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Original Research Article

Can the sensitivity of the histopathological diagnosis of coeliac disease be increased and can treatment progression be monitored using mathematical modelling of histological sections? – A pilot study

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ABSTRACT

Purpose: The aim of this pilot study was to attempt to define a set of equations from histological observations of tissue affected with coeliac disease (CD) to predict Marsh score.
Material/methods: Tissue from 15 patients with untreated CD, 6 patients with treated CD and 9 healthy control patients were stained using the standard H&E, Giemsa's staining for tissue sections and Alcian Blue protocols. A number of histological measures were then taken from each section and the data was used to ultimately design a set of linear predictive algorithms to calculate Marsh score.
Results: Using MANOVA and discriminant analysis, two linear functions were defined which could accurately predict the Marsh score of patients 62.5% (full Marsh score) to 79.2% (simplified Marsh score) of the time.
Conclusions: This pilot study has shown that a set of objective histological measures can be used to define algorithms to predict Marsh score in CD patients and also monitor treatment compliance and progression.

1. Introduction

Coeliac disease (CD) is an autoimmune enteropathy which is increasing in prevalence [1] and is characterised by an inappropriate immune response to gluten, a protein found in many of the major grains which are consumed in modern diets [1–3]. The diagnosis of CD is based on serological and histopathological assessments; with the latter being the most conclusive current test for the condition and based on the Marsh grading system [4–6]. However, due to subjective interpretation of histological sections, this grading system may result in mis-diagnosis of CD [7]. Furthermore, several studies [8–12] have also highlighted the variable, patchy nature of CD presentation and the difficulties associated with the diagnosis of these patients. Therefore, it was hypothesised in this pilot study that by quantifying the cell types and tissue structures within duodenal histological sections that algorithms could be defined to objectively classify patients into Marsh score categories with minimal observer bias. Hence, the aim of this study was to examine duodenal CD sections using three standard histological stains (H&E, Giemsa's stain and Alcian Blue) and to use quantitative histological metrics to define predictive algorithms in an attempt to accurately place CD patients on the Marsh score scale.

2. Methods

To define the equations, single proximal duodenal biopsy samples were collected from a total of 29 patients (25 females and 4 males; mean age 45.7 ± 15.49 years, range 19–78 years) who underwent an upper GI endoscopy at one of two regional gastroenterology clinics in Tamworth or Lismore, NSW, Australia. Tables 1 and 2 give an overview of the study cohort and also include information about biopsies from 6 treated CD patients which were used to test the equations, please note that patients TF6 and TM1 (randomly assigned patient pseudonyms) donated tissue samples at the time of their initial diagnosis and at their follow-up biopsy after

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Table 1
Number of patients within each group for the study. The healthy control group consisted of 9 patients with Marsh type 0 pathology. The untreated CD Group consisted of a total of 14 patients with varying Marsh type pathology as indicated whilst the treated CD group consisted of a total of 6 patients also with varying degrees of Marsh type pathology (see Table 2). A single biopsy specimen was obtained from each patient in the study.

Group	Marsh type	*N*
Healthy controls	Marsh type 0	9
Untreated CD	Marsh type 1	2
	Marsh type 3a	6
	Marsh type 3b	5
	Marsh type 3c	1
Treated CD	Variable, see Table 2	6

Table 2
Details of treated CD patients in this study; including approximate time on treatment and the diagnosis after the treatment period. Please note patients TF6 and TM1 provided two biopsies to this current study, one at their initial diagnosis and one after the specified amount of time on a treatment programme. This allowed for the visualisation of the treatment progression for these two patients. The other treated CD patients in this study were diagnosed before the commencement on the study and thus did not provide an initial biopsy sample.

Treated patients information			
Patient identifier	Initial diagnosis	Time on treatment (approx.)	Diagnosis after treatment
TF1	No initial biopsy	24 months	Marsh type 1
TF6	**Marsh type 3a**	**17 months**	**Marsh type 0**
TF7	No initial biopsy	>36 months	Marsh type 2
TF10	No initial biopsy	18 months	Marsh type 3a
TF21	No initial biopsy	22 months	Marsh type 1
TM1	**Marsh type 3c**	**18 months**	**Marsh type 1**

undergoing treatment for more than 12 months. These patients are indicated in bold in Table 2 and in red in Fig. 3.

The study was approved by the University of New England Human Research Ethics Committee (Ethics number #HE13-184) and all patients gave written informed consent to participate in the study. Adequately orientated formalin-fixed paraffin-embedded (FFPE) duodenal sections 5 μM thick were taken from each patient sample and stained with either H&E, Giemsa's or Alcian Blue. Images of the entire section of the tissues were acquired using a Nano Zoomer 2.0-RS Digital Pathology Slide Scanner (Hamamatsu Photonics, Japan). High magnification (100×) images were collected using an Olympus BX41 Confocal Microscope with Olympus DPController Software (Olympus Corporation, Japan) if required to help identify cell types if an ambiguous image was collected under lower magnification.

Five randomly chosen representative villi from each H&E stained section were used to determine mean villous length (measured from the bottom of the crypt to the tip of the villus) and mean villous width (measured across the centre of the villus) from each sample. The mean total infiltrative leucocyte count (a combined measure of all of the infiltrative immune cells) was calculated from five randomly chosen representative 50 μm^2 regions of interest within each sample. These regions were randomly chosen by the observer and this size was chosen as it provided good representation of the cellularity of the lamina propria of the all the patients in the study and provided a good overall representation of the entire biopsy. Mean neutrophil, lymphocyte, monocyte, eosinophil, basophil and plasma cell numbers were then quantified from five randomly-chosen 50 μm^2 regions of interest in Giemsa's stained tissue sections in each patient. Cells were able to be quantified due to their unique presentation under the Giemsa stain with morphology as described previously [13,14].

Mean villous goblet cell numbers were quantified from five randomly chosen representative villi in each sample, similarly mean crypt goblet cell numbers were determined from five randomly-chosen representative crypts in each Alcian Blue stained sample. As the Alcian Blue stain binds to the mucous present within the Goblet Cells, cells which actively contained mucous were counted and included in the analysis. To standardise the Goblet cell counts, open thecae or "empty" cells were not quantified [15]. All analysis was performed using NDP Viewer 2 software (Hamamatsu Photonics, Japan).

To perform the statistical analysis and modelling; a Spearman's correlation analysis was completed to determine the relationships between each of the histological variables; as well as age, sex and Marsh score of each patient using GraphPad Prism version 6 for Windows (GraphPad Software, USA). Both MANOVA and hierarchical regression analysis were then performed to determine the optimum number of variables required to build meaningful algorithms from the data. Following this, discriminant analysis was undertaken to generate a set of predictive algorithms from the histological data. Finally, the accuracy of these putative equations was tested by attempting to classify the study group on the basis of their official Marsh score, generated by a trained histopathologist in the course of the patient's original diagnosis using standard techniques at either Pathology New England (Tamworth, NSW, Australia) or Sullivan Nicolaides Pathology (Lismore, NSW, Australia).

These classification analyses were performed using both a standard calculation and a holdout calculation of predictive accuracy, with the latter being used as a highly stringent and unbiased measure of predictive accuracy as it tests predictive power by removing one value and recomputing the result before repeating and withholding another value [16]. MANOVA and hierarchical regression analysis were performed using SPSS (Version 22.0, IBM Corp., USA). Discriminant analysis was performed using StatistiXL version 1.10 for Microsoft Excel (www.statistixl.com).

3. Results

Histological metrics were obtained from the paraffin-fixed sections from either normal (control biopsies) or CD patients with different Marsh scores which were determined independently by a qualified Histopathologist (Fig. 1). Overall, a statistically significant main effect was obtained from the MANOVA analysis (Wilk's $\lambda = 0.051$, F-value = 34.904, $p < 0.01$, partial $\eta^2 = 0.949$) of all of the histological parameters. Subsequent univariate analyses of each histological parameter (Table 3) revealed significant ($p < 0.033$) differences in eight out of twelve parameters examined and suggested that each of these eight parameters varied significantly across the CD Marsh score scale.

Spearman's correlation analysis of these histological parameters demonstrated significant ($p < 0.05$) positive correlations ($\rho > 0.7$) between Marsh score and mean infiltrative leukocytes, mean villous width, mean crypt goblet cells, mean lymphocytes and mean plasma cells. In contrast there were significant ($p < 0.05$) negative correlations ($\rho > 0.7$) between sample Marsh score and mean villous length and mean villous goblet cells. These correlations were expected. Thus, the analysis of individual parameters derived from the histological sections of CD patients correlated with the severity of CD pathology in these patients. It was therefore hypothesised that discriminant analysis using these significant histological parameters derived from CD patients would provide higher resolution classification within the Marsh score scale than a single parameter. To examine this, discriminant analysis was performed using all of the histological parameters.

To determine the contribution of each histological parameter to each function, a hierarchical regression analysis was then

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