.

The mean age at diagnosis of the 7648 patients was 28.4 years (median 25.0, SD: 24.9), which was younger than the age of diagnosis for those individuals who did not have a follow-up biopsy (Table 1). Females comprised 63% of this group. The initial histopathology demonstrated partial VA in 12%, while the remainder had either subtotal/total VA (27%) or VA without further characterisation (61%). Among those with no follow-up biopsy who died (n = 2669), the causes of death were cardiovascular in 1011 (38%), malignancy in 735 (28%), respiratory in 155 (6%) and other or not specified in 768 (28%). The overall mortality rate of patients who underwent follow-up biopsy was slightly less than those patients with CD who did not undergo follow-up biopsy

Table 1 | Characteristics of patients who did and did not undergo follow-up small intestinal biopsy 6 months to 5 years after initial diagnosis of CD

Characteristics	Follow-up biopsy (n = 7648)	No follow-up biopsy (n = 19 371)	*P*-value
Age at diagnosis (mean/median years)	28.4/25	33.4/32	<0.0001
Childhood diagnosis [age <20, *n* (%)]	3509 (46)	7649 (39)	
Gender [*n* (%)]			
Male	2816 (37)	7531 (39)	0.0017
Female	4832 (63)	11 840 (61)	
Calendar period of diagnosis [*n* (%)]			<0.0001
≤1989	1074 (14)	2409 (12)	
1990–1999	3274 (43)	7610 (39)	
≥2000	3300 (43)	9352 (48)	
Index biopsy result [*n* (%)]			0.0442
Partial villous atrophy	925 (12)	2415 (12)	
Subtotal/total VA	2055 (27)	4921 (25)	
Nonspecified VA	4668 (61)	12 035 (62)	
Duration of diagnosed CD at the time of repeat biopsy [*n* (%)]			
0.5–1 year	2024 (26)	–	–
Between 1 and 2 years	3429 (45)		
2–5 years	2195 (29)		
Died during follow-up [*n* (%)]	606 (8)	2669 (14)	<0.0001

(Adjusted HR: 0.88, 95% CI: 0.80–0.96, $P = 0.0036$). This difference was driven by the deaths occurring in the first 6 months after diagnosis of CD among those who did not undergo follow-up biopsy, and the mortality difference was null when those deaths were excluded (Adjusted HR: 0.97 95% CI: 0.89–1.06, $P = 0.5378$).

Patients were followed up for a median of 11.5 years from diagnosis, and 9.9 years after follow-up biopsy. For 1927 patients, 25% of the cohort, observation time after follow-up biopsy exceeded 15 years. The median duration of CD at the time of follow-up biopsy was 1.3 years, with 26% of the cohort undergoing follow-up biopsy between 6 months and 1 year after diagnosis (1–2 years after diagnosis: 45%; and 2–5 years after diagnosis: 29%).

Follow-up biopsy demonstrated persistent VA in 3317 patients, 43% of the cohort. The mean number of annual out-patient clinic visits among those with no follow-up biopsy (1.0) was similar to those with mucosal healing (1.1, $P = 0.0912$) and those with persistent villous atrophy (1.0, $P = 0.3636$). Patients with partial VA on the initial diagnostic biopsy were less likely to have persistent VA on follow-up biopsy (30%) than those who originally had subtotal/total VA (42%, $P < 0.0001$).

Serological status and mucosal healing

Among patients in whom serological results were available, 905 had a positive serology prior to the initial biopsy or within 6 months following the initial biopsy demonstrating CD (12% of the 7648 patients who underwent follow-up biopsy).

Of these 905 seropositive patients, 545 (60%) had repeat serology assessments within 6 months of the second biopsy. Of these 545 patients with a follow-up biopsy and follow-up serology, 224 (41%) had a persistently positive serology. The proportion of patients with persistent villous atrophy was greater in those with persistently positive serology (139/224, 62%) than in those with conversion to negative serology (67/321, 21%, $P < 0.0001$).

Mucosal recovery and overall mortality

Of the 7648 patients with a follow-up biopsy, 606 (8%) died during the observation period. Persistent VA was associated with an increased risk of death in this unadjusted analysis (Log rank test by Chi-square 14.3, $P < 0.0001$). On unadjusted Cox proportional hazard modelling, persistent VA was associated with an increased risk of death (HR: 1.37 95% CI: 1.16–1.62).

Adjusted Cox proportional hazards are shown in Table 2. Persistent VA was no longer associated with mortality after adjusting for age at follow-up biopsy, gender, calendar period of diagnosis, educational attainment and duration of CD (HR: 1.01, 95% CI: 0.86–1.19). This null association was present in adults (HR: 1.00, 95% CI: 0.85–1.18) as well as children (HR: 1.09, 95% CI: 0.37–3.16).

Increased age and male gender were independently associated with mortality. However, age did not modify the null association between persistent VA and mortality (P-value for interaction term 0.1284), nor did gender (P-value for interaction term 0.9960). The null association between persistent villous atrophy and mortality was seen in both men (HR: 1.00, 95% CI: 0.79–1.26) and women (HR: 1.01, 95% CI: 0.80–1.28). Risk

Table 2 | Cox proportional hazard model assessing the association of persistent histological activity with mortality rate

Characteristics	Adjusted* Hazard Ratio (95% CI)	P-value
Result of follow-up biopsy		
Mucosal healing	1.0 (reference)	(reference)
Villous atrophy	1.01 (0.86–1.19)	0.9208
Age at follow-up biopsy		<0.0001
0–2	0.03 (0.01–0.07)	
3–9	0.01 (0.004–0.04)	
10–19	0.14 (0.07–0.3)	
20–29	0.12 (0.05–0.28)	
30–39	0.19 (0.11–0.33)	
40–49	0.55 (0.39–0.78)	
50–59	1.0 (reference)	
60–69	2.65 (2.02–3.47)	
70–79	7.47 (5.76–9.68)	
≥ 80	16.64 (11.48–24.13)	
Gender		
Male	1.85 (1.57-2.17)	<0.0001
Female	1.0 (reference)	
Calendar period of diagnosis		0.0483
≤ 1989	1.0 (reference)	
1990–1999	1.04 (0.82–1.33)	
≥ 2000	0.80 (0.59–1.08)	
Education level		0.0031
< 2 years of high school	1.0 (reference)	
2 years of high school	0.85 (0.68–1.06)	
3 years of high school	0.69 (0.50–0.95)	
College/University	0.72 (0.55–0.93)	
Unknown	1.37 (1.02–1.86)	
Duration of diagnosed disease prior to 2nd biopsy		0.2184
0.5–1 year	1.0	
Between 1 and 2 years	0.96 (0.79–1.16)	
2–5 years	1.15 (0.94–1.40)	

*Adjusted for age at follow-up biopsy, gender, time period, duration since diagnosis, and educational attainment.

estimates were similar in patients aged ≥50 years at the time of follow-up biopsy (HR: 0.96, 95% CI: 0.80–1.14).

Mortality risk was not significantly increased in the three prespecified time period strata after follow-up biopsy (6 months to 1 year: HR: 0.82, 95% CI: 0.48–1.38; 1–5 years HR: 0.87, 95% CI: 0.65–1.17; greater than 5 years HR: 1.12, 95% CI: 0.91–1.39).

Redefining the time windows of follow-up biopsy did not influence the relationship between persistent VA and mortality (Table 3).

Serological status and mortality

In the subset of patients with baseline and follow-up serologies, 224 patients had persistent positive serologies, and there were 15 deaths. On multivariate Cox analysis, the HR for persistent positive serology (adjusting for other covariates including persistent villous atrophy) was 3.17 (0.8–12.6, $P = 0.1006$), while the HR for persistent villous atrophy was 2.05 (0.56–7.34, $P = 0.2772$).

Mucosal recovery and cause-specific mortality

Persistent VA on follow-up biopsy was not associated with an increased risk of death from cardiovascular, neoplastic, respiratory or other causes (Table 4).

DISCUSSION

In this nationwide population-based study, we found no association between mucosal healing and death rates in patients with CD when followed up for a median of 11.5 years after diagnosis and 9.9 years after follow-up biopsy. This null association was similar for adults (HR: 1.00, 95% CI: 0.85–1.18) as well as children (HR: 1.09, 95% CI: 0.37–3.16). This mortality estimate remained stable when defining the time frame for follow-up biopsy, and it remained unchanged in multiple time strata after follow-up biopsy.

Table 3 | Mortality risk, redefining follow-up biopsy window (all the same covariates as previous, except for disease duration)

Follow-up biopsy definition	Adjusted* hazard ratio for persistent villous atrophy (95% CI)	*P*-value
6 months–1 year after diagnosis ($n = 2024$)	1.30 (0.98–1.72)	0.0704
Between 1 and 3 years after diagnosis ($n = 4575$)	0.93 (0.73–1.19)	0.5832
Year 3–5 after diagnosis ($n = 1049$)	0.74 (0.48–1.14)	0.1766

* Adjusted for same covariates as in Table 2.

Table 4 | Association of follow-up biopsy result with cause-specific mortality

Cause-specific mortality	Adjusted* Hazard Ratio for persistent villous atrophy (95% CI)	*P*-value
Cardiovascular	1.03 (0.76–1.38)	0.8712
Malignancy	1.20 (0.88–1.66)	0.2542
Respiratory	0.78 (0.41–1.48)	0.4427
Other	0.94 (0.69–1.29)	0.7117

* Adjusted for same covariates as in Table 2.

This is the first study with adequate power to assess the mortality risk in patients with CD who undergo follow-up biopsy. Prior studies have suggested that patients with persistent VA were at risk for adverse outcomes.[9, 13]

Our finding of no increased mortality risk among patients with CD and persistent VA was hence unexpected, given the prior literature. Earlier studies suggesting a possible mortality risk may have been subject to selection bias, given their single-centre settings.

A prior study of this cohort found that after the first year of follow-up, the risk of death for patients with CD was the same as those with 'latent CD,' as defined by positive serology but normal small intestinal histology (HR for CD: 1.26 95% CI: 1.20–1.32; HR for latent CD: 1.27 95% CI: 1.02–1.52). As the mortality risk for those with mucosal healing would not likely be lower than those with latent CD (who have normal histology on their first biopsy), and as patients with latent CD have the same mortality risk as those with CD, one might therefore surmise that mucosal healing would not be associated with a decreased mortality.

Our results indicate that the results of the follow-up biopsy do not further risk-stratify patients with regard to mortality. Although persistent VA may reflect imperfect adherence to the gluten-free diet,[9, 11, 12, 23, 24] it may alternatively indicate variable rates of healing; prior studies of patients undergoing serial biopsy have shown that a substantial proportion of patients with persistent VA on initial follow-up biopsy demonstrate healing on subsequent biopsy.[9, 25] Although our null mortality finding was the same for the three prespecified time period strata after follow-up biopsy, it is possible that the first

follow-up biopsy represents only a 'snap shot' that does not portend long-term villous atrophy.

Another possible explanation for the null association between persistent villous atrophy and mortality is that any potential increase in mortality related to malabsorption or activated autoimmunity is offset by a protective effect; for example, patients with CD have a decreased risk of breast, ovarian, and endometrial cancer, which is probably related to lower adiposity and early menopause.[26]

The proportion of patients with persistent VA was 43%, which falls within the range reported previously.[8, 9, 10–12, 23–25, 27] Most studies have reported rates of persistent VA to be between 20% and 50%.[10, 11, 24, 25, 27] Our results confirm that persistent VA is common, but also indicate that this finding is not associated with increased mortality. Other clinically relevant outcomes possibly associated with a histological response to the gluten-free diet were not investigated in this study, such as quality of life, bone density or the development of further autoimmune diseases.[28] Thus, mucosal healing and its confirmation may still be desirable after treatment with a gluten-free diet in patients with CD.

This study has a number of strengths. In this population-based study, we were able to link data on follow-up biopsies in patients with CD from 28 pathology departments with mortality data from Swedish National Registers. A separate validation study involving the review of more than 1500 charts demonstrated the accuracy of using these diagnosis codes for identification of CD patients in this database.[15] During the follow-up of 7648 patients, there were more than 600 deaths, giving this study unprecedented power to examine not only overall mortality but also mortality in important subgroups such as children and mortality by calendar period. The Cause of death Register includes 99.7% of all Swedish death causes, and linkage through the unique personal identity number guarantees a virtually 100% follow-up.[14] Unlike previous estimates of morbidity and mortality associated with histopathological follow-up, this population-based study is not subject to referral bias or loss to follow-up with regard to mortality. The subgroup analysis that included patients with serological information corroborates prior studies that found a correlation between persistently elevated serology results and persistent villous atrophy.[9, 11, 12]

This study has several limitations. Despite the population-based source of sampling, selection bias is a possibility; we had data on follow-up biopsy in 7648, 26% of the 29 096 patients with CD. Swedish data suggest that follow-up biopsies in children were more common in the 1990s and in the early years after the year 2000.[29] As this cohort of patients who underwent follow-up biopsy is younger compared with the overall CD cohort (mean age at diagnosis 28.4 vs. 33.4, $P < 0.0001$), it is possible that the overall exposure to gluten in this group was relatively low, leading to a low mortality rate. When we restricted this population analysis to those aged ≥ 50 years, the null results persisted.

When assessing whether patients undergoing follow-up biopsy were characteristically different from those not being biopsied a second time (Table 1), we found a slightly (but significantly) lower mortality rate in patients undergoing a second biopsy (HR: 0.88, 95% CI: 0.80–0.96). This raises concern regarding ascertainment bias. One reason for this reduced mortality is that patients who underwent follow-up biopsy have all survived a window of elevated mortality risk: the first 6 months after diagnosis. In fact, after excluding deaths that occurred in this period, there was no difference in mortality risk between these two groups (HR: 0.97, 95% CI: 0.89–1.06),

The modest protective effect associated with follow-up biopsy may reflect a greater tendency to adhere to medical follow-up; those undergoing a follow-up biopsy might exhibit more health-seeking behaviour, relating to diet, lifestyle and adherence to medication and preventive care. Moreover, having a follow-up biopsy by necessity entails additional contact with the healthcare system, and this may indirectly lead to improved dietary adherence. As this group has an overall mortality that is lower than that of those who did not undergo follow-up biopsy, caution should be exercised before generalising these results to all patients with CD, given the potential for ascertainment bias.

Nevertheless, the patients in this population-based study are likely representative of CD patients across the clinical spectrum of severity. While the 'Swedish CD epidemic'[30] increased the awareness of CD among Swedish physicians, there has only been one major CD screening initiative in Sweden so far (the ETICS study).[31] Although that study identified 145 asymptomatic patients with CD, these patients would constitute less than 0.5% of the total population of our original cohort of 29 096 patients with CD[4] and have little influence on the risk of complications including death. In a random subset of CD patients,[15] 51% of patients (60/118) had severe CD defined as having either weight loss/growth failure, diarrhoea or both. This compares to 55% of patients in an Italian cohort of CD patients,[32] while 65% of American

CD patients in our earlier study on follow-up mortality had 'classical CD.'[9]

The time period allowable between CD diagnosis and follow-up biopsy was 6 months to 5 years; this cut-off was used so as to minimise the possibility that follow-up biopsy was prompted by clinical worsening. In a sensitivity analysis, changing the time period of the second biopsy inclusion criteria did not change our results (Table 3).

Misclassification is unlikely to be substantial in this analysis. In one centre, Örebro, the misclassification rate (i.e. biopsy was not a routine follow-up biopsy, but was rather prompted by a new clinical development) was 6.7% (7/105). We had data on the number of biopsy specimens in 65 of the remaining 98 individuals, and the mean number of specimens submitted was 3.6.[33]

Another limitation is the lack of comprehensive information data on dietary adherence in every patient. Earlier evidence suggests that questioning for symptom response to a gluten-free diet correlates well with intestinal damage.[12] However, data from our own research group have indicated that dietary adherence does not always lead to mucosal recovery.[8] When we reviewed the charts of 121 randomly selected patients with VA,[15] 22 patients had data on adherence and a follow-up biopsy. All 4 patients with poor dietary adherence had persistent VA, but of 18 with reportedly good dietary adherence, 7 (39%) nevertheless had persistent VA on their follow-up biopsy ($P = 0.090$). While persistent villous atrophy was greater in those with positive follow-up serologies, up to 21% with negative follow-up serologies had persistent villous atrophy, consistent with prior reports.[8–12] Accurate and non-invasive tests to predict mucosal healing are needed.

Among patients with persistent VA in the control biopsy, there may be some patients with refractory CD. Given the high excess mortality in refractory CD,[34–36] our data suggest that refractory CD is uncommon in a nonselected population as it would otherwise have affected the mortality risk in this study.

Despite the null result, it remains possible that mortality increases over a longer term follow-up period. The patients in this study were followed up for a median of 11.5 years after diagnosis, and 9.9 years after follow-up biopsy. We therefore cannot completely rule out a long-term effect on mortality among those with persistent VA. To address limitation, we derived separate hazard ratios as a function of time since the follow-up biopsy (see Results section), and found no association between persistent VA and mortality in the long-term time strata.

We conclude that there is no association between the histopathological appearance of follow-up biopsy and mortality risk in patients with CD when followed up for a median of 11.5 years after diagnosis and 9.9 years after follow-up biopsy. In the select population of patients who undergo repeat biopsy, persistent villous atrophy is not useful in predicting future mortality. Future studies are warranted to assess the role in follow-up biopsy in predicting subsequent morbidity in CD.

AUTHORSHIP

Guarantor of the article: J. F. Ludvigsson.

Author contributions: BL, JFL, FG, AE, SMM, JAM, ART and PG read and met the ICMJE criteria for authorship. JFL, BL and FG designed the experiments/the study. JFL collected the data. BL analysed the data. BL and JFL wrote the first draft of the paper. FG, AE, SMM, JAM, ART and PG contributed to the writing of the paper. JFL, BL, FG, AE, SMM, JAM, ART and PG contributed to the design of study and interpretation of the data analyses. JFL, BL, FG, AE, SMM, JAM, ART and PG interpreted the data. JFL was responsible for data integrity, supervised the project and obtained funding. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

Declaration of personal interests: BL was supported by The American Scandinavian Foundation, the Celiac Sprue Association, and the National Center for Research Resources, a component of the National Institutes of Health (KL2 RR024157). AE was supported by the Stockholm County Council. JAM was supported by the National Institutes of Health – DK071003 and DK057892. JFL was supported by the Örebro University Hospital, Karolinska Institutet, the Swedish Society of Medicine, the Swedish Research Council – Medicine (522-2A09-195) and the Swedish Celiac Society.

Declaration of funding interests: JAM has received grant support from Alba Therapeutics and has served on the advisory board of Alvine Pharmaceuticals, Inc. and Nexpep. He has been a consultant for Ironwood, Inc., Flamentera, Actogenix, Ferring Research Institute Inc., Bayer Healthcare Pharmaceuticals, Vysera Biomedical, 2G Pharma, Inc., ImmunosanT, Inc. and Shire US Inc. PG has served on the advisory board of Alvine Pharmaceuticals, Inc. and has been a consultant for Alba Therapeutics and Shire US Inc. This project (2006/633-31/4) was approved by the Research Ethics Committee of the Karolinska Institute, Sweden, on 14 June 2006.

REFERENCES

1. Green PH, Cellier C. Celiac disease. *N Engl J Med* 2007; **357**: 1731–43.
2. Dube C, Rostom A, Sy R, *et al.* The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review. *Gastroenterology* 2005; **128**: S57–67.
3. Tio M, Cox MR, Eslick GD. Meta-analysis: coeliac disease and the risk of all-cause mortality, any malignancy and lymphoid malignancy. *Aliment Pharmacol Ther* 2012; **35**: 540–51.
4. Ludvigsson JF, Montgomery SM, Ekbom A, Brandt L, Granath F. Small-intestinal histopathology and mortality risk in celiac disease. *JAMA* 2009; **302**: 1171–8.
5. Ciclitira PJ, King AL, Fraser JS. AGA technical review on Celiac Sprue. American Gastroenterological Association. *Gastroenterology* 2001; **120**: 1526–40.
6. Rostom A, Dube C, Cranney A, *et al.* The diagnostic accuracy of serologic tests for celiac disease: a systematic review. *Gastroenterology* 2005; **128**: S38–46.
7. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. *Gastroenterology* 2006; **131**: 1981–2002.
8. Lee SK, Lo W, Memeo L, Rotterdam H, Green PH. Duodenal histology in patients with celiac disease after treatment with a gluten-free diet. *Gastrointest Endosc* 2003; **57**: 187–91.
9. Rubio-Tapia A, Rahim MW, See JA, Lahr BD, Wu TT, Murray JA. Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. *Am J Gastroenterol* 2010; **105**: 1412–20.
10. Bardella MT, Velio P, Cesana BM, *et al.* Coeliac disease: a histological follow-up study. *Histopathology* 2007; **50**: 465–71.
11. Lanzini A, Lanzarotto F, Villanacci V, *et al.* Complete recovery of intestinal mucosa occurs very rarely in adult coeliac patients despite adherence to gluten-free diet. *Aliment Pharmacol Ther* 2009; **29**: 1299–308.
12. Ciacci C, Cirillo M, Cavallaro R, Mazzacca G. Long-term follow-up of celiac adults on gluten-free diet: prevalence and correlates of intestinal damage. *Digestion* 2002; **66**: 178–85.
13. Kaukinen K, Peraaho M, Lindfors K, *et al.* Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease. *Aliment Pharmacol Ther* 2007; **25**: 1237–45.
14. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol* 2009; **24**: 659–67.
15. Ludvigsson JF, Brandt L, Montgomery SM, Granath F, Ekbom A. Validation study of villous atrophy and small intestinal inflammation in Swedish biopsy registers. *BMC Gastroenterol* 2009; **9**: 19.
16. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992; **102**: 330–54.
17. Johannesson I. *The Total Population Register of Statistics Sweden*. New Possibilities and Better Quality. Örebro: Statistics Sweden, 2002.
18. Swedish Cause of Death Register. Available at: http://www.socialstyrelsen.se/register/dodsorsaksregistret.
19. de Faire U, Friberg L, Lorich U, Lundman T. A validation of cause-of-death certification in 1,156 deaths. *Acta Med Scand* 1976; **200**: 223–8.
20. Johansson LA, Westerling R. Comparing Swedish hospital discharge records with death certificates: implications for mortality statistics. *Int J Epidemiol* 2000; **29**: 495–502.
21. Peters U, Askling J, Gridley G, Ekbom A, Linet M. Causes of death in patients with celiac disease in a population-based Swedish cohort. *Arch Intern Med* 2003; **163**: 1566–72.
22. Logan RF, Rifkind EA, Turner ID, Ferguson A. Mortality in celiac disease. *Gastroenterology* 1989; **97**: 265–71.
23. Collin P, Maki M, Kaukinen K. Complete small intestine mucosal recovery is obtainable in the treatment of celiac disease. *Gastrointest Endosc* 2004; **59**: 158–9; author reply 9–60.
24. Hutchinson JM, West NP, Robins GG, Howdle PD. Long-term histological follow-up of people with coeliac disease in a UK teaching hospital. *QJM* 2010; **103**: 511–7.
25. Wahab PJ, Meijer JW, Mulder CJ. Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. *Am J Clin Pathol* 2002; **118**: 459–63.
26. Ludvigsson JF, West J, Ekbom A, Stephansson O. Reduced risk of breast, endometrial, and ovarian cancer in women with celiac disease. *Int J Cancer* 2012; **131**: 244–50.
27. Selby WS, Painter D, Collins A, Faulkner-Hogg KB, Loblay RH. Persistent mucosal abnormalities in coeliac disease are not related to the ingestion of trace amounts of gluten. *Scand J Gastroenterol* 1999; **34**: 909–14.
28. Cosnes J, Cellier C, Viola S, *et al.* Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet. *Clin Gastroenterol Hepatol* 2008; **6**: 753–8.
29. Stenhammar L, Hogberg L, Danielsson L, *et al.* How do Swedish paediatric clinics diagnose coeliac disease? Results of a nationwide questionnaire study. *Acta Paediatr* 2006; **95**: 1495–7.
30. Ivarsson A, Persson LA, Nystrom L, *et al.* Epidemic of coeliac disease in Swedish children. *Acta Paediatr* 2000; **89**: 165–71.
31. Myleus A, Ivarsson A, Webb C, *et al.* Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. *J Pediatr Gastroenterol Nutr* 2009; **49**: 170–6.
32. Corrao G, Corazza GR, Bagnardi V, *et al.* Mortality in patients with coeliac disease and their relatives: a cohort study. *Lancet* 2001; **358**: 356–61.
33. Lebwohl B, Kapel RC, Neugut AI, Green PH, Genta RM. Adherence to biopsy guidelines increases celiac disease diagnosis. *Gastrointest Endosc* 2011; **74**: 103–9.
34. Cellier C, Delabesse E, Helmer C, *et al.* Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. *Lancet* 2000; **356**: 203–8.
35. Malamut G, Afchain P, Verkarre V, *et al.* Presentation and long-term follow-up of refractory celiac disease: comparison of type I with type II. *Gastroenterology* 2009; **136**: 81–90.
36. Rubio-Tapia A, Kelly DG, Lahr BD, Dogan A, Wu TT, Murray JA. Clinical staging and survival in refractory celiac disease: a single center experience. *Gastroenterology* 2009; **136**: 99–107.