Citation: Clinical and Translational Gastroenterology (2017) 8, e218; doi:10.1038/ctg.2016.70
 2155-384X/17

www.nature.com/ctg

Outcome of Referrals for Non-Responsive Celiac Disease in a Tertiary Center: Low Incidence of Refractory Celiac Disease in the Netherlands

R.L.J. van Wanrooij, MD[1], G. Bouma, MD, PhD[1], H.J. Bontkes, PhD[2], A. Neefjes-Borst, MD[2], N.C. van Grieken, MD, PhD[2], B.M.E. von Blomberg, PhD[2] and C.J.J. Mulder, MD, PhD[1]

OBJECTIVES: Refractory celiac disease (RCD) is a severe cause of non-responsive celiac disease (CD) due to its association with the enteropathy associated T-cell lymphoma (EATL). Conflicting data exist on the prevalence and the clinical manifestations of RCD type I (RCD I) and type II (RCD II). The aim of the current study was to provide insight in the incidence of RCD and in the distinction with other causes of non-responsive CD.

METHODS: A total of 106 CD patients were referred to our tertiary referral center between January 2006 and December 2011 for evaluation of non-responsive CD. In addition, a questionnaire was sent to all 82 gastroenterology departments in the Netherlands to reveal whether a patient with RCD was currently being evaluated or had been treated between 2006 and 2012.

RESULTS: During a 6 year period, a total of 31 patients were diagnosed with RCD (19 RCD I and 12 RCD II). The nationwide survey revealed 5 additional patients with RCD I and one patient with RCD II. This leads to an annual incidence of RCD of 0.83/10.000 CD patients. The remaining patients were diagnosed with involuntary gluten ingestion (21.7%), delayed mucosal recovery (11.3%), enteropathy associated T-cell lymphoma (7.5%) and autoimmune enteropathy (1.8%).

CONCLUSIONS: This nationwide study reveals a low incidence of RCD in the Netherlands. Nevertheless, RCD is a clinically relevant disease entity in CD patients non-responsive to the gluten-free diet.

Clinical and Translational Gastroenterology (2017) **8,** e218; doi:10.1038/ctg.2016.70; published online 26 January 2017
Subject Category: Colon/Small Bowel

INTRODUCTION

A gluten-free diet (GFD) induces clinical improvement in the majority of celiac disease (CD) patients within weeks to months.[1] Nevertheless, in a substantial group of patients long-standing mucosal abnormalities can be found despite a strict GFD. This can be either due to inadvertent gluten intake or slow mucosal recovery, i.e., lasting longer than 1 year.[2] The latter may occur in up to 80% of adult onset CD patients, and this would decrease to a still considerable 40% after five years of treatment.[2–7] These patients should be distinguished from those who develop primary or secondary resistance to a gluten-free diet with persisting or recurring intestinal villous atrophy (VA) and symptoms of malabsorption. Patients with such refractory CD (RCD) can be distinguished based on the absence (type I RCD) or presence (type II RCD) of increased numbers (>20%) of intra-epithelial lymphocytes (IELs) with an abnormal phenotype.[8,9] The latter are characterized by the absence of cell surface CD3 expression yet have CD3 contained within the cytoplasm ($cytCD3^+sCD3^-CD45^+CD7^+CD4^-CD8^-$cells) and are considered lymphoma precursor cells.[10] Indeed, over 50% of patients with RCD type II (RCD II) develop overt lymphoma within 5 years.[11–13] Especially the distinction between RCD type I (RCD I) and slow response to a GFD can be a challenge in clinical practice.

RCD is considered a rare entity but the exact incidence and prevalence are not well known. Moreover, previous studies have shown discordant results regarding the distribution of the RCD subtype. Various reasons, including heterogenic definitions and diagnostic work-up have been suggested to be responsible, at least in part, for these differences.[14] This distinction is however crucial, as RCD I generally follows a benign course while RCD II is associated with high morbidity and mortality.[15]

The aim of this study was (1) to provide insight in the prevalence of RCD in the Dutch population and (2) to gain insight in the underlying causes of persisting VA in patients where RCD has been excluded.

METHODS

Patients. Patients included in this study visited the out-patient department of Gastroenterology at the VU University Medical Centre, Amsterdam, The Netherlands, for an one day diagnostic work-up for suspected complicated CD. Initial CD diagnosis was reassessed. Diet compliance was evaluated by a specialized dietitian and follow-up of anti-tissue transglutaminase antibody and anti-endomysium antibody (EMA) titers.

Furthermore, HLA genotyping, IgA serumlevels, anti-enterocyte IgA and IgG antibodies, as well as hematological

[1]Department of Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands and [2]Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
Correspondence: R.L.J. van Wanrooij, MD, Department of Gastroenterology and Hepatology, VU University Medical Centre, PO Box 7057, 1007 MB Amsterdam, The Netherlands. E-mail: rl.vanwanrooij@vumc.nl
Received 25 October 2015; accepted 28 November 2016

and biochemical parameters were determined. All patients underwent upper gastrointestinal endoscopy during which biopsies were collected from different locations in the duodenum. Four biopsies were scored according to the Marsh classification and evaluated for other causes of VA including giardiasis, collagenous sprue, eosinophilic duodenitis, absence of plasma cells and Whipple's disease. In addition, epithelial cell populations were evaluated as described below.

The diagnosis of RCD was based on persisting or recurring symptoms despite strict adherence to a gluten-free diet for at least one year and small intestinal VA in absence of other disease entities as mentioned above. To differentiate between RCD I and RCD II flow cytometric analysis of duodenal IEL subsets was used as it has shown to be the most accurate diagnostic tool currently available.[9,16] The diagnosis RCD II was based on the clinically validated cutoff of more than 20% aberrant IELs.[9] Computed tomography-scan, videocapsule imaging, magnetic resonance imaging-enteroclysis, colonoscopy and positron emission tomography-scan were performed on indication.

According to the WHO-classification, enteropathy associated T-cell lymphoma (EATL) was defined as a nonmonomorphic, pleomorphic, anaplastic or immunoblastic tumor, with a $CD3^+CD4^-CD8^-CD7^+CD5^-CD56^-$ phenotype with expression of Granzyme B and TIA.[17] It should be noted that immunhistochemistry is unable to differentiate between surface and cytoplasmatic expression of CD3.[18] The CD3 expression by EATL cells is therefore thought to represent cytoplasmic CD3 expression.

Flow cytometric analysis of intra-epithelial lymphocytes. Multiparameter flow cytometric immunophenotyping was performed on IEL suspensions, isolated as previously described.[9] In brief, biopsies were vigorously shaken at 37 °C for 60 min in phosphate-buffered saline supplemented with 1 mM dithiothreitol (Fluka BioChemika, Buchs Switzerland) and 1 mM ethylenediaminetetraacetic acid (Merck, Darmstadt Germany). The released IELs were washed twice with phosphate-buffered saline supplemented with 0.1% BSA (Roche Diagnostics) and subsequently stained for 30 min on ice, with fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein and allophycocyanin-labeled monoclonal antibodies directed against CD3, CD4, CD7, CD8, CD16+56, CD19, CD45 (all from BD Biosciences, San Jose, CA, USA) and CD52 (Serotec, Düsseldorf, GermanyCD103 (IQ products, Groningen, The Netherlands) Cytoplasmic staining of CD3 was performed after cell permeabilisation by Cytofix/CytoPerm Plus (BD Biosciences), according to the manufacturer's instructions. Stained cells were washed with phosphate-buffered saline containing 0.1% bovine serum albumin (BSA, Sigma, St. Louis, MO) and analysed on a standard 4-color flowcytometer (FACSCalibur, BD Biosciences). The data were analysed using Cellquest software (BD Biosciences). Care was taken to analyse only viable cellular events based on light scatter properties.

Inventory of RCD II prevalence in the Netherlands. To estimate the incidence of RCD in the Netherlands, all 82 gastroenterology departments in the Netherlands were sent a short questionnaire. The questionnaire included two questions: (1) whether a patient with (suspected) RCD was currently being treated by any of the gastroenterologist practicing in that department. (2) Whether a patient with RCD had been diagnosed during the last 6 years by any of the gastroenterologist practicing in that department. Responses were provided per department. When no response was obtained after 14 days, one or more gastroenterologist per department received a phone consultation. In case a patient had been diagnosed with RCD further information was acquired to verify if the patient fulfilled the diagnostic criteria for RCD.[19]

Ethical approval. This study was in accordance with the ethical guidelines of our institution.

Statistical analysis. Incidence was reported as number per 100.000 inhabitants. 95% confidence intervals (CI) were calculated based on a Poisson distribution.

IEL populations were reported as percentages of total, and per group as median and 10th–90th percentile. One-way analysis of variance analysis of the data, for comparison between the groups, was performed using SSPS software (version 20, SPSS Inc., Chicago, IL, USA). To correct for multiple testing, *post hoc* pair wise comparisons using Tukey's honestly significant difference test were carried out. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Referrals. From January 2006 until December 2011 a total number of 106 patients were evaluated for suspected complicated CD. These patients were referred from 66 hospitals in the Netherlands. Clinical and demographic data are summarized in **Table 1**. At time of referral the median age was 56.6 years (range: 22–77 years). The majority of patients (56%) presented with recurring symptoms, and had been on a GFD for a median of 4 years (range: 1–40 years; **Table 1**). CD patients with persisting symptoms since diagnosis had been on a strict GFD for a median of 2 years (range: 1–5 years).

The majority of patients (64.9%) presented with symptoms of diarrhea and-or weight loss. Anemia and hypoalbuminemia were present in 46.2% and 24% of patients, respectively (**Table 1**). Histological evaluation of duodenal specimens revealed VA (Marsh $\geq$ 3A) in 50.9% of patients. The remainder of patients did not fulfill the criteria for VA. Causes for their symptoms will be further discussed below.

Gluten contamination. Twenty-three (21.7%) CD patients were referred with persisting symptoms (**Table 2**). These symptoms were considered to be due to inadvertent gluten contamination as supported by positive serology and inadvertent gluten intake objectified by a specialized dietitian. In 20/23 (90.9%) of these patients histological evaluation of the duodenum was abnormal (Marsh $\geq$ 1). Two other symptomatic CD patients had no histological abnormalities yet positive serology and persistent gluten intake was substantiated by our dietitian.

Slow responders. Twelve patients were referred due to persisting mucosal abnormalities ($\geq$ Marsh 3a) upon upper

Table 1 Patient characteristics

	CD patients with suspected RCD	
	***n*=106**	
Sex		Female: 56.8 %
Age (median; range)		56.6 years (22–77)
Time since onset of GFD		4 years (1–40)
Symptoms		
Absent		14.2%
Present		85.8%
Symptomatic patients	*n*=91	
Symptoms: persisting—recurring		44–56%
Abdominal pain		28.6%
Diarrhea only		15.4%
Weight loss only		4.4%
Diarrhea and weight loss		45.1%
Fatigue		5.5%
Fever/night sweats		0.9%
HLA-DQ genotype	*n*=91	
HLA-DQ2 heterozygous		56%
Homozygous		26.4%
HLA-DQ8 heterozygous		8.8%
HLA-DQ2/DQ8 heterozygous		3.3%
HLA-DQ2/DQ8 negative		5.5%
Laboratory abnormalities	*n*=104	
Anemia		46.2%
Folic acid deficiency	89	6.7%
Vitamin B12 deficiency	88	8.0%
Hypoalbuminemia	100	24.0%
Duodenal histology	*n*=106	
Marsh 0		28.3%
Marsh 1		14.2%
Marsh 2		6.6%
Marsh 3A		29.2%
Marsh 3B		6.6%
Marsh 3C		12.3%
Ulcerative jejunitis		2.8%

CD, celiac disease; GFD, gluten-free diet; RCD, refractory celiac disease.

endoscopy despite the absence of clinical symptoms such as diarrhea, weight loss or abdominal discomfort (**Table 2**). These asymptomatic patients received follow-up endoscopy to confirm mucosal recovery, as recommended by local guidelines.[20] Median age of this group was 58 years (range: 34–77) and median time between index and follow-up endoscopy was 2 years (range 1–12). Eleven patients were human leukocyte antigen (HLA)-DQ2 and/or DQ8 positive, whereas one patient was homozygous for the HLA-DQ2 beta chain (*02). Serology was negative in all patients and dietary evaluation revealed no inadvertent gluten intake. In all these patients index and follow-up biopsies were re-evaluated by a specialized gastrointestinal pathologist. Follow-up biopsies at our institution showed minimal abnormalities (Marsh score <2) in 5 of these patients. In 7 patients persistent evident abnormalities (i.e., Marsh score >2) were observed, but histological scores had improved compared to the index biopsies. Based on these findings these patients were diagnosed as "slow responders".

Suspected aberrant IEL populations. Three patients were referred with a supposed aberrant IEL population as diagnosed elsewhere with the use of immunohistochemistry. Two patients received follow-up endoscopy because they were experiencing symptoms: one patient reported fatigue and the other patient had unexplained recurrent fever episodes. The third patient was asymptomatic but received follow-up endoscopy in accordance with the previously mentioned guidelines. Flow cytometric analysis revealed normal IEL populations and histological examination no other abnormalities.

Table 2 Diagnosis of CD patients with suspected complicated CD

Diagnosis of CD patients suspected for complicated CD	*n* (%)
Gluten contamination	23 (21.7)
Slow responders	12 (11.3)
No duodenal abnormalities	33 (31.1)
Microscopic colitis	3 (2.8)
Inflammatory bowel disease	1 (0.9)
Helicobacter pylory infection	2 (1.8)
Irritable bowel syndrome	24 (22.6)
Absence of CD	3 (2.8)
Immunodeficiency disorder	1 (0.9)
Autoimmune enteropathy	2 (1.8)
RCD I	14 (13.2)
RCD II	11 (10.4)
CD with clonal γδ-T-cells	2 (1.8)
Secondary EATL	8 (7.5)
Total	106

CD, celiac disease; EATL, enteropathy associated T-cell lymphoma; RCD, refractory celiac disease

Non-responsive disease without duodenal abnormalities. In 33 symptomatic patients (31.1%) duodenal examination revealed no persisting abnormalities. Most reported symptoms included abdominal discomfort (42%), diarrhea (37%) and fatigue (11%).

In three patients, the initial CD diagnosis was rejected based on absence of HLA-DQ2 or –DQ8 in combination with atypical histology and lack of CD-antibodies at time of diagnosis. In the other 30 patients the initial CD diagnosis could be confirmed. In two of these patients a *Helicobacter pylori* infection appeared to be the cause of their symptoms since symptoms disappeared after eradication. Nineteen of the remaining 28 patients underwent colonoscopy. Three patients were diagnosed with microscopic colitis, and one with inflammatory bowel disease. The rest of these patients were considered to suffer from CD-related irritable bowel syndrome and was treated accordingly.

Symptomatic patients with duodenal abnormalities on a strict GFD. In addition to the 12 patients that had been categorized as slow responder, 36 CD patients (34%) had evident duodenal abnormalities (Marsh $\geq$3A) despite being on a strict GFD, as indicated by the absence of CD-related antibodies and dietary evaluation (**Table 2**).

In eight patients a secondary EATL was present. Three patients suffered from concomitant other disease entities: two were diagnosed with an autoimmune enteropathy, and another with common variable immunodeficiency disorder. Twenty-five patients (23.6%) were eventually diagnosed with RCD. Fourteen patients were diagnosed with RCD I. Opposed to patients in the slow responder group, these patients were

experiencing malabsorption related symptoms and-or displayed iron- or vitamin deficiencies. Eleven patients were diagnosed with RCD II based on the presence of increased numbers of aberrant T-cells. The median percentage of these cells was 59% (10th–90th percentile: 22–88) in RCD II patients (**Table 3**). Two RCD I patients expressed exceptionally high percentages (>70%) of monoclonal γδ-T-cells in their epithelial layers, and were considered as a distinctive type of RCD, as more extensively described elsewhere.[21]

Nationwide questionnaire: prevalence of RCD. In order to further define the prevalence of RCD in the Netherlands, all Gastroenterology departments were sent a questionnaire and 14 (17%) received a follow-up telephone call after two weeks. As a result, a response was received from all hospitals (100% response rate). Eight patients were reported to be diagnosed with RCD elsewhere. After careful evaluation of the patient history, six patients fulfilled the criteria for RCD. These included five RCD I and one RCD II patients. These patients continued their treatment at their own institution.

Over a 6 year period a total of 31 patients were diagnosed with RCD: 19 with RCD I and 12 with RCD II. The annual incidence of RCD in the Dutch population (16.7 million inhabitants) is 0.031 per 100,000 inhabitants (CI 0.022–0.044). According to a recent study using the Dutch Pathology Registry (PALGA) that has full nationwide coverage, the incidence of biopsy proven CD is 6.65 (CI 6.27–7.06) per 100,000 inhabitants.[22] This indicates that 1,111 new patients were diagnosed with CD in the Netherlands in the year 2010, as compared to 5 patients with RCD (0.46%). Another study addressed the prevalence of recognized and unrecognized CD using serological markers and HLA-genotype in a study group representative for the Dutch population.[23] The prevalence of both recognized (0.016%) and unrecognized CD (0.35%) was 0.37% (CI 0.27–0.51%), which indicates that there are ~62,000 CD patients in the Netherlands. This indicates an annual incidence of RCD of 0.83 (CI 0.67–0.01) per 10,000 CD patients (both recognized and unrecognized).

Table 3 IEL phenotype per disease entity

	Active CD (%)	CD in remission (%)	RCD I (%)	RCD II (%)	EATL (%)
CD3+ T-cells					
Median	98	92	97	40[a]	44
10–90th percentile	84–99	78–99	69–99	9–82	15–98
CD8+ T-cells					
Median	69	74	70	19[b]	33
10–90th percentile	53–84	38–86	32–87	4–55	9–78
CD4+ T-cells					
Median	5.5	6	2	8	7
10–90th percentile	2.5–20	2–24	0.5–12	2–18	4–10
NK cells					
Median	1	3	2	5	3.5
10–90th percentile	0.1–5	1–11	0–5	0.3–11	1–7.8
γδ T-cells					
Median	26	18	22	11[c]	10
10–90th percentile	13–52	5–49	9–42	0.4–20	2–31
B-cells					
Median	0.1	0.02	0.1	0.2	0.2
10–90th percentile	0–1	0–0.8	0–0.9	0–1	0–1
Aberrant IELs					
Median	1	4	1	59[d]	48
10–90th percentile	0.1–8	0.6–12	0.2–14	22–88	0.1–75

CD, celiac disease; EATL, enteropathy associated T-cell lymphoma; IELS, intraepithelial lymphocytes; NK, natural killer; RCD I, refractory celiac disease type I; RCD II, refractory celiac disease type 2.
[a]Significantly less CD3+ T-cells in RCD II and EATL as compared to all other groups $P<0.001$.
[b]Significantly less CD8+ T-cells in RCD II as compared to active CD $P<0.01$.
[c]Significantly less γδ T-cells in RCD II and EATL as compared to all other groups $P<0.001$.
[d]Significantly more aberrant T-cells in RCD and EATL as compared to all other groups $P<0.001$.
Median of percentages of various cell subsets present in the duodenal epithelium.

DISCUSSION

RCD is an extremely rare yet feared complication of CD. In our tertiary referral center, 23.6% of non-responsive CD patients were eventually diagnosed with RCD, which is higher than reported in other studies (0 to 10%).[24–27] This is most likely due to differences in the referral population. Inadvertent gluten contamination was observed in 21.7% of our referred patients, whereas this number was much higher in other studies, ranging between 35–45%.[24–27] In a substantial number of patients (21.7%) duodenal abnormalities were absent at time of RCD work-up, an observation that is consonant with other studies[24–27] Autoimmune enteropathy was diagnosed in two patients and in one patient a variable immunodeficiency disorder was identified. This reiterates the variety of sometimes rare causes that can underlie persistent VA and may mimic RCD.[28]

Despite the relatively high percentage of RCD in our referral population, our findings indicate that RCD is an extremely rare disorder with an annual incidence of 0.031 per 100,000 Dutch inhabitants and 8.4 in CD patients (both recognized and unrecognized). As far as we are aware other studies so far have reported on the prevalence, but not incidence, of RCD. This included three population-based studies. In an unselected, population-based cohort study from Derby, United Kingdom, five out of the 713 (0,7%) diagnosed CD patients fulfilled the criteria for RCD between 1978 en 2005.[29] In another population-based study encompassing 204 biopsy proven CD patients in Omsted County, United States, three patients were diagnosed with RCD over a 56 year time period.[19] A Finnish study reported the lowest prevalence (0.31%) of RCD in CD patients.[30] Finland has the highest prevalence (0.7%) of clinically diagnosed CD in a population, and has a high (88%) dietary adherence. Based on these observations, the authors suggested that early diagnosis and treatment of CD may result in a lower incidence of RCD. Other studies from tertiary referral centers have reported much higher prevalence's, ranging from 1.7–10%.[7,19,27,31–33] However, these study populations might have been subject to selection bias, and are difficult to compare.

RCD I appears to be more common than RCD II; in the published case series so far 56–92% of patients was diagnosed with type I RCD.[11–13,24,25,27,31,33–35] We have recently shown that for the diagnosis of RCD, immunohistochemistry may underestimate the number of aberrant cells in duodenal tissue and RCD II patients with moderately increased numbers of aberrant IELs may erroneously be classified as type I RCD.[16] This may also explain the variety in outcome in RCD I patients between different centers.

In conclusion, this study underlines the wide variety of causes underlining non-responsive CD. This nationwide study on the prevalence of RCD shows that the incidence of RCD in the Netherlands, and in other European and North-American populations are more similar than previously thought. The likelihood of developing refractory disease is extremely low which may be reassuring and of help in the counseling of patients. Understanding why and identification of which patients may develop this severe complication of CD remains a major challenge. Collaboration between specialized centers to standardize diagnostic procedures and treatment protocols is therefore urgently needed.

CONFLICT OF INTEREST

Guarantor of the article: R.L.J. van Wanrooij, MD.
Specific author contributions: Study design and conduction of the study, laboratory studies and writing of the manuscript: R.L.J. van Wanrooij; study design and supervision, and writing of the manuscript: G. Bouma and C.J.J. Mulder; laboratory study: H.J. Bontkes and B.M.E. von Blomberg; histological analysis: A. Neefjes-Borst and N.C. van Grieken.
Financial support: Supported by the Coeliac Disease Consortium, The Netherlands.
Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Refractory celiac disease (RCD) is a feared cause of non-responsive celiac disease (CD).
- ✓ There is conflicting data on the prevalence of RCD.

WHAT IS NEW HERE

- ✓ This nationwide study reveals a low incidence of RCD.
- ✓ Insight in the aetiologies of non-responsive CD in an European center.

1. Murray JA, Watson T, Clearman B *et al.* Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease. *Am J Clin Nutr* 2004; **79**: 669–673.
2. Kaukinen K, Peraaho M, Lindfors K *et al.* Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease. *Aliment Pharmacol Ther* 2007; **25**: 1237–1245.
3. Dickey W, Hughes DF, McMillan SA. Disappearance of endomysial antibodies in treated celiac disease does not indicate histological recovery. *Am J Gastroenterol* 2000; **95**: 712–714.
4. 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**: 1299–1308.
5. Lee SK, Lo W, Memeo L *et al.* Duodenal histology in patients with celiac disease after treatment with a gluten-free diet. *Gastrointest Endosc* 2003; **57**: 187–191.
6. Rubio-Tapia A, Rahim MW, See JA *et al.* Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. *Am J Gastroenterol* 2010; **105**: 1412–1420.
7. 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**: 459–463.
8. Cellier C, Patey N, Mauvieux L *et al.* Abnormal intestinal intraepithelial lymphocytes in refractory sprue. *Gastroenterology* 1998; **114**: 471–481.
9. Verbeek WH, Goerres MS, von Blomberg BM *et al.* Flow cytometric determination of aberrant intra-epithelial lymphocytes predicts T-cell lymphoma development more accurately than T-cell clonality analysis in Refractory Celiac Disease. *Clin Immunol* 2008; **126**: 48–56.
10. 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**: 203–208.
11. Al-Toma A, Verbeek WH, Hadithi M *et al.* Survival in refractory coeliac disease and enteropathy-associated T-cell lymphoma: retrospective evaluation of single-centre experience. *Gut* 2007; **56**: 1373–1378.
12. 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.
13. Rubio-Tapia A, Kelly DG, Lahr BD *et al.* Clinical staging and survival in refractory celiac disease: a single center experience. *Gastroenterology* 2009; **136**: 99–107.
14. Malamut G, Cellier C. Is refractory celiac disease more severe in old Europe? *Am J Gastroenterol* 2011; **106**: 929–932.
15. Rubio-Tapia A, Malamut G, Verbeek WH *et al.* Creation of a model to predict survival in patients with refractory celiac disease using a multinational registry. 44 ed. *Aliment Pharmacol Ther* 2016; **44**: 704–714.
16. van Wanrooij RL, Muller DM, Neefjes-Borst EA *et al.* Optimal strategies to identify aberrant intra-epithelial lymphocytes in refractory coeliac disease. *J Clin Immunol* 2014; **34**: 828–835.
17. Swerdlow SH, Campo E, Pileri SA *et al.* The 2016 revision of the World Health Organisation classification of lymphoid neoplasms. *Blood* 2016; **127**: 2375–2390.
18. van Wanrooij RL, Schreurs MW, Bouma G *et al.* Accurate classification of RCD requires flow cytometry. *Gut* 2010;**59**:1732.
19. Rubio-Tapia A, Murray JA. Classification and management of refractory coeliac disease. *Gut* 2010; **59**: 547–557.
20. Nieuwenhuis WP, Kneepkens CP, Houwen RH *et al.* Guideline Coeliac disease and Dermatitis Herpetiformis. *Ned Tijdschr Geneeskd* 2008; **154**: A1904.
21. van Wanrooij RL, de Jong D, Langerak AW *et al.* Novel variant of EATL evolving from mucosal yd-T-cells in a patient with type I RCD. *BMJ Open Gastroenterol* 2015; **2**: e000026.
22. Burger JP, Roovers EA, Drenth JP *et al.* Rising incidence of celiac disease in the Netherlands; an analysis of temporal trends from 1995 to 2010. *Scand J Gastroenterol* 2014; **49**: 933–941.
23. Schweizer JJ, von Blomberg BM, Bueno-de Mesquita HB *et al.* Coeliac disease in The Netherlands. *Scand J Gastroenterol* 2004; **39**: 359–364.
24. Abdulkarim AS, Burgart LJ, See J *et al.* Etiology of nonresponsive celiac disease: results of a systematic approach. *Am J Gastroenterol* 2002; **97**: 2016–2021.
25. Dewar DH, Donnelly SC, McLaughlin SD *et al.* Celiac disease: management of persistent symptoms in patients on a gluten-free diet. *World J Gastroenterol* 2012; **18**: 1348–1356.
26. Fine KD, Meyer RL, Lee EL. The prevalence and causes of chronic diarrhea in patients with celiac sprue treated with a gluten-free diet. *Gastroenterology* 1997; **112**: 1830–1838.
27. Leffler DA, Dennis M, Hyett B *et al.* Etiologies and predictors of diagnosis in nonresponsive celiac disease. *Clin Gastroenterol Hepatol* 2007; **5**: 445–450.
28. Akram S, Murray JA, Pardi DS *et al.* Adult autoimmune enteropathy: Mayo Clinic Rochester experience. *Clin Gastroenterol Hepatol* 2007; **5**: 1282–1290.
29. West J. Celiac disease and its complications: a time traveller's perspective. *Gastroenterology* 2009; **136**: 32–34.
30. Ilus T, Kaukinen K, Virta LJ *et al.* Refractory coeliac disease in a country with a high prevalence of clinically-diagnosed coeliac disease. *Aliment Pharmacol Ther* 2014; **39**: 418–425.
31. Arguelles-Grande C, Brar P, Green PH *et al.* Immunohistochemical and T-cell receptor gene rearrangement analyses as predictors of morbidity and mortality in refractory celiac disease. *J Clin Gastroenterol* 2013; **47**: 593–601.
32. O'Mahony S, Howdle PD, Losowsky MS. Review article: management of patients with non-responsive coeliac disease. *Aliment Pharmacol Ther* 1996; **10**: 671–680.
33. Roshan B, Leffler DA, Jamma S *et al.* The incidence and clinical spectrum of refractory celiac disease in a north american referral center. *Am J Gastroenterol* 2011; **106**: 923–928.
34. Biagi F, Gobbi P, Marchese A *et al.* Low incidence but poor prognosis of complicated coeliac disease: a retrospective multicentre study. *Dig Liver Dis* 2014; **46**: 227–230.
35. Daum S, Ipczynski R, Schumann M *et al.* High rates of complications and substantial mortality in both types of refractory sprue. *Eur J Gastroenterol Hepatol* 2009; **21**: 66–70.