Complicated Coeliac Disease

Diagnosis and Management

Abdul-Baqi Al-Toma

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door

Abdul-Baqi Al-Toma

geboren te Basrah, Irak

Promotor: prof.dr. C.J.J. Mulder

Dedication

To: My wife Amera,
My daughters Dania and Minen,
My mother and father.

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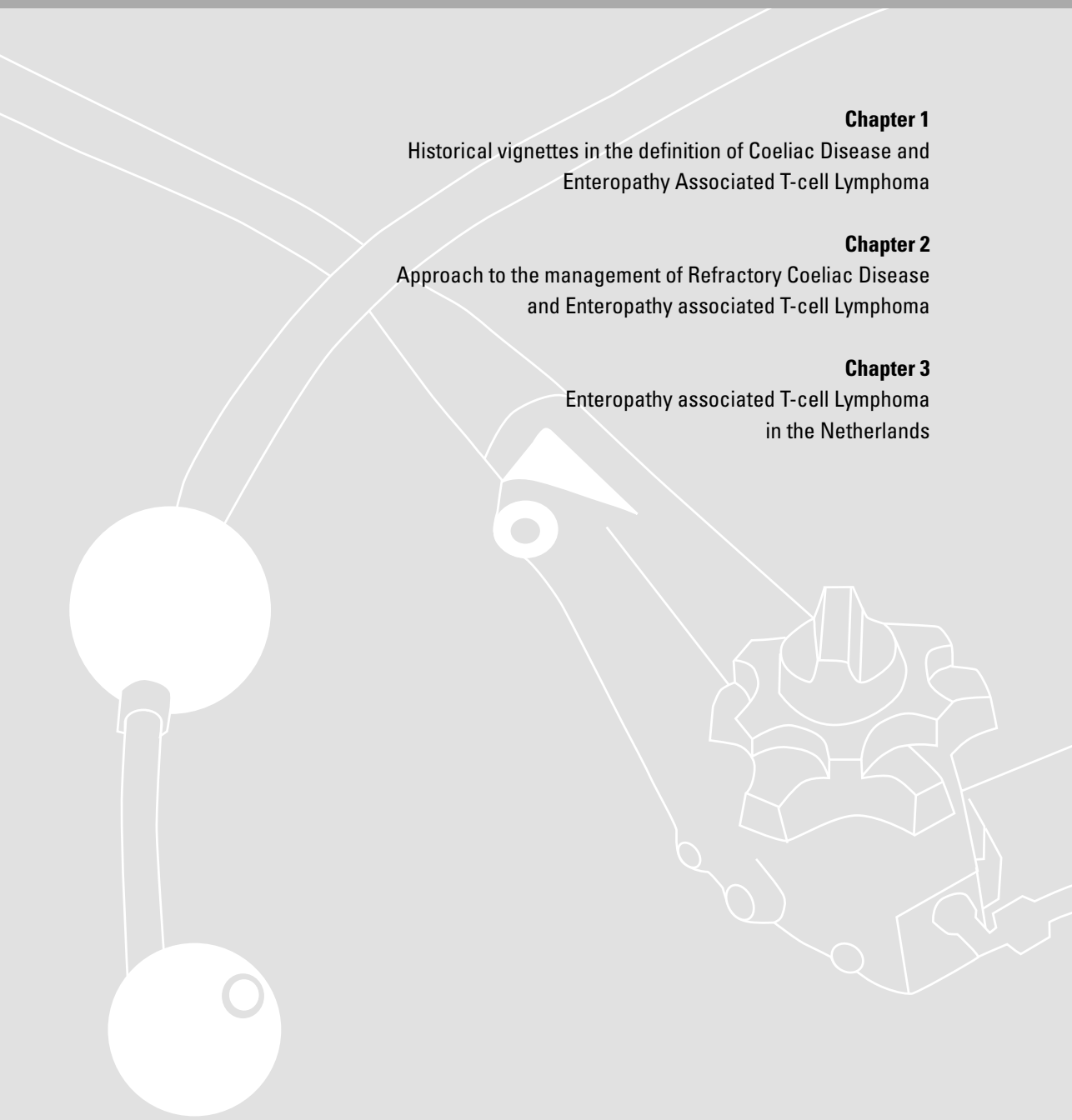
INTRODUCTION



1 Historical vignettes in the definition of Coeliac Disease and Enteropathy Associated T-cell Lymphoma

The discovery of the gluten link

In 1888, in the Saint Bartholomew's Hospital Reports, Dr. Samuel Gee published the first detailed account in "modem" times of what he called "*The coeliac affection*".[1] Gee did not consider that he had discovered a new disease, but rather that he was describing a disorder that had been known since classical antiquity, as reported by the Greek physician Arataeus the Cappadocian. He recognized the importance of diet in the management of coeliac patients ("To regulate the food is the main part of treatment; ...the allowance of farinaceous food must be small...").

"To regulate the food is the main part of treatment"

Several observations concerning the coeliac affection in children appeared in the medical literature in the years to follow. In 1908 Dr. O.A. Herter first described in the American literature the disease reported by Gee [2], and for a time the disease was known as *Gee-Herter's* disease. This was in fact the term used by Willem Karel Dicke, another pioneer in the history of coeliac disease, in his first report on the wheat-free diet, published in *Het Nederlands Tijdschrift voor Geneeskunde* in 1941 ("A simple diet for Gee-Herter's syndrome").
Diet had been the main treatment for coeliac disease since the late 1920s and early 1930s: in 1924 Haas reported a banana diet to be successful, and Fanconi's diet based on fruit and vegetables was also recommended. Dicke was convinced of the beneficial effect of a wheat-free diet long before 1940. A case of a patient in whom diarrhoea recurred after the consumption of bread encouraged his first experiments with wheat-free diets, starting in 1934-1936. The subsequent period of war convinced him further that eating fewer cereals improved the clinical condition of his patients. After the war, Dicke worked closely together with J. H. van de Kamer, a Dutch biochemist, who was the first to develop an accurate and easy-to-perform method to measure faecal fat content. On the basis of experiments performed using standardized diets and measuring fat absorption, Dicke concluded that wheat and rye flour, but not wheat starch, were responsible for the steatorrhoea observed in his patients. Subsequently, by the same approach, he identified the toxic moiety of wheat flour in the alcohol-soluble or gliadin component. Since the introduction of a gluten free diet, mortality has decreased dramatically. By this time it had been realized that children with coeliac disease and patients with so-called idiopathic steatorrhoea of adult life were suffering from the same condition, with the majority of adult patients also responding to the omission of wheat and rye from their diet.[3]

The evolution of diagnostic criteria

Until the 1950s, the diagnosis of coeliac disease was made when a child or adult had malabsorption in the absence of infection. When techniques for peroral small bowel biopsy were introduced during the 1960s, patients with malabsorption were found to have either a normal or a grossly abnormal jejunal biopsy.[4] In 1969, the diagnostic criteria for coeliac disease were proposed at the Interlaken Meeting of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN).[5]

These criteria were further elaborated at the Second International Symposium of Coeliac Disease in 1974, and are as follows (A) structurally abnormal jejunal mucosa when taking a diet containing gluten; (B) clear improvement of villous structure when taking a gluten free diet; (C) deterioration of the mucosa during gluten challenge.[6, 7]

These criteria were reviewed by ESPGAN in 1990.[8] Challenge was no longer required except for children under 2 years of age.

Through in vitro studies, Marsh demonstrated in 1992 a spectrum of consecutive stages of mucosal abnormalities that can be seen in gluten sensitivity.[9] Subsequently, immunological studies demonstrated that in active coeliac disease antibodies to endomysium (EMA) and tissue transglutaminase (tTG) can be detected, which opened the way for new diagnostic strategies and population screening.[10]

The criteria for the diagnosis of coeliac disease in adults have been defined in 2001 by a working group during the United European Gastroenterology Week (UEGW) in Amsterdam.[11] It was concluded that the diagnosis of coeliac disease does not require further confirmation if it is based on duodenal histology showing villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis while using a gluten containing diet, which normalizes on a gluten free diet. Findings of circulating antibodies such as EMA and/or tTG before a gluten free diet support the diagnosis, but are not essential.

Human Leucocytes Antigen (HLA)-DQ_2 or HLA-DQ_8 are still considered circumstantial evidence. Gluten challenge might be useful for minor histological abnormalities, such as intraepithelial lymphocytosis.[12] Immunogenetic research located coeliac related patterns on the HLA region on the short arm of chromosome 6, giving way to the formation of new concepts of the aetiology of the inflammatory response that is seen in coeliac disease. Subsequently, more research have been conducted to verify the relationship between different HLA-DQ haplotypes and the risk of developing a complicated form of coeliac disease, including the progression to malignancies particularly enteropathy associated T-cell lymphoma (EATL).[13,14,15] In *chapter 4* of the present thesis, we will present our results on the correlation between HLA-DQ2 homozygosity and the development of serious complications of coeliac disease, in particular RCD II and EATL.[16]

A landmark development in the workup of coeliac disease was the application of immunophenotyping and the analysis of T-cell receptor gene using PCR studies.[17, 18, 19] These studies paved the way to the current understanding and classification of coeliac disease in its different forms. In *Chapter 2* we will discuss the impact of these informations on the classification of refractory coeliac disease.

Recent advances in small intestine enteroscopy, particularly the video capsule endoscopy [20] and the double-balloon enteroscopy [21] have revolutionized the diagnostic approach of refractory coeliac disease and EATL. *Chapter 5* of the present thesis deals with the impact of the introduction of the double-balloon enteroscopy on the management of refractory coeliac disease [22], while *chapter 6* is devoted to discuss the applicability of computed tomography in the diagnostic work-up of refractory coeliac disease and EATL.[23]

Historical phases in Coeliac Disease diagnosis	
Before 1950s	Coeliac disease is diagnosed when a child or adult had malabsorption in the absence of infection.
In 1960s	Discovery of peroral small bowel biopsy.
1969/1974	ESPGAN criteria the diagnostic criteria for Coeliac Disease.
1990 ESPGAN	Challenge was no longer required except for children under 2 years of age.
1992	Marsh demonstrated a spectrum of consecutive stages of mucosal abnormalities of gluten sensitive enteropathy.
In 1990s	Immunological discoveries and HLA typing
2001	UEGW in Amsterdam: the diagnosis of Coeliac Disease is based on duodenal histology showing villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis while using a gluten containing diet, which normalizes on a gluten free diet. Gluten challenge might be useful for minor histological abnormalities, such as intraepithelial lymphocytosis.
2000s	Small bowel endoscopy and radiology have made great steps: Double-Balloon Enteroscopy, Video Capsule endoscopy, CT and MR-enteroclysis.

The quest for effective medical therapy

Several trials and case reports were published dealing with medical therapies of the refractory forms of coeliac disease, [24, 25, 26] in addition to gluten free diet. Steroids have been tried since the eighties of the last century with mixed results. The best results were achieved in those patients with refractory forms of coeliac disease currently classified as refractory coeliac disease type I (without aberrant T-cells). On the other hand those patients with aberrant T-cells (refractory coeliac disease type II) were refractory to steroids and most of the subsequently studied immunosuppressives, particularly azathioprine.
Our results on treating those refractory patients with new medications, specifically cladribine (2-CDA) are to be presented in *chapter 7*.[27] Furthermore, *chapter 8* deals with a novel and a promising approach to try change the natural history of the refractory coeliac by using high dose chemotherapy followed by autologous stem cell transplantation.[28]

Enteropathy Associated T-cell Lymphoma

The relation of coeliac disease and lymphoma has been recognised since the first reported case in 1937 by Fairley et al.[29], while the association of coeliac disease with adenocarcinoma of the small bowel was described in the late fifties of the last century.[30,31,32] Prior to 1950 malabsorption in lymphomatous patients was considered to be the result of either mesenteric lymphomatous lymphadenopathy (with lymphatic block) or diffuse lymphomatous infiltration of the small bowel.[33] In 1950 the presence of mucosal abnormalities in the small bowel of such patients was suspected by Bjerkelund,[34] and subsequently cases of lymphoma and malabsorption with jejunal mucosal changes resembling those in adult coeliac disease have been recorded.[35, 36] In these patients the lymphoma has been considered to be the primary disease. In 1962 the hypothesis that small bowel reticulosis may develop as a complication in patients with coeliac disease (at that time called idiopathic steatorrhoea) was offered by Gough et al.[37] It is not until the late seventies when the terminology has been changed to lymphoma and called coeliac- related lymphoma.

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Approach to the management of Refractory Coeliac Disease and Enteropathy associated T-cell Lymphoma

2

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Definition/ Criteria of diagnosis of Refractory Coeliac Disease

Coeliac disease is life-long inflammatory condition of the small intestine in genetically susceptible individuals. On small bowel biopsy there is a characteristic, although not specific, mucosal lesion that impairs nutrient absorption by the involved bowel. Prompt improvement of nutrient absorption and healing of the characteristic intestinal mucosal lesion is seen upon withdrawal of dietary gluten.[1]
Non-responsive coeliac disease can be described in terms of the clinical scenario of a lack of initial response to a prescribed gluten free diet, or the recurrence of symptoms despite maintenance of gluten free diet in a patient who responded initially to gluten free diet.[2] Although clinical improvement is usually followed by histological improvement most of the time, on occasions there is evidence for histological improvement with persistence of clinical symptoms that could be related to other causes.[3] Clinical improvement is usually evident within the first few weeks of starting gluten free diet; however, it might take up to 2 years before a complete restoration of intestinal mucosa is evident.

Non-responsive coeliac disease

a lack of initial response to a gluten free diet, or the recurrence of symptoms despite adherence to diet in a patient who responded initially

Currently, true refractory coeliac disease (RCD) is being defined as persisting or recurring villous atrophy with crypt hyperplasia and increased intraepithelial lymphocytes in spite of a strict gluten free diet for more than 12 months or when severe persisting symptoms necessitate intervention independent of the duration of the dietary therapy.[2] RCD may not respond primarily or secondarily to gluten free diet.[4] All other causes of malabsorption must be excluded and additional features supporting the diagnosis of coeliac disease must be looked for, including the presence of antibodies (tTG) in the untreated state and the presence of HLA-DQ markers.[3, 5, 6]

Two categories of RCD are being recognized: type I without aberrant T-cells and type II with aberrant T-cells detected by immunophenotyping by flowcytometric analysis or immunohistology of the intestinal mucosa.[2, 7, 8] Arbitrarily, based on our experience, a percentage of aberrant cells $CD7^{+}CD3^{-}$ of $CD103^{+}$ IEL or cytoplasmic $CD3^{+}$ surface $CD3^{-}$ % of $CD103^{+}$ IEL of ≤ 10% can be regarded as normal, and more than 20% as definitively abnormal.

	Diagnostic criteria
RCD I	– Villous atrophy persisted or recurred despite strict adherence to a gluten free diet. – At least partial villous atrophy (Marsh IIIA) according to the modified Marsh criteria – Excluding other causes of villous atrophy. – When ≤ 10% aberrant T-cells in intestinal biopsy. – Intraepithelial lymphocyte phenotype is normal with the expression of surface CD3, CD8 and TCR
RCD II	– The same as RCD I, in addition to the presence of ≥ 20% aberrant T-cells in intestinal biopsy. – The intraepithelial lymphocytes have normal morphology, but exhibit an aberrant phenotype (normal expression of CD103 and CD7, downregulation of surface CD3 to intracytoplasmic CD3, and the lack of surface T-cell markers: CD4, CD8 and TCR). – EATL has been confidently excluded.

Genetic and environmental factors in the pathogenesis of RCD

In the last few years, great progress has been made in understanding the pathogenesis of coeliac disease. It has become clear that ingestion of gluten by coeliac disease patients results in an improper T-cell mediated immune response.
The environmental factor is mainly ingestion of gluten, while several genes contribute to the genetic predisposition. The main genetic factors, as mentioned before, are given HLA-DQ genes, i.e. the genes encoding DQ_2 or DQ_8 in the HLA complex on 6p21.[9,10] Approximately 95% of coeliac patients have a DQ_2 heterodimer comprised of DQB1*02 and DQA1*05 and most of the remaining 5% have a DQ_8 heterodimer comprised of DQB1*302 and DQA1*03. A small number of individuals lacking either of those heterodimers have DQB1*02 or DQA1*05 alone. Gene dosage also affects coeliac disease susceptibility, e.g., homozygous individuals who carry DQB1*02 and DQA1*05 in *cis* on both chromosomes have a greater risk of disease.[9] Non-HLA complex genes seem to contribute, but the nature and effects of these genes are less well known. The identification and knowledge of the function of additional genetic factors should improve the understanding of the actual pathogenesis of coeliac disease and lead to new diagnostic strategies in case-finding and screening high risk groups.[11, 12]

The pathogenesis of coeliac disease is illustrated in figure 1. In coeliac disease, HLA-DQ molecules bind and present gluten peptides to antigen-specific T-cells. These HLA-DQ-peptide complexes induce inflammatory responses in the small intestine consisting of lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte population, hyperplasia of the crypts and atrophy of the villi. In RCD the number of intraepithelial lymphocytes is markedly raised and it is from these cells that EATL may arise. There is now strong molecular and immunophenotypic evidence showing that a monoclonal

neoplastic T-cell population may emerge from intraepithelial lymphocytes in RCD. Clonal expansion of this monoclonal T-cell population eventually leads to frank EATL.[7] The genesis and expansion of these monoclonal T-cells involve both inappropriate immune responses to gluten and acquisition of genetic abnormalities. Although the monoclonal intraepithelial lymphocytes in patients with RCD are neoplastic, they are not cytologically abnormal and do not form tumour masses which differentiate these patients from EATL patients, in addition to the absence of radiological and bone marrow evidence of lymphoma.[13, 14]

Much less is known about non-HLA genes in this disorder.[11,12] There are several reports that imply involvement of the gene for the negative co-stimulatory molecule CTLA4, or a neighbouring gene (such as those encoding CD28 or ICOS). However, the overall effect of this gene is small.[11, 12]

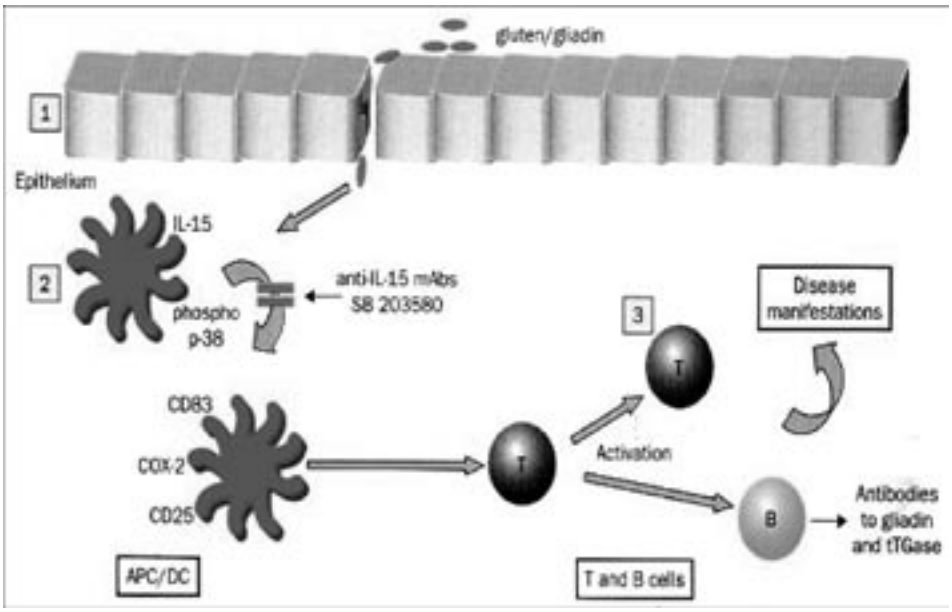

Figure 1. pathogenesis of coeliac disease. 1 and 2: innate response; 3: adaptive response. IL-15=interleukin-15. MAbs=monoclonal antibodies. (From *Maiuri et al*)[15]

Diagnostic Approach to Refractory Coeliac Disease

Revision of the initial diagnosis of coeliac disease

In a patient with villous atrophy refractory to a gluten free diet, the first step requires to reassess the initial diagnosis of coeliac disease, in order to exclude other diseases (Table 1). The presence of circulating antigliadin, EMA or tTG antibodies before the institution of the gluten free diet, an HLA DQ2 or DQ8 status and an initial clinical and histological improvement after a strict gluten free diet are strongly suggestive of coeliac disease. Regarding

the histological features, an increased number of intraepithelial lymphocytes (more than 30 lymphocytes per 100 epithelial cells) is seen in almost all active coeliac disease patients.[4]

Table 1. Other causes than refractory coeliac disease for persisting villous atrophy (*Adapted from Daum et al.*) [16]

Mostly increased number of Intraepithelial lymphocytes
Giardiasis
Tropical sprue
Postinfectious diarrhoea
Collagenous sprue
Protein intolerance (cows milk, soya)
Mostly normal number of Intraepithelial lymphocytes
Tuberculosis (including atypical)
AIDS
Common variable immunodeficiency syndrome
Whipple's disease
Radiation enteritis
Immunoproliferative small intestinal disease
Crohns disease
Eosinophilic gastroenteritis
Autoimmune enteropathy

Assessment of the gluten-free diet

The most important cause of non-responsive coeliac patient is failure to adhere to a gluten free diet, which has been reported in up to 50% of adult coeliac disease patients.[17, 18] The presence of persisting circulating EMA or tTG antibodies is strongly suggestive of dietary mistakes.[15] However, the absence of circulating antibodies cannot rule out minor, inadvertent or voluntary, ingestion of gluten in the diet. On the other hand persisting antibody titres, especially EMA, may also be found in rare patients on a strict gluten free diet with RCD.[2] tTG-antibodies mostly return to normal within 2-3 months. A careful dietary inquiry performed by a skilled dietician in coeliac disease should be performed as the first line of investigation in a supposed RCD patient.

Exclude other causes of diarrhoea with/without villous atrophy

In case of persisting diarrhoea despite demonstrable improvement in the histologic lesion and exclusion of dietary mistakes, other associated disorders should be considered. Well known causes responsible for symptoms mainly include microscopic colitis and more rarely intermittent pancreatic insufficiency in coeliac disease, secondary lactase deficiency, bacterial overgrowth, coexisting inflammatory bowel disease, irritable bowel syndrome but also anal incontinence.[5, 6, 19] (Table 1)

There are many other causes of villous atrophy besides coeliac disease. Clinical history should investigate longer stays near the equator for detection of tropical sprue. Small bowel enteropathy seems to occur often in southern parts of Africa.[20] Giardiasis should be excluded by immunofluorescence of stool samples and may be diagnosed by duodenal histology.[21] Crohn's disease with involvement of the duodenum may mimic or even coexist with coeliac disease.[22] The term collagenous sprue should be used with caution, as this disease is not an established independent entity. There exists no definition for collagenous sprue unlike for collagenous colitis. However, a subepithelial matrix broader than 10-20 µm should point to the diagnosis of collagenous sprue. Deposition of excess of extracellular matrix underneath the basement membrane is an unspecific reaction, which can be seen in gluten-responsive coeliac disease, as well as in several other entities of RCD and also in EATL.[23, 24] Collagenous band like structures regress to a large part in responsive coeliac disease.[23] Autoimmune enteropathy is seen mainly in children and young adults, but may occur also in elderly patients.[25, 26] The histological picture often shows a diminished number of paneth cells. The number of intraepithelial lymphocytes often is normal and patients present frequently with concurrent autoimmune diseases.[27]

We have to realize that villous atrophy has also been reported in association with the presence of a thymoma, with protein intolerance, in conjunction with common variable immunodeficiency syndromes [28, 29] and eosinophilic enteritis.[30] In common variable immunodeficiency antibody testing for coeliac disease-associated antibodies is not useful. Only histological and clinical improvement on a strict GFD may reveal underlying coeliac disease in single cases of common variable immunodeficiency.[31]

Exclude malignant complications of coeliac disease

Unexplained weight loss, abdominal pain, fever and night sweating should alarm physicians of an overt EATL. Other markers for overt EATL may be positive stool blood tests, increased LDH or beta2-microglobulin.[32, 33] In patients on gluten free diet, EATL need not necessarily be accompanied by duodenal villous atrophy.[34] A high index of suspicion for an overt lymphoma should lead to an extensive work-up including upper and lower endoscopy, ENT-workup, CT-scan of thorax and abdomen with enteroclysis, video-capsule enteroscopy (VCE) and double-balloon enteroscopy (DBE) in order to obtain histological specimens. In some cases laparotomy, intra-operative enteroscopy and full thickness biopsies are necessary, as the operative procedure may come to an earlier diagnosis what may be essential.

Positron Emission Tomography (PET) scan has been investigated in patients with EATL and RCD. Hadithi et al [35], in a prospective cohort of 8 EATL patients and 30 patients with RCD,

demonstrate that PET can visualize in all patients sites affected by EATL as confirmed on biopsy. Patients with complicated coeliac disease showed more bowel wall thickening, lymphadenopathy and intussusception, less increase in number of small mesenteric vessels and a smaller splenic volume compared with uncomplicated coeliac disease.[36, 37]

The diagnosis of overt T-cell lymphoma is based on histological and immuno-histochemical features with mainly evidence of large or medium size T-cell proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$ phenotype. The majority presents as $CD3^+$, $CD8^-$, $CD30^+$ large cell lymphoma, however small cell lymphomas often are $CD3^+$, $CD8^+$, $CD30^-$.[32, 33]

Diagnosis of small bowel adenocarcinoma may even be more difficult than lymphoma. Especially tumours located in the jejunum and ileum, which are not reached by standard endoscopic techniques, require extensive investigations. Diagnosis may often be made only after operative procedures.[38] Obscure gastrointestinal bleeding, obstructive symptoms, stenotic lesions on radiological examinations and retention of a video capsule enteroscope should raise the suspicion of these malignancies.

The evolving role of Double-balloon Enteroscopy

First described by Yamamoto and colleagues in 2001, DBE is a new endoscopic technique with the potential to allow complete visualization of the entire small bowel.[39]

The full description of the scope specifications and the technique of enteroscope insertion and withdrawal are provided in our review article.[40] In the European retrospective study, enteroscopy was diagnostic in all patients suspected of having refractory coeliac disease.[41]

We have performed double-balloon enteroscopy in a total of 21 consecutive patients with refractory coeliac disease. EATL was found in five patients (24%) as circumferential, discrete, or confluent ulcerations, while excluding EATL in another 4 patients.[42]

Establishing the diagnosis of RCD

Finally, RCD is a diagnosis of exclusion, defined as a persisting villous atrophy that does not respond to a strict gluten free diet. Demonstration of an aberrant clonal intraepithelial T-cell population and/or loss of antigen on intraepithelial lymphocytes seem to characterize this patient population on high risk for development of overt lymphoma and differentiates RCD II from RCD I, which shows low or almost absent aberrant T-cells. RCD II is also referred to as cryptic intestinal T-cell lymphoma; Sprue-like intestinal T-cell lymphoma). Detection of a clonal T-cell population by testing for TCR rearrangement was thought to be highly predictive of EATL development. However, oligo- or monoclonal intraepithelial lymphocytes populations can be detected in the large majority of both RCD I and RCD II patients, also in patients that do not develop an EATL. Clonality is therefore of limited use in establishing the diagnosis of RCD and to predict the development of EATL.[14, 43- 45]

Steps required to establish the diagnosis of Refractory Coeliac Disease	
Revision of the initial diagnosis of coeliac disease	The presence of antigliadin, EMA or tTG antibodies before the institution of gluten free diet, an HLA DQ2 or DQ8 status and an initial clinical and histological improvement after a strict diet
Assessment of the diet	The presence of persisting EMA or tTG antibodies is strongly suggestive of dietary mistakes
Exclude other causes of diarrhoea ±villous atrophy	See table 1
Exclude malignant complications of coeliac disease	EATL or small bowel adenocarcinoma
Establishing the diagnosis and differentiate RCD I from RCD II	Clinical behaviour, presence/absence of aberrant clonal intraepithelial T-cell population and/or loss of antigen on intraepithelial lymphocytes

Comparison between RCD I versus RCD II

Clinical and biological behaviour

Patients with RCD I may represent an earlier stage of the disease than RCD II and the prognosis may be better, and the risk of developing an overt lymphoma is almost non-existent. In RCD I adherence to the gluten free diet should be carefully investigated since a strict gluten free diet may induce remission in some patients.[46]

In RCD I patients often develop concomitant autoimmune diseases, infectious and thrombembolic complications. Retrospective data from our patient population suggest that RCD I patients have a low mortality rate, which is not different from that of the general population.[47]The presence of mucosal ulcerations (ulcerative jejunitis) should alert the doctor for the possible presence of an early EATL.[48]

RCD II is seen mostly in adults and the mean age at diagnosis of RCD II is between 50 and 60 years old but younger cases may be observed.[7] Most of the patients develop severe malabsorption with weight loss, abdominal pain and diarrhoea. Some patients may also have skin lesions mimicking pyoderma gangrenosum or ulcerations mostly in legs, arms and face, chronic chest or sinusoidal infections or unexplained fever.[7] The link between coeliac disease and RCD II is usually suggested by the detection of circulating antigliadin, anti-EMA or anti-TG antibodies before the initiation of the gluten free diet in almost two-thirds of patients, an HLADQ2 or DQ8 status in almost all patients [47] and an initial response on GFD in about one-third of patients with RCD II.[7]

Endoscopic and radiological features

The same pattern of endoscopic abnormalities is usually seen in RCD I and II as in classical active coeliac disease. The finding of mucosal ulcerations, mostly in the jejunum, defines the clinical picture of ulcerative jejunitis.[48] In some cases of RCD II also stomach and/or colonic ulcerations may be found.[50] Enteroscopy using push- or DBE or VCE should be performed in such patients with RCD II in order to search for overt lymphoma and ulcerative jejunitis.[50] CT-scan and/or MRI-enteroclysis may be useful to exclude overt lymphoma and may demonstrate a mesenteric cavitation syndrome and hyposplenism (volume<100 cm^3) in 30% of cases.[36, 37] Enlarged mesenteric lymph nodes often accompany RCD, without necessarily being specific for a T-cell lymphoma.

Histopathology of small bowel biopsies

At least 10 duodenal biopsies are to be taken for histological, immunohistochemical and flow cytometric examination. Four to six biopsies are fixed and preserved in 10% formalin for histopathological and immunohistochemical evaluation. Three-4 biopsies for TCR gene rearrangement studies are taken separately, preserved on histocon and frozen at -20°C. For immunophenotypical evaluation 3-4 biopsies are taken and preserved in RPMI medium. Usually the same spectrum of villous atrophy is observed as in active uncomplicated coeliac disease.

Immunophenotyping of Intraepithelial lymphocytes

Lymphocytes and enterocytes are isolated from small bowel biopsies. The released cells are subsequently washed and labelled by 4-color staining with various combinations of fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein and allophycocyanin labelled monoclonal antibodies against CD3, CD4, CD8, CD7, CD103, CD19, CD45, CD16/56, γδTCR and cytoplasmic CD3.

Cell surface immunophenotyping of Intraepithelial lymphocytes are performed on a 4 colour FACS Calibur flow cytometer. Nonviable cells and debris are excluded based on forward and sideward light scatter properties and a gate on CD45 positive cells is used for selecting lymphocytes. Intraepithelial localisation of lymphocytes is confirmed by surface expression of CD103 (αEβ7 integrin, a gut homing receptor for E-cadherin). Intraepithelial lymphocytes are analysed based on their expression of cell markers: cytoplasmic CD3, surface CD3, CD4, CD7, CD8, CD16/56, CD19, CD103 and TCR-γδ on $CD45^+$gated intraepithelial lymphocytes.

Arbitrarily, from our own experience, a percentage of aberrant cells $CD7^+CD3^-$ of $CD103^+$ Intraepithelial lymphocytes or cytoplasmic $CD3^+$ surface $CD3^-$ % of $CD103^+$ Intraepithelial lymphocytes of ≤ 10% has been regarded as normal, and more than 20% as definitively abnormal (Figure 2).

TCR gene rearrangement study

DNA is extracted from cryosections of duodenal biopsies. T-cell receptor (TCR) - gamma (TCR-γ) gene rearrangements are analysed by multiplex PCR amplification under standardized conditions. A monoclonal and polyclonal control is included in each experiment.

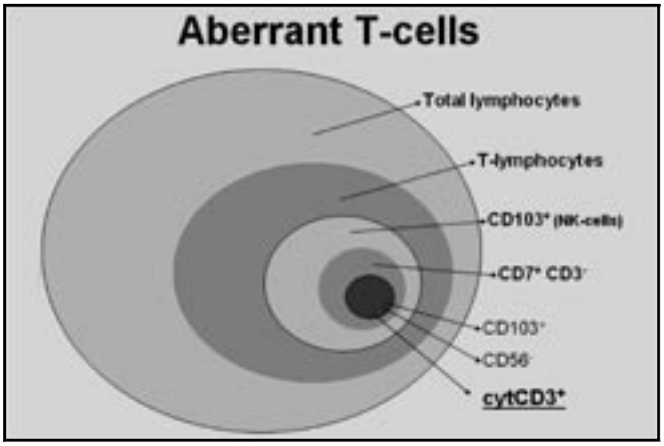

Figure 2. Showing approximate differntial percentages of lymphocytes.

HLA-DQ typing

Typing of HLA-DQA1*and DQB1* alleles can be performed on whole blood samples. In our immunology laboratory, this typing is being performed with a combined single stranded conformation polymorphism/heteroduplex method by a semi-automated electrophoresis and gel staining method on the Phastsystem (Amersham-Pharmacia-Biotech, Uppsala, Sweden).[49]

Reports by Zubillaga et al [9] and Congia et al [10] have shown that DQ2 homozygosity may predispose a person to an earlier onset and to more severe disease manifestations. We found a highly significant correlation between HLA-DQ2 homozygosity and the development of serious complications of coeliac disease, in particular RCD II and EATL.[47] The link between HLA-DQ2 homozygosity and development of RCD II and coeliac disease-associated lymphoma of intraepithelial origin thus suggests that the strength of the gluten-specific T-cell response in the lamina propria directly or indirectly influences the likelihood of RCD II and lymphoma development. It has been reported earlier by Vader et al [50] that HLA-DQ2 homozygous antigen-presenting cells induce higher T-cell proliferation and cytokine secretion than HLA-DQ2/non-DQ2 heterozygous antigen-presenting cells. This may explain the strongly increased risk for disease development in HLADQ2 homozygous individuals. This would indicate that the adherence to a GFD is particularly important for CD patients who are HLA-DQ2 homozygous.

Summary of data on RCD I

Histology of the small bowel mucosa in most cases is indistinguishable from active untreated coeliac disease. The number of intraepithelial lymphocytes may be lower than in RCD II and active coeliac disease although this has not been proven in prospective studies.[45] The intraepithelial lymphocytes phenotype is normal with the expression of surface CD3,CD8 and TCR-β as in classical active coeliac disease.[7,45] The number of CD8– and TCR-β positive intraepithelial lymphocytes should exceed 50% of intraepithelial lymphocytes; a lower

expression seems to be quite sensitive for distinction of RCD I from RCD II but not very specific.[45,53]

Summary of data on RCD II

An aberrant clonal population of intraepithelial lymphocytes may be observed in 80% of patients with RCD on small bowel biopsies. Although these intraepithelial lymphocytes have a normal cytological feature, they exhibit an abnormal phenotype with the expression of intracytoplasmic CD3e, surface CD103 and the lack of classical surface T-cell markers such as CD8, CD4 and TCR-αβ. Furthermore the aberrant intraepithelial lymphocytes phenotype is associated with clonal TCR gene rearrangement. This aberrant intraepithelial lymphocytes population usually represents more than 50% of the intraepithelial lymphocytes and may also be observed in gastric and/or colonic epithelium in around 2/3 of patients and may be found in the peripheral blood in 1/3.[7, 53] It may be also detected in skin lesions or in the chest in single patients, suggesting that RCD II is a diffuse gastrointestinal disease.[50] The use of CD3 and CD8 on fixed biopsies is a very reliable method in order to assess the presence of this aberrant IEL phenotype, even retrospectively.[45, 53]

More recently, it has been shown that recurrent chromosomal abnormalities including a recurrent 1q trisomy may be found in these patients.[53] The diagnostic yield of these cytogenetic features has not been evaluated so far. These chromosomal abnormalities, the clonality of the T-cell receptor gene and the loss of antigens on intraepithelial lymphocytes, indicate that this clonal intraepithelial lymphocytes population can be considered as a cryptic intraepithelial lymphoma.[8]

Therapeutic options

Supportive treatment

The first line of treatment should be to correct nutritional status, if necessary through parenteral nutrition. The gluten free diet should be maintained as strict as possible and reassessed since a minority of patients may have a long-term benefit of a strict gluten withdrawal. Corticosteroid therapy is often useful to induce clinical remission.[7] There exist no prospective or even randomized trials in the use of corticosteroids. Locally acting corticosteroids like budesonide may be clinically effective and may spare corticosteroid side effects.[55] Corticosteroids often induce a clinical remission, although small bowel morphology does not improve significantly in almost all patients. Relapses during tapering of corticosteroids are observed in the majority of cases.[8]

Oligopeptide diet has not only been shown to reduce increased cytokine synthesis of the mucosal immune system, but also to improve clinical and morphological parameters in single patients with RCD.[56] However, patients do not tolerate long-term nutrition with oligopeptide diet and other treatment concepts had to be sought. Parenteral nutrition has to be considered in patients with persistent malabsorption and severe weight loss in spite of maximal medical treatment. Vitamins have to be checked and often iron, zinc, copper, Magnesium, folic acid

and vitamin B12 have to be supplemented. For protection against osteopenia, vitamin D and calcium should be supplied. In single cases with proven osteopenia intravenous therapy with biphosphonates has to be considered.

Treatment of Refractory Coeliac Disease I

In contrast to patients with high percentage of aberrant T-cells, patients with RCD I seem to profit from an immunosuppressive treatment.[46] According to the data of Goerres et al [46], azathioprine should be first line therapy after induction of clinical remission with corticosteroids. In contrast to RCD II, long-term treatment with corticosteroids or locally acting budesonide may be considered only in patients who have contraindications to other immunosuppressants.
Cyclosporine A, infliximab and tacrolimus have been reported to be effective in case reports, but further data are required particularly in the light of severe side effects.[57, 58] These agents should only be considered in case of clinical deterioration despite corticosteroid therapy or intolerance to azathioprine. Intestinal absorption of cyclosporine A is worse than that of tacrolimus, what has to be considered in the acute treatment. Also parenteral administration of tacrolimus has to be considered. Close monitoring of renal function is inevitable. Treatment with infliximab may induce prompt clinical and histological response but this effect has to be weighed against its possible acute allergic and chronic immunosuppressive side effects.[58]

Treatment of Refractory Coeliac Disease II

RCD II is usually resistant to medical therapies. Response to corticosteroid treatment does not exclude underlying EATL. In case of RCD II with persistent clinical symptoms and/or high percentage of aberrant T-cells in bowel biopsies in spite of a corticosteroid treatment, more aggressive therapeutic options should be considered. However, no therapy seems to be curative in RCD II. Some patients may benefit from azathioprine.[46] Cautious is needed in instituting immunosuprressive therapy, as this may induce a high risk of progression to an overt lymphoma.[46, 47] In most cases CHOP-like regimens have been applied, but also other agents used for nodal NHL may be applied. Maurino et al [59] reported the results of treating 7 RCD II patients with azathioprine. Clinical and histologic improvement was noted in 5 of 7 treated patients, although 3 patients died (1 from leucopenic fever and 2 died early). However, in their follow-up report on treating 13 patients with azathioprine, they reported a 46% mortality rate.[60] A recent report on the anti-tumor necrosis factor agent infliximab for treatment of RCD has been published, but no data were provided on aberrant T-cells (T flow cytometry or immunohistology).[61] Recognizing that some patients with RCD II, and especially with ulcerative jejunitis, are suffering from a low-grade EATL, we treated a group of these patients with cytotoxic chemotherapy. Cladribine (2-chlorodeoxyadenosine [2-CDA]) is a synthetic purine nucleoside with cytotoxic activity. In the past few years clinical trials with 2-CDA have confirmed its effectiveness in selected autoimmune disorders. We have treated 17 patients with 2-CDA therapy.[62] This therapy was well tolerated without serious side effects. Six of 17 patients (35.8%) responded with clinical improvement and another 6 had a significant decrease in aberrant T-cell percentages. Interestingly, one of our patients developed a complete clinical, immunologic (aberrant T-cell percentage decreased from 70%

to 15%), and histologic recovery (Marsh classification MIIIC- M-I) and remained symptom free during more than 4 years of follow-up evaluation. Furthermore, ulcerative jejunitis, an endoscopic feature of RCD-II, was seen to disappear in the 5 patients (29.4%) who had it initially. Seven patients (41.1%) developed EATL within 6-38 months after starting treatment and subsequently died despite multiagent chemotherapy (cyclophosphamide, adriamycin, vincristine, and prednisone). Although EATL was excluded adequately at inclusion, 3 patients died of EATL within 5-7 months after therapy. Thus therapy with 2-CDA seems to have a role, although based on our data it is less than optimal in the treatment of RCD with aberrant T-cells. It may be considered, however, as the only medical treatment thus far studied that showed significant reduction of aberrant T-cells, seems to be well tolerated, and may have beneficial long-term effects in a subgroup of patients showing significant reduction of the aberrant T-cell population. The antiCD52 (alemtuzumab) has been used in one patient with RCD II with a good response demonstrated by histological recovery and a clear decrease in the aberrant T-cell population.[63]

Autologous Stem Cell Transplantation (ASCT) in RCD II. Is preventing EATL development a reality or a myth?

In the last decade, stem cell transplantation has become an increasingly accepted treatment option for patients with severe autoimmune diseases refractory to conventional treatment.

The application of this treatment option in gastroenterology is being explored in the last few years. Its applicability has been tested and proved successful in treating refractory Crohn's disease.[64] We have tested the applicability of ASCT in a selected group of refractory coeliac patients with aberrant T-cells.[65]

The concept of intensive immunosuppression is based on findings in animal models of autoimmune diseases, as well as results obtained in patients who underwent SCT for haematologic or oncologic diseases while coincidentally having an autoimmune disease.[66]

Clinical observations in patients with autoimmune disease who were treated with bone marrow transplantation because of a concomitant severe haematologic disorder have paralleled the results from experimental animal studies.[67, 68] These experimental studies and clinical observations paved the way for collaborative efforts to further explore the clinical potential of stem-cell grafting in human autoimmune disease. The lack of alternative treatment options for severe, uncontrolled autoimmune disease prompted development of this treatment strategy.

We have treated seven patients [4M, 3F, mean age 61.5 years (range 51-69 years)] with ASCT. After conditioning with fludarabine and melphalan, ASCT was performed. All 7 patients completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning and transplantation. Engraftment occurred in all patients. No major non-haematological toxicity or transplantation-related mortality was observed. There was a significant reduction in the aberrant T-cells in duodenal biopsies associated with improvement in clinical wellbeing, and normalization of haematological and biochemical markers (mean follow-up 15.5 months, range 7-30 months). These preliminary results showed that high-dose chemotherapy followed by ASCT seems feasible and safe, and might result in long-term

improvement of RCD II patients whose condition did not respond promptly to available drugs. Controlling the inflammatory reaction and resetting the immune response might theoretically prevent or delays the development of EATL.

Follow-up of patients with RCD

RCD, particularly RCD II, is a serious disorder with a 5-year survival less than 50% and the most frequent cause of death is the occurrence of an overt T-cell lymphoma and recurrent infections. The presence of a clonal IEL population is significantly associated with a poor survival and a high risk of progression to overt lymphoma. The same clonal TCR-gene rearrangement initially identified in patients with clonal RCD may be subsequently observed in lymphomatous specimens suggesting a continuum between RCD and high-grade lymphoma.[7, 69] The risk of developing an overt T-cell lymphoma in patients with RCD II seems to be favoured by immunosuppressive drugs.[70]

It remains to be proved whether a close monitoring with vigilent clinical and laboratory observation, video capsule, double-balloon enteroscopy and/or PET-scan are capable of detecting earlier lesions in RCD before development of an overt lymphoma and consequently result in a better outcome.

Treating EATL with Autologous Stem Cell Transplantation

EATL has a very poor outcome with 1- and 5-year survival rates in the range of 31-39% and 11-20% respectively.[71, 72] In a prospective multicentre study of 35 patients with EATL treated with high dose chemotherapy, the cumulative 2-year survival was only 28%.[73]

It has been stated that adherence to a gluten free diet in coeliac disease for more than 5 years has been shown to reduce the incidence of EATL to that of the general population.[74]

EATL can present in two ways. There are patients with well-established coeliac disease who have responded to a gluten free diet but then deteriorate because of the development of RCD II and/or EATL. In the other group, the diagnoses of coeliac disease and EATL are made more or less simultaneously (*de novo* EATL).

In the largest case series investigating treatment and clinical outcomes in EATL, more than half of the patients could not complete treatment secondary to poor nutritional status.[75] Chemotherapy was complicated by small bowel perforation, gastrointestinal bleeding, and development of enterocolic fistulae.

The results from thus far reported case studies of EATL treated with ASCT after high dose chemotherapy are disappointing and conflicting.[75-79] These reports described very small groups of patients with a complete remission and disease-free survival ranging from (0 - 64 months post ASCT). Encouraging results came from a recent report [79] describing the treatment of six patients with ASCT after receiving two cycles of IVE (ifosphamide, etoposide, epirubicin), followed by two cycles of high-dose methotrexate (3 g/m^2) with folinic acid rescue and BEAM (carmustine, etoposide, cytarabine, melphalan). Four patients remain alive in complete remission at 1.83-4.32 years; two have relapsed.

Between 2001 and 2006, we have treated four adult patients (2Males:2Females) with EATL with high dose chemotherapy followed by ASCT.[80] The first patient has complete remission 30

months post transplantation, while the other 3 patients had died because of recurrence few months post transplantation. Combining immunotherapy (adalimumab) with chemotherapy in the preconditioning regimen was also not successful in the single patient who received that combination. It seems that our current chemotherapy and preconditioning regimens are not effective.

In conclusion, the current treatment of patients with intestinal T-cell lymphomas is unsatisfactory with only a few long-term survivors. Therefore, earlier diagnosis, the development of more effective treatments including antiCD52 agents, better preconditioning regimens and possibly the use of T-cells-depleted grafts or allogeneic stem cell transplantation with or without primary central nervous system prophylaxis are urgently required to improve the prospects of these patients.

General Conclusions

Before diagnosing patients as having RCD, other specific diseases like autoimmune enteropathy or tropical sprue and especially gluten contamination have to be excluded. In recent years molecular, phenotypic and clinical characterization of patients with RCD has found this entity to comprise a heterogeneous group of diseases. Based on immunohistological and TCR-gene rearrangement investigations, mainly two entities RCD I and RCD II have been differentiated.

In RCD I the intraepithelial lymphocytes show a normal phenotype and absence of aberrant T-cells. In contrast RCD II is characterized by the presence of high percentage of aberrant intraepithelial lymphocytes and in some cases development of overt EATL.

New therapeutic strategies for RCD II consider early chemotherapeutics, but also ASCT after high dose chemotherapy seems promising.

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Stem Cell Transplantation in Gastroenterology: Current applications and future perspectives

Review Article

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Abstract

Haematopoietic Stem cell transplantation (HSCT) can be used to cure or ameliorate a wide variety of non-malignant diseases. These range from inherent defects of haematopoietic cell production or function, through metabolic diseases (where blood cells are providing in vivo enzyme therapy to solid organs), to severe autoimmune diseases.
In the last decade, SCT has been explored in treating patients with severe autoimmune diseases refractory to conventional treatment.
The rationale for this strategy is based on the concept of immunoablation by intense immunosuppression using high dose chemotherapy, with subsequent regeneration of naïve T lymphocytes derived from reinfused haematopoietic progenitor cells. Possibly the use of SCT allows the administration of high dose chemotherapy resulting in a prompt remission in these therapy refractory patients.
The application of HSCT in gastroenterology is being explored in the last few years. We have tested its applicability in a selected group of refractory coeliac patients with aberrant T-cells. These patients are usually resistant to any known therapy and has a high risk of developing enteropathy associated T-cell lymphoma (EATL) (60-80% within 5 years). The short term results thus far are promising. HSCT has also been tested and proved successful in treating refractory Crohn's disease. In cryptogenic cirrhosis, basic research and unpublished data concerning mesenchymal SCT are encouraging.
In gastrointestinal oncology, there are reported cases and case series on the use of SCT in gastric, colorectal and cholangiocarcinomas, mucosa- associated lymphoid tissue lymphomas and EATL.
In refractory autoimmune gastrointestinal diseases, it seems that high-dose chemotherapy followed by HSCT is feasible and safe, and might result in long-term improvement of disease activity.
This review highlights the major scientific developments and defines the areas of successful use of HSCT in gastrointestinal disorders and gives a perspective of possible future developments and applications.

Introduction

For more than 40 years, radiation or chemotherapy or both have been given in myeloablative doses to cancer patients while their autologous haematopoietic cells were stored for infusion to restore bone marrow function. The initial preclinical studies were performed in canine and murine models.[1-5] It is important to use agents (radiation, drugs) that are associated with a steep dose-response curve and a relatively short half-life. In addition, excellent supportive care measures are required to bridge the time between high-dose chemotherapy (HDC) followed by haematopoietic stem cell transplantation (HSCT) and recovery of bone marrow function.

During the past 4 decades, important observations were made in animal models; several anticancer drugs suitable for use at high doses have been developed; a vast amount of new knowledge concerning the safer administration of total body irradiation (TBI) has been generated; extensive information concerning the characteristics, collection, and manipulation of haematopoietic stem cells (HSC's) from marrow and peripheral blood has been gained; potent antibiotics to prevent or treat serious infections during the time of marrow aplasia have become available; transfusion support has become available; and haematopoietic growth factors to "mobilize" HSC's into the circulation and to shorten the time to recovery of marrow function have been developed. With these important support modalities, HDC followed by HSCT is increasing rapidly with more than 200,000 patients treated worldwide.[6]

Autologous cells were initially obtained by multiple bone marrow needle aspirations while the patient was under general or regional anaesthesia.[7-9] The observations that peripheral blood from mammals and humans contain viable HSC's [10-14] subsequently made the clinical use of circulating HSC's for haematological reconstitution a reality.[15, 16] The potential of peripheral blood HSC's was further enhanced by the fact that these cells could be "mobilized" from the marrow into the bloodstream, where they can easily be collected by aphaeresis. High doses of cyclophosphamide (CY), growth factors (granulocyte colony stimulating factor [G-CSF], granulocyte-macrophage colony stimulating factor [GM-CSF], stem cell factor, or combinations of these agents greatly increase the number of HSC's in the peripheral blood.[17,18]

A prospective randomized trial demonstrated that "mobilized" blood HSC's are superior to unstimulated marrow cells with respect to speed of engraftment, requirements for platelet transfusions, and duration of hospitalization.[19]

In the last decade, SCT is becoming an increasingly accepted treatment option for patients with severe autoimmune diseases refractory to conventional treatment.

The application of this treatment option in gastroenterology is being explored in the last few years. Its applicability has been tested and proved successful in treating refractory Crohn's disease. We have tested the applicability of ASCT in a selected group of refractory coeliac patients with aberrant T-cells. The short term results thus far are promising.

In hepatology, the efficacy of mesenchymal SCT in patients with cryptogenic cirrhosis is being evaluated and seems promising.

In gastrointestinal oncology, there are case reports and small series showing some success.

This review highlights the major scientific developments and defines areas of successful use of SCT in gastrointestinal disorders and gives a perspective of possible future developments and applications.

Gastrointestinal inflammatory diseases

Background

The concept of intensive immunosuppression is based on findings in animal models of autoimmune diseases, as well as results obtained in patients who underwent SCT for haematologic or oncologic diseases while coincidentally having an autoimmune disease.

HSCT as a treatment option in autoimmune diseases was first evaluated in lupus models in rodents.[20, 21] It was subsequently shown that infusion of bone marrow derived from non-susceptible donors could prevent autoimmune diseases after immunoablation of the host predisposed to develop autoimmune diseases.[22, 23]

Following these observations, animals with induced forms of AD were treated with bone marrow transplantation (BMT) from a healthy donor and pseudo-autologous BMT (i.e., bone marrow from affected animals of the same strain).[24]

In the case of adjuvant arthritis in rats, heat-killed Mycobacterium tuberculosis is used for induction of disease. A myeloablative regimen followed by allogeneic and also, unexpectedly, by autologous and syngeneic SCT not only prevented the disease but also induced remission.[25] Spontaneous relapses after syngeneic BMT occurred more frequently in experimental allergic encephalomyelitis, a model for multiple sclerosis (30% after syngeneic vs. 5% after allogeneic transplantation).[26] Subsequent studies suggested that relapses in encephalomyelitis were due to residual host activated T lymphocytes and lymphocytes in the graft, respectively.[27, 28] This was supported by studies in adjuvant arthritis demonstrating superiority of more intense conditioning.[29]

In summary, valuable lessons have been learned from the animal studies that may be relevant in the application of SCT in autoimmune diseases: *(1)* myeloablative therapy, followed by BMT, has curative potential, *(2)* allogeneic SCT may be more effective than autologous SCT if a graft-vs.-autoimmunity effect exists and if intrinsic stem-cell defects play a role in the disease pathogenesis, and *(3)* in vivo T-cell depletion may be a prerequisite for a sustained response.

Clinical observations in patients with autoimmune diseases who were treated with BMT because of a concomitant severe haematological disorder have paralleled the results from experimental animal studies.[30-32]

These experimental studies and clinical observations paved the way for collaborative efforts to further explore the clinical potential of stem-cell grafting in human autoimmune diseases. The lack of alternative treatment options for severe, uncontrolled autoimmune diseases prompted development of this treatment strategy. Until recently, mortality and morbidity of HSCT were considered too high to justify such a procedure in patients with chronic autoimmune diseases where prevention of morbidity instead of mortality is the major goal. This is particularly the case with allogeneic SCT (allo-SCT), with its attending transplant-related morbidity and mortality rates of 15% -30%. Autologous SCT (ASCT), however, carries a transplant-related mortality rate of less than 5%.[33-35] Therefore, priority was given to ASCT.

In 1995 an international committee was established that developed guidelines on entry criteria and transplant protocols for severe autoimmune diseases.[36] Furthermore, a database was created to collect clinical data that would enable monitoring of feasibility, toxicity, and efficacy of the different treatment protocols. Analysis of the pooled data has yielded relevant information on trends with regard to mortality, type of protocols used, and diseases targeted.

Patients who undergo autologous HSCT proceed along a 4-step sequence: autologous cells are secured, processed, and cryopreserved (*step I*); myeloablative therapy is administered (*step II*); the graft is infused (*step III*); the patient receives supportive care until recovery (*step IV*).

Reports in inflammatory diseases

Crohn's disease

Crohn's disease is a relapsing- remitting disorder causing life long impairment of health and quality of life. It has a strong genetic component as evidenced by a high proportion of patients with a family history and the specific contribution of mutations of the NOD2-gene. The mechanisms by which interactions between genetic and environmental factors lead to Crohn's disease are unclear.[37] Crohn's disease involves a loss of immune tolerance, characterized by an overactive TH-1 immune response.[38, 39] Given that HSCT has been of some value in diseases characterized by loss of immune tolerance and/or a TH-1 predominant immune response, it is possible that it could be of value in Crohn's disease.

On such an analysis, allo-SCT could be of benefit by replacing the genetic predisposition to Crohn's disease in circulating leucocytes, while ASCT might be of benefit because clearing the body of committed lymphocyte clones might restore the patient to the *status quo ante* of being predisposed to but not suffering from Crohn's disease.

An alternative explanation is that ASCT simply allows more intense immunosuppression to be given. Immunosuppressive agents are of considerable value in Crohn's disease although relapse occurs on their cessation. It is possible that more intense immunosuppression in some way achieves a more fundamental switch in immune activity. On this basis, the transplant plays a secondary role in enabling intense immune suppression to be given relatively safely.

Recently, interesting insights into possible mechanisms by which HSCT could affect the gut have emerged. In both animal and patient studies, sex mismatched transplants have been given, enabling donor cells to be identified by staining for the Y chromosome. These have shown in both mice and women that a population of myofibroblast derived from the donor populates the intestinal mucosa, particularly in the sub-epithelial segment.[40] Given the importance of myofibroblasts in orchestrating the function of epithelial cells, these data suggest a mechanism other than one targeted at immunosuppression that could beneficially reset patient functions, for example enhancing barrier function following SCT.

Evidence for the effectiveness of SCT in Crohn's disease

The possibility that SCT might be an effective treatment for Crohn's disease arose initially from reports of improvements in Crohn's patients who had SCT or BMT for other reasons [41] followed by case reports and case series of transplantation specifically performed for Crohn's patients. Tables 1 and 2 summarise the literatures on direct and indirect evidence of efficacy.

Indirect evidence

Allogenic Transplantation

In 1993 there was a report of a patient with Crohn's disease receiving an allo-BMT for lymphoma.[42] Following the transplant Crohn's disease improved although evaluation was not fully systematic and the follow up restricted to 6 months. Five years later a report of 6 patients undergoing allo-SCT, who incidentally had Crohn's disease, substantially increased interest

in the possibility that SCT could be of value in this disease.[43] Of 5 patients whose disease was active before transplantation, 3 achieved long lasting remission, despite discontinuation of immunosuppression. Other patients did less well. One patient developed recurrence18 months after transplantation and required surgery.
From these data it is clear that allo-SCT could benefit Crohn's disease but that benefit is not universal.
A report by Ditschkowski et al concerning 11 patients (7 Crohn's disease, 4 ulcerative colitis) has been published.[44] These patients had inflammatory bowel disease for a median of 10 (range 0.5 - 22) years with a post-transplant follow-up of 34 (range 3 -117) months. Ten of the 11 patients became, and remained, inactive following transplantation.
Because these studies were not designed to investigate Crohn's disease itself, follow-up was less systematic than if they had been. However, they support the concept that the patients' immune system plays a central role in Crohn's disease and that replacing it with a transplanted one may be beneficial. This concept is further supported by a case report in which a healthy patient undergoing allo-SCT developed aggressive Crohn's disease soon afterwards.[45] Investigations showed that the transplanted stem cell had a pathogenetic mutation of the NOD2-gene, compatible with the notion that the Crohn's disease developed when a healthy immune system was replaced by one, which, from the point of view of Crohn's disease, was an unhealthy one.

Autologous Transplantation
Several studies have reported the clinical course of patients with Crohn's disease receiving ASCT for another condition.[46-49] Perhaps the most impressive concerned a patient who was diagnosed with Crohn's disease at age of 13 year and who required substantial treatment over the next 7 years.[46] Following an ASCT for non-Hodgkin's lymphoma it was reported that there was no evidence of recurrence of his Crohn's disease in the next 7 years.

SCT done specifically for Crohn's disease
Burt et al published in 2003 the first report of SCT for Crohn's disease concerned 2 patients.[50, 51] In both patients Crohn's disease activity index (CDAI) was more than 250 (normal range <150, pathological range 220 - 600) despite treatment with infliximab. Peripheral blood stem cells (PBSC) were mobilized and enriched ex-vivo by CD 34^+ selection. Cyclophosphamide and anti-thymocyte globulin (ATG) were used for immune conditioning. The first patient was a 22 year old woman with a history of a right hemicolectomy and had severe ileocolic Crohn's disease causing intractable diarrhoea, fistula and peri-anal sepsis. The second was a 16 year old boy who had been ill for 6 years and receiving immunosuppressives with Crohn's colitis. In both patients, following transplantation, diarrhoea resolved, CDI normalized, CRP was within normal limits and the albumin remained or became normal.
These cases were accompanied by extensive colonoscopic evidence. Prior to transplantation both patients had areas of severely active Crohn's disease. Following transplantation the bowel remained abnormal though changes were much more trivial.
A second case report [52] illustrates many of the issues surrounding Crohn's disease and SCT in that on the one hand the clinical course is relapsing-remitting and on the other that complete

histological remission was maintained only with low dose prednisone and methotrexate. Overall, the prospect of transplantation for Crohn's disease ranges from no difference compared to conventional management through substantial improvement to possible cure.
Rectovaginal fistulas in Crohn's disease are difficult to resolve, and surgical failure is very frequent. Recent studies have shown that mesenchymal adult stem cells extracted from certain tissues, such as adipose tissue, can develop into different tissues, such as muscle. Garcia-Olmo et al [53] reported a young Crohn's patient who had a recurrent rectovaginal fistula that was treated by ASCT with a lipoaspirate as the source of stem cells. Three months after transplantation, the patient has not experienced vaginal flatus or faecal incontinence through her vagina. Subsequently, they reported a prospective Phase I clinical trial to test the feasibility and safety of ASCT in the treatment of Crohn's fistulas.[54] They inoculated nine fistulas in four patients with autologous adipose tissue-derived stem cells. In six fistulas, the external openings were covered with epithelium at the end of week 8, and were considered healed (75 percent) at the end of the follow-up period (average 22 months; range 12- 30 months). In the other two fistulas, there was only incomplete closure of the external opening, with a decrease in output flow (not healed; 25 percent). This was the first report of a clinical trial of cell therapy using autologous stem cells obtained from a lipoaspirate. The results indicate that the protocol is feasible, safe and encouraging to perform further studies in Phase II.

Table 1. Indirect evidence for efficacy of SCT in Crohn's disease

Author/ year	No.	Type of SCT	Original indication	Conditio-ning	Results	Remarks
Drakos/ 1993	1	Allo- SCT	NHL	NA	Remission	Only 6 months follow up
Castro/ 1996	1	ASCT	NHL	NA	Asymptomatic, recurrence after 3yrs	Before ASCT diffuse pancolitis
Kashyap/ 1998	1	ASCT	NHL	Cy, VP16 + TBI	No recurrence 7 yrs	Crohn's at age of 13 yr, ASCT at 20 yrs
Lopez-Cubero/ 1998	5	Allo- SCT	Leukaemia	NA	3 pts had lasting remission	--
Soderholm/ 2002	1	ASCT	AML	Cy +TBI	Remission for 5 yrs	Persisting subclinical inflammation
Ditschkowski/ 2003	7	BMT, ASCT (±CD34 deplete)	AML,CML, MDS	Variable	6/7 Crohn's became/ remained inactive	+ 4 UC* all in remission. Follow up 34 m (3-117m)

UC= ulcerative colitis, NA=not available, NHL=non-Hodgkin lymphoma, CY=cyclophosphamide, TBI=total body irradiation, AML=acute myeloid leukaemia, CML= chronic myeloid leukaemia, MDS=Myelodysplastic syndrome

Oyama et al [55] conducted a phase I HSCT study in 12 patients with refractory Crohn's disease. Candidates were younger than 60 years with a CDAI of 250-400 despite therapies including infliximab. The initial median CDAI was 291 (range= 250-358). Symptoms and CDAI improved before hospital discharge, whereas radiographic and colonoscopy findings improved gradually over
months to years following HSCT. Eleven patients entered a sustained remission. After a median follow-up of 18.5 months (range, 7-37 months), only one patient has developed a recurrence 15 months after HSCT.
From all these reports we can conclude that ASCT may be performed safely and has a marked salutary effect on Crohn's activity. Randomized multicentre studies will be needed to confirm the efficacy of this therapy.

Table 2. Direct evidence for efficacy of SCT in Crohn's disease

Author/ year	No.	Type of SCT	Indication	Conditio-ning	Results	Remarks
Burt/2003	2	ASCT	– Fistulas – Colitis CDAI >250	Cy +ATG	CDAI, CRP, Albumin normalized. Remission for more than 1 yr	CD34 enriched stem cells. The first ever report on SCT specifically for Crohn's
Kreisel/2003	1	ASCT	Ileocolic Crohn's	Cy*	9 m complete remission without treatment	T-cell depleted SCT
Garcia-Olmo/ 2003	1	ASCT/ lipo-aspirate	Recurrent rectovaginal fistula	None necessary	3 m asymptomatic	Adipose tissue obtained by liposuction under local anesthesia.
Scime/2004	1	ASCT	Severe colitis	Cy+ ATG**	improved	Disappearance of extraintestinal manifestations
Garcia-Olmo/ 2005	5	Mesench-ymal SCT	Fistulas	None necessary	75% healing	Follow up 22m (12-30 m). Different types of fistulas.
Oyama/2005	12	ASCT	Refractory Crohn's	Cy +ATG	11 sustained remission	-

*Cy=cyclophosphamide. **ATG=Anti-thymocyte globulin

Ulcerative colitis

Two patients with a long history of psoriasis and ulcerative colitis underwent an ASCT for leukaemia. The colitis, psoriasis, and leukaemia remained in remission for four years following transplantation.[56] Another 4 patients remained in remission after receiving ASCT for leukaemia.[44]

Refractory Coeliac Disease

In coliac disease (CD), HLA-DQ molecules bind and present gluten peptides to antigen-specific T-cells. These HLA-DQ-peptide complexes induce inflammatory responses consisting of lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte population, hyperplasia of the crypts and atrophy of the villi.[57] In a small percentage (2-5%) of coeliacs diagnosed as adults a refractory state develops despite strict adherence to a gluten-free diet (GFD).[58] In RCD the number of IEL is markedly raised and it is from these IEL's that enteropathy associated T-cell lymphoma (EATL) may arise.[58, 59] Immunophenotyping of the intraepithelial lymphocytes [60- 62] identifies two groups of RCD patients: those with normal intraepithelial lymphocytes (RCD I) and those with aberrant intraepithelial lymphocytes, lacking surface expression of CD3 and often of CD8 (RCD II).[59] The latter group (RCD II) is usually resistant to any known therapy [63-68] and has a high risk of developing EATL (60-80% within 5 years).[69]

We have assessed the feasibility, safety and efficacy of HDC followed by ASCT in patients with RCD type II.[70]

Between March 2004 and August 2005 four patients [(3 males, 1 female, mean age 64,2 years (range 61-69 years)] with RCD-II underwent ASCT. EATL has been excluded by endoscopic examination, computed tomography, body positron emission tomography and bone marrow biopsy.

HSC were harvested from the peripheral blood after mobilization using G-CSF. The conditioning regimen consisted of T-cell depletion with fludarabine and myeloablation with melphalan.

Within 3 months of ASCT all patients had normalization of stools frequency, disappearance of abdominal pain and improvement in biochemical markers. Also improvement of body mass index and serum albumin level was documented. Endoscopically there was disappearance of erosions and ulcerations in the jejunum in all patients, and histology of the small intestine showed significant regeneration as documented by down-staging of the Marsh class.

Aberrant [$CD7^{+}CD3^{-}$] T-cell percentage of $CD103^{+}$ lymphocytes decreased from a mean of 61,8% (range 36-95%) at baseline to 33,5% (range 7-47%) three to four months post-transplantation.

Two years after transplantation, our first patient is showing further improvement in his immunopathology status as demonstrated in further decline in the percentage of aberrant T-cells and histologically improved from Marsh III-A to Marsh-I.

Although the short term results in these patients are very promising, follow up at present is too short to permit firm conclusions as to efficacy. The selection of patients for this treatment should be restricted to those patients with a substantial population of aberrant T-cells.

HDC followed by ASCT seems feasible and safe, and might result in long-term improvement of disease activity in RCD II whose condition previously did not respond to treatments, and possibly prevents or delays the development of EATL. Longer-term follow up and additional pilot studies with larger groups of patients are needed to confirm the efficacy of this therapy.

Reports in hepatology

Cirrhosis represents a late stage of progressive fibrosis characterized by distortion of the hepatic architecture and formation of regenerative nodules. Liver transplantation is considered the standard treatment in advanced decompensated cirrhosis. However, it has several limitations such as long waiting list, high cost and several complications.[71-73]

Studies have shown that circulating stem cells can differentiate into mature hepatocytes or cholangiocytes in the human body.[74] Bone marrow is a reservoir of various stem cells, including HSC, mesenchymal stem cells (MSC), and multipotent adult progenitor cells. While MSC's have been shown to be capable of mesodermal and neuroectodermal differentiation, but they have endodermal differentiation potential; and their differentiation into functional hepatocyte-like cells has been demonstrated very well in animal experiments.[74] Recent animal studies have shown that infusion of MSC's can protect against experimental rat liver fibrosis.[76] Also, infusion of non-haematopoietic bone marrow stem cells can lead to regression of liver fibrosis in mice.[77] Thus, bone marrow SCT is a potential strategy in liver cirrhosis. Four patients thus far received MSC through a peripheral vein, and another 4 received CD 34^+ through the hepatic artery. (Dr. M Mohammadnejad, DDRC, TUMS, Tehran, Iran, *personal communication*). All patients had Child B/C cirrhosis with some elevation of liver enzymes. Patients who received MSC's had better response than CD 34^+ve stem cell recipients with significant improvement of albumin level, prothrombin time, and Model End Stage Liver Disease (MELD) score in 3 of 4; although the follow up has not been completed.[78]

Reports in Gastrointestinal malignancies

The literature is rich in reports dealing with HSCT after HDC in some of gastrointestinal cancers. Reports have been summarized in table 3

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type

Due to their homing properties, extranodal marginal zone B-cell lymphoma (MZBL) of mucosa-associated lymphoid tissue (MALT) type remain localized for long periods of time, and therefore have an excellent prognosis. However, if generalization and/or transformation into a diffuse large-cell lymphoma occur, the prognosis deteriorates and no established treatment concepts are yet available. One report [79] described a patient with relapsed transformed stage-IV extranodal MZBL of MALT type of the entire gastrointestinal tract who was successfully treated using salvage chemotherapy followed by ASCT. Follow-up revealed a sustained complete remission for 22 months.

Another case report by Rosler et al described complete remission of lymphoma after HSCT in a patient with Sjogren syndrome.[80]

Table 3. Summarizes reports in gastrointestinal oncology

Author/ year	No.	Type of SCT	Indication	Conditio-ning	Results	Remarks
Rosler/ 1998	1	ASCT	MALT type lymphoma + Sjogren syndrome	BEAM	CR* of lymphoma. Died 20 m post-ASCT from PCP**	Steroid dependent Sjogren
Neumeister/ 2000	1	ASCT	Stage-IV MZBL of MALT type	BEAM	CR 22 m	-
Suzuki/ 1990	3	ASCT	Advanced gastric carcinoma	EAP	Stenosis, lymph node metastasis, pancreas invasion were reduced	-
Ueda/ 2004	1	ASCT	Gastric carcinoma	EAP	HDC+HSCT and dendritic cells (DC)-based immuno-therapy is feasible	One pt underwent ASCT followed by cancer vaccine therapy with DC
Hentschke/ 2003	6	Allo-SCT	Colorectal carcinoma	Fludarabine +TBI***	Poor	Low intensity conditioning
Jantunen/ 2003	5	ASCT	EATL	BEAM	Median survival 2 m	Surgery + CHOP
Author's experience	5	ASCT	EATL	BEAM/ or Fludarabine +Melphalan	Poor results. Only one long term survival (>30m)	Unpublished data

*CR= Complete remission ** PCP= *Pneumocystis carinii* pneumonia ***TBI=Total body irradiation

Gastric carcinoma

The EAP combination of etoposide, doxorubicin and cisplatin has been reported to be highly active for advanced gastric cancer. However, it is associated with severe myelotoxicity. Few reports examined whether PBSC's could be mobilized during haematopoietic recovery after EAP, and assessed the possibility of using multimodal cell therapy with PBSC's for the treatment of advanced gastric cancer.

The first report [81] dates back to 1990 and describes three patients with advanced gastric cancer were treated with EAP and then ASCT. All patients were recovered from aplastic period without any severe complications. The second report [82] described five men with advanced gastric adenocarcinoma. EAP was given to each patient, and myelotoxicity was carefully monitored. G-CSF was administered after the neutrophil nadir, and PBSC's were collected by leucopheresis during haematopoietic recovery. Sufficient numbers of PBSC's [CD34 $^{(+)}$ cells] could be mobilized in 4/5 patients. A 45-year-old patient with extended lymph node metastasis received high-dose EAP with HSCT, followed by cancer vaccine therapy with dendritic cells

(DC's), induced from cryopreserved PBSC's. Although associated with severe myelotoxicity, EAP can mobilize sufficient numbers of PBSC's during haematopoietic recovery. Multimodal cell therapy combining HDC with HSCT and DC-based immunotherapy is feasible and can be a reasonable approach in advanced gastric cancer.

Berdel et al [83] reported a phase-II-pilot trial on preoperative HDC with stem cell rescue in patients with locally advanced cancers of the upper gastrointestinal tract. This pilot trial in16 patients shows that the approach is feasible with tolerable toxicity and can lead to prolonged disease-free survival in responding patients.

Colorectal carcinoma (CRC)

The immune system is known to induce tumour regression [84] following HSCT, graft-versus-host disease (GVHD) has been found to contribute to an antileukaemic effect.[85, 86] An alloresponse of donor T lymphocytes is most probably the cause of GVHD and the graft-versus-leukaemia (GVL) effect.[87] An allogenic graft-versus-tumour (GVT) effect has also been reported in breast and renal metastatic carcinoma.[88, 89]

This approach of using lethal myeloablative conditioning has been challenged by using lower doses and less toxic conditioning to induce immunosuppression and take advantage of the GVT effect later rather than the antitumour effect of chemoradiotherapy.[90-92]

Hentschke et al [93] used a low-intensity conditioning protocol in treating six patients with CRC. They noted response in two patients. Since a high tumour load may increase the risk of progression, tumour debulking before transplantation was done in all but two of the patients.

Interestingly, they saw regression of metastases mainly in the lungs and lymph nodes, but not in the liver and bone. The tumour response in the lymph nodes may be expected if haematopoietic recipient cells are replaced by haematopoietic donor cells, which may also induce antitumour activity. The discrepancy between the higher responses seen in the lungs than in the liver is unclear.

To improve the results and perhaps even have complete responses, patients should probably be selected at an earlier stage with a lower tumour burden.

Enteropathy associated T-cell Lymphoma (EATL)

EATL is a rare lymphoma type frequently associated with CD and originating from intraepithelial lymphocytes of small bowel mucosa. It has a very poor outcome with 1- and 5-year survival rates in the range of 31-39% and 11-20% respectively.[94, 95]

Techniques of immunocytochemistry, together with gene rearrangement studies, now make the histological diagnosis of EATL more straightforward.[96-98] Once diagnosed, combination chemotherapy should be considered for all patients. A variety of regimens, including CHOP [99], have been used with limited success. Prognosis, in part, reflects late diagnosis and poor performance status at the time of presentation. Associated malabsorption and malnutrition make tolerance of chemotherapy difficult, and treatment-related complications such as gastrointestinal bleeding and perforation also worsen prognosis.[100, 101] Nutritional support should always be considered in these patients. Efforts should therefore be made to diagnose

EATL early. The diagnosis should be considered in all patients who present in midlife with CD and in those who experience a clinical deterioration after a period of stability on a GFD.[102] In a proportion of patients there is no preceding celiac history (*de novo EATL*), making diagnosis more difficult.

Jantunen et al [103] reported a series of 5 patients with EATL and histologically proven CD. Four patients had been treated with partial small bowel resection. Subsequently all five received HDC followed by ASCT. Two patients died early from transplant-related complications. The 3 patients who survived the immediate post-transplant period relapsed and progressed 0-14 months after HSCT. The median survival is only 2 months.

Another report [104] from the United Kingdom in an abstract form revealed no early mortality in 8 patients but no long term results reported yet.

We have transplanted a total of 5 patients, four of them after performing debulking of lymphoma with partial small bowel resection. The patients received HDC followed by ASCT. The short term results are not encouraging. (*Unpublished data*)

Problems with transplantation

The age limit of transplantation candidates has been raised over time, especially with improved supportive care. It is now clear that cancer patients in their eighth decade of life can be considered for ASCT provided they have no other serious comorbid conditions.

If one uses a cytotoxic drug at maximum tolerated dose, often in combination with other agents or TBI, regimen - related toxicity becomes obvious, especially in the form of oropharyngeal mucositis, gastroenteritis, diffuse alveolar haemorrhage, veno-occlusive disease, or other organ function impairment. Such toxicity can sometimes lead to fatal multiorgan failure syndromes. Fortunately, through judicious use of regimens, such serious problems have been reduced to less than 5%, even into the 1% to 2% range.

Graft failure leads only rarely to early or late post-transplantation pancytopaenia. If an adequate quantity of $CD34^+$ cells has been cryopreserved and infused, this problem occurs most frequently in patients who develop severe viral infections with cytomegalovirus or human herpes virus type 6. The incidence of opportunistic infections after ASCT is much lower than in allo-SCT recipients. Occasionally, pre-existing infections with *Aspergillus* species are reactivated.

It is the goal to integrate patients as soon as possible after ASCT into their personal and professional environment. Quality-of-life studies show that patients are able to return to their daily lives within 6 to 12 months and achieve a high quality of life.[105]

Because inflammatory disease does not fulfil the criteria of clearly increased mortality to justify HSCT, it seems prudent that the patient must be fully informed and ongoing results should be analyzed by a safely board. Allo-SCT has a transplant-related mortality rate of 15% -30%. Autologous SCT (ASCT), however, carries a transplant-related mortality rate of less than 5% [33-35] and therefore gains more preference over allo-SCT.

Future perspectives

Prospective multicenter studies are needed to confirm the results of case reports and small case series. Criteria for selection of patients, in addition to timing of transplantation particularly in malignant and premalignant conditions are strongly needed. Furthermore, new technologies of tracking the stem cells, determining the number of cells to be transplanted and the need for T-cell depletion or CD34 lymphocytes enrichment need to be further elaborated. Still more work need to be done mainly on intensification of immunosppression regimens, graft manipulation and optimizing conditioning regimens.

We think that HSCT in gastroenterology gains steadily more grounds in the treatment of inflammatory disease, lymphomas and possibly also malignancies. Possible indications that deserve more attention are mainly autoimmune liver diseases, e.g., autoimmune hepatitis, primary biliary cirrhosis and primary sclerosing cholangitis.

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Milestone in Gastrointestinal Endoscopy
Double-balloon Enteroscopy of the small bowel

Review Article

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Abstract

The small bowel has been largely bypassed by flexible endoscopy because of inaccessibility. Push enteroscopy is now in the past, with recent innovations now making visualization of the small bowel possible. Wireless capsule endoscopy (VCE) and double-balloon endoscopy (DBE) have been introduced. In this review, we focus on the diagnostic and therapeutic modalities of DBE, which may be a suitable replacement for push enteroscopy, preoperative endoscopy and to some extent of small bowel follow through and CT scan. DBE is a new method of endoscopy developed and described by Yamamoto et al. in Jichi, Japan, in cooperation with Fujinon®. Introduced to the market in 2003, it is possible with this endoscope to observe the entire small bowel in steps of 20-40 cm. Measuring the depth of insertion is also possible. Obscure gastrointestinal bleeding can be explained and treated in the majority of cases. Biopsy sampling, hemostasis, polypectomy, dilatation and tattoo are possible in the small bowel.
Guidelines for familial adenomatosis polyposis (FAP) and Peutz-Jeghers syndrome will probably be reviewed in the next few years. The safety and efficacy of DBE have been demonstrated. DBE improves small bowel disease management and can substitute for more complex investigations. Additional data will come to light in years to come. Combining DBE with VCE, CT/MRI enteroclysis in a new era for SB work-up and treatment is the likely future.

Introduction

Endoscopy has a pivotal role to play in the diagnosis and treatment of gastrointestinal diseases, including those of the oesophagus, stomach, duodenum and colon. However, the small bowel has been largely bypassed because of the relative inaccessibility of the flexible endoscope. The small bowel accounts for 75% of the total length of the gastrointestinal tract, measuring about 580 cm, and has some of the most important functions.[1] Until recently, the only technique available for observing the entire SB was intra-operative endoscopy. Sonde-type enteroscopy, introduced in the early 1970s for total enteroscopy, has been abandoned.[2,3] Difficulty in handling and a long preparation time have hampered its universal use. Since then, the push method, with use of a long endoscope, has been regarded as the standard procedure and no major improvement has been made in regard to enteroscopy until recently. Although push enteroscopy can usually reach as far as the upper jejunum, about 50 cm from the ligament of Treitz, most of the small bowel is beyond reach.[4] Features about position and anatomy of the small bowel limit how far the endoscope can be introduced. Specifically, the tight curve of the bulb and its relatively fixed retroperitoneal posterior position as it proceeds to the ligament of Treitz, where it passes downwards as a loosely supported looped structure on a mesentery, challenges the endoscopist. Recent innovations in endoscopy have made it possible to visualize the entire small bowel. Two new methods have been introduced: wireless capsule endoscopy (VCE) and double-balloon endoscopy (DBE). In this article,

we review recent reports, focusing on DBE, because of the possibility to take biopsies and intervene therapeutically.

Double-balloon Enteroscopy

Technique

First described by Yamamoto and colleagues in 2001 [5], DBE is a new endoscopic technique with the potential to allow complete visualization of the entire small bowel. The first commercially available enteroscope (Fujinon EN-450P5/20; Corporation, Saitama, Japan) is 200 cm in length, with an 8.5 mm diameter and a 2.2 mm working channel, and is equipped with a 145 cm soft overtube with a diameter of 12.2 mm (Fujinon®). For the oral route, an extremely flexible enteroscope is necessary, i.e. the EN-450P. For the anal route, by contrast, a slightly greater degree of rigidity is desirable in order to pass the colon and reach a stable position in the ileum. In addition, improved suction is mandatory because of faecal debris. This is met by the EN-450T with its external diameter of 9.5 mm and 2.8 mm working channel. Latex balloons are attached at the tip of both the enteroscope and the overtube, and can be inflated and deflated with air using a specifically designed pressure-controlled pump system. By inflating the overtube balloon and thereby obtaining a stable position in the intestine, which can occur at a balloon pressure of 45 mmHg or 6 kPa, the endoscope can be inserted further in the SB without creating redundant loops. After inflating the endoscope balloon and deflating the overtube balloon, this overtube can be advanced to the distal end of the endoscope (push). Subsequent inflation of the overtube balloon straightens the system (pull), thereby pleating the intestine onto the overtube. This method effectively uses the working length of the endoscope and allows for insertion deep into the small bowel in steps of 0-40 cm, with a mean of 20 cm, either by the anterograde (oral) approach or by the retrograde (anal) approach. The preferred approach depends upon the suspected location of the responsible lesions (usually achieved by prior VCE or other small bowel imaging techniques). This technique, also known as "push-and-pull" enteroscopy, allows the endoscopist to visualize the small bowel, to obtain tissue biopsy specimens and to perform therapeutic interventions. Experience with DBE in Japan [6] and Europe [7, 8] illustrates how total small bowel enteroscopy can be performed using a combination of oral and/or anal approaches, and with a good safety profile and patient tolerance for the procedure.

Feasibility

The DBE can be inserted via either the oral or anal route, reaching about two-thirds or one-third, respectively, of the entire small bowel. Intubation of the caecum via the oral approach is in general not possible, but was achieved by Yamamoto [6] in 2/123 (1.6%) patients and by May [7] in 2/137 (1.5%) patients. However, observation of the total small bowel is frequently possible by a combination of both routes.Yamamoto reported that the entire small bowel could be observed in 24/28 (86%) of the cases in which total enteroscopy was attempted, documented by India ink injection at the most distal point during the oral or anal approach

and advancement of the endoscope to the tattoo during the opposite approach.[6] This result is comparable to the 79% success rate obtained by VCE.[9] May et al. reported a lower, but probably more realistic success rate of 45% (25/55) total enteroscopies in the subgroup where total enteroscopy was aimed for.[7]

Failure of total enteroscopy can be due to technical difficulties with the DBE method caused by adhesions following abdominal surgery, i.e. as reported by Yamamoto in 4/28 procedures.[6] Furthermore, the floppy nature of the endoscope makes the retrograde approach more difficult, and passing the caecal valve can be cumbersome. Ell reported a failure of 5/35 (14%) and May a failure of 7/87 (8%) of introducing the endoscope in the ileum.[7,8] The new EN-450T enteroscope might be advantageous in the case of the anal access route, but comparative studies are lacking. Termination of the procedure because of patient discomfort due to a lack of sedation or because of the insufflated air can also be a reason for failure. For this reason, it can be advantageous to commence with the oral approach unless prior imaging techniques predict a more distal small bowel lesion.

Although there is a lack of data regarding patient tolerability, the published studies attribute unsuccessful procedures merely to technical problems, thus suggesting acceptable or good tolerability.

Table 1. Dosage of medication (mean ± SD) used for conscious sedation in DBE studies.

Propofol	Midazolam	Diazepam	Meperidine	Fentanyl	Reference
630 ± 310 mg range 100-1420	9 ± 4 mg range 2-5	10 ± 4 mg range 2.5-20	60 ± 20 mg range 25-100	350 ± 190 µg range 100-600	Ell [8]
575 ± 250 mg range 200-1200	9 ± 3 mg range 3-15	9 ± 3 mg range 2.5-20	57 ± 20 mg range 25-150	Not used	May [7]
Not used	10.3 ± 4 mg	Not used	Not used	60 ± 0,03 µg	Heine [10]

Only May reported a prematurely stopped oral procedure caused by intolerance 1/153 (0.6%).[7] The sedation and analgesia used in the different studies were heterogeneous: propofol, meperidine, midazolam, diazepam and fentanyl. One major complication in the May study was attributed to sedation; aspiration pneumonia after an epileptic attack induced by propofol sedation.

Complications and safety

Yamamoto et al. reported two major complications out of 178 examinations.[6] However, surgical treatment was required in only one case: perforation after an endoscopy for post-chemotherapeutic evaluation of a malignant lymphoma of the small bowel. The other complication occurred in a patient diagnosed with Crohn's disease of the small bowel by DBE. The subject had abdominal pain and fever after the endoscopy, requiring fasting and administration of antibiotics, but the symptoms subsided in a few days and laparotomy was not necessary.

The European Multicentre Study, with a total of 100 patients and 147 procedures, showed no major complications such as perforation, bleeding of relevant injury to the small bowel tissue or mesentery. Minor complications, occurring in 12% of the patients, included abdominal pain, throat-ache with a need for medical treatment, fever without an infection focus and therefore not treated with antibiotics, and vomiting after the procedure. Reddening of the mucosal tissue with or without intramucosal haemorrhage was noted in 26%.[8]
May et al. reported comparable results with no major complications and minor procedure-related symptoms such as mucosal reddening (10%) abdominal pain (9%) of the patients and spontaneously vanishing fever.[7] Both studies reported a propofol-related epileptic attack resulting in aspiration pneumonia, probably mentioning the same patient from Wiesbaden.[7, 8]
Heine et al. documented two mild and one moderate severe pancreatitis as complications in addition to the already mentioned mild procedure related symptoms.[10]
In conclusion, since no other cases of complications have been reported, DBE seems to be a safe procedure.

Indications and diagnostic and/or therapeutic yield

Obscure gastrointestinal blood loss

The major indication for enteroscopy is OGIB (obscure and/or overt gastrointestinal blood loss). The Yamamoto et al. series contained 66/123 (54%) patients with OGIB.[6]

Table 2. Medication, combinations or single use, used for sedation in DBE studies

Sedation and analgesia used	Ell et al.[8] (145 procedures)	May et al.[7] (248 procedures)	Yamamoto[6]	Heine et al.[10] (316 procedures)
Propofol + fentanyl	N=5		N	
Propofol + meperidine	N=5		O	
Propofol + midazolam	N=9		D	
Propofol		N=no numbers	A	
Midazolam and/or diazepam + merepidine	N=82	N=no numbers	T A	
Midazolam + fentanyl				N=316
Non-combination (drug not mentioned)	N=46			

The source of bleeding was found in 76% of these patients. Ulcerations and/or erosions were found in 22 (40%) patients (NSAIDs 7/22, blind loop 3/22, Crohn's disease 2/22, Meckel's diverticulum 2/22, Behcet disease 2/22 and other ulcers 6/22), polyps or tumours in 10 (20%) and arteriovenous malformations in 7 (14%). In the prospective study by May et al [7], the majority of the patients had obscure gastrointestinal bleeding (90/137; 66%). Pathologic lesions thought to be the origin of the bleeding were identified in 109/137 (79%) patients. The most common finding was angiodysplasia treated with argon plasma coagulation. A

new diagnosis was established in 47/137 (34%) of the patients, confirmation of a diagnosis established by other methods in an additional 30%, determination of the extent of a diagnosis in 12%, and correction or exclusion of a previous diagnosis in 10%. Twenty percent of the patients had no pathological findings. DBE resulted in a change of therapy in 104/137 (76%) patients. Therapeutic endoscopic interventions were administered in 41.5% of the patients, 17% received new or a change in medication and surgical therapy was indicated in 17.5%. The European Multicentre Study included 64/100 (64%) patients for OGIB.[8] The data from this article did not really describe the relationship between the indication and diagnostic yield. In general, most patients suffered from angiodysplasia 34/100 (34%), ulcerations and/or erosions 16/100 (16%), and polyps or tumours (13%). Endoscopic intervention was possible in 42/100 (42%) patients and consisted of argon plasma coagulation 37%, polypectomy 2%, dilatation 2%, and foreign body extraction in 1%. Changes with regard to medical treatment were made in 12 patients and surgical resection was indicated in 8 patients. The European retrospective study included 38/62 (61%) patients with OGIB and in 30/38 (79%) the enteroscopy was diagnostic.[11] Treatment was performed in 42% of the patients. The outcome of this study is also competitive with the outcome of VCE (30%-80%). DBE has changed our management of small bowel bleeding and has taken over the lead in treating bleeding sources endoscopically and/or by guiding surgeons to the marked lesions (submucosal tattoo).

Crohn's disease and coeliac disease

In the group of patients included in Yamamoto's series for OGIB, 2 patients from a total of 50 were diagnosed with Crohn's disease and 1/50 with Behcet disease. In the group of ''other'' indications there were 5/32 with suspected Crohn's disease. No data of a diagnostic affirmation are available in the report.[6] May included 6/137 patients with known Crohn's disease and subileus or severe abdominal pain.[7] The group in which a diagnosis was established or confirmed contained 18/109 patients with Crohn's disease and 1 patient with Behcet disease. Ell et al. reported a diagnostic yield of 72%, and in this group 11 patients were diagnosed with Crohn's disease. It was not stated with which presenting symptoms these patients were included.[8] In the European retrospective study, enteroscopy was diagnostic in all patients suspected of having refractory coeliac disease and in 2/3 patients with Crohn's disease.[11]

Hereditary polyposis syndromes

Small bowel problems with Peutz-Jeghers syndrome could only be handled by push enteroscopy and/or intraoperative endoscopy. Recurring intussusception of benign, hamartomatous polyps, sometimes with ischaemia of the small bowel and necrosis, results in surgical interventions. Even the surgeon estimating that there are no polyps remaining is no guarantee for the future. Intraoperative endoscopy was a breakthrough for this condition in the 1980s.[12] DBE appears superior to this approach in a recent case report.[13] Adequate data about the influence of DBE on further management and outcome in comparison with MRI enterography and/or CT enteroclysis are mandatory.

Miscellaneous

No data are available about patients referred for malabsorption, protein-losing enteropathy and staging of B- and T-cell lymphomas. Potential indications for DBE, such as difficult colonoscopy, will be investigated as well.

Interventions

Since a stable position in the whole small bowel can be obtained by the inflated balloon at the tip of the overtube, the endoscope can be moved back double-balloon enteroscopy of the small bowel 35 and forth past a point of interest for repeated observation. Moreover, the endoscope has a working channel enabling targeted biopsy and/or endoscopic treatment.[6] Diagnosis using a DBE has been reported for a GIST [14], an inflammatory fibroid polyp [15] and a lymphoma.[16] Endoscopic treatment includes obtaining hemostasis in bleeding, polypectomy, endoscopic mucosal resection, balloon dilatation and stent placement in the SB. When lesions are located in the distal portion of the SB, a DBE can shorten the intestine, leading to the lesions with the overtube and simplifying the endoscopic route. Endoscopic observation and biopsy sampling are useful in the diagnosis of small-intestinal tumours. In addition, the endoscope can be inserted even in a bypassed intestine, including the afferent loop in a Roux-en-Y anastomosis.[17] The access in the afferent loop enabled EMR of early carcinoma; ERCP, too, has been shown feasible. Selective contrast-enhanced X-ray examinations can be performed by occluding the intestine with a balloon to prevent reflux of the contrast medium. Three case reports have been published describing the retrieval of entrapped capsules from the small bowel.[18-20] The capsules were entrapped in Crohn's patients (n=3) and a patient with a surgically removed solitary EATL who had the capsule entrapped in a blind loop. The report shows that the foreign bodies can be removed by means of the double-balloon, thereby preventing laparotomy.

Polypectomy

Polypectomy of the entire small bowel has been reported for Peutz-Jeghers polyps of polyposis syndrome in all series without complications. Ohmiya et al. described two patients with Peutz-Jeghers syndrome in which 18 pedunculated polyps (10-60 mm in size) were resected endoscopically.[13] Resection was not complicated by subsequent bleeding or perforation. These results show that polypectomy by DBE is a relatively safe procedure and might prevent the known complications of small bowel polyps, including intussusceptions, bleeding and tumour genesis, which often require multiple laparotomies. Data about larger patient groups with polyps managed by DBE are mandatory.

Argon plasma coagulation

Argon plasma coagulation is a safe procedure/treatment for angiodysplasia. Unfortunately, no data have been reported about the settings of the ERBE module.

Tattooing

Aiming for total enteroscopy, with the knowledge of a low success rate in intubating the caecum via the oral approach, makes it necessary to mark the most distal site reachable in the small bowel with ink. Also, lesions observed during enteroscopy can be marked with ink for the surgeon after biopsies are obtained for histological diagnosis. In the study by Heine et al., Indian ink was used for tattooing after injecting a small amount of saline into the submucosal space prior to ink injection, so avoiding transmural injection of ink into the abdominal space resulting in pain or colouring the total abdominal space.[10]

Dilatation

DBE can be performed when stenosis of the small bowel is suspected as a result of a neoplasm or as in Crohn's disease or refractory coeliac disease. Sunada et al. reported 17 cases of stenosis of the small bowel for which DBE was used.[21] The series included three patients with a neoplasm, eight with inflammatory disease and five with a narrowed lumen without mucosal changes, suggesting adhesions or postoperative stricture. In four cases, including Crohn's disease, post-traumatic stricture and inflammatory stricture, an endoscopic balloon dilatation was performed. All the procedures were completed without complications.

Table 3. Comparison of the data from the published DBE studies

	European retrospective [11]	European prospective [8]	Wiesbaden [7]	Yamamoto [6]	Amsterdam [10]
Patients (N)	62	100	137	123	275
DBE procedures	89	147	248	178	316
Panendoscopy	10/62 (16.2%)	16%			
One session	-	-	2/137 (1.4%)	2/128 (1.5%)	14/275 (5.1%)
Two sessions	-	-	25/55 (45%)	24/28 (86%)	12/36 (33%)
Insertion depth in cm					
Oral	254 ± 174	220 ± 90	240 ± 100	No data	270 ± 121
Anal	180 ± 150	130 ± 80	140 ± 90	No data	120 ± 110
Duration of procedure	-	75 ± 19 (32-150)	73 ± 25 (25-131)		
Oral	70 ± 30	-	72 ± 23 (30-131)	47 (27-100)*	90 ± 47
Anal	90 ± 35	-	75 ± 28 (25-130)	73 (30-123)*	110 ± 37
Diagnostic yield	80%	72%	79 %	76 %	83%
Endoscopic therapy	42%	62%	76%	20%	No data
Complications	None	None 1 propofol-related	None 1 propofol- related	2/178 (1.1%)	3/316 (0.9%) §

* median and range of initial (2 oral and 20 anal) and opposite approach (2 anal and 20 oral) in the group with a total enteroscopy § 3/316; 2 mild and 1 moderate severe pancratitis

Video Capsule Endoscopy and Double-balloon Enteroscopy

The VCE was introduced in the late 1990s and since then several studies have demonstrated the diagnostic value of this method in patients with OGIB, inflammatory bowel diseases and coeliac disease.[22, 23] Two recent studies compared the results of VCE with DBE in obscure gastrointestinal bleeding.[24, 25] Matsumoto et al. examined 22 patients with OGIB (13/22) and polyposis (9/22) using both modalities. Positive findings were identified with DBE in 12 patients (54.5%). VCE detected positive findings in the area explored by DBE in 8 patients (36.4%) and in the unexplored area in 11 patients (50.0%). The overall diagnostic yield between the procedures was comparable. DBE appeared to be superior to VCE in identifying polyps.

Moreover, there was a trend towards a higher number of polyps detected with DBE as compared with VCE. Hadithi et al. evaluated 35 patients OGIB.[25] In 28 patients (80%), positive findings were identified by VCE in comparison with DBE in 21 patients (60%). DBE could not confirm or identify the polyps diagnosed by VCE in two patients, suggesting a false-positive finding.

In conclusion, DBE and VCE show a comparable overall diagnostic yield, whereas DBE is superior to VCE in patients with polyposis.

Conclusions

In the past, surgeons have been confronted with problematic blood loss from the gastrointestinal tract, and unrewarding ''blind'' resections of stomach or colon have been the standard. Gastroscopy and colonoscopy have changed this attitude since the 1960s. DBE, 40 years after the introduction of endoscopy, is now changing this for the small bowel.

Indications for DBE are now well recognized.

- Obscure blood loss of unknown origin with negative proximal and distal endoscopy is a reason for DBE with or without prior screening. Overt bleeding of the small bowel is an indication for DBE because of its superior diagnostic (suction, rinsing, and biopsies) and therapeutic properties.
- Suspected inflammatory bowel diseases such as Crohn's disease and coeliac disease for establishing a diagnosis and or treatment (dilatation).
- Locating and tattooing lesions, not suitable for endoscopic treatment, can guide surgeons in theater.
- Therapeutic removal of foreign bodies such as capsules, etc., bridging stenotic areas with dilatation, insertion of tubes, endoprosthesis, etc.
- Proper management of hereditary polyp syndromes such as FAP and Peutz-Jeghers.
- Staging B- and T-cell small bowel lymphomas.

Capsule endoscopy for screening and DBE for histology, confirmation and treatment, whether or not in combination with coagulation, argon plasma coagulation and polypectomy, are more than promising in daily practice. Chronic blood loss and the need for multiple

transfusions are now manageable.Achieving a ''clean'' polyp-free small bowel in FAP and Peutz-Jeghers will result in fewer operations and probably better quality of survival. Polypectomy of small bowel polyps by DBE can prevent the complications of larger small-intestinal polyps, including intussusceptions, bleeding and dedifferentiation to cancer. The duration of interventions during DBE demands mutual understanding between endoscopist, nursing staff and patients. Since the small bowel is stretchable, and no landmarks such as the hepatic flexure, etc., are recognized, the method of measuring the depth of the introduction by May et al. is the standard for the moment. The literature of the years to come will guide us about when to use the DBE as first approach and when to start with VCE as screening. The complication rate reported in the current literature is promising; however, the pancreatitis rate reported by the Amsterdam group has to be kept in mind.[10] Meta-analysis of the first 10,000 cases, to be reported in literature in the near future, will help us to determine the actual complication risks. Small bowel, the terra incognita of flexible endoscopy, is rapidly being discovered and false answers of the past will be corrected in the coming years. DBE has established itself in record time and is recognized as a milestone in endoscopy.

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Enteropathy associated T-cell Lymphoma in the Netherlands

3

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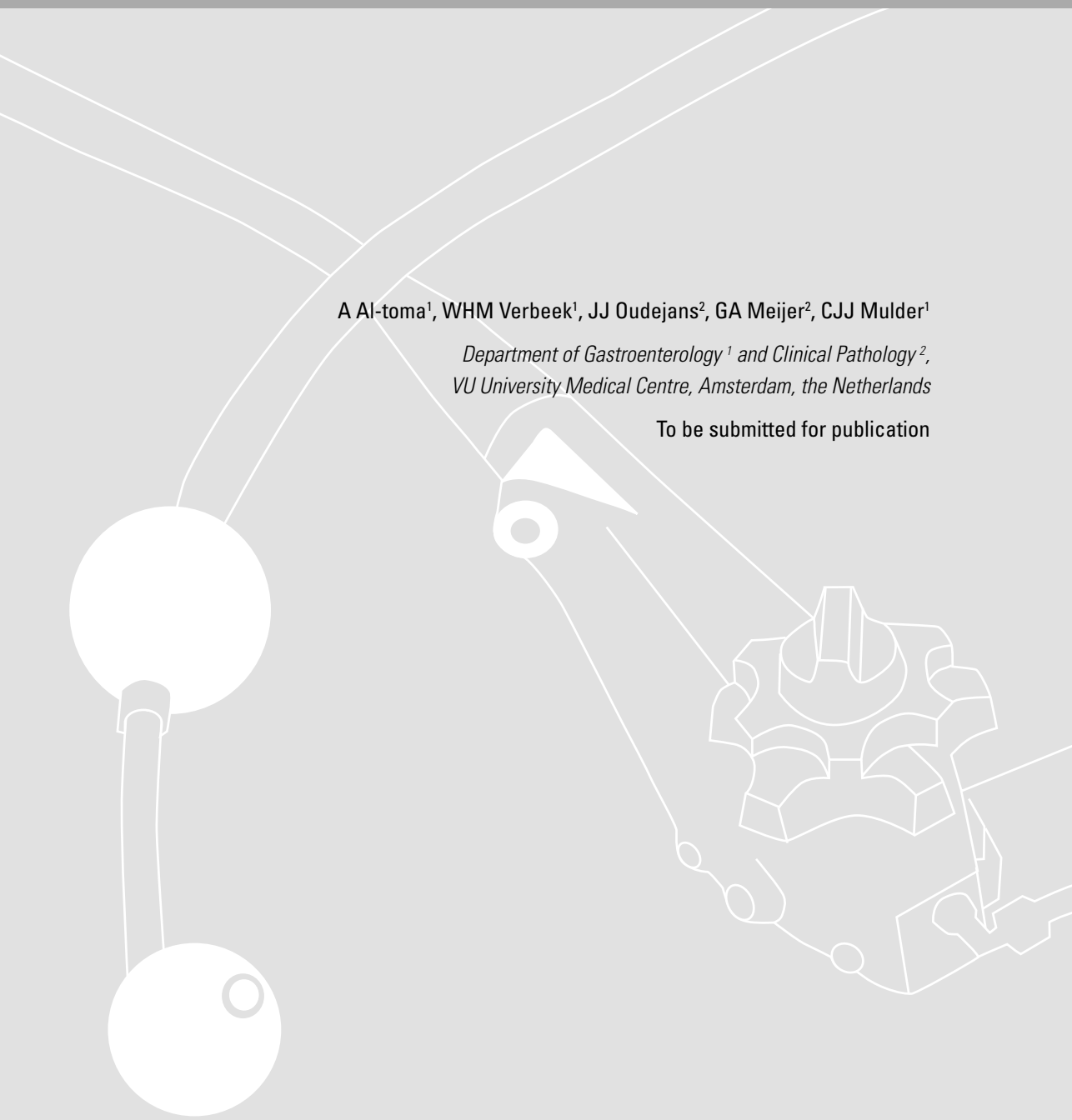

Abstract

Background: Enteropathy-associated T-cell lymphomas (EATL) are T-cell non-Hodgkin lymphomas of the small bowel, which are associated with coeliac disease. EATL's arise in patients with either previously known or newly diagnosed coeliac disease. In a subgroup of patients EATL is preceded by a progressive deterioration, a refractory form of coeliac disease. There is little information about the incidence of EATL's.
Aim: To study the demographic characteristics of patients with EATL and its association with coeliac disease.
Design and Methods: A survey in the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) was performed including all T-cell lymphomas with an initial presenting tumour in the small bowel. The data on patients diagnosed with small bowel T-cell lymphomas between 2000 -2004 were analysed.
Results: Clinico-pathological data were gathered for 91 cases of EATL; 19% occurred in the duodenum, 20% in the jejunum, 7% in the ileum, 5% in more than one site and 49% in non-specified sites of the intestine. There is a predominance of males (65%) versus females (35%) (p=1.00). The overwhelming majority (89%) of patients are 50 years and over.
Conclusions: This study indicates that EATL is a rare complication of therapy- refractory coeliac disease with an incidence of 0.12 per 100.000 inhabitants per year. EATL occurs mainly in patients over the age of 50, mean age 60 years (range 39-75, SD± 8,6), primarily in the proximal small bowel. Because coeliac disease in old age patients is sometimes not noted, it is possible that the actual incidence is considerably higher. It would be interesting to look for coeliac disease associated T-cell lymphomas outside the gastrointestinal tract.

Introduction

Enteropathy-associated T-cell lymphoma (EATL) is a rare form of high-grade, T-cell non-Hodgkin lymphoma (NHL) of the small intestine that is specifically associated with Coeliac disease.[1] EATL in coeliac disease patients can present in two ways.[2; 3] There are patients with well-established coeliac disease who have responded to a gluten free diet but later deteriorate because of the development of refractory coeliac disease with aberrant T-cells. In the other group, the diagnosis of coeliac disease is made following the diagnosis of EATL (*de novo* EATL). In 60-80% of patients with refractory coeliac disease with aberrant T-cells, EATL develops within 5 years.[4]
A prospective, multi-centre, case-control study in 10 European countries [5] has found that patients with coeliac disease have an increased risk of developing NHL (both T and B lymphomas) [odds ratio (OR) 2,6, 95% confidence interval (CI) 1,4-4,9] and that clinically silent coeliac disease is rare in patients with NHL. This risk was only present in patients with coeliac disease diagnosed clinically before the study (OR 3,3, 95% CI 1,4-7,9), but not in those with silent coeliac disease detected by screening (OR 1,3, 95% CI 0,6-2,7).
EATL has almost always dismal prognosis. In a British national lymphoma investigation, 16 cases of EATL were described with survival of only 25% at 18 months.[6] Egan et al [7] reported

30 patients with EATL having survival of only 31% at one year and 11% at 5 years. In our own series of 39 EATL patients, the 2 year survival is only 15-20% (*CJJ Mulder: submitted*).
The magnitude of the prevalence of EATL and the association with coeliac disease has never been established through a national survey. To address this, we have carried out a search in *the nationwide network and registry of histo- and cytopatholgy in the Netherlands* (abbreviated as *PALGA*), which is a central database for all histopathological diagnosis in the country.

Design and Methods

The PALGA registry

The PALGA database is a central archive containing the abstracts of all histopathological and cytological reports from all hospitals in the Netherlands since 1991. Every record contains a summery of the reports and diagnostic codes similar to the Systematized Nomenclature of Medicine (SNOMED).[8]

Analysis of the data

The present survey was based on the data recorded between 2000 and 2004.
All lymphomas diagnosed as EATL were included. In addition all T-cell lymphomas with a first presentation in the small intestine (duodenum, jejunum, and ileum) were included, whether or not an association with coeliac disease was noted.
The following terms were used in the search: Small bowel, small intestine, lymphoma, enteropathy associated lymphoma, coeliac disease, villous atrophy, gluten sensitive enteropathy.
From the data gained we have looked for the combination EATL or T-cell lymphoma and coeliac disease. The records were checked for reporting specific anatomical sub-sites and the presence of features associated with coeliac disease, such as enteropathy, gluten sensitive enteropathy, sprue and villous atrophy. The data were further analysed looking for age-specific incidence (for 10-years age group), gender and gender-specific incidence, method of establishing the diagnosis (endoscopic, surgical resection, autopsy or a combination of these), localization and the association with the presence of coeliac disease or villous atrophy.

Results

Between 2000 and 2004, a total of 640 entries of small bowel lymphoma were found, only 91 (59 males: 32 females) entries of them fulfilled the criteria for EATL. Data on a total of 549 patients were discarded because of the following: the diagnosis of mantle cell, MALT, Burkitt and B-cell lymphoma.
Patients' characteristics by diagnostic year are shown in table 1. The mean age at diagnosis of EATL is 60 years (range 39-75, SD= 8,6, CI= 59,8-65,3). Eighty-nine percent of the patients were diagnosed after their 50th birthday, with a male: female ratio of 1,8 : 1.

Table 1. Patients characteristics by diagnostic year

	2000		2001		2002		2003		2004		Total	
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Age (yrs)												
<50	2	14	2	15	2	8	3	14	1	6	10	11
50-59	4	29	4	31	7	28	7	33	8	44	30	33
60-69	7	50	4	31	6	24	6	29	6	33	29	32
70-79	1	7	3	23	10	40	5	24	3	17	22	24
Gender												
Male	9	64	6	46	17	68	15	71	12	67	59	65
Female	5	36	7	54	8	32	6	29	6	33	32	35
Total	14	100	13	100	25	100	21	100	18	100	91	100

The age-specific incidence is shown in figure 1. The incidence is 0,4/100,000 population in those between 40 and 49 years old, 1,5/ 100,000 in those between 50-59 years, 2,1/100,000 in those between 60-69 years and 2.0/100,000 in those between 70-79 years old. Furthermore, the gender-specific incidence is 0,74/100,000 males and 0,39/100,000 females.

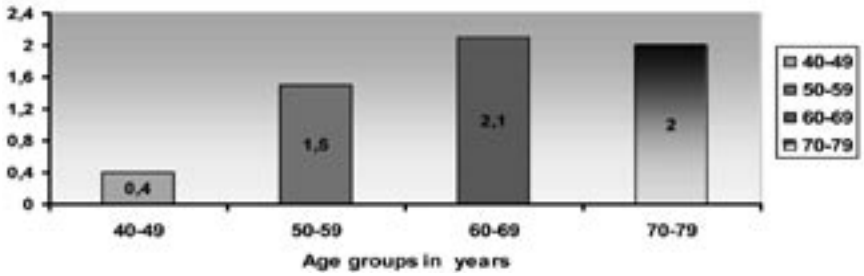

Figure 1. Shows the age-specific incidence

Table 2 shows Localization of EATL by diagnostic year. EATL is mostly localised in the proximal small intestine, 39% in duodenum and jejunum compared to only 7% localised primarily in the ileum. No localisation was provided in 49% of patients.
The relation of EATL to coeliac disease is shown in table 3 and 4. Twenty-four of patients had Marsh IIIA-C at diagnosis of EATL (Table 3), meanwhile in 76% of the cases only a diagnosis of coeliac disease was made, but no marsh classification was provided. We studied the time-lag between the diagnoses CD and EATL in the 40 intestinal T-cel lymphomas registered as EATLs in PALGA. Of these 40 cases registered 50% of the patients were known to have coeliac disease at forehand, while a diagnosis of coeliac disease was established in a total of 47.5% (range 20-40%) simultaneously with the diagnosis of EATL (de novo EATL) (Table 4).

In the other 51 intestinal T-cell lymphomas villous atrophy was not mentioned, thus studying the time-lag was not possible. In only two patients (2.5%) no data is available on the time interval between coeliac disease diagnosis and EATL development. Furthermore, most cases of EATL developed in the first 5 years after coeliac disease diagnosis.

Table 2. Localization of EATL by diagnostic year

	2000		2001		2002		2003		2004		Total	
Localization	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Duodenum	3	22	4	31	4	16	3	14	3	17	17	19
Jejunum	2	14	2	15	7	28	4	19	3	17	18	20
Ileum	1	7	0	0	2	8	2	10	1	5	6	7
Multifocal	1	7	0	0	2	8	1	5	1	5	5	5
Not specified	7	50	7	54	10	40	11	52	10	56	45	49
Total	14	100	13	100	25	100	21	100	18	100	91	100

Table 3. Marsh stage by diagnostic year

Marsh	2000		2001		2002		2003		2004		Total	
Classification	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Marsh IIIA	0	0	1	7.6	2	8	0	0	0	0	3	3
Marsh IIIB	1	7	4	30.7	4	16	2	9.5	2	11	13	14
Marsh IIIC	0	0	1	7.6	1	4	3	14	1	5.5	6	7
Not specified	13	93	7	53.8	18	72	16	76	15	83	69	76
Total	14	100	13	100	25	100	21	100	18	100	91	100

Table 4. Time lag in years between the diagnoses CD and EATL by diagnostic year (total= 40)

Time lag (yrs)	2000		2001		2002		2003		2004		Total	
CD-> EATL	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	1	20	1	25	4	28.5	5	41.6	2	40	19	47.5
1-5	3	60	3	75	4	28.6	4	33.3	3	60	17	42.5
6-10	1	20	0	0	2	14.3	2	16.6	0	0	1	2.5
10+	0	0	0	0	3	21.4	0	0	0	0	2	5
Not available	0	0	0	0	1	7.1	0	0	0	0	1	2.5
Total	5	100	4	100	14	100	12	100	5	100	40	100

Discussion

Patients with refractory coeliac disease and aberrant T-cells are at a greater risk of developing malignancy, particularly EATL (60-80% within 5 years).[4] EATL ultimately develops in 7-10% of patients with long-standing coeliac disease with a relative risk of 42,7.[2, 9]

The annual incidence rate of EATL has been reported to be 0,5-1 per million people in Western countries.[1] We report here an annual incidence in the Netherlands of 0,8-1,5/ million people. We found a male: female ratio of 1,8:1. Other studies also reported a predominance for EATL in males, with a peak in the 6th decade of life, [1,11,12] however the majority of uncomplicated coeliac disease patients are females.[13]

Here we report that 89% of the patients were diagnosed after their 50th birthday (between 50-79 years). Patients with coeliac disease who develop worsening of symptoms of diarrhoea and malabsorption, particularly patients older than 50 years should be considered for complete endoscopic and radiologic evaluation to exclude EATL. In patients without a prior diagnosis of coeliac disease, EATL is a very rare disorder and the diagnosis in such cases is often difficult and delayed due to the non-specific nature of the symptoms and a very low index of clinical suspicion.[2-4]

It seems that older age, male sex and the presence of HLA-DQ2 homozygosity [14] are associated with a higher risk for development of EATL in coeliac disease.

We found that EATL is mostly localised in the proximal small intestine, 39% in duodenum and jejunum compared to only 7% localised primarily in the ileum. This is consistent with reports of others[1] that EATL commonly develops in the jejunum but may also be found in the ileum and lymph nodes and less frequently in the stomach and colon. It is often multifocal with ulcerative lesions, which explains the high perforation rate at presentation or during chemotherapy.[3]

Concerning the time lag between diagnosis of coeliac disease and subsequent development of EATL, variable intervals have been reported reported intervals ranging from 3 to 21 years.[16, 17] In this survey, we found that most cases (90%) are diagnosed within 5 years from the diagnosis of coeliac disease. This is consistent with the findings of Holmes et al[10] that GFD for more than 5 years will reduce the risk of intestinal lymphoma to that of the general population.

Several studies reported that in about 50% of cases, the patient presented with an EATL, while the underlying coeliac disease was still silent and coeliac disease was only recognized after the diagnosis of lymphoma had been made.[1, 3] In this survey we found that the diagnosis of coeliac disease was established in a total of 47.5% simultaneously with the diagnosis of EATL (*de novo* EATL). This underscores the importance of taking endoscopic biopsies even from grossly normal appearing mucosa to exclude coeliac disease since GFD needs to be instituted.

Only in a minority of patients with localization of lymphoma in the proximal small bowel can the diagnosis be established on endoscopic biopsies. In most cases laparotomy is necessary to establish the diagnosis as well as being part of the treatment.[18] Approximately 50% of the patients require laparotomy for complications of haemorrhage, perforation or obstruction.[7]

With the advent of the new small bowel endoscopy techniques, particularly the video capsule endoscopy [19] and the double-balloon enteroscopy (DBE) [20] and the developments in small bowel radiological imaging (PET scan, MR enteroclysis and CT enteroclysis) [21,22] we think that will improve earlier detection of malignant and pre-malignant lesions and will boost the standard of care of the patients with currently dismal outcome.

Methodological considerations

There might be some degree of underreporting of EATL cases in PALGA since only 91 cases were reported during a period of 5 years. This might be due to the fact that patients with EATL usually present with non-specific worsening symptoms of diarrhoea and malabsorption in their fifth or sixth decade, and that imaging techniques do not recognize abnormalities in a subgroup at this stage.[3,7] In this subgroup of patients EATL is not recognized. If no surgery for obstruction or perforation takes place, these patients might die of malnutrition or cachexia and not be registered in the PALGA database unless autopsy is done.

Another possible explanation of the underreporting might be that, as put forward earlier, almost 50% of these patients are treated with partial resection without due consideration to take biopsies from the normal appearing mucosa. In these cases coeliac disease would be overlooked; hence these patients are not registered in PALGA as EATL's.

Lastly, EATL's can present as extra-intestinal T-cell lymphomas in 20% of patients.[3] In this case the presence of coeliac disease will probably be overlooked and these cases will be labelled as T-cell lymphomas but not specifically EATL. In these cases, considering the search used in the present study, there will be underreporting in PALGA. Furthermore, a better characterisation of the Marsh stage at diagnosis, reporting the immunophenotype of EATL are needed.

Conclusions

Taking the possibility of an underreporting under consideration, this study reflects the incidence of EATL registered by all hospitals in the Netherlands. The incidence reported in this study is rather low and EATL is seen almost always in patients of 50 years and over.

Acknowledgment

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ADVANCES IN DIAGNOSTIC APPROACH II

Human Leukocyte Antigen-DQ2 Homozygosity and the Development of Refractory Coeliac Disease and Enteropathy-Associated T-Cell Lymphoma

4

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Abstract

Background & Aims: Coeliac disease (CD) is a common gluten-sensitive enteropathy associated with human leukocyte antigen (HLA)-DQ2 and HLA-DQ8. The aim of this study was to determine if a particular HLA-DQ subtype predisposes to complications such as refractory CD with (RCD II) or without aberrant T-cells (RCD I), and enteropathy-associated T-cell lymphomas (EATL).
Methods: Molecular HLA-DQ typing was performed on 43 RCD I, 43 RCD II, and 30 EATL patients, and compared with age-matched groups of 121 patients with histologically defined uncomplicated CD and 183 healthy controls. All individuals were Dutch Caucasians and were at least 21 years of age.
Results: HLA-DQ2 was present in 79% of RCD I, 97.7% of RCD II, and 96.6% of EATL patients. The differences were significant when compared with 28.9% in controls but not with 91.7% in uncomplicated CD. Homozygosity for HLA-DQ2 was observed in 25.5% of RCD I, 44.1% of RCD II, and 53.3% of EATL patients vs 20.7% of uncomplicated CD patients and 2.1% of controls. HLA-DQ8 was present in 10.7% of CD, 16.2% of RCD I, 9.3% of RCD II, and 6.6% of EATL patients vs 20.2% of controls.
Conclusions: Homozygosity for HLADQ2 is associated with RCD II and EATL. Early identification of HLA-DQ2 homozygous CD patients may help to recognize CD patients at risk for developing these severe complications.

Introduction

Coeliac disease (CD) is a common gluten-sensitive enteropathy affecting 1/150 to 1/300 individuals worldwide.[1, 2] CD is strongly associated with the class II human leukocyte antigen (HLA)-DQ2 heterodimer encoded by the DQA1*0501 and DQB1*02 alleles. The DQ2 glycoprotein is present in 90-95% of Caucasian CD patients.[3, 4] The majority of DQ2-negative CD patients are positive for the haplotype DQA1*03-DQB1*0302 (HLA-DQ8).[5-7] A small number of CD patients lacking these heterodimers have either DQA1*05 or DQB1*02 alone.[8] CD-associated HLA-DQ molecules bind and present gluten peptides to antigen-specific T-cells. These HLA-DQ-peptide complexes induce inflammatory T-cell responses in the small intestine with villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis.[4, 9] HLA-DQ2 homozygous antigen-presenting cells (APC) induce higher T-cell proliferation and cytokine secretion than HLA-DQ2/non-DQ2 heterozygous APC.[9] This may explain the strongly increased risk for disease development in HLA-DQ2 homozygous individuals.[10-13]
In a small percentage of patients serious complications develop. CD patients may be regarded as suffering from refractory disease (RCD) when symptoms persist or recur after a former good response despite strict adherence to a gluten-free diet. When normal expression of T-cell antigens and polyclonal TCR-gene rearrangement occur (RCD I) the prognosis is less dismal than when an aberrant clonal intraepithelial T-cell (IEL) population and/or loss of antigen on IELs is present (RCD II). These patients have a high risk of developing intestinal lymphoma.[14-17] Patients with refractory CD are at a greater risk of developing malignancy.[18] EATL has

histological and immuno-histochemical features of large or medium size T-cell proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$ phenotype. The majority of these lymphomas present as $CD3^+$ $CD8^-$ $CD30^+$ large cell lymphoma, however small cell lymphomas, often $CD3^+$ $CD8^+$ $CD30^-$ may occur.[19]
We have investigated whether a distinct HLA-DQ subgroup represents a risk factor for the development of refractory disease and the development of EATL.

Materials and Methods

Subjects

Forty-three patients with RCD I (12 males and 31 females; mean age at diagnosis 49 years, range 23-86), 43 patients with RCD II (19 males and 24 females; mean age at diagnosis 59 years, range 47-88) and 30 patients with EATL (16 males and 14 females; mean age at diagnosis 61.5 years, range 52-79) were studied. Patients were referred to the Rijnstate Hospital or the VU University Medical Centre, tertiary referral centres for CD and were recruited from all provinces in the Netherlands from 1992 to 2003. The patients with RCD I and II were followed over a mean follow-up period of 5 years (range 2-12 years) for evidence of transition to a more severe state, i.e., the transition from RCD I to RCD II and /or EATL, and from RCD II to EATL.
We have used for comparison data on a total of 121 unrelated and uncomplicated Dutch Caucasian CD patients (24 males and 97 females; mean age at diagnosis 45.9 years, range 22-75) selected on age to match the age groups under study. All these patients had villous atrophy (Marsh type III) on a normal gluten containing diet who responded with histological and clinical improvement to withdrawal of gluten from the diet.[20] Table 1 shows the demographic characteristics of these patients and the age at diagnosis of both CD and the complicated state (RCD and EATL). A group of 183 unrelated healthy Dutch Caucasians (85 males and 98 females, mean age at participation 38.7 years, range 24-89), previously typed for HLA-DQ served as controls.[20] In complicated CD, possible underlying diseases (except EATL), such as bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple's disease, hypogamma-globulinemia, eosinophilic enteritis, inflammatory bowel disease were excluded. In addition to endoscopic and histopathological evaluation, all patients with complicated coeliac disease underwent clinical, laboratory and radiological assessment including: intraepithelial lymphocytes phenotyping for signs of (cytotoxic-) monoclonality, small bowel x-ray and/or magnetic resonance, serological results for anti-gliadin, anti-endomysium and tissue transglutaminase (tGA), thyroid function tests, stool examination for giardia and other parasites, HIV serology and DEXA-scan as part of a routine work-up.[1, 17] When indicated, computed tomography scans of the abdomen, positron emission tomography, video capsule endoscopy and/or double-balloon enteroscopy were performed.

Criteria for diagnosis

The histology in the gluten-sensitive spectrum was categorized according to the modified Marsh's criteria adapted by the working group of the 2001 United European Gastroenterology Week (UEGW) in Amsterdam.[21] The diagnosis of CD was confirmed by histological examination with a documented histologic response to gluten withdrawal.[21] Patients with CD were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a gluten-free diet. The diagnosis of RCD was established as type 1 when no aberrant T-cells were present in intestinal biopsy specimens and type II with aberrant T-cells detected by immunophenotyping using flow-cytometric analysis or immunohistology of the intestinal mucosa.[17, 21] In RCD I the IEL phenotype is normal with the expression of surface CD3 CD8 and TCR-β. In RCD II the IELs have normal cytological features, but they exhibit an abnormal IEL phenotype with the expression of intracytoplasmic CD3ε, surface CD103 and the lack of classical surface T-cell markers such as CD4, CD8 and TCR-αβ.[18] The diagnosis of EATL was established according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[19, 22, 23] The immunohistochemical features of EATL are evidence of large or medium size T-cell proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$. The majority are $CD3^+$ $CD8^-$ $CD30^+$ large cell lymphoma; however, small cell lymphomas often are $CD3^+$ $CD8^+$ $CD30^-$.[19]

HLA-DQ typing

Whole blood was obtained for typing of HLA-DQA1* and DQB1* alleles, performed with a combined single-stranded conformation polymorphism (SSCP)/heteroduplex method by a semi-automated electrophoresis and gel staining method on the Phastsystem™ (Amersham-Pharmacia-Biotech, Sweden).[20, 24]

Statistical analysis

Statistical data were analyzed by the student *t* test, and results were considered statistically significant at a P value < 0.05. Odds ratios (OR) and their 95% confidence intervals (CI) were used to assess the significance of association between the dosage of haplotypes HLA-DQ2 and/or DQ8 and the risk of having RCD I, RCD II and EATL.

Results

Table 1 shows the age and sex distribution of the patients with histologically-defined uncomplicated CD (Marsh III), RCD I and RCD II, EATL, and controls.

Table 2 shows the distribution of carriers of HLA-DQ2 and DQ8 allelic combinations in the patients with histologically-defined uncomplicated CD, RCD I and RCD II, EATL, and controls. Fourty-nine out of 183 (26.8%) controls were found to be HLA-DQ2 heterozygous. Four (2.1%) controls were homozygous for DQ2. Thirty-seven controls (20.2%) carried HLA-DQ8, among them two individuals (1.1%) were homozygous for HLA-DQ8. Ten controls were positive for both HLA-DQ2 and DQ8. Altogether, eighty controls (43.7%) were positive for

HLA-DQ2 and/or DQ8. Of note, in 103 controls (56.3%) HLA-DQ2 and DQ8 are absent.
In the patients with histologically-defined uncomplicated CD (Marsh III), 111 out of 121 (91.7%) patients carried at least one copy of the combination of genes DQA1*0501 and DQB1*02, i.e., were HLA-DQ2-positive, and 25 out of 121 (20.7%) were DQ2 homozygous. Thirteen patients (10.7%) carried the haplotype HLA-DQ8 and 8 (6.6%) were positive for both HLA-DQ2 and DQ8. Therefore, 95.9% of patients with uncomplicated CD carried DQ2 and/or DQ8 markers.
In the RCD I group, 34 out of 43 (79%) patients were DQ2-positive, and 11 out of 43 patients (25.5%) were DQ2 homozygous. Seven patients (16.2%) carried the haplotype HLA-DQ8. Overall, 86% of RCD I patients carried HLA-DQ2 and/or DQ8. Six out of 43 (14.0%) patients with RCD I had neither HLA-DQ2 nor DQ8, however, three of them had the allele DQB1*02.

Table 1. Age and sex distribution of the patients with histologically defined uncomplicated CD (Marsh III), refractory CD (RCD I and RCD II), EATL, and controls

	Mean age at diagnosis CD in years (range)	Mean age at diagnosis RCD I, RCD II, and EATL in years (range)	Male:Female
Uncomplicated CD (n=121)	45.9 (22-75)	-	24:97
RCD I (n=43)	47 (21-75)	49 (23-86)	12:31
RCD II (n=43)	57 (40-69)	59 (47-88)	19:24
EATL (n=30)	59 (46-69)	61.5 (52-79)	16:14
Controls (n=183)	38.7 (24-89) at participation	-	85:98

In the RCD II group, 42 out of 43 (97.7%) patients were HLA-DQ2-positive and 19 patients (44.1%) were HLA-DQ2 homozygous. Four patients (9.3%) carried the haplotype DQ8. Therefore, all RCD II patients carried HLA-DQ2 and/or DQ8.
In the EATL group, 29 out of 30 (96.6%) patients were HLA-DQ2-positive and 16 (53.3%) were HLA-DQ2 homozygous. Two patients (6.6%) carried the haplotype DQ8. Therefore, all patients with EATL carried HLA-DQ2 and/or DQ8.
The mean age for CD diagnosis in the RCD II subgroup was 57 years (range 40-69) and 59 years (range 46-69) in EATL. Over a mean follow-up period of 5 years (range 2-12), none of the RCD I patients had progressed to RCD II or EATL. Furthermore, of the 30 patients with EATL, the diagnosis was established in five without preceding known history of CD and/or RCD II, in other words, 25 patients have progressed from RCD II to EATL.

Table 2. Distribution of carriers of HLA-DQ2 and DQ8 allelic combinations in the patients with histologically defined uncomplicated CD (Marsh III), refractory CD (RCD I and RCD II), EATL, and controls. X = non-DQ2 and non-DQ8

	Controls (n=183) (%)		Uncomplicated CD (n=121) (%)		RCD-I (n=43) (%)		RCD II (n= 43) (%)		EATL (n=30) (%)	
DQ2/X	39	(21.3)	78	(64.5)	19	(44.1)	20	(45.4)	12	(40.0)
DQ2 homozygous	4	(2.1)	25	(20.7)	11	(25.5)	19	(44.1)	16	(53.3)
DQ2/DQ8	10	(5.5)	8	(6.6)	4	(9.3)	3	(6.9)	1	(3.3)
DQ2 positive	53	(28.9)	111	(91.7)	34	(79)	42	(97.7)	29	(96.6)
DQ8/X	25	(13.6)	4	(3.3)	3	(6.9)	1	(2.3)	1	(3.3)
DQ8 homozygous	2	(1.1)	1	(0.8)	0		0		0	
DQ8 positive	37	(20.2)	13	(10.7)	7	(16.2)	4	(9.3)	2	(6.6)
Non DQ2/non DQ8	103	(56.3)	5	(4.1)	6	(14.0)	0		0	
DQ2 or DQ8 positive	80	(43.7)	116	(95.9)	37	(86.0)	43	(100)	30	(100)

Data on HLA-DQ2 homozygosity in RCD I, RCD II and EATL and a comparison made to the histological-defined uncomplicated CD (Marsh III) and controls are presented in Table 3. The difference in HLA-DQ2 homozygosity between RCD II (44.1%) and EATL (53.3%) was not significant (P >0.05). RCD II and EATL patients have a statistically significant higher frequency of DQ2 homozygosity compared to uncomplicated CD (20.7%), (P = 0.0046; OR= 3.04 (95 % CI: 1.44 - 6.41) and P=0.0003; OR =4.39 (95% CI: 1.91 - 10.08), respectively). No statistically significant differences were found in the carrier frequencies of HLA-DQ8 between uncomplicated CD, RCD I, RCD II and EATL. In relation to carriage of HLA-DQ2, the difference between RCD I and uncomplicated CD is significant (P=0.048, OR 3.0, 95% CI: 1.169 to 7.700), the difference between RCD II and RCD I does reach significance (P=0.0148; OR=11.118, 95% CI: 1.341 to 92.198) and the difference between EATL and RCD I is significant (P=0.0399, OR=7.676, 95% CI: 0.917 to 64.28). The difference in frequency of DQ2 homozygosity between RCD I (25.5%) and uncomplicated CD (20.7%) is not statistically significant (P=0.503, OR =1.32 (0.59-2.94).

Table 3. HLA-DQ2 homozygosity and the risk of having RCD I, RCD II and EATL compared to the patients with histologically-defined uncomplicated CD (Marsh III) and controls as defined by OR and 95% Confidence Intervals

Disease complication	Odds ratios (95% CI) vs. uncomplicated CD	P- value vs. uncomplicated CD	Odds ratios (95% CI) vs. controls	P-value vs. controls
RCD I	1.32 (0.59-2.94)	0.503	15.3 (4.8 - 48.5)	<0.0001
RCD II	3.04 (1.44-6.41)	0.0046	35.4 (11.5 - 107.6)	<0.0001
EATL	4.39 (1.91-10.08)	0.0003	51.1 (15.5 - 165.8)	<0.0001

Discussion

The relationship between HLA-DQ2, DQ8 and CD has become clearer in recent years. Through the activity of the enzyme tissue transglutaminase (tTG) glutamine residues in gluten are converted into glutamic acid. Subsequently, a multitude of gluten-derived peptides is generated that when bound to either HLA-DQ2 or DQ8 can induce T-cell responses in CD patients.[25, 26] A particular glutamine and proline rich 33-mer α-gliadin peptide that contains 6 different T-cell stimulatory sequences and is resistant to gastric and duodenal proteolysis might be the primary initiator of the inflammatory response to gluten.[27, 28] In the large majority of patients, even in children with CD, inflammatory T-cell responses to other gluten peptides are also observed, implicating multiple gluten peptides in the disease process.[29]

Although reports by Zubillaga et al.[7] and Congia et al.[30] have shown that DQ2-homozygosity may predispose a person to an earlier onset, and to more severe disease manifestations, Greco et al.[31] found no correlation of clinical features of CD with different HLA-DR/DQ genotypes. Howell et al. [32] using frozen or paraffin-embedded biopsy tissue from 43 British EATL patients found that EATL arises in individuals with the DQA1*0501, DQB1*02 CD-predisposing genotype, however, the patients were not homozygous for HLA-DQ2. In the present study, we found a highly significant correlation between HLA-DQ2-homozygosity and the development of serious complications of CD, in particular RCD II and EATL. We have no explanation for this discrepancy. However, Howell et al. found 40% of EATL patients to possess the HLA-DRB1*0304 genotype. Since 32.6% of the EATL patients carries allele HLA-DQB1*0302 that is in strong linkage disequilibrium with HLA-DRB1*04 a similar frequency of the haplotype DQA1*03-DQB1*0302 (HLA-DQ8) was to be expected in these Caucasian patients. The authors do not comment on the unexpectedly low frequency (16.3%) of carriers of DQA1*03 suggesting some problems with genotyping these samples.

The link between HLA-DQ2-homozygosity and development of RCD II and CD-associated lymphoma of intraepithelial origin thus suggests that the strength of the gluten specific T-cell response in the lamina propria directly or indirectly influences the likelihood of RCD II and lymphoma development. It has been reported earlier by Vader et al that HLA-DQ2 homozygous antigen-presenting cells (APC) induce higher T-cell proliferation and cytokine secretion than HLA-DQ2/non-DQ2 heterozygous APC.[9] This may explain the strongly increased risk for disease development in HLA-DQ2 homozygous individuals.[10-13] This would indicate that the adherence to a gluten-free diet is particularly important for CD patients who are HLA-DQ2 homozygous.

Interestingly, none of the RCD II and EATL patients have been diagnosed with these complications below the age of 45 years and only one of these patients was diagnosed with CD below the age of 45. These observations suggest that the specific tests such as CD3 cytoplasmic positive T-cells (IEL's) with immunohistology or T-cell flow cytometry should be indicated in all patients with CD who are not responding to a gluten-free diet above the age of 45 years. Since the prognosis is very serious we propose to evaluate all "old-age coeliacs" diagnosed with coeliac disease above the age of 50 years.[18] The availability of a simple and reliable immunohistochemical method makes the distinction between CD and RCD

feasible.[33] Although in this study we have not observed a transition from RCD I to RCD II, a prospective follow-up with immunohistochemical techniques is indicated in particular in HLA-DQ2 homozygous patients. We strongly advise that for the time being at least full low resolution HLA-DQA1 and DQB1 typing in CD is performed. Techniques that recognize only the presence of HLA-DQ2 or DQ8 miss the few patients with CD that are non-HLA-DQ2 non-HLA-DQ8 and the possibility to diagnose HLA-DQ2 or DQ8 homozygotes.
Our observations require further confirmation in a larger group of patients and the set up of prospective studies. HLA-DQ typing is feasible and it may be an efficient test to recognize individuals at risk for these conditions with a poor prognosis, in particular nowadays that some evidence has been given to support the hypothesis that autologous haematopoietic stem cell transplantation can alter disease progression in severe autoimmune disease.[34, 35]

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The value of Double-Balloon Enteroscopy in patients with Refractory Coeliac Disease

5

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Abstract

Objective: Patients with refractory coeliac disease can develop enteropathy-associated T-cell lymphoma (EATL) or ulcerative jejunitis. Double-balloon enteroscopy allows examination of the entire small bowel. We investigated prospectively the value of this technique in patients with refractory coeliac disease.
Methods: The small bowel enteroscopy was performed in a total of 21 consecutive patients for lesions like ulcerations. Biopsy specimens were taken from macroscopical lesions and from three different levels from the examined small bowel. Tissue specimens were evaluated for the modified Marsh classification and for the presence of EATL.
Results: Twenty-four procedures were successfully performed without complications. EATL was found in five patients (24%) as circumferential, discrete, or confluent ulcerations. In three of them Marsh III was found while in the other two patients with EATL Marsh I was found. Another two patients (9%) had ulcerative jejunitis in absence of EATL and histology was compatible with Marsh III. In the remaining 14 patients (54%) no high-risk lesions were found. Double-balloon enteroscopy could exclude the presence of EATL in four patients that was suggested by abdominal computerized tomography.
Conclusions: Complications of refractory coeliac disease like ulcerative jejunitis or EATL could efficiently be detected, its extent delineated or excluded by double-balloon enteroscopy. This technique should be reserved for patients with refractory coeliac disease or patients with a past history of EATL.

Introduction

Coeliac disease is characterized by villous atrophy of the small bowel secondary to gluten ingestion which recovers upon gluten withdrawal from the diet in the majority of patients.[1] However, 2-10% of patients with coeliac disease seem to develop refractory coeliac disease.[2-4] In this specific population, the relative risk for developing ulcerative jejunitis [5] or enteropathy-associated T-cell lymphoma (EATL) [6, 7] is markedly increased.
Endoscopic examination of the small bowel in patients with coeliac disease, including direct visualisation and the possibility of obtaining biopsy specimens for histological assessment has previously been done using conventional upper gastrointestinal endoscopy [8-11] or push enteroscopy.[5, 12-14] Using these techniques, it is possible to examine a limited extent of the small bowel, whereas only the distal ileum can be reached with classical ileo-colonoscopy.[15] Endoscopy of the remaining small bowel in patients with coeliac disease could only be performed using surgical enteroscopy in theatre.[16] Video capsule endoscopy was recently introduced as a diagnostic method for patients with coeliac disease.[17] Double-balloon endoscopy allowed a new era in small bowel endoscopy with its potential for full-length examination of the small bowel, obtaining biopsies, and performing endoscopic interventions, however the procedure describes lesions without the ability to obtain samples for histological examination.[14]

Double-balloon enteroscopy allowed a new era in small bowel endoscopy with its potential for full-length examination of the small bowel, obtaining biopsies, and performing endoscopic interventions.[18-21] In the present study we investigated for the first time whether double-balloon enteroscopy detects mucosal lesions, including ulcerative jejunitis and EATL in a population of refractory coeliac disease patients in segments of the small bowel that are usually not accessible to standard endoscopy. Biopsies of the lesions were taken for histological confirmation of the presence of ulcerative jejunitis or EATL.

Patients and Methods

Patients

The base-line characteristics of 21 patients with coeliac disease who were referred for double-balloon eteroscopy between February 2004 and July 2005 are summarized in Table 1. All patients were symptomatic on a strict gluten-free diet for a median period of 60 months (range 3-384 months). Surgical removal of EATL took place in seven patients and additional therapy consisting of cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) [22, 23] in six, within a median interval of 13 months (range 3-48 months) before double-balloon enteroscopy. In three of these seven patients, histological evidence of EATL was found in surgically removed tissue just 3-5 months prior to double-balloon enteroscopy. These patients were referred for small bowel endoscopy to detect or exclude other EATL lesions. The other 14 patients were suffering from persistent villous atrophy in duodenal histology despite adherence to gluten-free diet for at least 12 months. Previous medical treatment included steroids (n=6) and azathioprine (n=2).

All patients underwent a diagnostic work-up, before undergoing double-balloon enteroscopy, which included the measurement of the coeliac disease specific serological tests [IgA anti-transglutaminase antibodies (TGA) and IgA anti-endomysium antibodies (EMA)] [24], HLA-DQ typing [HLA-DQ2 (encoded by DQA I*0501 and DQB I*02 alleles) and HLA-DQ8 (encoded by DQA I*0301 and DQB I*0302 alleles)] [25] as well as gastroduodenoscopy.[1] Additional diagnostic examinations included abdominal computerized tomography (CT) scan (n=14), and video capsule endoscopy (n=7).

Criteria for diagnosis of RCD

Patients with coeliac disease were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a GFD for at least one year. Furthermore, possible underlying diseases such as autoimmune enteritis, bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple's disease, hypogamma-globulinemia, eosinophilic enteritis, EATL and inflammatory bowel disease were excluded.[3]

Immunophenotyping of the IEL's identifies two groups of RCD patients: those with normal IEL's (RCD I) and those with aberrant IEL's, lacking surface expression of CD3 and CD8 (RCD II).[2, 3]

Table 1. Base-line characteristics of patients with refractory coeliac disease referred for double-balloon eteroscopy.

Characteristic	Total number 21*	
Age, y †	61	(41-89)
Men, n (%)	11	(52%)
Body mass index †	21.2	(16-27)
Family history of coeliac disease, n (%)	1	(5%)
Caucasians, n (%)	20	(95%)
Duration of coeliac disease, y †	5	(0.3-33)
Autoimmune diseases, n (%) ‡	6	(28%)
Surgery for EATL, n (%) §	7	(33%)
Chemotherapy, n (%)	6	(28%)
Duration of gluten free diet, m †	60	(3-384)
Symptoms, n (%)		
Diarrhoea	17	(81%)
Weight loss	11	(52%)
Abdominal pain	3	(14%)
Anaemia	3	(14%)

* Twenty-one patients were evaluated by double-balloon enteroscopy. † Values are the median (range).
‡ Autoimmune diseases consisted of autoimmune thyroid disease (n=4), Sjögren's syndrome (n=1), and dermatitis herpetiformis (n=1). § Enteropathy-associated T-cell lymphoma.

The diagnosis of RCD was established as type II when ≥ 20% aberrant T-cells were present. Detection of a clonal T-cell population by testing for TCR rearrangement was thought to be highly predictive of EATL development. However, oligo- or monoclonal IEL's populations can be detected in the large majority of both RCD I and RCD II patients, also in patients that do not develop an EATL. Clonality is therefore of limited use in establishing the diagnosis of RCD and to predict the development of EATL.[26, 27]
The diagnosis of T-cell lymphoma was based on histological features with mainly evidence of large or medium size T-cell proliferation and immuno-histochemical features with expression of $CD3^+$, $CD8^{+/-}$, $CD103^+$, TIA-1/GranzymB 7, TCRγ gene rearrangements and lack expression of CD30, CD21, bcl-2 and bcl-6, by immunofenotyping of T-cell infiltrate in biopsy specimens or resection material.[4]

Double-balloon enteroscopy

All patients underwent double-balloon enteroscopy within 4-8 weeks of the initial gastroduodenoscopy. The double-balloon enteroscopy system (Fujinon EN-450 P5, Fuji Photo Optical Incorporated Company Fujinon Inc., Japan) was used for examination of the small bowel. The system and method, previously described in detail [18, 19, 21, 27], consists of an endoscope (length 200 cm, outer diameter 8.5 mm, working channel 2.2mm), and a flexible

overtube (length 140 cm, outer diameter 12 mm) that are both provided with soft latex balloons connected through a built-in air route to a controlled pump system. Advancement or withdrawal of the scope is achieved by, deflating (- 45 mmHg) or inflating (+ 45 mmHg) the balloons, respectively. The endoscope was introduced antegradely (orally) in all patients. The length of the visualized small bowel was estimated by calculating the sum of each sequential progressive extension of the scope through the overtube (10-40 cm each sequence), starting the calculation from the pylorus onwards.[28] The calculation of scope advancement was corrected for looping. Patients were prepared for proper endoscopic examination by prior bowel cleansing (1 liter Klean prep ®). A median dose of 7.5 µg (range 5-10µg) of fentanyl and 10 mg (range 5-15 mg) of midazolam was used for conscious sedation. Diagnostic biopsies were taken via a 2.2 mm in diameter working channel. Following the procedure, all patients were monitored in a recovery room for at least 2 hours, and were discharged in the absence of complications.

The small bowel was assessed for a priori defined low-risk lesions including; a reduction (≤3 per endoscopic field of view) or loss of folds; [8] scalloping, that is a coarse and notched appearance of small bowel folds; [28, 29] nodularity of mucosa or mosaicism that is cobblestone or micronodular appearance of the mucosal surface [30]; and presence of visible vessels, [31] after air insufflation.[32] Ulcerations, defined as mucosal defects of at least 5 mm in diameter, and stenosis were considered high-risk lesions for their potential risk of harboring malignancy.[5] Endoscopic findings were considered jejunal if they were found in the proximal 2-3 meters of the calculated endoscopic insertion depth.[27] In addition, the small bowel being examined and visualized by double-balloon enteroscopy after the ligament of Treitz was subdivided into proximal, middle and distal segments where four biopsies were taken from each segment.

Histology of small bowel biopsies

Small bowel biopsy specimens were fixed and preserved in 10% formalin for histopathological and immunohistochemical evaluation. Besides hematoxylene-eosin staining, anti-CD3 (DakoCytomation, Glostrup, Denmark) staining was performed for optimal assessment of the number of intra-epithelial lymphocytes (IEL). Duodenal and small bowel biopsies were evaluated according to the modified Marsh criteria.[35] Tissue samples from suspicious lesions were assessed for the presence of EATL according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[23]

Immunophenotyping of IEL

IEL's were isolated from 3 duodenal biopsies by passing them through nylon filters (1x100µm, 1x 40µm, BD Falcon). Cells were stained with fluorescent labeled monoclonal antibodies to CD3, 7, 8, 45, 103, and TCRγδ, as well as with relevant isotype controls.

All monoclonal antibodies were from BD Falcon (BD biosciences, CA USA), except for CD103, which was from IQ-products, Groningen, The Netherlands) and analyzed by 4-color flow-cytometry (FACS-Calibur, BD). Leucocyte common antigen (CD45) was always included to identify the lymphocyte population. In some tubes cell surface CD3 staining (anti-CD3-APC) was followed by permeabilization (Cytofix /cytoperm, BD Biosciences Pharmingen, CA USA) and subsequent cytoplasmic staining with anti-CD3-FITC or isotype control.

Aberrant T-cells were defined either as $CD7^+$ surface $CD3^-$ cells (expressed as % of $CD103^+$ lymphocytes) or as cytoplasmic $CD3^+$, surface CD3 negative cells (expressed as % of $CD103^+$ lymphocytes).[36, 37]

All flow-cytometry analyses were performed by an analyst and interpreted by the same medical immunologist, while histopathology was performed by the same pathologist to ensure uniformity, reproducibility and consistency of results.

Assessment of TCR gene rearrangement by Polymerase Chain Reaction (PCR)

T-cell receptor-gamma (TCR-γ) gene rearrangements studies were performed in separate three-four duodenal specimens that were preserved on histocon and frozen at -20°C. DNA was extracted from cryosections of duodenal specimens by a standard procedure using proteinase-K digestion and ethanol precipitation of the genomic DNA. TCR-γ gene rearrangements were analyzed by multiplex polymerase chain reaction (PCR) amplification under standardized conditions. A monoclonal and polyclonal control was included in each experiment. Clonality assessment for TCR-γ gene rearrangements was done using the BIOMED-2 multiplex TCR PCR protocol.

Informed consent

Informed consent was obtained from all patients after receiving information about the experimental nature of the procedure and its risks according to institutional review board guidelines.

Statistical analysis

Results are presented in median (range) for continuous data and in frequencies (percentages) for categorical data, respectively. McNemar's test was used to compare histology between different small bowel segments in the same patient. A p value of less than 0.05 was considered to be statistically significant.

All statistical analysis was performed using the Statistical Software Package version 11.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Procedure characteristics

Twenty-four double-balloon enteroscopy procedures were successfully performed in 21 patients. No major procedure-related complication occurred. Self-resolving abdominal pain was observed following the double-balloon enteroscopy procedure in six (29%; 95% CI 13 to 49) patients. No relevant technical problems were encountered. The scope could be advanced into the small bowel to a median depth of 310 cm (range 160-440 cm) beyond the pylorus. The median examination time required was 100 minutes (range 30-200 minutes).

Endoscopic and histologic features

Jejunal ulcerations, histology of which revealed the presence of EATL, were found in five of 21 patients (24%). Three of them have already been operated upon for EATL EATL and in two patients the diagnosis was made by double balloon enteroscopy. The endoscopic features of EATL (colour plate A-1) consisted of circumferential, discrete, or confluent ulcerative lesions, which were associated with stenosis in one patient. In three of these patients, Marsh III was found, while Marsh I was found in mucosal biopsies adjacent to EATL ulcerations in the other two patients.

Ulcerative lesions in absence of histological evidence of EATL were found in another two patients (9%) (colour plate A-2). In these two patients, small bowel histology of non-ulcerative mucosa was classified as Marsh III and therefore they were considered to have ulcerative jejunitis.[5]Table 2 summarizes the clinical, endoscopic and histologic features of patients with coeliac disease complicated by development of EATL or ulcerative jejunitis.

In the remaining 14 patients (66%; 95% CI 45 to 82), low-risk endoscopic features like flattened villi, loss of folds, scalloping, and nodularity were found by double balloon enteroscopy (colour plate A-3). Table 3 summarizes the clinical characteristics, proportions (average) of aberrant T-lymphocytes ($CD7^+sCD3^-CD103^+$ and $CD7^+sCD3^-cytCD3^+$), endoscopic and histologic features of these patients. These 14 patients were considered to suffer from refractory coeliac disease according to the currently accepted definition since persistent villous atrophy despite gluten free diet for at least 12 months was documented in duodenal biopsies.[4]

Small bowel histology revealed Marsh III in all 14 patients, while eight of them had the same changes more distally. Figure 4 illustrates that 19 patients (90%) of all included had villous atrophy in proximal bowel segments while only 13 patients (62%) had villous atrophy in distal bowel segments (p= 0.031; odds ratio 0.17, 95% CI 0.03 to 0.93). Whitish villi were observed in three patients. Extensive histological examination of these lesions revealed mucosal changes characterized by the presence of dilated lymph channels in the villi with different levels of inflammation (Marsh I-III) and the absence of EATL.

Gastroduodenoscopy could demonstrate low-risk lesions in duodenum, but could neither detect EATL nor ulcerative jejunitis in any patient.

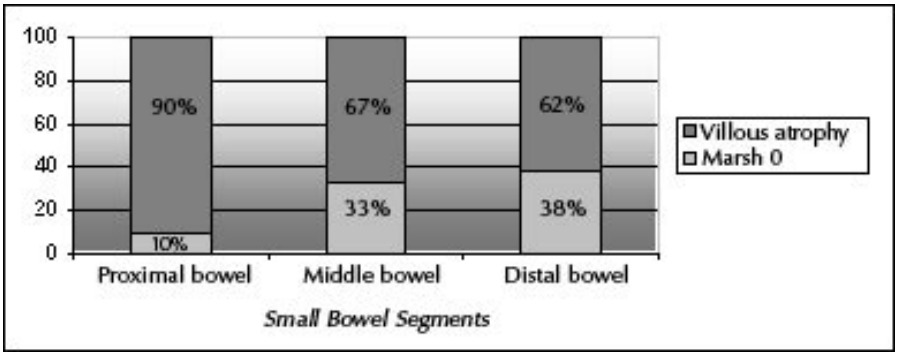

Figure 4. Small bowel histology from proximal, middle and distal bowel segment biopsies taken by double-balloon endoscopy in 21 patients with coeliac disease.

Table 2. Clinical, endoscopic, and histologic features of patients with enteropathy-associated T-cell lymphoma or ulcerative jejunitis.

N	Age	Sex	Diagnosis	Surgery for EATL *	Antibody tests TGA†	EMA‡	HLA-DQ§	FACS || $CD103^+$	$cytCD3^+$	Average	TCR-γ ¶	Duodenal histology`	CT-scan findings**	Double-balloon enteroscopy findings	Small bowel histology†† Proximal	Middle	Distal
1	65	M	EATL	Yes (5 m)	-	-	DQ2	12	7	9	Positive	IIIa	Normal	Ulcerations	I	I	I
3	89	M	EATL	No	-	-	DQ2	28	27	27	Positive	IIIa	Abnormal	Ulcerations	I	I	I
4	58	F	EATL	No	-	-	DQ2	27	25	26	Positive	IIIa	Abnormal	Loss of folds, scalloping, nodularity, ulcerations, and stenosis	IIIa	IIIa	IIIa
5	67	M	EATL	Yes (3 m)	+	+	DQ2	10	8	9	Positive	IIIa	Abnormal	Loss of folds, scalloping, nodularity, and ulcerations	IIIb	IIIa	IIIa
2	74	F	EATL	Yes (3 m)	-	-	DQ2	47	30	38	Positive	IIIb	Abnormal	Loss of folds, scalloping, nodularity, and ulcerations	IIIb	IIIa	IIIa
6	61	M	Ulcerative jejunitis	No	+	-	DQ2	4	4	4	Positive	IIIa	Normal	Loss of folds, scalloping, nodularity, visible vessels, and ulcerations	IIIa	IIIa	IIIa
7	50	M	Ulcerative jejunitis	No	-	-	DQ2	30	27	29	Positive	IIIa	Normal	Loss of folds, scalloping, nodularity, ulcerations, stenosis and prestenotic dilation	IIIa	IIIa	IIIa

* Entropathy-associated T-cell lymphoma. Number of months

anti-endomysium antibodies. Positive + , negative -, § Human leukocyte antigen-DQ2 &/or -DQ8. || T-cell flow cytometry showing the percentage of T-lymphocytes expressing $CD7^+sCD3^-$ $CD103^+$, $CD7^+sCD3^-$ $cytCD3^+$, and average of both subgroups respectively. ¶ Clonality assessment for TCR-γ gene rearrangements. ` Duodenal biopsy samples were taken during upper gastrointestinal endoscopy for histological assessment according to the modified Marsh classification. ** Abdominal computerized tomography. †† small bowel biopsy samples were taken during double-balloon enteroscopy for histological assessment according to the modified Marsh classification.

Table 3. Clinical, endoscopic, and histologic features of patients with refractory coeliac disease.

			Surgery for	Antibody tests			FAC S ||				Duodenal	CT-scan	Double-balloon	Small bowel histology††		
N	Age	Sex	EATL *	TGA	EMA	HLA-DQ	$CD103^+$ $cytCD3^+$		Average	TCR-γ¶	histology`	findings**	enteroscopy findings	Proximal	Middle	Distal
2	50	F	No	+	+	DQ2	5	4	4	Negative	IIIb	ND¶	Loss of folds, scalloping, and nodularity	IIIa	IIIa	IIIa
3	42	M	No	+	+	DQ2	2	1	1	Negative	IIIa	ND	Loss of folds and scalloping	IIIa	IIIa	IIIa
4	57	F	No	-	-	DQ2	2	1	1	Negative	IIIa	ND	Loss of folds and scalloping	IIIa	I	0
7	69	F	No	+	+	DQ2	2	1	1	Negative	IIIa	Abnormal	Loss of folds, scalloping, and nodularity	IIIa	IIIa	IIIa
5	67	F	No	+	+	DQ2	3	1	2	Negative	IIIb	Normal	Loss of folds, scalloping, and nodularity	IIIb	I	I
6	64	F	No	-	-	DQ2DQ8	13	18	16	Positive	IIIa	Abnormal	Loss of folds, scalloping, and nodularity	IIIa	IIIa	0
1	62	M	No	-	-	DQ2	58	70	65	Positive	IIIb	Normal	Loss of folds, scalloping, nodularity, visible vessels,and erosions	IIIb	IIIa	IIIa
8	64	F	No	+	-	DQ2	42	61	52	Positive	IIIb	Abnormal	Loss of folds, scalloping,nodularity, and visible vessels,	IIIa	IIIa	IIIa
9	54	F	No	-	-	DQ2	68	70	69	Positive	IIIa	Abnormal	Loss of folds and scalloping	IIIa	II	I
10	61	M	No	-	+	DQ2	54	42	48	Positive	IIIb	ND	Loss of folds, scalloping, and nodularity	IIIb	IIIa	IIIa
11	68	F	Yes	-	-	DQ2	3	2	2	Negative	IIIa	ND	Loss of folds and scalloping	IIIa	I	I
12	69	M	Yes	-	-	DQ2	51	50	50	Negative	IIIb	ND	Loss of folds and scalloping	IIIa	I	0
13	65	M	Yes	-	-	DQ2	41	49	45	Positive	IIIc	ND	Loss of folds, scalloping, and nodularity	IIIb	IIIa	IIIa
14	65	M	Yes	+	+	DQ2	55	30	40	Positive	IIIb	Normal	Loss of folds, scalloping, and nodularity	IIIa	IIIa	IIIa

*Entropathy-associated T-cell lymphoma. Months before undergoing double-balloon enteroscopy are indicated in parenthesis. || T-cell flow cytometry showing the percentage of T-lymphocytes expressing $CD7^+sCD3^-$ $CD103^+$, $CD7^+sCD3^-$ $cytCD3^+$, and average of both subgroups respectively. ¶ Clonality assessment for TCR-γ gene rearrangements. ` Duodenal biopsy samples were taken during upper gastrointes . †† small bowel biopsy for histological assessment according to the modified Marsh classification. ND = not determind.

Abdominal CT-scan was abnormal in four patients with EATL, but was normal in one patient with EATL and both patients with ulcerative jejunitis. Moreover, CT scan and video capsule endoscopy have suggested the presence of EATL in another 4 of 7 and one in 7 respectively. Double-balloon enteroscopy, however, did not find high-risk lesions in these specific patients. After a median interval of 36 months of follow-up, no evidence of lymphoma appeared in these patients. Video capsule endoscopy showed low-risk lesions like scalloping or nodularity, as reported previously [38], in the other six patients.

Double-balloon enteroscopy was repeated in two patients after three chemotherapy courses (standard CHOP-therapy). These patients had initially EATL diagnosed during double-balloon enteroscopy examination. In one patient previously documented ulcerative EATL lesions had disappeared while in the other patient ulcerative EATL lesions remained unchanged. Double-balloon endoscopy was further repeated in one patient with ulcerative jejunitis, stenosis, and prestenotic intestinal dilatation (80-150 cm distal to pylorus) in order to exclude the presence of EATL. This patient underwent surgical resection of stenotic segments and subsequently high dose chemotherapy followed by autologous peripheral stem cell transplantation. He showed an impressive clinical, histological an immunological recovery.[38]

Immunophenotyping of intraepithelial lymphocytes and TCR gene rearrangement

All patients with RCD had significantly high percentage of aberrant T-cells (ranging from 25-80%) at the time of diagnosis of the refractory state. In addition, all patients had a clonal rearrangement of the TCRγ gene.

Discussion

Small bowel examination by double-balloon enteroscopy in patients with coeliac disease established the diagnosis of ulcerative jejunitis in two patients and ulcerative EATL lesions in five. In 2 of these 5 patients the diagnosis was made by double-balloon enteroscopy. Due to its limitations in introducing the endoscope beyond the ligament of Treitz, the usual standard gastroduodenoscopy failed to diagnose those patients. Moreover, double-balloon enteroscopy, with the help of tissue sampling, could adequately exclude the presence of EATL that was suggested by abdominal CT scan in 4 patients and by video capsule endoscopy in one. Small bowel endoscopy seems to be valuable even in patients who are already known to have EATL especially when therapeutic options are considered or effect of surgery and/or chemotherapy requires evaluation. Furthermore, localizing and marking with ink can be indispensable to help the surgeon to plan and perform partial small bowel resection. All three patients who were previously operated for small bowel EATL were destined to receive additional chemotherapy upon the exclusion of other EATL lesions in the small bowel by double-balloon enteroscopy.

Contrary to the low-risk lesions of coeliac disease, ulcerations are more severe lesions that can harbor EATL [5]. EATL usually affects the jejunum and grossly presents as multiple ulcers without the formation of definite tumor masses. Double-balloon enteroscopy is suitable examination for patients with refractory coeliac disease in order to detect multifocal or

distally located lesions because ulcers are more commonly located in the jejunum [5], and the ileum rather than the duodenum.[40]

Remarkably, EATL has been found in small bowel segments next to mucosa with normal villous/crypt ratio and lymphocytic intraepithelial infiltrate in two patients (Marsh I). In most cases, the changes in the histology of the small bowel remote from the site of the tumor are identical to those of coeliac disease with villous atrophy, crypt hyperplasia, and increase in intraepithelial lymphocytes.[36, 40-42] In some cases of coeliac disease, the mucosal changes are much less severe. The villous architecture may be normal or near-normal and the only hint of coeliac disease is an increase in intraepithelial lymphocytes.[42] This finding, previously thought to argue against a strict association of coeliac disease and EATL, indicates that Marsh I can be a premalignant condition witout interim progression through Marsh III.

Some authors showed agreement between histological findings of duodenal biopsies as compared with jejunal biopsies [41] while others have demonstrated that histology could reveal more severe mucosal changes in jejunal biopsies as compared with those of duodenal biopsies.[13] Although the length of the small bowel being examined was not equal in all patients, Marsh III was in this series more frequently found in proximal than distal small bowel biopsies in agreement with earlier report.[5] It is unclear if the five patients who fulfilled the definition criteria of refractory coeliac disease and yet showed signs of histological recovery in deeper segments of the small bowel virtually suffer from refractory coeliac disease or simply represent cases of slow recovery. The variability in histology of small bowel segments in several patients, who according to current definition were considered to have refractory coeliac disease, and the variability phenotypes of intraepithelial T-lymphocytes immunophenotypes among patients with ulcerative jejunitis, EATL, or refractory coeliac disease necessitate a consensus to define refractory coeliac disease and to define the value of T-cell flow cytometry in the management of patients with refractory coeliac disease. Such a consensus is required especially when new treatment lines are to be investigated.

Abdominal CT scan has shown shown limited effectiveness in detecting ulcerative jejunitis and EATL in patients with refractory coeliac disease or assessing the tumor load in affected patients [44, 45] because they develop initially as mucosal lesions that only in a later stage proceed into disseminated lymphoma with mass lesions. 18F-FDG PET scans, however, has been demonstrated higher sensitivity and specificity for detection of EATL compared to CT scanning technique.[46]

Push enteroscopy, a known widely available endoscopical method of the proximal small bowel [47], can be assumed to have identified most or all the lesions found by double-balloon enteroscopy in this series. However, it has recently been shown that double-balloon enteroscopy is superior to push enteroscopy in patients with suspected mid-gastrointestinal bleeding with regard to the length of small bowel visualized, as well as the diagnostic yield.[48] Complications such as perforation [49], mucosal stripping [48, 50], or pancreatitis [51] have been reported to occur in 0.6-2% of patients undergoing push enteroscopy.[47, 52] For patients undergoing double-balloon enteroscopy, a risk of perforation and pancreatitis has been reported in 1%, and 1.1% respectively.[8, 21] Fortunately, no such complications were encountered in any of the double-balloon enteroscopy procedures in the present series despite

the high propensity for perforation at presentation in EATL.[41] Therefore, the invasive nature of push enteroscopy and the restricted length of the proximal small bowel being examined (70 cm with push enteroscopy [12, 53] versus 310 cm with double-balloon enteroscopy in this series) disqualify this procedure as first method of choice when double-balloon enteroscopy is available.

Full-length small bowel examination in patients with coeliac disease can currently be achieved by video capsule endoscopy.[54] Moreover, the value of video capsule endoscopy has recently been reported in seven patients with small bowel lymphoma.[55]Villous atrophy, erythema, and whitish villi were described as early manifestations of small bowel lymphoma in this series.[55] However, these conclusions were based on interpretations of macroscopic images obtained by video capsule endoscopy. In our series, the histological examination was the only reliable means to verify the nature of these indicative, but non-pathognomonic, endoscopic features. Screening patients with refractory coeliac disease by video capsule endsocopy followed by double-balloon enteroscopy for abnormal findings may be a reasonable and patient-friendly approach that warrants further investigation. Finally, laparoscopy is preserved for obtaining full wall thickness biopsy of small intestine or mesenteric lymph nodes resection when they appear suspicious on imaging studies. Double-balloon enteroscopy, however, that can examine deeper segments of the small intestine in a relatively safe and minimally invasive nature with associated morbidity of approximately 1% [21], should be considered before open surgery and intraoperative endoscopy.

Ulcerative jejunitis is considered as a premalignant condition due to the demonstration of TCRγ monoclonality in the ulcers and intervening mucosa in ulcerative jejunitis in the absence of overt lymphoma [35], while others have demonstrated, by careful histological, immunophenotypical and molecular examination, the presence of T-cell lymphoma in cases of ulcerative jejunitis.[2] As a result, many recommend a common management approach of ulcerative jejunitis like a haematological malignancy that requires to be treated by devised strategies.[58] In this series one patient underwent surgical resection of stenotic segments and subsequently high dose chemotherapy followed by autologous peripheral stem cell transplantation. He showed an impressive clinical, histological an immunological recovery.[38]

With respect to our previous report describing the broader use of double-balloon enteroscopy in a variety of situations, there was no difference with the technical details.[21] However, more patients were considered to have ulcerative jejunitis due to the inclusion of patients with ulcers less than 5 mm. Furthermore, one patient with T-cell lymphoma was excluded from this series because the diagnostic work-up revealed the presence of normal small bowel histology and absence of the coeliac specific antibodies and HLA-DQ heterodimers.

This study is the largest series of patients with refractory coeliac disease being investigated by a new method of small bowel endoscopy for detection or exclusion of EATL. The relatively limited number of patients, the patient selection in this referral center and the extended but not complete enteroscopy in most studied patients may be considered confounding factors to the main outcome. However, the prospective nature of the study validates to our opinion the reported results.

The present study indicates that direct visualization of the small bowel and obtaining biopsy specimens for histological examination during double-balloon endoscopy in patients with refractory coeliac disease can efficiently detect or exclude complications like ulcerative jejunitis or EATL.

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Abdominal Computed Tomography in Refractory Coeliac Disease and Enteropathy Associated T-cell Lymphoma

6

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Abstract

Background: Adult onset coeliac disease (CD) has a high risk of developing refractory CD and enteropathy associated T-cell lymphoma (EATL).
Aim: We evaluated CT findings, useful to suggest the presence of RCD and EATL.
Design and Methods: CD patients were divided into two groups. Group I: uncomplicated CD (n=14) and RCD type I (n=10). Group II: RCD type II (n=15) and EATL (n=7).
Results: Both groups showed classic signs of CD on CT. Intussusception was seen in 1 patient in group I vs. 5 in group II (p=0.06). Lymphadenopathy was seen in 5 patients in group II vs. no patients in group I (p=0.01). Increased number of small mesenteric vessels was noted in 20 patients in group I vs. 11 in group II (p=0.02). Eleven patients (50%) in group II had a splenic volume < 122 cm3 vs. 4 in group I (14%), 10 patients in group I had a splenic volume > 196 cm3 (66.7%) vs. 5 in group II (33.3%) p=0.028.
Conclusions: CT scan is a useful tool in discriminating between CD and (pre)EATL. RCD II and EATL showed more bowel wall thickening, lymphadenopathy and intussusception, less increase in number of small mesenteric vessels and a smaller spleen ompared with CD and RCD I.

Introduction

Coeliac disease (CD) is one of the most common immunologically mediated gastrointestinal diseases. The prevalence varies between approximately 1:100 and 1:300 worldwide. Refractory coeliac disease (RCD) is considered when patients show persistent or relapsing symptoms and villous atrophy despite adherence to a GFD, especially those over the age of 50 years.[1] Two forms of RCD can be discriminated, RCD I which is defined as RCD with normal intra-epithelial T lymphocytes (IEL's) in intestinal biopsies and RCD II defined as RCD with aberrant IEL's.[2, 3] In RCD enteropathy associated T-cell Lymphoma (EATL) can evolve with a 20 time higher relative risk compared to the general population.[4, 5, 6] Therefore it is necessary to be able to discriminate uncomplicated CD from its malignant complications.
Computed tomography (CT) is one of the first radiological examinations performed for different indications in patients with CD, especially those with RCD to exclude malignancy. A variety of findings like jenunoileal fold pattern reversal [7], small bowel intussusception 8 and (benign) mesenteric lymphadenopathy [9] have been recognized on CT images in patients with CD. However, no discriminating or specific CT signs have been recognized and described regarding RCD II or EATL. Therefore, we evaluated both the spectrum of abdominal CT findings, useful for suggesting CD and those findings which might be useful to suggest the presence of EATL in adult coeliac patients

Patients and methods

Between January 2003 and January 2005 a total of 46 patients (18 M, 28 F; mean age 58 years, range 18-88 years) with proven CD according to UEGW criteria (2001), were enrolled. All included patients were previously diagnosed as having CD, RCD I, RCD II or EATL by clinical evaluation, serology and intestinal biopsy.10 Patients were divided into two groups: Group I consisted of 24 patients with uncomplicated CD (n=14) and RCD type I (n=10), group II consisted of 22 patients with RCD type II (n=15) and EATL (n=7).

The indications for CT scan were assessment of unexplained recurrent abdominal complaints and/or suspicion of EATL. After overnight fasting, examinations were performed either on a 4-detector (Somatom 'Volume Zoom', Siemens, Erlangen, Germany) or on a 64-detector ('Sensation 64', Siemens, Erlangen, Germany) CT scanner using either a 2.5 mm or a 0.6 mm collimation, reconstructed in 5 mm contiguous axial slices. Forty-three out of 46 patients received an orally administered diluted solution of barium sulphate suspension (46 mg/g, 49 mg/ml, 900 ml E-Z-CAT, E-Z-EM Canada Inc, Montreal Canada) divided into two doses (450 ml), the night before and the morning of the investigation. Forthy five minutes prior to imaging, patients received an extra 300-500 ml oral contrast. Because of severe abdominal symptoms or refusal of contrast, 3/46 patients did not receive oral contrast. Intravenous non-ionic contrast (Ultravist, Iopromide (300 ml/l), Schering, Berlin, Germany) was administered in 42/46 patients (2 patients refused intravenous contrast and 2 patients supposed to be allergic to contrast) at an injection rate of 3 ml/s (maximum total amount of 100 ml, depending on body weight) with CT acquisition after 70 seconds.

The following CT findings were evaluated: (a) abnormalities of intestinal fold pattern, (b) bowel dilatation, (c) air excess, (d) fluid excess, (f) bowel wall thickening, (g) intestinal intussusception, (h) ascites, (i) lymphadenopathy, (j) increased number of lymph nodes, (k) mesenteric vascular changes, (l) splenic size and (m) splenic vein thrombosis.

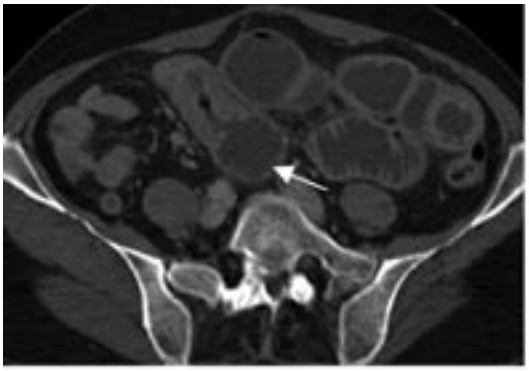
Abnormalities of the intestinal fold pattern were defined quantitatively as a decreased number of jejunal folds and/or an increased number ('jejunization') of ileal folds, measured as the mean number of folds per 2.5 cm in three segments at different locations.[7] Less than 4 jejunal folds per 2.5 cm were considered to be decreased and more than 4 ileal folds per 2.5 cm were considered to be increased.[7] The presence of an equal number of intestinal folds in ileum and jejunum (ileum/jejunum fold ratio ≥1) was defined as 'jejunoileal fold pattern reversal' (JFPR).[11, 12, 13] In cases of doubt, abdominal loops in the left upper quadrant were considered to be jejunal and loops in the right lower quadrant were considered to be ileal. Intestinal loops were considered dilated if more than three segments measured equal or more than 3 cm in diameter on transverse images.[14] Fluid excess and air excess were scored directly in patients with dilated intestinal loops on a Likert scale (none/mild/moderate/severe). Fluid excess was indirectly assessed by dilution of oral contrast (flocculation). Air excess was scored as present in case more than 3 segments were dilated with air. The bowel wall was considered thickened when it measured more than 3 mm in the transverse plane of a fully distended loop.[15] Intussusception was denoted as a target mass or as a more complex layered mass within the bowel lumen.[16] Lymph node enlargement was considered present if

nodes measured greater than 1 cm in diameter in their shortest axis. The number of lymph nodes within the mesenterium was scored on a Likert scale (none/mild/moderate/severe). Cavitation of nodes was present by showing a low-density center within the lymph node. Ascites was evaluated by visual inspection. Increase in splanchnic circulation was scored as the transverse diameter 2-3 cm caudal of the origin of the superior mesenteric artery. Also an increase of number of small vessels within the mesenterium was noted on a Likert scale (none/mild/moderate/severe). Splenic volume was calculated by the following formula; (30+0.58*(length x width x height). The longest axis in the transversal plane is considered as length, the perpendicular distance is considered the width and the longest cranio-caudal distance is considered as the height of the spleen.[17] Splenic vein thrombosis was scored visually when a mass was noted within the lumen of a contrasted splenic vein.

All CT scans were analyzed by two dedicated radiologists in consensus (MM and JHvW). Informed consent was obtained from all the patients who participated in this study. All procedures followed in this study were in accordance with the standards of the institutional ethical committee.

Statistical analysis

Student's paired t-test, Mann-Whitney, or Fisher's exact test was used for data analysis when indicated. P values of less than 0.05 were considered to be statistically significant. All statistical analysis was performed using the Statistical Software Package version 11.0 (SPSS Inc., Chicago, Illinois, USA).



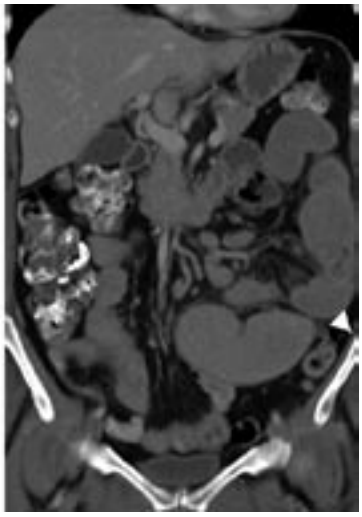


Figure 1a. Axial CT image demonstrates EATL (arrow) and prestenotic dilation of jejunal loops. **Figure 1b.** Coronal reformation demon-strates dilated jejunum with infiltrated mesenterial fat (*) and multiple enlarged lymph nodes (arowheads)

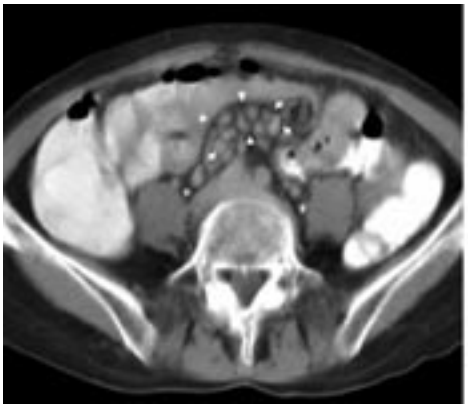


Figure 2a. Transverse CT images of a RCD I patient demonstrate multiple small lymph nodes (arrowheads).

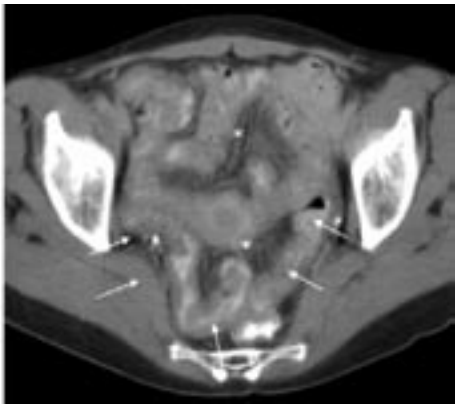


Figure 2b. Transverse CT images in same patient. Thickening of the ileal wall (arrows) with infiltration of the mesenteric fat (*).

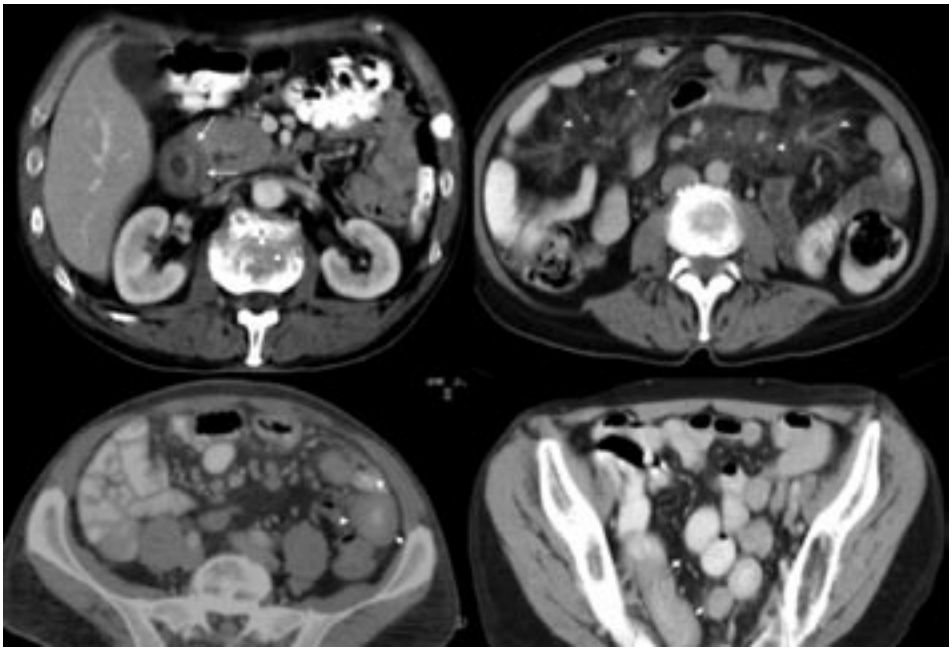
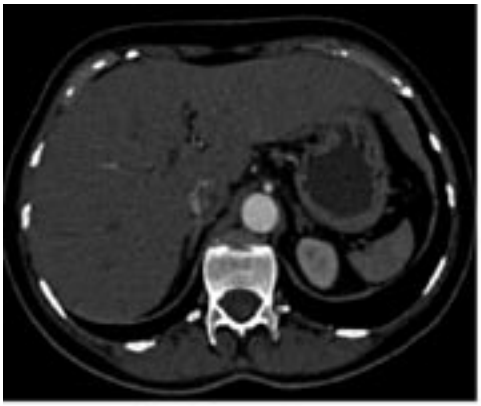


Figure 3a-d. Axial CT images demonstrating abdominal EATL location. **Figure 3a.** duodenal localization of lymphoma (arrow). **Figure 3b.** Mesenterial localization showing "misty mesenterium" (*) and multiple lymph nodes **Figure 3c.** Jejunal localization of lymphoma (arrowhead) and multiple lymph nodes. **Figure 3d.** Ileal localization (arrowhead).



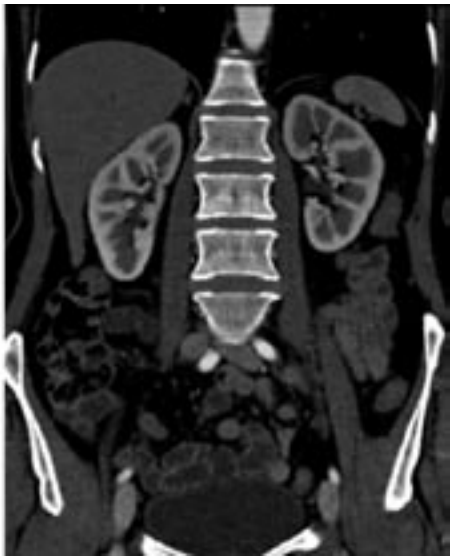
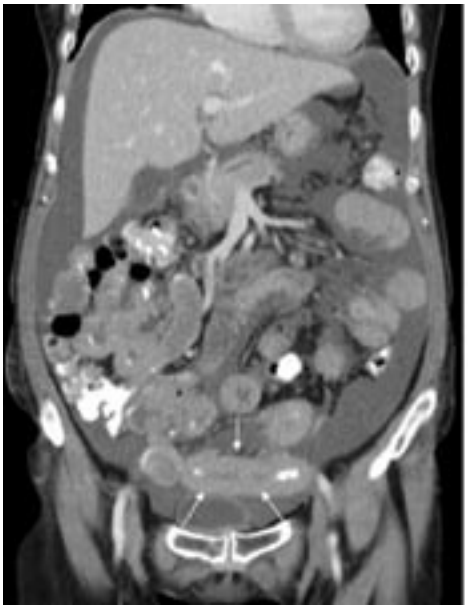


Figure 4a & b. Hyposplenism in RCD I patient demonstrated on axial image and coronal reconstruction.



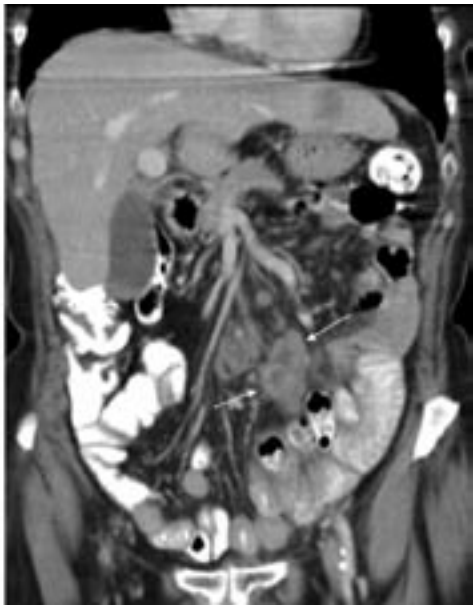


Figure 5a. Coronal reconstructed image of RCD II patient demonstrating ascites and thickening of ileal loops (arrows). **Figure 5b.** Coronal reconstruction in (other) RCD II patient showing cavitation of lymph nodes and smaller non cavitated lymph nodes in combination with infiltrated mesenterial fat (arrows).

Results

CT findings of all patients included in the study are summarized in Table 1. Because of lack of intraluminal contrast or lack of distension of the small bowel loops, jejunal fold pattern could only be analyzed in 26 patients whereas ileal fold pattern could be assessed in 29 patients. Ten out of 26 (38%) patients showed a decreased number of jejunal folds, 16 (62 %) showed an increased number of folds. Ileal folds were found increased in 5 out of 29 (17%) patients and decreased in 24 (83%) patients. No significant difference was found in JFPR between who both groups. Small bowel dilatation ranged from 30-35 mm and was found in 11/46 patients in total (p=ns). Excess of air was not visible in 24/46 patients, mild in 13 patients, moderate in 7 patients and severe in 2 patients in total. Fluid excess and flocculation were scored as none in 20 (43 %) patients, mild in 8 (17 %) patients, moderate in 12 (26 %) patients and severe in 6 (13 %) patients. All findings were equally distributed between the groups. Increased wall thickness ranged from 4 to 11 mm in group I (mean 7.7 mm, median 7 mm) and from 5 to 15 mm (mean: 9.6 mm, median 10 mm) in group II. Nine patients in group II showed a thickness of more than one cm versus only 4 in group I (p=ns). Intussusception was observed in only 1 patient in group I, compared to 5 patients in group II (p=0.06). Only one patient (RCD II, 67 years old male) showed a small amount of intra-abdominal fluid in the rectovesical pouch.

Table 1. CT findings according to the groups. All signs are described by the number of patients affected and mentioned otherwise in case of deviation

CT findings	CD & RCDI	RCD II & EATL	Total
Number of patients	24	22	46
Gender (F;M)	18;6	10;12	28;18
Mean age (years)	56	61	58
Jejunal/ileal fold ratio (No of folds/2.5cm)	4.5/3.0	3.7/2.9	4.1/3.0
JFPR	3	6	9
Bowel dilatation	5	6	11
Air excess	14	8	22
Fluid excess	12	14	26
Increased wall thickness	10	14	24
Intussusception	1	5	6
Ascites	0	1	1
Lymphadenopathy	0	5	5
Increased No of lymph nodes	16	12	28
Lymph node cavitation	0	1	1
Increased splanchnic circulation	20	11	31
Splenic vein thrombosis	0	4	4

Enlarged lymph nodes were only found in 5 patients in group II (p = 0.013). Both groups showed an increase in non-enlarged lymph nodes (p=0.295). Only one lymph node showed cavitation (59 year old male with RCD II).
An increase in the number of small mesenterial vessels was observed in 20/24 (83%) patients in group I vs. 11/22 (50%) patients in group II (p=0.02). The diameter of the superior mesenteric artery was measured in a total of 23 patients and this varied from 4-7 mm.
Splenic volume of all patients ranged from 37-321 cm^3 (mean 162 cm^3) in normal distribution. No significant differences were found between mean volumes in both groups I and II (178 cm^3 vs. 144 cm^3). However, after allocating the patients into 3 arbitrary groups according to the splenic volume as shown in table 2, the RCD II and EATL group showed significant more patients with a smaller spleen than RCD I and uncomplicated CD (p = 0.028).

Table 2. Splenic volume according to the groups

Splenic volume (cm^3)	CD & RCDI	RCD II &EATL	All
Group A: 37-122 cm^3 (n=15)	4 (27%)w	11 (73%)	15
Group B: 124-196 cm^3 (n=16)	10 (63%)	6 (38%)	16
Group C: 196-321 cm^3 (n=15)	10 (67%)	5 (33%)	15
Mean volume (cm^3)	178	144	162

Discussion

In patients clinically suspected of having CD, biopsies are mandatory to confirm or exclude the diagnosis.[18] In uncomplicated cases, radiological examination is not required. However, in clinical practice pre-malignant and malignant complications of CD have to be excluded in patients who have persistent complaints despite strict adherence to a GFD. Furthermore CT, performed in patients presenting with atypical abdominal symptoms, can suggest a diagnosis of CD.[13] The most striking features found in CD are jejunoileal fold pattern reversal, small bowel intussusception, and benign mesenteric lymphadenopathy.[7, 8, 9, 12, 13] However, to our knowledge, no discriminating findings between CD/RCD I and (pre)EATL have been decribed using CT.
Regarding jejunal and ileal fold abnormalities; especially jejunoileal fold pattern reversal and total loss of jejunal folds may be considered specific findings in CD.[7, 12, 13] In our study, only in 52% (24 out 46 patients) both jejunal and ileal folds could be assessed because of lack of contrast or lack in distention of small intestinal loops, probably due to suboptimal bowel preparation because of progressive abdominal complaints. In only 9 out 24 (38%) patients a jejunoileal fold pattern reversal was observed. This is a low percentage compared to that reported by Tomei et al[7], however we included a high percentage of patients with RCD I, RCD II and EATL. Furthermore, increased ileal folds, decreased jejunal folds, and jejunoileal fold pattern reversal were equally distributed between the subgroups, which demonstrate that the number of folds is not a good discriminator between both groups.

Transient intussusception of the small bowel was present in 5 patients in group II compared to one patient in group I (p=0.06). The majority of patients showed an increase in the number of nodes (<1 cm), which was not significantly unequally distributed between both groups CD. However, mesenteric lymphadenopathy (short axis > 1 cm) was only found in the (pre) EATL group, whereas cavitation, which is considered to be a rare complication associated with a poor outcome [22, 23, 24], was found in one patient with RCDII. In this patient additional examinations, including 18F-FDG-PET scan and laparascopic mesenteric lymph nodes resection, did not show any evidence of EATL.

Regarding non-specific signs, bowel dilatation and excess of fluid (with flocculation of contrast) and air, [13,19] bowel dilatation and increased splanchnic circulation, as measured using the diameter of the superior mesenteric artery 2-3 cm distal to its origo, we found no significant differences between the two subgroups. However mesenteric vascularity, as measured using a semi-quantative scale, was significantly increased in group I. We hypothesize that this increase of small vessels and small lymph nodes in group I may be due to the acute inflammatory process in this group. Also no significant difference in number of patients with increased wall thickness was found. However more patients in group II showed a wall thickness of more than 1 cm (p = ns).

Splenic atrophy occurs frequently in patients with CD and is related to the severity of the disease and degree of dietary control and shows a significant correlation with an impaired function with the incidence rising with increasing age of starting treatment.[26] Although no correlation was observed in literature between splenic size and the duration of the GFD as well as the percentage of splenic size recovery after gluten withdrawal, hyposplenism in adult CD was improved by a GFD.[27] Furthermore regarding group II, hyposplenism was not related to the development of malignant disease in small samples.[28] In this study however, significantly more patients in the RCD II/EATL group showed a smaller splenic size.

In conclusion, both groups showed classic signs of CD on CT. Though small groups were analysed, group II showed more bowel wall thickening, lymphadenopathy, intussusception and more hyposplenism and less increase in splanchnic circulation than group I. Therefore, we conclude that bowel wall thickening, lymphadenopathy, intussusception and hyposplenism should raise suspicion for RCD II and the development of EATL

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NEW THERAPIES III

Cladribine therapy in Refractory Coeliac Disease with aberrant T-cells

7

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Abstract

Background: Refractory Coeliac Disease (RCD) may be subdivided into RCD-I and type II with phenotypically normal and aberrant intraepithelial T-cell population respectively. In RCD-II transition into Enteropathy Associated T-cell Lymphoma (EATL) is frequently seen. We have evaluated the effect of cladribine (2-CDA), a purine analogue inducing T-cell depletion, on clinical, histopathological and immunological parameters as well as the toxicity and side effects in a group of RCD II patients.

Methods: Between 2000 and 2005, 17 patients were included (8 Males/9 Females). All patients had a clonal rearrangement of the TCRγ gene and immunophenotyping showed an aberrant T-cell population lacking surface expression of CD3, CD8 and TCR-αβ, in the presence of expression of surface CD103 and intracytoplasmic CD3. Treatment consisted of 2-CDA (0,1 mg/kg/day) intravenously for 5 days, given in 1-3 courses every 6 months depending on the response.

Results: All patients tolerated 2-CDA without serious side effects. Six patients (35.8%) showed a clinical improvement (weight gain, improvement of diarrhoea and hypoalbuminemia). In 10 patients (58.8%) a significant histological improvement and in 6 patients (35.2%) a significant decrease in aberrant T-cells was seen. Seven patients (41.1%) developed EATL and died subsequently. One patient died from progressive refractory state with emaciation.

Conclusions: 2-CDA-treatment in RCD type II is feasible, well tolerated and can induce clinical and histological improvement as well as a significant decrease of aberrant T-cells in a subgroup of patients, albeit does not prevent EATL-development. However, the earlier reported potential risk of precipitating an overt lymphoma should be taken in consideration.

Introduction

In a subgroup of patients with coeliac disease (CD) the clinical features and histological abnormalities persist or recur after initial improvement despite strict adherence to a gluten free diet (GFD). This syndrome is classified as "Refractory Coeliac Disease" (RCD), when other causes of malabsorption with a flat mucosa, such as tropical sprue, bacterial overgrowth, giardiasis, autoimmune enteropathy, eosinophilic enteritis, hypogammaglobulinemic sprue, intestinal lymphoma and carcinoma are ruled out.[1] The prevalence of RCD is estimated to be 2-5% of all coeliacs diagnosed as adults.[2, 3] In follow-up of these RCD patients a high incidence of enteropathy associated T-cell lymphoma (EATL) is reported (60-80% within 5 years).[4]

The small bowel mucosal inflammation in CD consists of lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte population, hyperplasia of the crypts and atrophy of the villi. In patients with CD there is ample evidence that strict adherence to GFD results in a decrease of chronic intestinal inflammatory activity.[5] In RCD the number of intraepithelial lymphocytes (IEL) is markedly raised and it is from these IEL's that EATL may arise.[6, 7] Immunophenotyping of the IEL's identifies two groups of RCD patients: those with

normal IEL's (RCD I) and those with aberrant monoclonal IEL's (RCD II).[1, 2, 6, 7] The former group seems to have a low incidence of EATL and has a good response to azathioprine/prednisone therapy.[1] The latter group (RCD II) is usually refractory to any known therapy, including azathioprine/prednisone, cyclosporine and IL 10 therapy.[1, 3, 7-11] In our population of RCD II patients the majority of patients developed EATL and died, despite chemotherapy with Cyclophosphamide, Adriamycin, Vincristine and Prednisone (CHOP).[12] Therefore, we evaluate new treatment strategies for these RCD II patients before they develop overt EATL.
Recognizing that some patients with RCD-II, and especially with ulcerative jejunitis are suffering from a low-grade EATL, we propose that these patients need to be treated with cytotoxic chemotherapy. Cladribine (2-Chlorodeoxyadenosine,2-CDA) is a synthetic purine nucleoside with cytotoxic activity. It is probably transformed by deoxycytidine kinase to cladribine triphosphate in cells with high levels of this enzyme, such as lymphocytes and monocytes.[13] Accumulation of intracellular cladribine triphosphate causes cell death. Cladribine is of proven value in the treatment of Hairy Cell Leukaemia.[13] Pathological cells in Hairy Cell Leukaemia are CD103 positive as in T-cells in RCD II.[12] In the past few years clinical trials with 2-CDA have confirmed its effectiveness in selected autoimmune disorders.[14, 15]
2-CDA has a relatively low toxicity profile. Haematological toxicity consists of transient monocytopenia, prolonged and profound lymphopenia (especially of $CD4^-$positive cells), and modest lowering of the platelet count and granulocyte count and of haemoglobin, with development of long-lasting macrocytes.[16]
We evaluated the clinical efficacy and tolerance of 2-CDA in a group of RCD II patients as well as its effect on histopathological and immunological parameters.

Patients and Methods

This study was performed as an open-label prospective phase II pilot study. Between January 2000 and April 2005, 17 patients with RCD II were included (8 males, 9 females, mean age 63.9 years (range 54-76). They were referred for treatment to 2 tertiary referral centres for CD in the Netherlands- the Rijnstate Hospital in Arnhem and the VU University Medical Centre in Amsterdam.

Diagnosis of RCD II and EATL

The diagnosis RCD II was based on clinical relapse or persisting malabsorption after at least a year of strict adherence to GFD and histopathology showing at least partial villous atrophy (Marsh IIIA, B, C, or ulcerative jejunitis) after excluding other causes of villous atrophy.[2, 6, 16]
Immunophenotyping by T-cell flow- cytometry showed aberrant T-cell populations (surface CD3 negative, CD8 negative, CD30 positive and intracytoplasmatic CD3 positivity) of ≥ 25% of IEL.[18, 19, 20] All patients had a clonal rearrangement of the TCRγ gene (see below).
In those patients who developed or died from EATL, the diagnosis was established according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[21]
The immunohistochemical features of EATL are evidence of large or medium size T-cell

proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$. All those who developed EATL had $CD3^+$ $CD8^-$ $CD30^+$ large cell lymphoma.

Inclusion

Patients included were only those with aberrant T-cells ≥ 25% of IEL's. The presence of EATL or other malignancies has been excluded using radiological (small bowel follow through, computed tomography (CT scan) scanning of thorax and abdomen and whole body positron emission tomography (PET scan),[22, 23] endoscopic [upper gastrointestinal endoscopy, video capsule endoscopy (VCE) and/or double-balloon enteroscopy (DBE)], as well as bone marrow aspirates.[16] Those diagnosed in 2003 or earlier had negative small bowel follow through and CT scan (8 patients), while those diagnosed after 2003 had negative CT scan, PET,VCE and DBE (9 patients).

All patients had WHO performance status 0-1.[24] Most of the patients had formerly been treated with prednisone for months before inclusion which was stopped before treatment with 2-CDA. Other immunomodulatory drugs were not allowed.

Exclusion

Patients were excluded: if they had a history of any type of malignant disease; if they were known to have serious cardiovascular, renal disease or active infection; if abnormalities were found on imaging, endoscopy, histology and or bone marrow examination suggestive of malignant lymphoma; when having haemoglobin level < 5.0 mmol/l, WBC < 4000 /mm^3, Platelets < 100,000 /mm^3;female patients who are pregnant or breast feeding; serum anti-HCV positive, serum HBsAg positive, serum anti-HIV positive; cyclosporin therapy in the last 6 months; or the use of any experimental drug within 30 days of study entry.

Assessment

Patients were evaluated two weeks before treatment, including clinical evaluation, laboratory tests, endoscopy, histopathology/ immunophenotyping of IEL, TCR gene rearrangement study, CT scanning of the abdomen and thorax, whole body PET scan and bone marrow aspiration. Body mass index (BMI), haemoglobin (Hb) and albumin were used as clinical malabsorption parameters and evaluated prior to treatment and at weeks 12, 24 and 48. HLA-DQ status of all patients was determined using Polymerase Chain Reaction (PCR) typing method.[25] Similarly, serological tests including anti-endomysium antibodies and anti-tissue transglutaminase (anti-tTG) were performed at weeks 0, 24, 48.

Small bowel biopsies

Upper gastrointestinal endoscopy was performed in all patients. At least 10 duodenal biopsies were taken for histological, immunohistochemical and flow-cytometric examination. Four to six biopsies were fixed and preserved in 10% formalin for histopathological and immunohistochemical evaluation. Three-4 biopsies for TCR gene rearrangement studies were taken separately, preserved on histocon and frozen at -20°C.[26] For immunophenotypical evaluation 3-4 biopsies were taken and preserved in RPMI medium.

Isolation of IEL and cell-staining for immunophenotyping

IEL and enterocytes were isolated from 3-4 small bowel biopsies (SIBs) by homogenising tissue samples and passing fragments through 100µm nylon sew (Becton Dickinson®, Cell strainer) in RPMI medium supplemented with 1% FCS. The released cells were subsequently washed and labelled by 4-color staining for 30 minutes on ice with various combinations of fluoescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein and allophycocyanin labelled monoclonal antibodies against CD3, CD4, CD8, CD7, CD103, CD19, CD16/56, γδTCR and cytoplasmic CD3. The FACS method used in this study was in accordance with the manufacturer's guidelines.

Cell surface immunophenotyping of IEL was performed on a 4 colour FACS Calibur flow cytometer (Becton Dickinson, BD, immunocytometry systems, San Jose, CA). Nonviable cells and debris were excluded based of forward and sideward light scatter properties and a gate on CD45 positive cells was used for selecting lymphocytes from all epithelial cells. Intraepithelial localisation of lymphocytes was confirmed by surface expression of CD103 (αeβ7 integrin, a homing receptor). IEL's were analysed, using CellQuest tm (KS Stat) based on their expression of cell markers: cytoplasmic CD3, surface CD3, CD4, CD7, CD8, CD16/56, CD19, CD103 and TCRγδ on $CD45^{+}$gated IEL's.[18, 19, 20]

Assessment of TCR gene rearrangement by Polymerase Chain Reaction (PCR)

DNA was extracted from cryosections of duodenal biopsies by a standard procedure using proteinase-K digestion and ethanol precipitation of the genomic DNA. T-cell receptor (TCR-γ) gene rearrangements were analysed by multiplex PCR amplification under standarized conditions. Monoclonal and polyclonal controls were included in each experiment. Clonality assessment for TCR-γ gene rearrangements was done using the BIOMED-2 multiplex TCR PCR protocol.[26-30]

Study protocol

Patients were hospitalized, and 2-CdA was given (0,1 mg/kg) intravenously for 2 hours daily for 5 days. Cotrimoxazole, 960 mg (gluten free suspension) orally two times daily and for two days a week starting at week 0 until 6 months after 2-CDA therapy, was used as prophylaxis against pneumocystis pneumonia. When indicated, patients had supplemental folic acid, vitamin B12 and/or iron.

Ethical approval and informed consent

Approval for the study protocol was obtained from the local ethics committee, and all patients gave written informed consent.

Assessment of response

The patients were seen and evaluated at the outpatient clinic at weeks 4, 8, 12, 24 and 48. Upper gastrointestinal endoscopy and small bowel biopsies were taken at weeks 12 and 48 after therapy.

Primary efficacy parameters of the study were: clinical improvement; change of histopathology

in the small bowel (modified Marsh classification) analyzed in 4 specimens taken from the distal duodenum [6] and decrease of aberrant IEL's of the small bowel.
Clinical improvement was defined as disappearance of diarrhoea, improvement in the performance status according to the WHO scale, and at least 2 of the following three criteria: an increase of BMI >1 point; an increase in albumin level >10% of the baseline level and an increase of Hb >1 point.
A decline of ≥ 20% in the percentage of aberrant T-cells was regarded as significant.
Secondary efficacy parameters were: EATL development and mortality.
Safety parameters were registered, haematological as well as non-haematological laboratory data, febrile and infectious episodes, bleeding events and the need for transfusion of blood products.

Results

Table 1 shows the clinical and laboratory characteristics of the treated patients at baseline and 12 weeks after completing the first treatment course. Ten out of 17 (58.8%) patients were homozygous for HLA-DQ2 haplotype, 7 (41.2%) were DQ2 heterozygous.[31]

Side effects of treatment

All patients tolerated the 2-CDA treatment without significant adverse events. Three patients had nausea and vomiting, one developed diarrhoea, and one bronchitis. No significant haematological side effects were noted (table 2); particularly no significant lymphopenia, monocytopenia or anaemia requiring transfusion was reported.

Response to treatment:

All patients received 1 courses of therapy. Patients who had a clinical, histological and/or immunogical response received additional second course (6 patients) or third 2-CdA course (7 patients). The mean follow up period was 22 months (range 7- 67 months). The mean body mass index of all patients increased from 20.6 (±SD =2.12) to 21.2 (±SD =3.14) kg/m^2. The mean albumin level increased from 30.0 (±SD=7.20) to 33.7 (±SD=7.49) g/l. The mean haemoglobin level remained unchanged 7.65 (±SD=1.35).
Table 3 shows the clinical condition and response of the patients on follow up 12 weeks after completing the first treatment course.

Clinical response

Six of 17 patients (35.8%) showed a clinical response, including weight gain and improvement of diarrhoea and hypoalbuminaemia. Two patients (10.5%) showed no improvement in their clinical condition and remained emaciated. Seven patients (41.1%) developed EATL (6 months –38 months, mean 14 months) after the start of treatment and subsequently died. One patient (*patient no.1*) died from bronchiectasis caused by persisting postnasal discharge in association with EATL localization in the paranasal sinuses (>90% aberrant T-cells). Another patient (*no.3*) died from progressive refractory state with emaciation.

Table 1. The characteristics of the patients. All patients had a clonal rearrangement of the TCRγ gene on TCRγ-PCR analysis

Patients	Sex	Age	HLA-DQ	Age		BMI		Albumin		Hb		SIB		%Aberrant T-cells	
				CD	RCD	S	E	S	E	S	E	S	E	S	E
1	F	54	DQ2 homo	45	46	22	22	27	35	7.6	6.6	IIIC	IIIA	73	43
2	M	69	DQ2 homo	62	64	21.4	26	26	34	9.7	9.1	UJ	IIIB	45	43
3	M	70	DQ2 homo	59	63	17.8	18	20	18	6.5	6.0	UJ	IIIA	92	90
4	F	71	DQ2 homo	58	59	25.5	25.6	19	22	6.6	6.7	IIIB	IIIA	90	91
5	M	66	DQ2 heter	58	59	23.6	23.8	34	40	10	9.5	IIIC	IIIC	88	90
6	M	65	DQ2 homo	60	61	17.3	17.6	16	29	6.7	6.7	IIIA	IIIA	90	73
7	M	61	DQ2 homo	55	59	21.5	22.5	32	35	7.8	8.2	IIIC	IIIC	33	4
8	M	61	DQ2 homo	56	59	20	21	34	39	8.7	8.8	IIIA	IIIA	98	40
9	F	63	DQ2 homo	59	60	18.7	19.8	40	46	8.9	9.8	IIIC	I	70	15
10	F	64	DQ2 heter	62	63	19.3	20.2	29	35	6.0	8.0	UJ	IIIA	40	60
11	M	63	DQ2 homo	59	63	23.4	24	36	40	8.7	9.4	IIIA	IIIA	70	70
12	M	67	DQ2 homo	66	67	22	23	30	38	6.6	5.7	IIIC	II	40	40
13	F	76	DQ2 heter	75	76	13.8	13.5	34	30	5.6	6.8	IIIC	IIIA	94	94
14	F	58	DQ2 heter	46	56	19.4	19.5	40	43	7.6	7.7	UJ	IIIB	82	35
15	F	63	DQ2 heter	56	58	20	20	23	30	6.2	6.5	IIIA	IIIB	88	55
16	F	65	DQ2 heter	63	64	22.5	22.8	35	35	9.1	8.0	IIIA	IIIC	30	60
17	F	58	DQ2 heter	47	58	22.1	21	35	24	7.8	7.2	UJ	IIIC	70	60

M=male, F=female, SIB=small bowel biopsy according to Marsh classification, S=before treatment, E= after treatment, BMI=body mass index (Kg/m^2), Hb=haemoglobin mmol/l, albumin in g/l, UJ= ulcerative jejunitis.

Table 2. Haematological parameters at baseline and at follow up after the first course of therapy

Parameters	At baseline, mean (±SD)		At follow up, mean(±SD)	
Haemoglobin (mmol/L)	7.65	(1.35)	7.69	(1.29)
MCV *(FL)	85	(6.3))	92	(6.9)
Leucocytes	7.27 $\times 10^9$ /L	(1.85)	7.07 $\times 10^9$ /L	(1.48)
Neutrophils	56%	(12.0)	60%	(13.0)
Lymphocytes	19.5 %	(10.2)	21.7%	(7.68)
Monocytes	7.9%	(5.8)	8.2%	(6.00)
Platelets	334$\times 10^9$ /L	(94.9)	296$\times 10^9$ /L	(89.5)

*MCV= Mean corpuscular volume

Table 3. Condition of the patients on follow up after completing treatment

Patients	Survival (months)	Status on follow up	Cause of death	Date ASCT
1	24	Died	EATL, localization in the paranasal sinuses with bronchiectasis	-
2	67	Improved clinically and histologically	-	August 2004
3	34	died	Progressive refractory state with emaciation	-
4	23	Died	EATL	-
5	38	Died	EATL	-
6	15	Died	EATL	-
7	22	Clinically stable	-	-
8	22	Clinically stable	-	March 2003
9	24	Complete remission	-	-
10	11	Clinically stable, improved histologically	-	April 2005
11	12	Clinically stable	-	August 2005
12	6	Died	EATL	-
13	7	Died	EATL	-
14	44	Clinically stable, histologically improved	-	November 2005
15	5	Died	EATL	-
16	14	Clinical and histological deterioration	Unsuccessful (CD34) leukopheresis. Unsuccessful treatment with alemtuzumab (Mabcampath)	
17	10	Clinically stable, histologically improved	-	December 2005

ASCT= Autologous Haematopoietic Stem Cell Transplantation.

Histological response

Ten patients (58.8%) showed improvement of the histological status including the disappearance of ulcerative jejunitis in all 5 patients (29.4%) who initially presented with this disease state (*Patients 2, 3, 10, 14 and 17*). Patient number 9 had complete clinical and histologic recovery (Marsh classification M-IIIC to M-I) and remained symptom free during 3 years of follow up. Five patients (29.4%) had no change in the histology status and two patients deteriorated histologically (*patients 15, 16*).

Immunological response

The mean percentage of aberrant T-cells changed from 72.7% (SD=23.3) at baseline to 57.7 % (SD= 26.9) thereafter. In six patients (35.2%) a significant decrease in aberrant T-cell percentages was seen (≥20%). Two patients (*patients 10 and 16*) had a significant increase in aberrant T-cell percentages.

Development of EATL

Concerning the 7 patients who developed an EATL and died: *patient no.1* had a significant decrease of aberrant T-cell percentages (from 73% to 43%); *patient no. 4* had a histological improvement but unchanged high percentages of aberrant T-cells (>90%); *patient no. 5* showed unchanged study parameters with high percentages of aberrant T-cells (>90%); *patient no. 6* showed no significant change in study parameters although a decrease of aberrant T-cell percentages was seen (from 90% to 73%); *patient no.12* showed a clinical improvement and histological normalization of villi, but aberrant T-cell percentages remained unchanged (40%); *patient no.13* had a histological improvement, but unchanged high percentages of aberrant T-cells (>90%); *patient no. 15* showed a significant decrease of aberrant T-cell percentages (from 88% to 55%). All EATL's were of the $CD3^+$ $CD8^-$ $CD30^+$ large cell type.

Discussion

Closer investigation of refractory coeliacs suggests two subgroups, involving patients with and without aberrant, T-cell lines in the small bowel mucosa. Particularly patients with aberrant T-cells (RCD type II) seem to be at high risk of developing an EATL and subsequent death.[1, 2,7,14] As the outcome of disease in patients with EATL is usually very poor, despite chemotherapy, it seems obvious that clinical focus should be on earlier diagnosis and intervention before progression to overt malignancy. The association between CD and malignancy is long established. The most frequent malignant complication is EATL.[2,4,25] Most patients with EATL present with malaise, anorexia, weight loss and diarrhoea. A substantial number require diagnostic and sometimes therapeutic laparotomy to deal with complications such as perforation or obstruction.[12]

Earlier studies on treatment of RCD showed that steroids, cyclosporine and IL-10 had no effect on outcome in RCD II patients.[1, 3, 7-11, 32-36] Table 4 summarizes the results of earlier reports on therapies in RCD patients. However, positive results in some of these studies can

not be adequately interpreted because no distinction has been made between RCD I and RCD II. Maurino et al [32] reported the results of treating 7 RCD II patients with azathioprine. Clinical and histologic improvement was noted in 5 of 7 treated patients, although 3 patients died (one from leucopenic fever and 2 died early). However, in their follow up report on treating 13 patients with azathioprine, they reported 46% mortality.[33]

Table 4. Summery of therapies on refractory coeliacs

Therapy/Reference	Type of report	Total	Type RCD	Results
Cyclosporine [8]	Case report	1 (2 yrs old)	ND	Initial improvement, relapse after stopping treatment
Cyclosporine [9]	Case report	1	ND	Remission
Cyclosporine/ Azathioprine [34]	Case report	1	ND	Short term improvement
Azathioprine [35]	Case report	1	ND	Steroid tapering, died from infection
Cyclosporine [10]	Open- label	13	ND	Clinical & histologic improvement (61%)
Interleukin 10 [11]	Open -label	10	ND	Inconsistent response
Azathioprine [32,33]	Open-Label prospective	7	Yes	Short term clinical and histologic improvement in 5 of 7 treated patients. Three died (one leucopenic fever). Long term follow up 46% mortality
Prednison/ Azathioprine [1]	Open-label	10 RCD/ 8 RCD II	Yes	7 of 8 RCD II died (6 from EATL), while 10/10 RCD I have long term survival
Infliximab [36]	Case report	1	ND	Remission

One recent report on using the anti-tumour necrosis factor (anti-TNF) agent Infliximab in treating RCD has been published, but again no data were provided on aberrant T-cells (T flow-cytometry or immunohistology).[36,37] In the present study, 2-CDA therapy was well tolerated without serious side effects. Six of 17 patients (35.8%) responded with clinical improvement and another 6 had a significant decrease in aberrant T-cell percentages.

Interestingly, one of our patients developed a complete clinical, immunological (aberrant T-cells percentage decreased from 70% to 15%) and histologic recovery (Marsh classification M-IIIC to M-I) and remained symptom free during more than 4 years of follow up. Furthermore, ulcerative jejunitis, an endoscopic feature of RCD-II, was seen to disappear in the 5 patients (29.4%) who had it initially and interestingly none of these 5 patients thus far developed EATL.

Seven patients (41.1%) developed EATL within 6-38 months after starting treatment, subsequently died despite multi-agent chemotherapy (CHOP). Although EATL has been adequately been excluded at inclusion, three patients died of EATL within 5-7 months

after therapy. Whether 2-CDA has accelerated the development of lymphoma or not can not be confidently concluded. Few cases of secondary malignancies after 2-CDA through T-cell immunodepression have been reported.[38] All our EATL- patients had a $CD3^+$, $CD8^-$, $CD30^+$ large cell lymphoma. We might expect that patients who present with a surface $CD3^-$, cytoplasmic $CD3^+$, $CD8^+$ and $CD30^-$ aberrant T-cells clone will develop a $CD3^+$, $CD8^+$, $CD30^-$ small cell lymphoma.[12]

Therapy with 2-CDA, thus, seem to have a role, although based on our data less than optimal in the treatment of RCD with aberrant T-cells. It may be considered however as the only treatment thus far studied that demonstrated significant reduction of aberrant T-cells, seems to be well tolerated and may have beneficial long term effects in a subgroup of patients demonstrating significant reduction of the aberrant T-cell population.

However, a search for a more successful treatment is strongly needed. Encouraging reports have been published on the effectiveness of high dose chemotherapy followed by Autologous Haematopoietic Stem Cell Transplantation (ASCT) in the treatment of patients with severe autoimmune disease refractory to conventional treatment.[39] Therefore, seven of the treated patients have been included in an ASCT program in our institution.[40]

In conclusion, 2-CDA is successful in a minority of RCD II patients. It remains to be seen whether more intensive treatment (like ASCT) would be helpful in 2-CDA resistant or partially responsive RCD II patients. Furthermore, we think that a likely future indication for the use of 2-CDA in RCD-II may be stabilization of the clinical condition of patients as a bridge to ASCT.

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Autologous Haematopoietic Stem Cell Transplantation in Refractory Coeliac Disease with aberrant T-cells

8

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Abstract

Background and aim: Autologous Haematopoietic Stem Cell Transplantation (ASCT) is an increasingly accepted treatment for refractory autoimmune diseases. Refractory Coeliac Disease with aberrant T-cells (RCD-type II) is unresponsive to available therapies and carries a high risk of transition into Enteropathy Associated T-cell Lymphoma (EATL). This study reports on the feasibility, safety and efficacy of ASCT in RCD type II.
Methods: Thirteen patients with RCD-II were evaluated. Seven patients [4M, 3 F, mean age 61.5 years (range 51-69 years)] were transplanted. After conditioning with fludarabine and melphalan, ASCT was performed. Patients were monitored for response, adverse effects and haematopoietic reconstitution.
Results: All 7 patients completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning and transplantation. Engraftment occurred in all patients. No major non-haematological toxicity or transplantation-related mortality was observed. There was a significant reduction in the aberrant T-cells in duodenal biopsies associated with improvement in clinical wellbeing, and normalization of haematological and biochemical markers (mean follow-up 15.5 months, range 7-30 months). One patient died 8 months post-transplant form progressive neurocoeliac disease.
Conclusions: These preliminary results showed that high-dose chemotherapy followed by ASCT seems feasible and safe, and might result in long-term improvement of RCD II patients whose condition did not respond promptly to available drugs.

Introduction

Autologous Haematopoietic Stem Cell Transplantation (ASCT) is an increasingly accepted effective treatment option for patients with severe autoimmune diseases refractory to conventional treatment [1] and has been used successfully in patients with multiple sclerosis,[2] rheumatoid arthritis,[3] systemic sclerosis [4], systemic lupus erythematosus [5] and Crohn's disease.[6] The rationale for this strategy is based on the concept of immunoablation by intense immunosuppression using high dose chemotherapy, with subsequent regeneration of naïve T lymphocytes derived from reinfused haematopoietic progenitor cells.[7]
In coeliac disease (CD), HLA-DQ molecules bind and present gluten peptides to antigen-specific T-cells. These HLA-DQ-peptide complexes induce inflammatory responses in the small intestine consisting of lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte population, hyperplasia of the crypts and atrophy of the villi.[8] In a small percentage (2-5%) of coeliac disease patients diagnosed as adults a refractory state develops despite strict adherence to a gluten-free diet (GFD).[9] In refractory coeliac disease (RCD) the number of intraepithelial lymphocytes (IEL) is markedly raised and it is from these IEL's that enteropathy associated T-cell lymphoma (EATL) may arise.[9,10] Immunophenotyping of the IEL's identifies two groups of RCD patients: those with normal IEL's (RCD I) and those with aberrant IEL's, lacking surface expression of CD3 and CD8 (RCD II).[10,11] RCD II can be

regarded as a 'cryptic' lymphoma.[9] There is now strong molecular and immunophenotypic evidence showing that a monoclonal neoplastic T-cell population may emerge from IEL's in RCD. Clonal expansion of this monoclonal T-cell population eventually leads to frank EATL. The genesis and expansion of these monoclonal T-cells involve both inappropriate immune responses to gluten and acquisition of genetic abnormalities. Although the monoclonal IEL's in patients with RCD are neoplastic, they are not cytologically abnormal and do not form tumour masses which differentiate these patients from EATL patients, in addition to the absence of radiological and bone marrow evidence of lymphoma.[10,12,13,14]

RCD II is usually resistant to any known therapy, including azathioprine/prednisone, cyclosporine and IL-10 therapy [15-18] and has a high risk of developing EATL (60-80% within 5 years).[10,19] This specific type of peripheral T-cell lymphomas has a very poor outcome with 1- and 5-year survival rates in the range of 31-39% and 11-20% respectively.[19,20,21] In a prospective multicentre study of 35 patients with EATL treated with six cycles of CHOP (Cyclophosphamide, Doxorubicine, Vincristine and Prednisone), the cumulative 2-year survival was only 28%.[11] Therefore, new treatment strategies for patients with ''premalignant'' CD (RCD-II) are urgently needed to improve their clinical condition with the ultimate goal of resetting the immune response which might prevent or delay development of overt EATL.

This study reports on the feasibility, safety and efficacy of high dose chemotherapy followed by ASCT in patients with RCD type II.

Methods

Patients

Between March 2004 and March 2006 thirteen patients were evaluated for ASCT. Seven patients [4 males, 3 female, mean age 61.5 years (range 51-69 years)] with RCD-II underwent ASCT. Six other patients were excluded because of the presence of coexistent coronary artery disease and heart failure (NYHA classification III) (two patients), EATL found on pre-transplantation evaluation (3 patients), and low performance status (one patient). One patient could not be treated due to unsuccessful leucophaeresis; she developed EATL and died subsequently despite chemotherapy and immunotherapy with antiCD52 (Alemtuzumab).[22]

The 2 patients with congestive heart failure died from: progressive disease, cachexia (one patient) and bronchiectasis (second patient). The 3 patients with EATL all died within few months, while the patient with low performance status died from cachexia.

The baseline characteristics of the patients are shown in table 1. All patients received therapy with prednisone and cladribine (2-CDA) several months before receiving ASCT (not within 6 months of transplantation).

The first 3 patients (patients A, B and C) were diagnosed with CD at relatively advanced age, had persistent diarrhoea, weight loss and failed to respond to GFD, steroids and immunosuppressives. Because of the presence of active disease and high percentage of aberrant T-cells in the small bowel mucosa, they were included in this study protocol. Patient D was diagnosed with CD at the age of 48 years in association with dermatitis herpetiformis.

Furthermore, he had a clinical picture of neurocoeliac disease with ataxia. After exclusion of structural brain and infectious disorders, he received ASCT at the age of 63.5 years.
Patient E has, in addition to CD with ulcerative jejunitis, Hashimoto's thyroiditis, while patient F has CD with ulcerative jejunitis.
One patient (patient G) was included because of the presence of very extensive ulcerative jejunitis with multiple small bowel strictures necessating repeated resections although initially biopsies showed a low percentage of aberrant T-cells. He had clinically short bowel syndrome (remaining small bowel approximately100-150 cm) requiring total parenteral nutrition.

Criteria for diagnosis of RCD

Patients with CD were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a GFD for at least one year. Furthermore, possible underlying diseases such as autoimmune enteritis, bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple's disease, hypogamma-globulinaemia, eosinophilic enteritis, EATL and inflammatory bowel disease were excluded.[11] The diagnosis of RCD was established as type II when ≥ 20% aberrant T-cells were present.[10,11,15]

Inclusion criteria

Patients were only included when the diagnosis of true RCD with aberrant T-cells was confirmed (except one patient (patient G) was included on the basis of extensive ulcerative jejunitis with short bowel syndrome despite having only 10% aberrant T-cells), after verifying their strict adherence to a GFD, performance status according to the WHO criteria needed to be 0-2, if there was no severe concomitant cardiac, pulmonary, renal or hepatic disease. EATL was excluded by endoscopic examination with multiple biopsies, CT-scan, PET and a trephine bone marrow biopsy. Furthermore, neither active uncontrolled infection nor HIV positivity was permitted.

Evaluation

Before proceeding to ASCT, the patients were extensively evaluated as to their performance status, the presence of concomitant diseases and extraintestinal disease or EATL.
This evaluation included:

- Clinical assessment noting particularly signs and symptoms of malabsorption, body mass index (BMI) and performance according to the WHO score, [23]
- Evaluating the adherence to a GFD: Frequent consultation with dietician (advice and follow up); in addition to checking serology (anti-endomysium (EMA) and anti-tissue transglutaminase-antibody (anti-tTG), both of which usually revert to negative after strict adherence to the GFD,
- Endoscopic evaluation by upper gastrointestinal (UGIE), video capsule endoscopy (VCE) and double-balloon enteroscopy (DBE). Duodenal biopsies (4 biopsies) were classified according to the modified Marsh criteria.[24, 25] T-cell Receptor (TCR-) gene rearrangement study, [12, 13, 14] T-cell flow-cytometry and IEL phenotyping were performed, [15, 26, 27]

- Laboratory evaluation: whole blood cell counts, serum levels of creatinine, bilirubin, liver enzymes, lactate deydrogenase, albumin, electrolytes, iron, ferritin, folic acid and vitamin B12. Anti-endomysium (EMA) and anti-tissue transglutaminase-antibody (anti-tTG) assays, HLA-DQ typing, thyroid function tests, stool examination for Giardia and other parasites and HIV serology were also performed.[28]
- Radiological evaluation: the patients underwent whole body computed tomography (CT-scan), whole body positron emission tomography (PET) to exclude intestinal and extra-intestinal localization of EATL.[29, 30]

Immunophenotyping of IEL's

IEL's were isolated from 3 duodenal biopsies by passing them through nylon filters (1x100µm, 1x 40µm, BD Falcon). Cells were stained with fluorescent labeled monoclonal antibodies to CD3, 7, 8, 45, 103, and TCRγδ, as well as with relevant isotype controls.

All monoclonal antibodies were from BD Falcon (BD biosciences, CA USA), except for CD103, which was from IQ-products, Groningen, The Netherlands) and analyzed by 4-color flow-cytometry (FACS-Calibur, BD). Leucocyte common antigen (CD45) was always included to identify the lymphocyte population. In some tubes cell surface CD3 staining (anti-$CD3^-$ APC) was followed by permeabilization (Cytofix /cytoperm, BD Biosciences Pharmingen, CA USA) and subsequent cytoplasmic staining with anti-CD3-FITC or isotype control.

Aberrant T-cells were defined either as $CD7^+$ surface $CD3^-$ cells (expressed as % of $CD103^+$ lymphocytes) or as cytoplasmic $CD3^+$, surface $CD3^-$ cells (expressed as % of $CD103^+$ lymphocytes).[12, 26]

All flow-cytometry analyses were performed by an analyst and interpreted by the same medical immunologist, while histopathology was performed by the same pathologist to ensure uniformity, reproducibility and consistency of results.

Assessment of TCR gene rearrangement by Polymerase Chain Reaction (PCR)

T-cell receptor-gamma (TCR-γ) gene rearrangements studies were performed in separate three-four duodenal specimens that were preserved on histocon and frozen at -20°C. DNA was extracted from cryosections of duodenal specimens by a standard procedure using proteinase-K digestion and ethanol precipitation of the genomic DNA. TCR-γ gene rearrangements were analyzed by multiplex polymerase chain reaction (PCR) amplification under standardized conditions. A monoclonal and polyclonal control was included in each experiment. Clonality assessment for TCR-γ gene rearrangements was done using the BIOMED-2 multiplex TCR PCR protocol.[12, 13, 14]

Peripheral blood stem cells mobilization and collection

Mobilization of haematopoietic progenitor cells from the bone marrow into the peripheral blood was achieved using granulocyte-colony stimulating factor (G-CSF) 2×5 µgm/kg by subcutaneous injection for at least four days. Haematopoietic stem cells were harvested from the peripheral blood by leucopheresis and kept frozen until ASCT. The target $CD34^+$ count was $> 2\times10^6$/kg.

Conditioning and ASCT

The conditioning regimen consisted of fludarabine given orally for five days (40 mg/m^2/day) and melphalan (intravenous, two days 70 mg/m^2/day) (Figure 1). At day 0, the frozen stem-cell suspension was thawed and reinfused. The rationale for this conditioning regimen was based on T-cell depletion by a purine analogue combined with a modified dose of melphalan (total dose 140 mg/m$^{2)}$ for myeloablation.

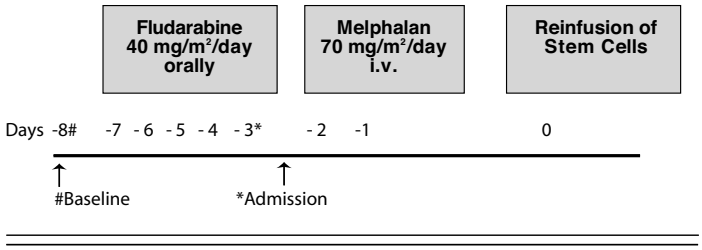

Figure 1. Scheme of transplantation protocol

Supportive care

Patients A, C and D were supported with parenteral feeding during the two week period of oral mucositis after ASCT, while patient G was receiving parenteral nutritional support before receiving the transplant. After discharge, all patients except one (patient G) were able to be fed enterally. Patient G was supported to gain weight for several months with a duodenal feeding tube and limited TPN (twice a week).

During admission, all patients received standard antibacterial and antifungal prophylaxis. Pneumocystis jiroveci pneumonia prophylaxis was initiated (Trimethoprim-sulphamethoxazole gluten-free syrup 480-960 mg daily) until six months posttransplant.

No patient received antidiarrhoeal or narcotic medications in the peri-transplantation period. Blood and platelet transfusions were given as indicated.

Follow - up and criteria of response

During follow up, WHO performance, nutritional status, changes in weight and stool frequency were noted, as well as relevant biochemical markers. An endoscopic and histological examination of the small bowel was performed (3, 12 and 24 months post ASCT). From the second part of duodenum, 4 biopsies were taken for histological assessment and 4-6 for T-cell flow-cytometry study. Haematological data [Haemoglobin, White cell count (WBC), differential and platelets] were registered before inclusion, after preconditioning and after transplantation until recovery. The nadir WBC, duration of neutropenia, infectious complications, bleeding tendency, and the need for supportive therapies such as blood and platelet transfusions were documented.

Ethical approval and Informed consent

Approval of the medical ethics committee was obtained and all treated patients signed an informed consent.

Results

Table 1 summarizes the demographic and clinical characteristics of the patients before ASCT. The mean age at diagnosis of CD was 52.5 years (range 47- 62 years) and for RCD-II was 59 years (range 51-64 years). Four patients were DQ2 homozygous and three were heterozygous.[31] The mean follow-up is 15.5 months (range 7-30 months). All patients had a WHO performance status of one except patient G (WHO performance 2).

Four of our patients (patients B, E, F and G) had ulcerative jejunitis. Three patients (patients A, C and D) had splenic atrophy on CT scan. PET scan showed an increased uptake in the small intestine in 3 patients (patients A, B and C). At the time of diagnosis of CD, all patients were positive for anti-tTG and EMA, but all reverted to negative after GFD. Before and after ASCT all patients remained negative for anti-tTG and EMA.

There was no transplantation-related mortality. The conditioning regimen seems feasible in this group of patients. The mean duration of hospitalization was 19,5 days (range 18-22 days). ASCT-related toxicity was relatively mild. Patient B had transient diarrhoea and fever of undetermined origin which was treated with intravenous antibiotics. Three weeks after discharge from the hospital, he suffered from a transient visual disturbance caused by minor retinal bleeding, which was not related to thrombocytopenia. Patient D experienced fever of undetermined origin and recovered after intravenous antibiotics.

One month after ASCT, patient E developed self-limiting erythematous plaque skin lesions with central necrosis. Detailed histopathological tests excluded EATL and showed aberrant T lymphocyte infiltration ($CD8^-$ $CD7^+$ $CD30^+$).

The mean time from the day of transplantation to neutrophil recovery was 17.8 days (range 10-21 days). Only one patient (patient B) had a transient a 5 days period of severe thrombocytopenia of 5×10^9/l, while all other patients had nadir platelets counts between 17-32 $\times10^9$/l without need for platelet transfusions.

Clinical and laboratory tests before and after ASCT are shown in table 2. Patients A, C and D were supported with parenteral feeding during the period of oral mucositis. No patient received antidiarrheal or long term narcotic medications. Within 3 months after ASCT, all patients showed impressive clinical improvement with normalization of stools frequency, disappearance of abdominal pain and improvement of biochemical markers. Also improvement of BMI was documented [from mean 20,2 at baseline to 24,1 after ASCT]. Mean serum albumin level increased from 29 g/l to 40,7 g/l.

Patient G showed a remarkable clinical improvement 3-4 months after ASCT and was able to be fed partly enterally with parenteral nutritional support twice a week.

Table 3 shows the endoscopic and immunological results. All patients were monoclonal for the TCR-γ. Endoscopically there was disappearance of erosions and ulcerations in the jejunum in all patients (patients B, E, F and G) who had ulcerative jejunitis before ASCT, and histology of the small intestine showed significant regeneration as documented by down-staging of the Marsh class (patients A, B, C, E, F and G).

Table 1. Baseline characteristics of the patients.

Patients	A	B	C	D	E	F	G
Age/gender	62/M	70/M	65/F	63/M	64/F	59/F	51/M
Age CD (yrs)	56	62	61	48	44	47	50
Age RCD II (yrs)	59	64	63	63	56	58	51
Age ASCT (yrs)	60	68	64	63	64	59	51
Date ASCT	*March 2004*	*August 2004*	*May 2005*	*August 2005*	*Nov.2005*	*Dec. 2005*	*March 2006*
HLA-DQ 2	Homozygous	Homozygous	Heterozygous	Homozygous	Heterozygous	Heterozygous	Homozygous
Marsh at RCD diagnosis	IIIA	IIIB	IIIA	IIIA	IIIC	IIIC	IIIA
BMI (Kg/m^2)	19,4	18,9	17,1	24,1	20,1	21,3	20,5
Performance	1	1	1	1	1	1	2
Pred/2-CDA	Pred/2-CDA	Pred /2-CDA	Pred / 2-CDA	Pred / 2-CDA	Pred/2-CDA	Pred / 2-CDA	Pred / 2-CDA
Symptoms/Associations	Diarrhoea, pain, weight loss	Pain, diarrhoea	Diarrhoea, Weight loss, Hypocalcemia	Diarrhoea, Weight loss, Dermatitis herpetiformis, neurological symptoms (ataxia)	Weight loss, skin rash, Hashimoto's thyroiditis	Weight loss, diarrhoea	Diarrhoea, Hypocalcemia, weight loss, extensive small bowel resection
Serology at CD diagnosis	EMA+, anti-TTG+	EMA+, Anti-TTG+	EMA+, anti-TTG+	EMA+, anti -TTG+	EMA+, anti- TTG+	EMA+, anti- TTG+	EMA+, anti- TTG+
Serology at RCD diagnosis	EMA-, anti-TTG-	EMA-, Anti-TTG-	EMA-, anti-TTG-	EMA-, anti -TTG-	EMA-, anti- TTG-	EMA-, anti- TTG-	EMA-, anti- TTG-
Endoscopy (GDS,VCE, DBE)	Nodular mucosa	Mosaic mucosa, erosions and ulcerations	Mosaic mucosa, visible vessels, no ulcerations	Nodular mucosa, disappearance of folds, erosions	Ulcerative jejunitis	Ulcerative jejunitis	Ulcerative jejunitis with multiple stenosis
CT scan	Splenic atrophy, thickened SI wall	Thickened SI loops	Splenic atrophy, dilated SI loops	Splenic atrophy	No abnormality	No abnormality	Small intestine ileus
PET scan	Increased uptake in SI	Increased uptake in SI	Increased uptake in SI	No abnormality	No abnormality	No abnormality	No abnormality

M=male, F= female, Pred=prednisone, 2-CDA=Cladribine, SI= small intestine, GDS= Gastroduodenoscopy VCE=Video capsule enteroscopy, DBE= double-balloon enteroscopy

Table 2. Clinical and laboratory tests before and at the last follow up after ASCT.

Patients	A		B		C		D		E		F		G	
Duration of follow up(months)	30		27		16		8		11		10		7	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
BMI (Kg/m^2)	19,4	25,6	18,9	26,1	22	24,1	24	24,1	20,1	22	22,1	23	20,5	25,8
Diarrhoea	+	-	+	-	+	+	+	-	+	-	+	-	+	-
Performance status	1	0	1	0	1	1	1	0	1	0	1	0	2	0
Albumin	32	43	33	41	30	46	32	41	20	41	30	44	26	46
Serum iron	18	26	11	18	14	10	14	15	17	13	7	17	13	18
Serum calcium	2,20	2,36	2,33	2,45	2,26	2,26	2,26	2,32	2,33	2,41	2,02	2,35	2,00	2,29
Serum folic acid	10	44	10,4	24	14	91	14	15	14	78	4,4	35	29	18
Serum B12	470	560	169	307	440	290	440	790	206	666	107	317	269	221

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Table 3. Histological and phenotypical flow-cytometric analysis of IEL's in duodenal biopsies before (1-6 months) and after (3, 12 and 24 months) ASCT. All patients had a clonal rearrangement of the TCRγ gene on TCRγ-PCR analysis

	Marsh category		CD7$^+$CD3$^-$ % of CD103$^+$ ly		Cyt CD3$^+$surf CD3$^-$% of CD103$^+$ ly		CD8$^+$% of CD103$^+$ ly	
Patient	Before	After 3- 12- 24m	Before	After 3- 12- 24m	Before	After 3- 12- 24m	Before	After 3- 12- 24m
A	IIIA	IIIA - IIIA - I	95	47- 48 -15	94	89 - 86 - 3	1	20- 7 - 52
B	IIIB	I - I - I	51	7 4 - 8	51	2 - 6 - 4	28	68 - 62 - 67
C	IIIC	IIIA - IIIA	62	33 - 24	59	34 - 27	15	41 - 36
D	IIIA	IIIA	54	47	81	78	7	13
E	IIIB	I	44	68	30	36	22	2
F	IIIB	IIIA	71	40	50	31	23	11
G	IIIC	IIIA	11	30	10	27	63	52
Mean	-	-	63	38	61	42	23	30

ly= lymphocytes. Normal range for Cyt CD3$^+$surf CD3$^-$ % of lymphocytes ≤ 10%. TCRγ-PCR analysis =T-cell receptor γ-polymerase chain rearrangement. Mean* = calculated for values at 3 months post ASCT.

Overall, the aberrant [CD7$^+$CD3$^-$] T-cell percentage of CD103$^+$ lymphocytes decreased from a mean of 63% (range 11-95%) at baseline to 38% (range 7-68%) three to four months post-transplantation. Aberrant cytoplasmic CD3$^+$ surface CD3$^-$ T-cell percentage of CD103$^+$ lymphocytes has decreased from a mean of 61% (range 10-94%) to 42% (range 2-89%). Furthermore, the mean percentage of CD8$^+$ cells has increased from 23% to 30% after ASCT. This was particularly noticeable in the first 3 patients. Patient D did not show a significant increase in CD8$^+$ cells and the last 3 patients have not yet shown a significant change. Individual responses to ASCT differed from each patient as shown in table 3. Patient B showed the most impressive response with a virtual complete disappearance of aberrant T-cells. The fluorescent-activated cell-sorting (FACS) data form patient B is shown in figure 2. Figure 3, Colour plate B-1 shows the trend of aberrant T-cells and body weight for the first 4 patients who have a follow-up period of at least one year. Follow up of patients E, F and G is yet limited.

Two years after transplantation, our first patient (patient A) is showing further improvement in his immunopathology status as demonstrated in further decline in the percentage of aberrant T-cells to 3% and histologically improved from Marsh III-A to Marsh-I and the second patient (patient B) still showing persistent complete clinical and histological response.

Patient D had no significant change in the percentage of aberrant T-cells, showed no histological improvement and also no significant improvement in CD8$^+$ percentage and he died 8 months post-transplantation. After ruling out structural and infectious (bacterial and viral) causes, we assumed that progressive disease of RCD-II with oligoclonal T lymphocytes infiltrating the brain was the cause of death in this particular patient. EATL could not be detected. Autopsy confirmed the presence of chronic encephalitis of the right temporal lobe with T-lymphocytes infiltration. Immunohistochemistry showed that the lymphocyte infiltrate

was CD3 positive and the majority of cells expressed CD8 positivity. TCR gene analysis showed the T-cells to be oligoclonal.

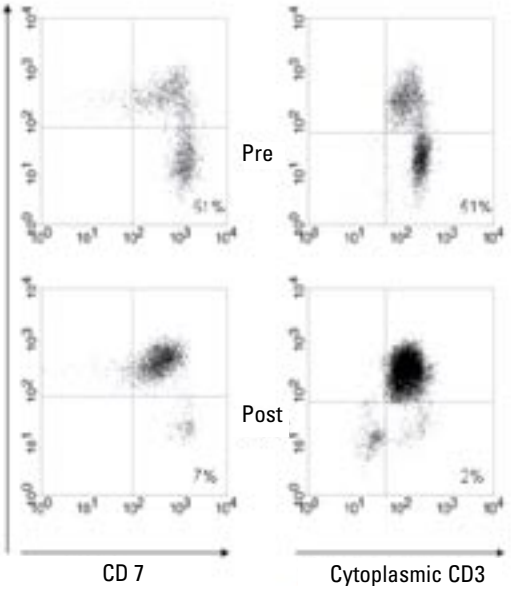

Figure 2. Flow-cytometric analysis of duodenal cells obtained from patient B, showing the change in the percentage of aberrant T-cell population pre- and post- ASCT. Aberrant population is shown as $CD7^{+}CD3^{-}$ within $CD103^{+}$ lymphocytes (left hand side) or as cytoplasmic $CD3^{+}$ surface $CD3^{-}$ within lymphocyte gate (right hand side). Normal range for Cyt $CD3^{+}$surf $CD3^{-}$ % of $CD103^{+}$ lymphocytes ≤10%

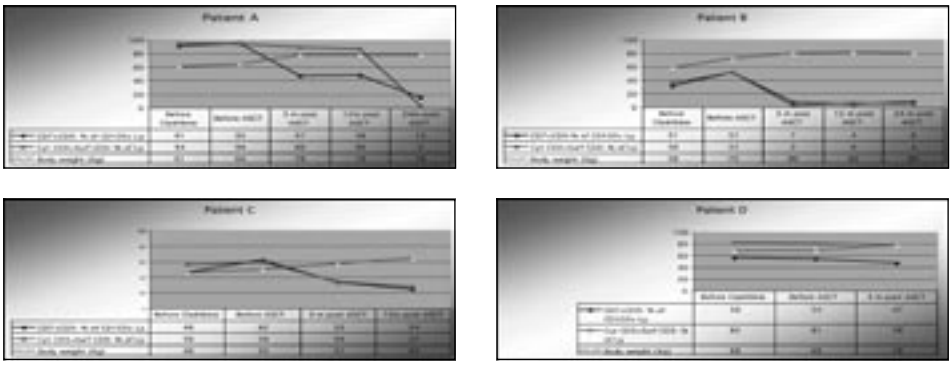

Figure 3. The trend of aberrant T-cells and body weight per patient. Ly= lymphocytes. Before = 1-3 months. Normal range for Cyt $CD3^{+}$surf $CD3^{-}$ % of lymphocytes ≤ 10%

Discussion

In this pilot study ASCT in RCD II patients was shown to be feasible. The conditioning regimen was well tolerated in all patients and there was a substantial clinical improvement. The rapid initial response (within 3 months) and the duration (2 years in patient A and B and 14 months in patient C) of the remission up to now are promising. Complications included the occurrence of neutropenic fever in 2 patients and retinal bleeding in one patient not related to thrombocytopenia, all with full recovery. The nadir leucocytes and platelets counts and the duration of leucopenia and thrombocytopenia were comparable to our experience in patients with Non-Hodgkin lymphomas and multiple myeloma receiving ASCT after a combination of carmustine, etoposid, cytarabine, and melphalan (BEAM) or high dose melphalan (HDM, 200 mg/m^2).[32] As there is no standard conditioning regimen for ASCT used in autoimmune disease [33], a standard regimen used in our institution was used. Fludarabine induces T-cell depletion while the alkylating agent melphalan was used to achieve myeloablation.

One patient was excluded due to unsuccessful leucopheresis. Although we could achieve successful leucopheresis in all patients despite earlier 2-CDA therapy, it is possible that the reason for failure of stem cell mobilization in one particular patient might be related to the use of 2-CDA.[34] T-cells play an essential role in the pathogenesis of CD and RCD-II / EATL.[8, 10, 15] Through the activity of the enzyme tissue transglutaminase (tTG) glutamine residues in gluten are converted into glutamic acid.[35, 36] Subsequently a multitude of gluten-derived peptides is generated, that, when bound to either HLA-DQ2 or DQ8 can induce T-cell responses in CD patients.[8, 24] A particular glutamine and proline rich 33-mer α-gliadin peptide that contains 6 different T-cell stimulatory sequences and is resistant to gastric and duodenal proteolysis might be the primary initiator of the inflammatory response to gluten. In the large majority of patients, even in children with CD, inflammatory T-cell responses to other gluten peptides are also observed, implicating multiple gluten peptides in the disease process.[26, 27]

The definition of RCD I/ II has undergone refinement in recent years. It seems that the most reliable available method to differentiate between RCD I and RCD II is flow-cytometry of intestinal biopsies revealing the presence of aberrant T-cells. Detection of a clonal T-cell population by testing for TCR rearrangement was thought to be highly predictive of EATL development. However, oligo- or monoclonal IEL's populations can be detected in the large majority of both RCD I and RCD II patients, also in patients that do not develop an EATL. Clonality is therefore of limited use in establishing the diagnosis of RCD and to predict the development of EATL.[14, 37, 38]

RCD II is usually resistant to any known immunosuppressive therapy, including azathioprine/ prednisone[15], cyclosporine [16] and IL 10 therapy.[17] Recently, we treated 17 patients with 2-CDA on intention to induce remission. Within a mean follow up period of 22 months (range 7- 67 months) 47% had a significant decrease in aberrant T-cell percentages with or without clinical response.[39] However, another 41% did not respond clinically, histologically nor immunopathologically and subsequently died from EATL.

Remissions of autoimmune diseases have been described in adults after both allogenic and autologous ASCT [1-7] most probably due to the extreme immunosuppressive effects of these

strategies[1], resulting in immunoablation with subsequent regeneration of naïve T lymphocytes derived from reinfused haematopoietic progenitor cells.[7] Furthermore, recently, interesting insights into possible unsuspected mechanisms by which stem cell transplantation could affect the gut have emerged. In both animal and patient studies, sex mismatched allogeneic stem cell transplants have shown in both mice and women that a population of myofibroblast derived from the donor populates the intestinal mucosa. Given the importance of myofibroblasts in orchestrating the function of epithelial cells, these data suggest a mechanism other than one targeted at immunosuppression that could beneficially reset patient functions, for example enhancing barrier function following stem cell transplantation.[40]

These positive results, the high risk of transforming into EATL and the absence of effective therapy for RCD with aberrant T-cells led us to introduce this new strategy with the ultimate goal of resetting the immune response which might prevent or delays development of overt EATL. On follow up, our patients showed improvement in the small intestinal histology, together with impressive clinical improvement as demonstrated by disappearance of diarrhoea and abdominal pain, normalization of serum albumin, electrolytes and haemoglobin, increase in BMI and improvement of the performance status. Two years after transplantation, our first patient is showing further improvement in his immunopathology status as demonstrated in further decline in the percentage of aberrant T-cells to 3% and histologically improved from Marsh III-A to Marsh-I. We propose that enhanced apoptosis of activated but aberrant T-cells has led to this late but remarkable decline.[41] One patient died 8 months post ASCT from progressive neurological manifestations in association with CD. Autopsy had excluded any structural or infectious cause. One patient had developed self-limiting erythematous plaque skin lesions with central necrosis two months post ASCT. Detailed analysis had excluded the presence of EATL. Our most recent patient with clinically short bowel syndrome is showing remarkable clinical, endoscopical and immunological improvement.

All our patients had negative serology before inclusion confirming their strict adherence to GFD and after ASCT all patients remained negative for anti-tTG and EMA.

Furthermore, the first 3 patients showed a significant increase in the percentage of $CD8^+$ lymphocytes, which is seen as a marker of lymphocyte regeneration after ASCT.[42] Patient D did not show a significant increase in $CD8^+$ cells and the last 3 patients have not yet shown a significant change. Absence of a demonstrable improvement in the surface expression of CD8 on the IEL might be regarded as a poor prognostic indicator of response; this is only to be proved or disproved on longer term follow up.

Although the short term results in these patients are promising, follow up at present is too short to permit firm conclusions as to efficacy. The selection of patients for this treatment should be restricted to those patients with a substantial population of aberrant T-cells, even after therapy with 2- CDA who have a greater tendency to progress to highly lethal EATL. High-dose chemotherapy followed by ASCT seems feasible and safe, and might result in long-term improvement of disease activity in RCD patients with aberrant T-cells whose condition previously did not respond to available treatments. Longer-term follow up and additional pilot studies with larger groups of patients are needed to confirm the efficacy of this therapy.

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Addendum

At the time of writing the thesis manuscript our first patient (patient A) developed an EATL and currently receiving chemotherapy. The other 5 patients are currently in a very good clinical condition. The last 3 patients have completed more than one year of follow up after transplantation.They are clinically asymptomatic; all three showed marked endoscopic, histologic and immunologic recovery. The following table summarizes the data at last follow up.

Updated data. Histological and phenotypical flow-cytometric analysis of IEL's in duodenal biopsies before and after ASCT. ly= lymphocytes.Normal range for Cyt CD3$^+$surf CD3$^-$ % of lymphocytes ≤ 10%.

Patient	Marsh category		CD7$^+$CD3$^-$ % of CD103$^+$ ly		Cyt CD3$^+$surf CD3$^-$ % of CD103$^+$ ly		CD8$^+$ % of CD103$^+$ ly	
	Before	After 3- 12- 24m	Before	After 3- 12- 24m	Before	After 3- 12- 24m	Before	After 3- 12- 24m
A*	IIIA	IIIA - IIIA - I	95	47- 48 -15	94	89 - 86 - 3	1	20- 7 - 52
B	IIIB	I - I - I	51	7 - 4 - 8	51	2 - 6 - 4	28	68 - 62 - 67
C	IIIC	IIIA - IIIA-	62	33 - 24-	59	34 - 27	15	41 - 36
D**	IIIA	IIIA	54	47	81	78	7	13
E	IIIB	I - 0	44	68 - 40	30	36 - 28	22	2 - 5
F	IIIB	IIIA - 0	71	40 - 41	50	31 - 26	23	11 - 11
G§	IIIC	IIIA - IIIB	11	30- 5	10	27 - 3	63	52 - 86

* developed EATL 32 months after receiving ASCT. ** died 8 months after ASCT. § data *after 6 months of ASCT*

Autologous Stem Cell Transplantation for Enteropathy-Associated T Cell Lymphoma

9

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Abstract

Background: Despite treatment, enteropathy associated T-cell lymphoma (EATL) has a very poor outcome. Chemotherapy can be complicated by small bowel perforation, gastrointestinal bleeding and development of enterocolic fistulae.
Her we report on the feasibility, safety and efficacy of autologous stem cell transplantation (ASCT) in EATL patients after undergoing cytoreductive therapy including high dose chemotherapy with or without partial small bowel resection.
Methods: Four patients [(2 males, 2 females, mean age 65 years (range 60-69 years)] have received ASCT (3 patients received upfront transplantation and one was transplanted only after relapse). After partial small bowel resection (3 patients), induction chemotherapy and conditioning, ASCT has been performed.
Results: All 4 patients completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning chemotherapy and transplantation. Engraftment occurred in all patients. No major non-haematological toxicity or transplantation-related mortality was observed. One patient has ongoing complete remission 32 months after transplantation. Three patients died from progressive disease within few months after ASCT.
Conclusions: ASCT for patients with EATL seems unsatisfactory. Therefore, earlier diagnosis and the development of more effective treatments are urgently required to improve the prospects of these patients. In patients with disease limited to the small bowel, pre-transplant partial small intestine resection might improve the response rate. However, more intensive conditioning, more aggressive chemotherapy with/or without targeted immunotherapy as well as allogenous SCT needs to be explored.

Introduction

Enteropathy associated T-cell lymphoma (EATL) is a specific type of peripheral T-cell lymphoma associated with coeliac disease and it is known for its very poor outcome: 1- and 5-year survival rates in the range of 31-39% and 11-20% respectively.[1, 2] In a prospective, multicentre study of 35 patients with EATL treated with six cycles of CHOP (Cyclophosphamide, Doxorubicine, Vincristine and Prednisone), the cumulative 2-year survival was only 28%.[3]
EATL is rare, except in the coeliac disease population, where the risk has been estimated to be as high as 19.2 times that of the general population.[4] The annual incidence rate of EATL has been reported to be 0.5-1 per million people in Western countries.[5]
Strict adherence to a gluten-free diet in coeliac disease for more than 5 years has been shown to reduce the overall cancer risk in the coeliac disease group to that of the general population.[6] EATL can present in two different ways. There are patients with well-established coeliac disease who have responded to a gluten free diet but then deteriorate because of the development of refractory coeliac disease (RCD) type II or EATL. In the other group, patients are not known with coeliac disease and coeliac disease and EATL are diagnosed more or less simultaneously (*de novo* EATL).[7]

An immunophenotypically aberrant clonal intraepithelial T-cell population has been found in up to 75% of patients with RCD.[8] Clonal T-cell receptor (TCR) gene rearrangements have been found in patients with refractory coeliac disease without histologic evidence of lymphoma.[9-11] It remains unclear whether chronic inflammatory conditions such as coeliac disease provoke an aberrant immune response or the underlying abnormal T-cell response is already present, creating the picture of refractory CD.[12] Furthermore, identifying patients at risk can be difficult, as establishing the diagnosis of RCD itself takes time.[13]

In the largest case series investigating treatment and clinical outcomes in EATL, more than halve of the patients could not complete treatment secondary to poor nutritional status and chemotherapy was complicated by small bowel perforations, gastrointestinal bleeding, and development of enterocolic fistulae.[14]

There have been few case studies of EATL patients treated with chemotherapy and upfront stem cell transplantation.[1, 14-18] These reports described very small groups of patients with a complete remission (CR) and disease-free survival ranging from (0 - 64 months post ASCT). Encouraging results came from a recent report [18] describing the treatment of six patients with upfront ASCT; four patients of them remain alive in CR at 1.83-4.32 years; two have relapsed.

Here we report on the feasibility, safety and efficacy of ASCT in patients with EATL (3 upfront ASCT and one received transplantation only at relapse), after high dose chemotherapy with or without partial small bowel resection.

Patients and Methods

Patients

Four patients (2 males, 2 females) with a diagnosis of EATL received high dose chemotherapy followed by ASCT.

Patient characteristics are summarized in table 1.

Patient A is a 69 year old female, known to have mononeuritis multiplex and Sjögren syndrome for more than 20 years. At the age of 64 years, a diagnosis of EATL and coeliac disease was established. She was treated with gluten free diet and partial small bowel resection followed by standard dose chemotherapy, which consisted of 8 courses CHOP therapy (without Vincristine because of the presence of peripheral neuropathy). Thereafter, she remained in complete remission (CR) for 18 months. Subsequently she developed relapse with localization in the jejunum. A second resection was necessary, and after recovery, second line high dose chemotherapy was initiated: Dexamethasone, Cytarabine and Cisplatinum (DHAP, two cycles), and Etoposide, Ifosfamide and Methotrexate (VIM, one cycle; DHAP-VIM-DHAP). This treatment was followed by conditioning with BEAM (BCNU, Etoposide, cytarabine, melphalan) chemotherapy and ASCT.

Patient B is a 60 year old female who was diagnosed with *de novo* EATL localized in the mesenteric lymph nodes. She was treated with 4 cycles CHOP chemotherapy and gluten free diet. Subsequently, she underwent ASCT preceded by conditioning therapy with fludarabine (40 mg/m^2/day for 5 days) and melphalan (dose 70 mg/m2 at day -2 and day -1).

Patient C is a 66 year old male. He was admitted because of pain in the epigastric region and

weight loss. On computed tomography (CT) scan of the abdomen, localized thickening of the small bowel wall was seen. He underwent *en bloc* resection of one meter small bowel segment and mesentery with primary anastomosis. Histopathologically, the diagnosis of EATL was confirmed. Subsequently, he was treated with CHOP chemotherapy (8 courses in total). Because of partial response after 4 courses (radiologic analysis showed multiple mesenteric lymph nodes), consolidation with ASCT after first line therapy followed. Conditioning was with fludarabine and melphalan.

Patient D is a 66 year old male, known with coeliac disease and osteoporosis. He was on gluten free diet for one year. Video capsule enteroscopy (VCE) and double-balloon enteroscopy (DBE) were performed because of persistent weight loss. Both of these methods showed ulcerative jejunitis of the distal jejunum and ileum, but histopathology of endoscopic biopsies was not conclusive. An emergency laparotomy was performed because of persistent melaena and hemodynamic instability. Partial resection of small intestine was performed. In the resection specimen multiple localisations of a lymphoma had been identified in the wall of the ileum, but not in enlarged lymph nodes. The lymphoma expressed CD2, CD3 and in a part of the cells TIA-1 and CD30, but was negative for CD5, CD4, CD8 and B-cell markers (figure 1, Colour plate C-1) and ulcerative jejunitis with Marsh IIIA villous atrophy (figure 2, Colour plate C-2). After recovery from laparotomy, he was treated with 8 cycles CHOP chemotherapy, combined with immunotherapy (antiCD52, alemtuzumab). Subsequently, he received conditioning with BEAM followed by ASCT.

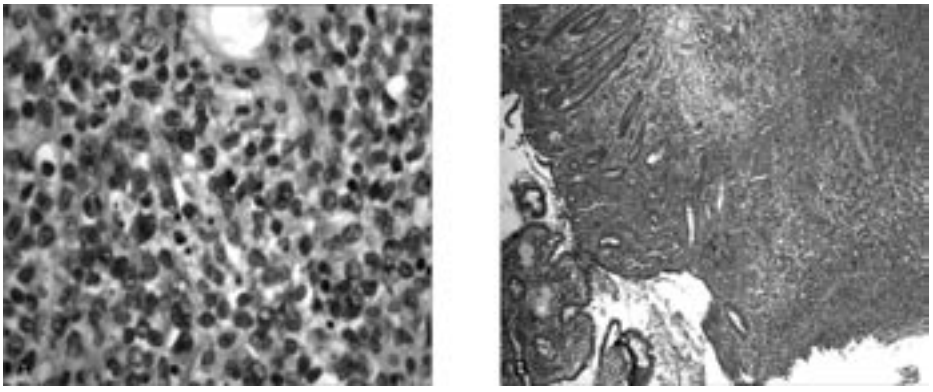

Figure 1. EATL in ileum resection specimen with a proliferation of middle-sized atypical lymphocytes (A) leading to villous atrophy and ulceration (B). (H&E, 12,5x and 630x)

Staging

The Ann Arbor staging system was used based on clinical assessment and the following investigations:

- Radiological evaluation: Chest x-ray, CT-scan [neck, chest, abdominal], Positron emission tomography (FDG-PET) [19], magnetic resonance (MR) enteroclysis,
- Evaluation by an Ear nose throat surgeon, to exclude nasopharyngeal and paranasal localization.
- Bone marrow trephine biopsy was performed to rule out bone marrow localization.

Criteria of diagnosis

The diagnosis of EATL was established according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[20] The immunohistochemical features of EATL are the presence of large or medium size T-cell proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$.

EATL can be $CD3^+$ $CD8^-$ $CD30^+$ large cell lymphomas, $CD3^+$ $CD8^+$ $CD30^-$ small cell lymphomas or γδ- lymphomas. Diagnosis of EATL was confirmed by an expert- panel of pathologists.

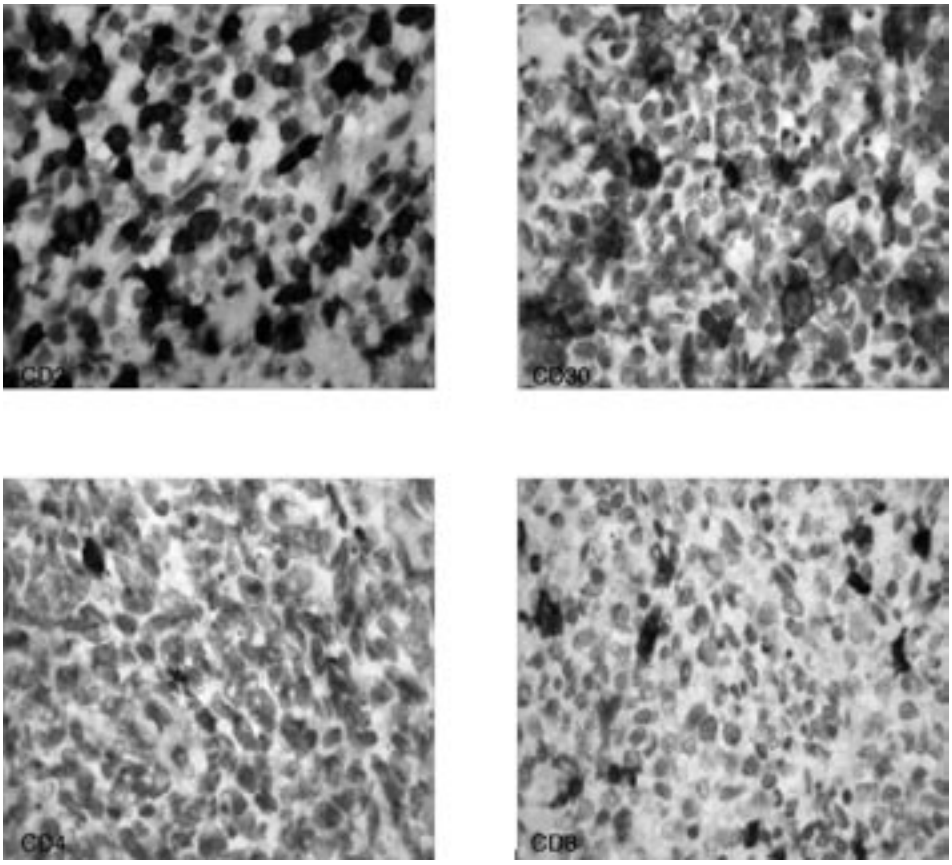

Figure 2. EATL in ileum resection specimen expressing CD3 and partly also CD30. Residual small T-lymphocytes express CD4 or CD8, lymphoma cells virtually all negative.

Peripheral blood stem cells mobilization and collection

Mobilization of haematopoietic progenitor cells from the bone marrow into the peripheral blood was performed using granulocyte-colony stimulating factor (G-CSF).

Haematopoietic stem cells were collected from the peripheral blood by leucopheresis.

Response criteria

Response to therapy was evaluated according to the Cheson criteria.[21]

Results

Patient characteristics

The baseline characteristics of the 4 patients are shown in table1. The mean age 65 years (range 60-69 years). Three patients had *de novo* type of EATL, while one patient was known to have coeliac disease one year before developing EATL (patient D). All patients had positive serology for coeliac disease at diagnosis. Endoscopically, 3 patients had evidence of ulcerative jejunitis. All patients had definite histological features of coeliac disease according to the Marsh criteria (3 had Marsh IIIA and one IIIB). A significant percentage (30-51%) of the intraepithelial lymhocytes was aberrant, defined as $CD7^+$ surface $CD3^-$ cells (expressed as % of $CD103^+$ lymphocytes) or cytoplasmic $CD3^+$, surface CD3 negative cells (expressed as % of $CD103^+$ lymphocytes) in all patients.

Immunophenotypical testing of lymphoma tissues showed that these EATL's were $CD3^+CD8^+CD30^+$ in 3 patients (patients A, B and C) and $CD3^+CD8^-CD30^+$ in one patient (patient D).

Patient B had bone marrow involvement at diagnosis and also localization in the cerebrospinal fluid during relapse. All patients had stage IV at diagnosis characterised by intestinal or extra-intestinal involvement.

Radiologically, the most prominent findings were mesenteric lymphadenopathy, bowel wall thickening and prestenotic dilatation. Mesenteric lymphadenopathy was seen in all patients; one patient (patient D) had also hilar lymphadenopathy. FDG-PET showed increased activity in all patients.

Stem cell mobilization and leucopheresis

Mobilization of haematopoietic progenitor cells from peripheral blood was achieved successfully and uncomplicated in all patients using G-CSF.

Toxicity of High dose chemotherapy

No transplantation-related mortality was reported. Blood and platelets transfusions were provided when needed. Conditioning regimens as used seemed feasible in this group of patients. The mean duration of hospitalisation was 20 days (range 18-24 days).

Engraftment

Haematopoiesis recovered in all patients. The median time to reach neutrophils > 0,5×106/l and unsupported platelets > 20 ×106/l were 12 days (8-15) and 14 days (9-16), respectively.

Table 1. Patients' characteristics. At diagnosis all patients have stage IV disease and positive TTG, EMA.

	Patient A	Patient B	Patient C	Patient D
Age/gender	69/F	60/F	66/M	66/M
Age CD (yrs)	64	60	65	65
Age ASCT (yrs)	66	60	66	65
DQ 2 haplotype	Homozygous	Homozygous	Heterozygous	Heterozygous
Marsh at Dx EATL	IIIA	IIIB	IIIA	IIIA
% aberrant T-cells at Dx	50%	51%	30%	NA
Immunohistochemical type of EATL	$CD3^+CD8^+CD30^+$	$CD3^+CD8^+CD30^+$	$CD3^+CD8^+CD30^+$	$CD3^+CD8^-CD30^+$
Extraintestinal localization	Mesenteric lymph nodes	Mesenteric lymph nodes	Mesenteric lymph nodes	Hilar, Retroperitoneal, and mesenteric nodes
Bone marrow involvement	No	Yes	No	No
Endoscopy (GDS,VCE, DBE, coloscopy)	Ulcerative jejunitis	Diffuse ulcerative jejunitis	Scalloping of folds	Ulcerative jejunitis, ulcerations in colon
CT scan/ MR enteroclysis	Dilated 2nd part with abrupt narrowing of distal duodenum	Diffuse lesions in both lungs	Dilated small bowel segment with localized thickening	Mesenteric lymphadenopathy with thickened jejunum loop
FDG-PET scan	Increased activity in upper abdomen	Increased activity in right lung and neck	Increased activity in upper abdomen	Increased activity in left hilar region

CD= coeliac disease, GDS=Gastroduodenoscopy, VCE=Video capsule endoscopy, DBE=double-balloon enteroscopy

Response and survival after ASCT

Three patients were in CR before receiving preconditioning regimens and one was in partial remission.

One patient (patient A) was in remission for 18 months after standard chemotherapy and received ASCT after having a relapse. Since then (32 months) she is still in CR.

Four weeks after ASCT, patient B developed severe neurological complaints. A cauda equina syndrome was diagnosed. CSF examination confirmed the presence of lymphoma cells carrying the same immunological markers ($CD3^+CD8^+CD30^+$). She received immediate palliative radiotherapy of the lower spine. Because of the rapid course of this relapse and her very poor clinical condition, no systemic therapy could be initiated. Unfortunately she died from CNS relapse two months after ASCT.

Table 2. Treatment results. CR= complete remission, PR=partial remission, Flud+Mel= fludarabine and melphalan

Patients	A	B	C	D
Follow up (ms) after SCT	32	2	6	9
Resection performed	Yes	No	Yes	Yes
Response to CHOP	CR (18 m) followed by relapse	CR	PR	CR
Other therapies	DHAP-VIM-DHAP	-	-	Alemtuzimab
Preconditioning regimen	BEAM	Flud+Mel	Flud+Mel	BEAM
Relapse after transplantation	No	Yes	Yes	Yes

Patient C was admitted six months after ASCT because of persistent gastrointestinal bleeding and pancytopenia. He received supportive care (blood and platelet transfusions). Relapsed EATL was suspected but after explicit request of the patient and his family, all supportive care was withdrawn and he died several days later. Autopsy examination C showed the presence of a large amount of blood in the gastrointestinal tract and multiple mesenteric pathological lymph nodes (Figure 3, Colour plate C-3). Microscopic examination of these nodes confirmed the presence of EATL in relapse.

Patient D had shown initial response, but he developed a relapse 9 months after transplantation. He was admitted with bleeding per rectum. Colonoscopy showed multiple deep ulcerations in the ascending colon and terminal ileum. Histopathological examination confirmed EATL relapse. He died. Autopsy examination showed the presence of a large amount of blood in the gastrointestinal tract due to numerous ulcers in the small intestine and the colon with multiple mesenteric pathological lymph nodes. Microscopic examination of these nodes showed considerable depletion of lymphocytes, but the pathological lymphoid population could not be identified with certainty in the lymph nodes.

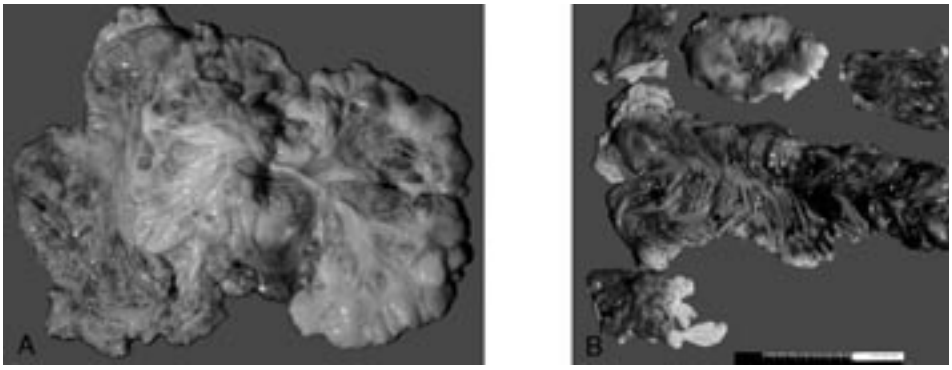

Figure 3. Macroscopic picture showing multiple pathological mesenteric lymph nodes (A) and large amount of blood in the small intestine (B)

Discussion

Intestinal T-cell lymphomas or EATL's are rare, but highly aggressive in their clinical course. They are generally diagnosed in advanced stages and presenting as surgical emergencies. They respond poorly to available anti-lymphoma regimens. Results were better in patients with limited stages of disease.[22] The most frequent complications of treatment were small bowel perforation, obstruction, gastrointestinal bleeding, and infection. The 1- and 5-year survival rates in the range of 31-39% and 11-20%, respectively.[1, 2]

We report here our results of the treatment of 4 patients with an advanced stage of EATL diagnosis (stage IV). The induction chemotherapy and "debulking" were feasible as the patients were in good condition before undergoing ASCT. The conditioning regimen and ASCT were well tolerated in all patients. The nadir leucocytes and platelets counts and the duration of leucopenia and thrombocytopenia were comparable to that in patients with Non-Hodgkin lymphomas and multiple myeloma receiving ASCT after BEAM or high dose melphalan.[23] There was no transplantation- related mortality.

Variable results were published dealing with ASCT in EATL and these are summarized in table 3. Encouraging results came form a recent report [18] describing the treatment of six patients with ASCT after receiving two cycles of IVE (ifosphamide, etoposide, epirubicin), followed by two cycles of high-dose methotrexate (3 g/m^2) with folinic acid rescue and a BEAM (carmustine, etoposide, cytarabine, melphalan). Four patients remain alive in complete remission at 1.83-4.32 years; two have relapsed.

It is a well known that T-cell malignancies do not respond adequately to conventional chemotherapeutic treatment.[24, 25] The introduction of monoclonal antibodies for the treatment of cancer has changed the outlook for patients with T-cell malignancies.[26] Recent studies with single-agent alemtuzumab, an anti-CD52 monoclonal antibody, have shown improved response rates and survival in patients with T-cell prolymphocytic leukaemia and cutaneous T-cell lymphoma.[27] Preliminary data also suggest that alemtuzumab may have

activity in patients with heavily pretreated peripheral T-cell lymphoma who are refractory to conventional chemotherapy.[28] Preclinical studies with mice bearing human adult T-cell leukemia/lymphoma cells suggest that alemtuzumab may have a potential therapeutic role in this setting.[29] Therefore, treatment of EATL with alemtuzumab in combination with chemotherapy could be promising.

In addition, an earlier institution of treatment and combination with chemotherapeutic agents may improve treatment outcome for patients with these patients and allow for consolidation with stem cell transplant strategies in selected patients.[24]

Furthermore, it has been shown that the use of intensified and high dose chemotherapy with ASCT may improve the outcome in high-risk NHL [30] and that patient who undergo allogeneic SCT for refractory or indolent NHL have lower relapse rates than those who undergo autologous SCT.[31, 32]

In this report, two different conditioning regimens were used. Because of the small cohort of patients treated, no conclusions could be made of the best conditioning regimen.

Cytoreductive therapy, using chemotherapy and partial small bowel resection, seems logical. We have recognized that the patients' condition improves before chemotherapy and also prevents the occurrence of complications as perforations, fistulas and bleeding.

The only long term survival we have is the patient with no bone marrow involvement at the time of diagnosis. The results of the other 3 patients were disappointing. Intervention at an earlier stage in the evolvement of lymphoma at the premalignant phase (RCD II) could theoretically prevent or delay the development of the malignant phase. Recently we reported on our experience in treating RCD II patients with high dose chemotherapy followed by ASCT and the results thus far are promising in six of the 7 transplanted patients.[33]

Conclusions

It seems that current chemotherapy, preconditioning regimens and ASCT do not improve the survival in this type of aggressive lymphoma. Relapse can occur within weeks to months post-ASCT. Therefore, earlier diagnosis, the development of more effective treatments including antiCD52 agents, better preconditioning regimens and possibly the use of T-cells depleted grafts or allogeneic stem cell transplantation with or without primary central nervous system prophylaxis are urgently required to improve the prospects of these patients.

Table 3. Summarizes the earlier reports on ASCT in EATL. *NHL= Non-Hodgkin's lymphoma. CR = complete remission.

Author/ reference	No. EATL patients	No. received ASCT	High dose chemotherapy	Preconditioning	Overall survival	Comments
Gale et al[1]	31	1	PEACE-BOM	BEAM	CR 64 months	overwhelming sepsis after SCT
Okuda et al [14]	1	1	8 cycles CHOP. AI relapse ESHAP (etoposide, methyl-prednisolone, cytarabine and cisplatin.	MCVC (ranimustine carboplatin, etoposide & cyclophosphamide)	8 months	Died after developing relapse (intestine and CNS)
Rongey et al [15]	1	1	4 cycles (Cyclophosphamide, Doxorubicine & Etoposide) and then 3 cycles CHOP	BEAM	CR18 months	EATL after having coeliac disease and follows gluten free diet irregularly
Jantunen et al [16]	5	5	CHOP	BEAC 3 patients/ BEAM 2 patients	median survival 2 ms (0-14m)	4 treated initially with partial small intestine resection
Blystad [17]	2 (total 40 NHL*)	2	Specific details over these 2 patients are not available	-	-	-
Bishton [18]	6	6	2 cycles of IVE (ifosphamide, etoposide, epirubicin), followed by two cycles of methotrexate (3 g/m2)+folinic acid rescue	BEAM	Four in CR at 1.83-4.32 yrs	2 relapsed

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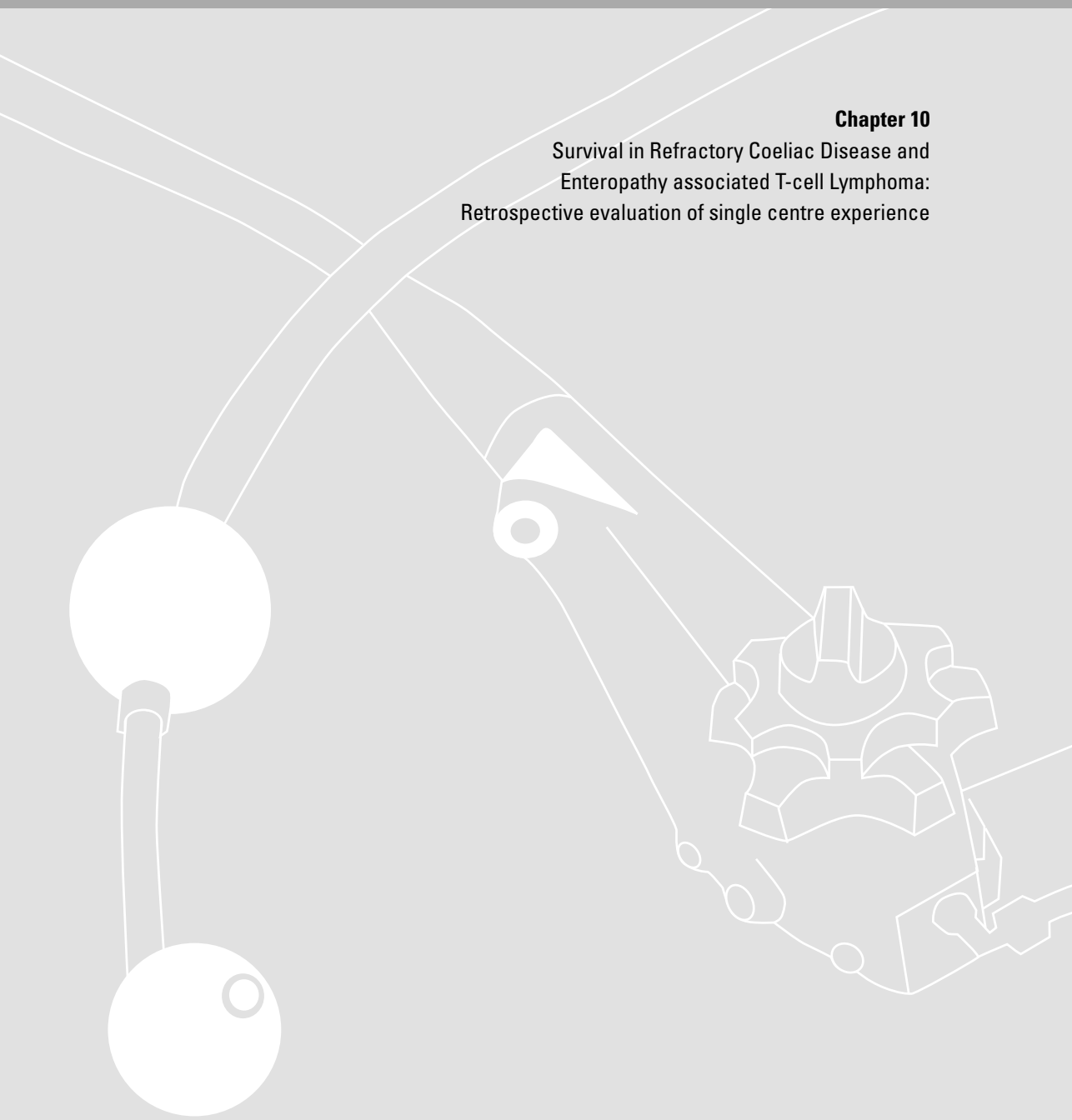
PROGNOSIS IV

Chapter 10

Survival in Refractory Coeliac Disease and Enteropathy associated T-cell Lymphoma: Retrospective evaluation of single centre experience



Survival in Refractory Coeliac Disease and Enteropathy associated T-cell Lymphoma: Retrospective evaluation of single centre experience

10

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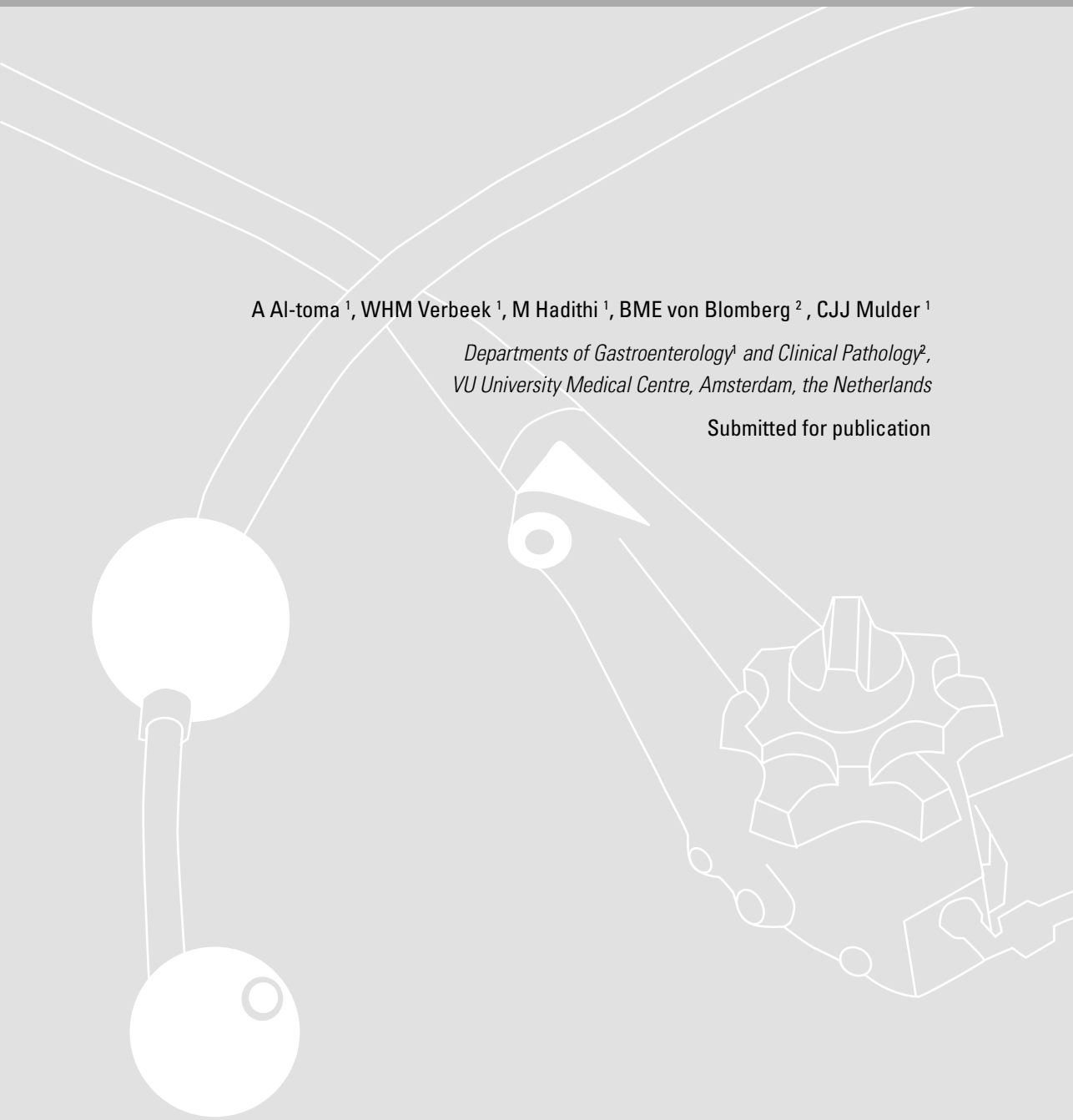

Abstract

Background: Coeliac disease (CD) may be regarded as refractory disease (RCD) when symptoms persist or recur despite strict adherence to a gluten free diet. RCD may be subdivided into types I and II with a phenotypically normal and aberrant intraepithelial T-cell population, respectively. RCD I seems to respond well to azathioprine/prednisone therapy. RCD II is usually resistant to any known therapy and transition into Enteropathy-Associated T-cell Lymphoma (EATL) is common.

Aim: The aim of this study is to provide further insight into RCD and the development of EATL, by reporting on long term survival, risk of transition of RCD into EATL in what is likely the largest cohort of patients with complicated coeliac disease in a single center.

Design and Methods: We have retrospectively compared responses to therapy in four groups of patients with complicated coeliac disease: 43 RCD-I, 50 RCD II (total), of whom 26 RCD II who developed EATL after a period of refractoriness to a gluten free diet (secondary EATL) and 13 EATL patients without preceding history of complicated coeliac disease (de novo EATL). Every effort was made to ensure correct classification and accurate patient allocation.

Results: No coeliac disease related mortality is recognized in the RCD I group. The overall five year survival in RCD I is 96%, in RCD II (total) is 58% and in RCD II after developing EATL is only 8%. The 2 year survival in the de novo EATL is 20% versus 15% in secondary EATL (P=0.63). Twenty eight (56%) from 50 patients with RCD-II died, 23 (46%) due to EATL and 4 due to progressive refractory state with emaciation and one from neurocoeliac disease.

Conclusion: Remarkably, no patient with RCD I developed RCD II or EATL within mean follow up of five years (range 2-15 years). Fifty two percent of the RCD II patients progressed to/ developed EATL within 4-6 years after the diagnosis of RCD II. More aggressive therapies seem necessary in RCD II and EATL.

Introduction

Coeliac disease is a life-long gluten sensitive enteropathy that commits the patient to a permanent gluten free diet, which is sufficient to treat the overwhelming majority of patients. However, in a small percentage (2-5%) of adult onset coeliac disease patients serious complications develop in the form of refractoriness or development of pre- and malignant complications.[1] Patients with coeliac disease may be regarded as suffering from refractory coeliac disease (RCD) when symptoms persist or recur after a former good response despite strict adherence to a gluten free diet.[1,2,3] We define RCD as persisting villous atrophy with crypt hyperplasia and increased intraepithelial T-lymphocytes in spite of a strict gluten free diet for more than 12 months or when severe symptoms necessitate intervention independent of the duration of the gluten free diet.[2,4] Immunologically, two types of RCD are recognized depending on the presence or absence of aberrant intraepithelial lymphocytes in the small bowel mucosa. When normal expression of T-cell surface markers occurs (RCD I), the prognosis is less dismal than when an aberrant intraepithelial lymphocyte population

is present (RCD II).[2,4,5] Patients with RCD II are known to be at a greater risk of developing malignancy, particularly enteropathy-associated T-cell lymphoma (EATL).[2,5,6,7]
RCD II can be regarded as a 'cryptic' lymphoma.[4] There is now strong molecular and immunophenotypic evidence showing that a monoclonal neoplastic T-cell population may emerge from IEL's in RCD. Clonal expansion of this monoclonal T-cell population eventually leads to frank EATL. The genesis and expansion of these monoclonal T-cells involve both inappropriate immune responses to gluten and acquisition of genetic abnormalities. Although the monoclonal IEL's in patients with RCD are neoplastic, they are not cytologically abnormal and do not form tumour masses which differentiate these patients from EATL patients, in addition to the absence of radiological and bone marrow evidence of lymphoma.[2, 8,9,10]
RCD II is usually resistant to any known therapy that has thus far been tested, including azathioprine/prednisone, cyclosporine, IL-10 and cladribine (2-Chlorodeoxyadenosine) therapy.[2,11-17] EATL has a very poor outcome with current therapies, with 1- and 5-year survival rates in the range of 31-39% and 11-20%, respectively.[18,19] In a prospective multicentre study from Germany on 35 patients with EATL treated with six cycles of CHOP (Cyclophosphamide, Doxorubicine,Vincristine and Prednisone) cumulative 2-year survival was only 28%.[19]
So far, no systematic analysis of the survival of this group of patients has been reported. The aim of this study is to provide further insight into the understanding of refractory coeliac disease and the development of EATL, by reporting on what is likely the largest cohort of patients with complicated coeliac disease in a single centre. We have retrospectively compared the survival in four groups of patients with complicated coeliac disease: RCD-I, RCD II total, secondary EATL and de novo EATL. Since correct disease allocation of these patients is crucial, we have applied state-of-the-art methodologies to ensure that our patients were classified according to the internationally accepted criteria by employing clinical, endoscopical, radiological and molecular techniques.

Patients and Methods

Patients

We performed a retrospective analysis of response to therapies, providing long term follow up data on 4 categories of patients with complicated forms of coeliac disease in a tertiary referral centre for coeliac disease. From 1992 to 2005 forty-three patients with RCD I (12 males and 31 females; mean age at diagnosis of RCD 49 years, range 23-86), 50 patients with RCD II (19 M: 31 F; mean age at diagnosis 59 years, range 47-88) of whom 26 patients with secondary EATL (11 M:15 F; mean age at diagnosis of EATL 61.5 years, range 52-79) and 13 patients with de novo EATL (11M:2 F; mean age at diagnosis 64.3, range 56-72) were studied. In the de novo EATL, there is no history of complicated coeliac disease and none of them have followed gluten free diet. A small group of patients with RCD have been excluded from the analysis, they were treated with cyclosporine or interleukin 10.[14, 15]
From the RCD-I patients 31 were treated with prednisone alone and 12 with a combination of prednisone and azathioprine.[11] Thirty four from RCD-II patients were treated with prednisone

and azathioprine [11] and subsequently 23 of them received cladribine because of persistent unsatisfactory responsiveness.[17] From the cladribine treated group 7 were included in our pilot study using high dose chemotherapy followed by autologous peripheral stem cell transplantation. These patients were in persistent refractory state with high percentage of aberrant T-cells and most of them (5/7) have ulcerative jejunitis. Stem cells were collected by leucopheresis. After conditioning with fludarabine and melphalan, autologous stem cell transplantation was performed.[17]

Patients with EATL were treated with multiagent chemotherapy according to standard lymphoma treatment protocols and when indicated partial small bowel resection has been performed. Four patients with EATL (3 de novo EATL and one with secondary EATL) received high dose chemotherapy followed by autologous peripheral stem cell transplantation. Three patients have partial small bowel resection before transplantation. (CJJ Mulder: unpublished data).

The patients with RCD I and II were followed for evidence of transition to a more severe state (i.e., the transition from RCD I to RCD II and /or EATL, and from RCD II to EATL) over a mean period of 5 years (range 2-14 years).

Diagnostic criteria

The diagnostic criteria of the different groups have been summarized in table 1. The diagnosis of coeliac disease was confirmed by histological examination with a documented histologic response to gluten withdrawal.[3, 18] Patients were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a gluten free diet, histopathology showing at least partial villous atrophy (Marsh IIIA) according to the modified Marsh criteria and after excluding other causes of villous atrophy.[3, 4] Taking in consideration that a significant number of patients (around 50%) with RCD I may indeed have inadvertent gluten ingestion.

The diagnosis of RCD was established as type I when no or less than 10% aberrant T-cells were present in small bowel biopsy specimens and type II with ≥ 20% aberrant T-cells detected by immunophenotyping using flow-cytometric analysis of the intestinal mucosa [2,11,22]. In RCD I the intraepithelial lymphocyte phenotype is normal with the expression of surface CD3, CD8 and TCR. In RCD II the intraepithelial lymphocytes have normal morphological features, but they exhibit an aberrant phenotype with the normal expression of CD103 and CD7 but with downregulation of surface CD3 to intracytoplasmic CD3, and the lack of classical surface T-cell markers such as CD4, CD8 and, as a consequence of CD3 downregulation, TCR.[2, 22]

In the RCD patients the presence of EATL has been confidently excluded using radiological and endoscopic methods (small bowel follow through, computed tomography scanning of thorax and abdomen [23], whole body positron emission tomography scan [24], upper gastrointestinal endoscopy, video capsule endoscopy and/or double-balloon enteroscopy [25] as well as trephine bone marrow biopsies. Those patients diagnosed in 2003 or earlier have negative small bowel follow through and computed tomography scan, while those diagnosed after 2003 have negative computed tomography scan, positron emission tomography scan, video capsule endoscopy and/or double-balloon enteroscopy.

The diagnosis of EATL was established according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[26, 27] The immunohistochemical features of EATL are evidence of large or medium size T-cell proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$. Thirty five patients out of 39 had $CD3^+$ $CD8^-$ $CD30^+$ large cell lymphomas, 2 patients had $CD3^+$ $CD8^+$ $CD30^-$ small cell lymphomas[16] and another 2 had γδ- lymphomas.

Table 1. Summarizes the diagnostic criteria

Disease category	Diagnostic criteria	References
RCD I	- Villous atrophy persisted or recurred despite strict adherence to a gluten free diet. - At least partial villous atrophy (Marsh IIIA) according to the modified Marsh criteria - Excluding other causes of villous atrophy. - When ≤ 10% aberrant T-cells in intestinal biopsy. - Intraepithelial lymphocyte phenotype is normal with the expression of surface CD3, CD8 and TCR	2,3,4,,8,19
RCD II	- The same as RCD I, in addition to the presence of ≥ 20% aberrant T-cells in intestinal biopsy. - The intraepithelial lymphocytes have normal morphology, but exhibit an aberrant phenotype (normal expression of CD103 and CD7, downregulation of surface CD3 to intracytoplasmic CD3, and the lack of surface T-cell markers: CD4, CD8 and TCR). - EATL has been confidently excluded.	2,3,4, 8,19
Secondary EATL	- WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. - The patient is already known to have coeliac disease or RCD.	23, 24
De novo EATL	- WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. - No previous history of coeliac disease or use of gluten free diet.	23, 24

Evaluation

Clinical, laboratorial (haematology, biochemistry and serology), endoscopic and histological examination of the small intestine was performed at regular intervals (3-6 months). Clinically, patients were followed at the outpatient clinic at regular intervals and their adherence to the gluten free diet was checked by a dietician. Particular attention was paid to clinical response including symptoms and signs of malabsorption, body mass index and performance status; and adverse effects of therapies.

Antiendomysium antibodies (EMA) and anti-tissue transglutaminase antibodies (anti-tTG) were tested at diagnosis and at follow up. HLA-DQ2/8 typing [28] and immunophenotyping of intraepithelial lymphocytes were performed in all patients.

Endoscopy using upper gastrointestinal endoscopy, video capsule endoscopy and/or double-balloon enteroscopy with small bowel biopsies was performed as indicated.

Computed tomography scan, positron emission tomography scan, magnetic resonance enteroclysis and dual energy X-absorptiometry were performed as indicated. The techniques for the video capsule endoscopy, double-balloon enteroscopy and MR enteroclysis are available in our centre only since the beginning of 2003.

Small bowel biopsies

Upper gastrointestinal endoscopy was performed in all patients. At least 10 duodenal biopsies were taken for histological, immunohistochemical and flow cytometric examination. Four to six biopsies were fixed and preserved in 10% formalin for histopathological and immunohistochemical evaluation. Three-4 biopsies for T-cell receptor gene rearrangement studies were taken separately, preserved on histocon and frozen at -20°C. For immunophenotypical evaluation 3-4 biopsies were taken and preserved in RPMI medium.

Isolation of intraepithelial lymphocytes and cell-staining for immunophenotyping

Lymphocytes and enterocytes were isolated from 3-4 small bowel biopsies by homogenising tissue samples and passing fragments through a 100μm nylon cell strainer (Becton Dickinson-) in RPMI medium supplemented with 1% FCS. The released cells were subsequently washed and labelled by 4-color staining for 30 minutes on ice with various combinations of fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein and allophycocyanin labelled monoclonal antibodies against CD3, CD4, CD8, CD7, CD103, CD19, CD45, CD16/56, γδTCR and cytoplasmic CD3. The FACS method used in this study was in accordance with the manufacturer's guidelines.

Cell surface immunophenotyping of intraepithelial lymphocytes was performed on a 4 colour FACS Calibur flow cytometer (Becton Dickinson, BD, immunocytometry systems, San Jose, CA). Nonviable cells and debris were excluded based of forward and sideward light scatter properties and a gate on CD45 positive cells was used for selecting lymphocytes. Intraepithelial localisation of lymphocytes was confirmed by surface expression of CD103 (αEβ7 integrin, a gut homing receptor for E-cadherin). Intraepithelial lymphocytes were analysed, using CellQuest^{tm} (KS Stat) based on their expression of cell markers: cytoplasmic CD3, surface CD3, CD4, CD7, CD8, CD16/56, CD19, CD103 and TCRγδ on CD45^{+}gated intraepithelial lymphocytes.[29] Arbitrarily, a percentage of aberrant cells CD7^{+}CD3^{-} of CD103^{+} intraepithelial lymphocytes or cytoplasmic CD3^{+} surface CD3^{-} % of CD103^{+} intraepithelial lymphocytes of ≤ 10% has been regarded as normal, and more than 20% as definitively abnormal. A percentage between 11% and 20% were regarded as borderline but none of our patients had such percentages.

Assessment of TCR gene rearrangement by Polymerase Chain Reaction (PCR)

DNA was extracted from cryosections of duodenal biopsies by a standard procedure using proteinase-K digestion and ethanol precipitation of the genomic DNA. T-cell receptor-gamma (TCR-γ) gene rearrangements were analysed by multiplex PCR amplification under standarized conditions. Monoclonal and polyclonal controls were included in each experiment. Clonality assessment for TCR-γ gene rearrangements was done using the BIOMED-2 multiplex TCR PCR protocol.[26, 27]

Statistical analysis

Kaplan-Meier survival curves were constructed using SSPS software (SPSS Inc. Chicago, Illinois, USA) for comparison between the groups. Chi square test, odds ratio, log rank and logistic regression tests were used to assess the statistical significance between variables. A p value ≤ 0.05 is considered statistically significant.

Results

Table 2 shows the baseline demographic characteristics according to the disease group. Regarding gender distribution in the studied groups, there is no difference between the RCD I and RCD II groups. In contrast the de novo EATL group showed a statistically significant increase in the male: female ratio as compared to the RCD I (P< 0.001) and secondary EATL group (P<0.025). Patients with RCD I are younger than RCD II and EATL patients, but no transition has been documented from RCD I to RCD II or EATL during the period of follow up.

HLA-DQ2 genotype is present in 80% of RCD I, 92% of total RCD II , 96% of secondary EATL and 92% of de novo EATL groups.

HLA-DQ2 homozygosity is significantly higher in secondary EATL and de novo EATL compared to RCD I, odds ratio= 2,6 (CI: 1,94 - 5,33) and OR=2,06 (CI: 1,88 - 4,78) respectively.[30]

Table 2. The baseline demographic characteristics and therapies received according to the disease group

Characteristic	RCD-I		RCD-II	Total	Secondary EATL		De novo EATL	
Total (Male: Female)	43	(12:31)	50	(19:31)	26	(11:15)	13	(11:2)
Age at Dx CD (±SD) (range) in years	47 (21-75)	(±13.5)	57 (40-69)	(±6.5)	59 (40-69)	(±11.2)	64.3 (56-72)	(± 4.5)
Age at Dx RCD/EATL (±SD) (range) in years	49 (23-86)	(±14)	59 (47-88)	(±9.5)	61.5 (52-79)	(±6.5)	64.3 (56-72)	(±3.4)
Follow up (mean, range) in months	72	(24-240)	44	(8-146)	10	(3 - 50)	12	(3-36)
DQ2 Total	34	(80%)	46	(92%)	25	(96%)	12	(92.3%)
- Heterozygous	23	(54%)	23	(46%)	7	(27%)	4	(30.7%)
- Homozygous	11	(26%)	23	(46%)	18	(69%)	8	(61.5%)
Aberrant T-cells at Dx of RCD and/or EATL (mean % ± SD)	3.0 ± 1.9		60 ± 25.9		68 ± 24.4		9 ± 13	

Dx= diagnosis, 2-CDA= cladribine. * P =0.025

Serologically, all patients with positivity for EMA and/or anti-tTG at the time of diagnosis of coeliac disease reverted to negative after the gluten free diet, confirming their strict adherence to diet (in addition to clinical assessment by a dietitian). Those with de novo EATL have negative serology at the time of diagnosis.

Concerning the mean percentage of aberrant T-cells at the time of diagnosis of the RCD and/or EATL, the difference between RCD I (3%) versus RCD II (60%) and between secondary EATL (68%) versus EATL de novo (9%) is extremely statistically significant ($P < 0.0001$).
Table 3 shows the treatments received and summarizes protocols. From all EATL patients 23 (59%) were treated with chemotherapy (Cyclophosphamide, Doxorubicine, Vincristine and Prednisone) (CHOP), while the other 16 patients were not eligible for chemotherapy because of a very bad general condition at the time of diagnosis. Fourteen (36%) have undergone partial resection of the small bowel. Laparotomy was needed in 3 patients to establish a diagnosis and 11 were operated for complications (5 for perforations and 6 for obstructive symptoms). Seven patients were treated both by chemotherapy and resection. Eight patients (61.5%) of the de novo EATL group have undergone partial small bowel resection compared to only 6 (23%) of secondary EATL group (P= 0.025). Three other RCD II patients had surgery because of ulcerative jejunitis with perforations. Four patients with EATL were treated with high dose chemotherapy followed by autologous peripheral stem cell transplantation (3 with de novo EATL and one with secondary EATL) and 3 of them died within few months thereafter (CJJ Mulder: unpublished data).

Table 3. Summary of treatments received and protocols. * P =0.025

Treatment	RCD I	RCD II	Secondary EATL	De novo EATL	Treatment protocol
GFD	43	50	26	0	-
Prednisone / topical steroids only	31 (72%)	16 (32%)	0	0	Prednisone 40 mg /day 6 weeks, tapered to 10 mg/day over 6 weeks and, if possible, tapered to 2.5-0 mg daily after 3 months
Prednisone + Azathioprine [8]	12 (28%)	34 (68%)*	0	0	Prednisone (as above) + Azathioprine 2 mg/kg/day for ≥ 52 weeks
Prednisone+ Azathioprine followed by 2-CDA [14]	0	23 (46%)	0	0	Prednisone + Azathioprine as above. 2-CDA (0,1 mg/kg/day) for 5 days, in 1-3 courses every 6 months depending on response
CHOP	0	0	16 (61.5%)	7 (53%)	Standard CHOP 6-8 cycles
ASCT [17]	0	6 (12%)	1	3 (30%)	Pretreatment with 1-3 courses of 2-CDA, leucopheresis, preconditioning (Melphalan+Fludarabine) + ASCT
Partial small bowel resection	0	9 (18%)	6 (23%) *	8 (61.5%)*	-
Total	43	50	26	13	-

Table 4 shows causes of death according to patients' categories. In the RCD I group, only 3 patients died during follow up, all of them from unrelated illnesses. In the RCD II 26 patients (52%) developed EATL within 4-6 years after the diagnosis RCD II had been made, 23 RCD II patients (46%) died after developing EATL. Four (8%) died due to progressive malabsorption with emaciation, one of them developed extensive multifocal squamous cell carcinoma of the skin(> 15 lesions). One patient died because of progressive neurocoeliac disease 8 months after stem cell transplantation. Nine of 13 (69%) patients with de novo EATL and 23 of 26 (88.4%) with secondary EATL died within months of diagnosis.
Figure 1 (A and B) shows the Kaplan -Meier curve of survival according to the disease group. The 5- year survival is 96% in RCD I versus 58% in RCD II (total) (P= 0.001). On the other hand, the 2-year survival in the de novo EATL group is 20% versus 15% in the EATL (RCD II) group (P=0.63). Interestingly, the most significant drop in survival in these groups is observed in the first 2 years after diagnosis. The longest survival thus far in the de novo EATL is 26 months.

Table 4. Causes of death according to the patients categories. * P value= 0.63

	RCD-I	RCD-II (total)	EATL (RCD II)	De novo EATL
EATL	0	23 (46%)	23 (88.4%)	9 (69%)
Refractory state and emaciation	0	4 (8%)	0	0
Other coeliac- related	0	1 neurocoeliac	0	
Unrelated	3 (6.9%) (1 alcoholic cirrhosis, 1 COPD, 1 lung cancer)	0	0	0
Alive	40 (93.1%)	22 (44%)	3 (11.6%)*	4 (21%)*
Total	43	50	26	13

Since this is not a randomized study, it is not possible to make definitive conclusions about the success of different treatments. However, in the prednisone alone group the 5 year survival is 25%, in the prednisone and azathioprine group 36% (P= 0.43) and the cladribine (2-CDA) group 22 % (43 % at 36 months) P=0.97. With respect to EATL development, there is no statistically significant difference between the groups.
RCD II patients who received autologous peripheral stem cell transplantation completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning and transplantation. Engraftment occurred in all patients. No major non-haematological toxicity nor transplantation related mortality was observed. The mean follow-up duration is 16 months (range 8-31 months). Within 3-4 months of autologous stem cell transplantation all patients had normalization of stools frequency, disappearance of abdominal pain and improvement in biochemical markers. Also improvement of the

body mass index, serum albumin, endoscopical findings and histology was documented. One patient with preexistent neuro-coeliac disease mimicking multiple sclerosis developed progression and died 8 months post transplantation. The protocol, inclusion and detailed results (clinical, laboratory and endoscopy) are described in our recent article [17].

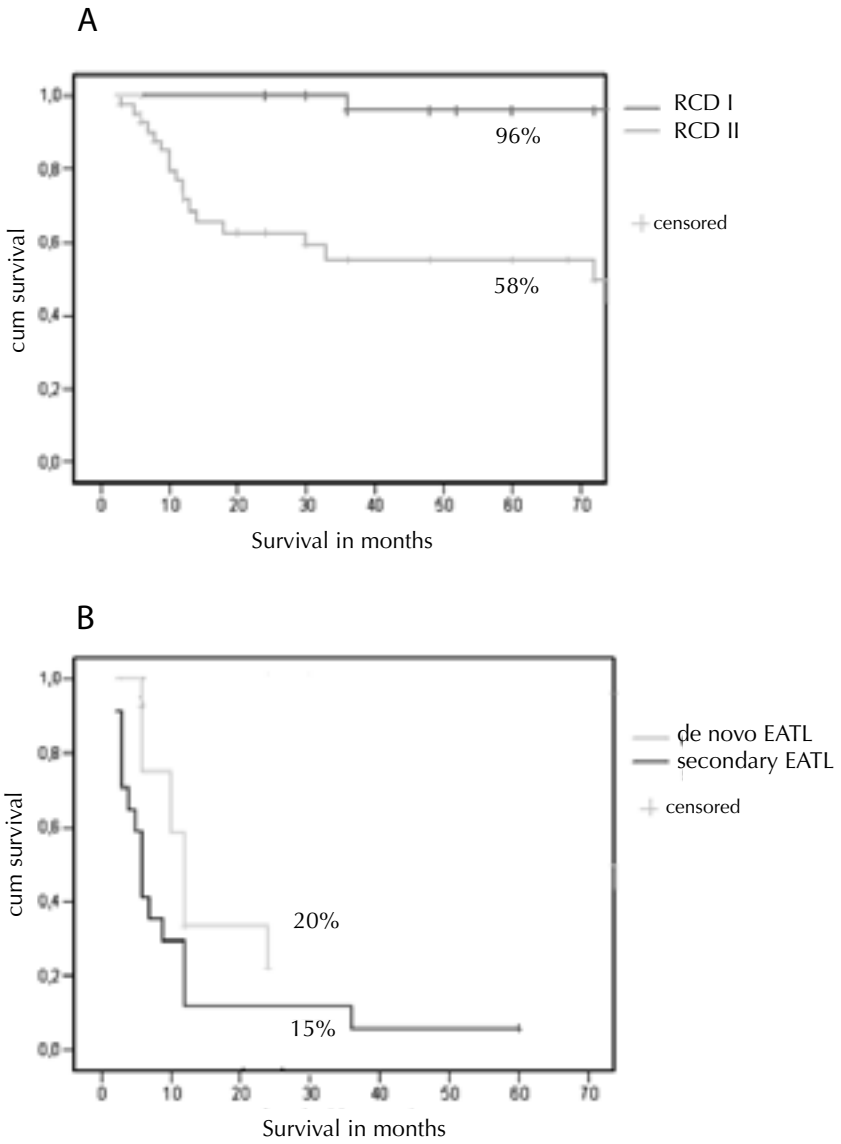

Figure 1. Shows the Kaplan -Meyer curve of survival according to the disease group. **(A)** The 5- years survival is 96% in the RCD I group versus 58% in RCD II (P= 000.1). **(B)** The 2 year survival is 15% in secondary EATL versus 20% in de novo EATL (P=0.63). Censored= Not lost for follow up

Discussion

The development of a refractory state in patients with coeliac disease may herald the start of a very serious stadium in the evolution of the disease state particularly in RCD patients with aberrant T-cells (RCD II).[1, 2] Particular attention should be paid to detect non-compliance or inadvertent gluten ingestion. Persistent positive serology may point to the last mentioned scenario.[31-33]

Regarding gender distribution in the studied groups, there is a female: male predominance in the RCD I, RCD II total and secondary EATL group, while in the de novo EATL group there is a reversed ratio with more males than females. The difference is not significant between the RCD I and RCD II groups (p= 1.05). However, it is highly significant in the de novo EATL versus RCD I (P< 0.001) and versus secondary EATL (P<0.025). Other studies also reported predominance for EATL in males, with a peak in the 6th decade of life [7, 33, 34]; however the majority of patients with uncomplicated coeliac disease are females.[35, 36]

HLA-DQ2 homozygosity is significantly higher in the secondary EATL and de novo EATL compared to RCD I, Odds ratio= 3,6 (CI:2,64 - 6,33) and OR=2,06 (CI: 1,88 - 4,78), respectively. As we reported earlier there is an association between DQ2 homozygosity and complicated forms of coeliac disease.[30]

A combination of prednisone and azathioprine is usually sufficient to treat RCD I patients.[11, 16] None of our RCD I patients has progressed to RCD II during follow up. This underscores the value of performing T-cell flow-cytometry in these patients, since the absence of aberrant T-cells in small bowel biopsies at diagnosis of the refractory state seems to indicate a favorable prognosis and conventional treatment with prednisone with or without azathioprine is usually sufficient.

In view of the poor prognosis of EATL, the question has always been: can the diagnosis of lymphoma be made earlier to allow more effective treatment, thereby improving the prognosis. Despite state-of the-art technologies used, we can only assume that Lymphoma has been excluded in these RCD II patients. The question remains whether a "subclinical" lymphoma was actually present and/or its development can be accelerated by available therapies.[37]

RCD II seems to be ''prodromal', a prelymphoma condition and should be treated aggressively. Different therapies have been evaluated, but there is no effective therapy available for these patients yet.[2, 11-17] Reports claiming good response are difficult to interpret because of absence of clear distinction between RCD I and RCD II in these case reports and small series. Maurino et al [16] reported clinical and histological improvement in 5-7 patients, treated with azathioprine, although 3 patients subsequently died (one from leucopenic fever after therapy, and 2 died during therapy). However, in their follow up report on treating 13 patients with azathioprine, they reported 46% mortality.[38] Cladribine (2-CDA) therapy might be promising in stabilizing patients' condition and improves the performance status and the histological picture as seen in 58% of our group. However, it does not prevent EATL.[17] Nine patients (39%) of 23 treated patients died from EATL. High dose chemotherapy followed by autologous stem cell transplantation after stabilization with cladribine might be an alternative approach

in these prelymphoma patients. Our experience with the first 7 patients is encouraging in improving the clinical condition, but it remains to be proved if development of EATL can be delayed or prevented.[17]

Factors that seem to be associated with a high risk for EATL development in coeliac disease are old-age presentation, male sex, ulcerative jejunitis, presence of aberrant T-cells in biopsies and DQ2 homozygosity.[30,39,40] However, we have seen disappearance of the ulcerative lesions after cladribine therapy in five patients and none of them has developed EATL thus far.[17] Thus, in case of early intervention in the high risk RCD II group, specifically ulcerative jejunitis, EATL development might be prevented.

Patients with EATL can present in two ways. There are patients with well-established coeliac disease who have responded to a gluten free diet but then deteriorate because of the development of RCD II and/or EATL. In the other group patients develop EATL without a preceding history of complicated coeliac disease, these patients often present with perforation or obstruction (de novo EATL). At the time of establishing the diagnosis, the percentage of aberrant T-cells in patients with de novo EATL is significantly lower than that in EATL developing after known RCD II ($P<0.0001$) possibly suggesting a different pathogenesis pathway.

Nine of 13 (69%) of de novo EATL and 23 of 26 secondary EATL (88.4%) died despite therapy. The 2-year survival in the de novo EATL group is 20% versus 15% in the EATL (RCD II) group ($P=0.63$). Interestingly, in our patients 61.5% of de novo EATL had undergone resection compared to 23% of secondary EATL ($P= 0.025$). Howdle et al [7] reported a laparotomy rate of 73% in lymphomas associated with coeliac disease. It may be necessary to resort to laparotomy when malignant lymphoma is suspected and the diagnosis can not be established with less invasive methods. Overall, surgery was needed in 3 patients to establish a diagnosis and 11 had surgery for complications (5 for perforations and 6 for obstructive symptoms). Surgery, radiotherapy and chemotherapy may be used depending on stage and clinical condition. EATL is often disseminated at diagnosis and has almost always a dismal outcome. However, if EATL is confined to part of the small bowel and if the affected segment (or segments) can be resected, the prognosis might be reasonable; some patients survive more than 5 years.[41, 42] Debulking by surgery might be mandatory; however prospective studies are lacking in current literature. Three of 4 EATL patients who received high dose chemotherapy and autologous stem cell transplantation died within months after transplantation (CJJ Mulder: unpublished data).

Although the studied groups of patients seem to be rather heterogeneous and not entirely exclusive but we think that we provided here a detailed description of the prognosis and response to currently available therapeutic options in the whole spectrum of complicated forms of coeliac disease, ranging from RCD I with its relatively benign course, RCD II which has a definite pre-lymphoma potential to the frankly malignant EATL. In addition, these groups of patients are the largest ever experience reported from a single centre dealing with coeliac disease and its complications.

In conclusion, an aggressive management approach and extensive evaluation using recently available small bowel endoscopy, radiology and immunophenotyping on small bowel biopsies

might be helpful in dealing with complicated forms of coeliac disease. Studies are needed to define more precisely the cut-off point between acceptable normal and pathologically increased percentage of aberrant T-cells. Multicentre cooperation and studies are required to further increase the understanding of RCD in general.

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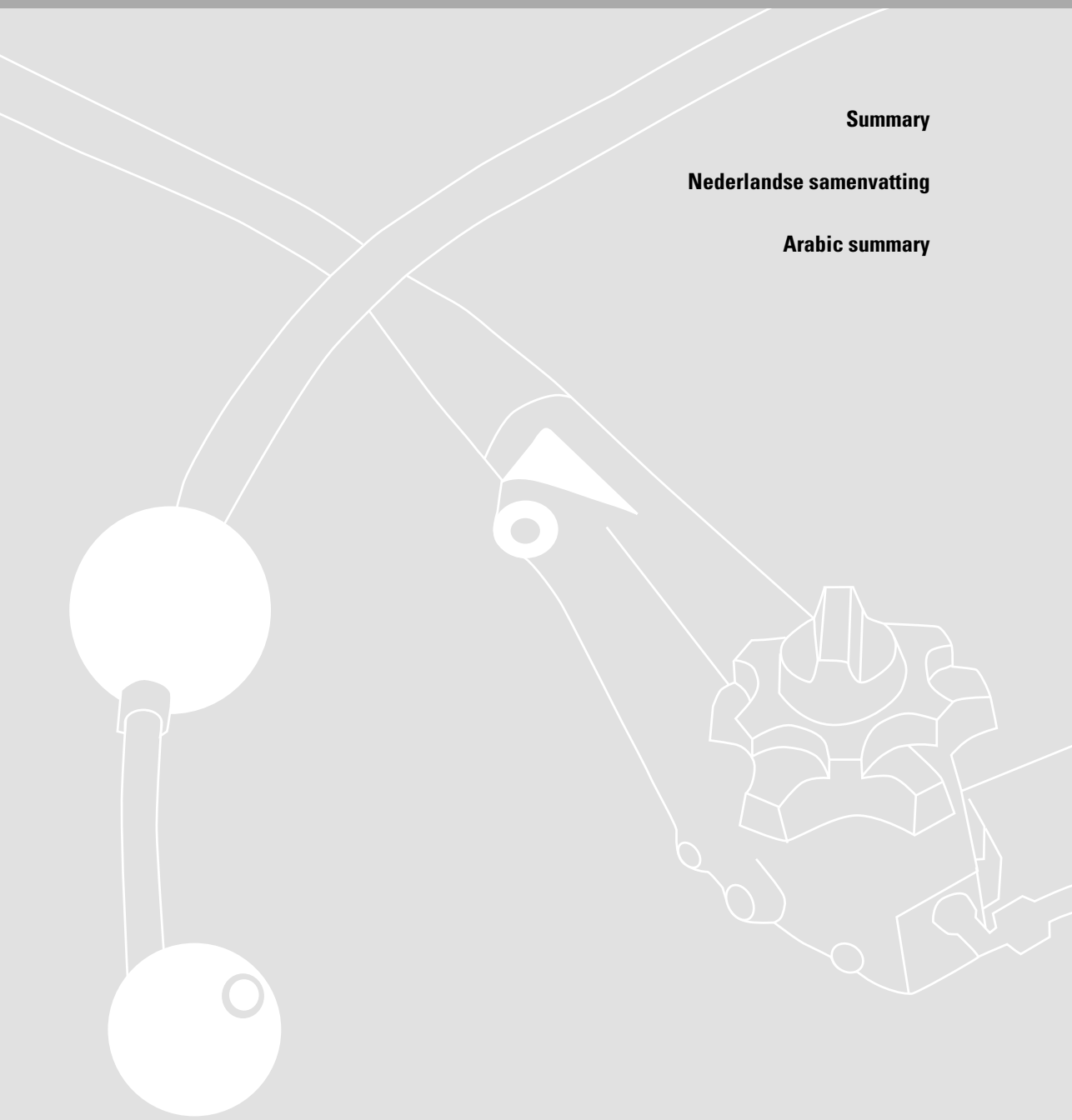
SUMMARY AND GENERAL DISCUSSION V



Summary

This thesis provides a detailed overview on the management of complicated forms of coeliac disease, particularly refractory coeliac disease in its currently recognized two major categories (RCD types I and II) and enteropathy associated T-cell lymphoma (EATL). The distinction between RCD I and II is currently based on T-cell flow- cytometry analysis of small bowel biopsies looking for the presence of significant percentage of aberrant T-cells. RCD I patients have very low or undetectable percentage of aberrant cells compared to RCD II. The latter is regarded as a pre-lymphoma because of the high potential to progress to EATL.
Chapter 1 provides an overview of the historical landmarks in the diagnosis, classification and treatment of coeliac disease and RCD; in addition to the recognition of the link or relationship between coeliac disease and EATL.
Chapter 2 gives a detailed account of the current management of RCD and EATL, incorporating all recent advances in diagnostic techniques and therapeutic measures. Concerning diagnosis, particular attention was devoted to recent advances in small bowel enteroscopy (both video capsule endoscopy and double-balloon enteroscopy) and computed tomography of the small bowel. Therapeutically, the use of immunosuppressives is discussed. More prominently, we introduced our pioneer work in the management of refractory coeliac disease type II in the form of using high dose chemotherapy followed by Autologous Stem Cell Transplantation. The 2 accompanying articles are respectively: a general and detailed review of the technique and application of double-balloon enteroscopy in the first article, while the second article reviews the current applications and gives a future perspective of stem cell transplantation in the field of gastroenterology in general and RCD in particular.
Chapter 3 looks at the epidemiology of EATL in the Netherlands. A survey in *the nationwide network and registry of histo- and cytopathology in the Netherlands* (abbreviated as *PALGA*) was conducted. The data on patients diagnosed with EATL between 2000 -2004 were analysed. EATL is seen in patients older than 50 years old. In the majority of patients, it has been found in the proximal small bowel.
Chapters 4, 5 and 6 deal with the advances in diagnostic approach in both RCD and EATL.
Chapter 4 describes our findings on the relationship between HLA-DQ2 haplotyes and the risk of developing a complicated form of coeliac disease. Molecular HLA-DQ typing was performed on 43 RCD-I, 43 RCD-II, and 30 EATL patients, and compared with age-matched groups of 121 patients with histologically defined uncomplicated coeliac disease and 183 healthy controls. HLA-DQ2 was present in 79% of RCD I, 97.7% of RCD II, and 96.6% of EATL patients. Homozygosity for HLA-DQ2 was observed in 25.5% of RCD I, 44.1% of RCD II, and 53.3% of EATL patients versus 20.7% of uncomplicated coeliac disease patients and 2.1% of controls. We concluded that homozygosity for HLADQ2 is associated with RCD II and EATL. Early identification of HLA-DQ2 homozygous coeliac disease patients may help to recognize patients at risk for developing severe complications.
Chapter 5 deals with the role of the double-balloon enteroscopy in the diagnosis of RCD and EATL. The small bowel endoscopy was performed in a total of 21 consecutive patients for lesions like ulcerations. Twenty-four procedures were successfully performed without

complications. EATL was found in five patients (24%). In three of them Marsh III was found while in the other two patients with EATL Marsh I was found. Double-balloon endoscopy could exclude the presence of EATL in four patients that was suggested by abdominal computerized tomography. We concluded that complications of refractory coeliac disease like ulcerative jejunitis or EATL could efficiently be detected, its extent delineated or excluded by double-balloon endoscopy.

Chapter 6 deals with the role of computed tomography in the diagnosis of RCD and EATL. CT scans of 28 women and 18 men were analysed. Our findings confirmed the useful role of CT scan in discriminating between uncomplicated coeliac disease and (pre)EATL. Patients with complicated coeliac disease showed more bowel wall thickening, lymphadenopathy and intussusception, less increase in number of small mesenteric vessels and a smaller splenic volume compared with uncomplicated coeliac disease.

Chapter 7 provides an account of our results in treating RCD II patients with cladribine (2-CDA), a purine analogue inducing T-cell depletion. Between 2000 and 2005, 17 patients were included (8 Males/9 Females). All patients tolerated 2-CDA without serious side effects. Six patients (35.8%) showed a clinical improvement. In 10 patients (58.8%) a significant histological improvement and in 6 patients (35.2%) a significant decrease in aberrant T-cells was seen. Seven patients (41.1%) developed EATL and died subsequently. 2-CDA-treatment in RCD type II is feasible, well tolerated and can induce clinical and histological improvement as well as a significant decrease of aberrant T-cells in a subgroup of patients, albeit does not prevent EATL-development.

Chapter 8 provides a detailed description of the first ever cohort of RCD II patients treated with autologous stem cell transplantation (ASCT) after receiving high dose chemotherapy. Thirteen patients with RCD-II were evaluated. Seven patients [4M, 3 F, mean age 61.5 years (range 51-69 years)] were transplanted. After conditioning with fludarabine and melphalan, ASCT was performed. All 7 patients completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning and transplantation. Engraftment occurred in all patients. No major non-haematological toxicity or transplantation-related mortality was observed. There was a significant reduction in the aberrant T-cells in duodenal biopsies associated with improvement in clinical wellbeing, and normalization of haematological and biochemical markers (mean follow-up 15.5 months, range 7-30 months). These results showed that high-dose chemotherapy followed by ASCT seems feasible and safe, and might result in long-term improvement of RCD II patients whose condition did not respond promptly to available drugs. The ultimate goal of resetting the immune response which might prevent or delay development of overt EATL remains to be proved.

Chapter 9 deals with the application of ASCT in EATL patients. Between 2001 and 2006, we have treated four adult patients (2Males:2Females) with a diagnosis of EATL with high dose chemotherapy followed by ASCT. The mean age was 65 years (range 60-69 years). The first patient still has complete remission 30 months post transplantation, while the other 3 patients died because of recurrence within few months post transplantation. Combining immunotherapy (adalimumab) with chemotherapy in the preconditioning regimen was also not successful in the one patient who received this combination. We concluded that the

current treatments of patients with intestinal T-cell lymphomas are unsatisfactory with only a few long-term survivors. Earlier diagnosis, the development of more effective treatments including antiCD52 agents, better preconditioning regimens and possibly the use of T-cells depleted grafts or allogeneic stem cell transplantation with or without primary central nervous system prophylaxis are urgently required to improve the prospects of these patients.

Chapter 10 provides a comprehensive analysis of the response of different groups of complicated coeliac disease to available therapies and giving insight on the long term survival in these patients. In our coeliac disease patients, no patient with RCD I developed RCD II or EATL within mean follow up of five years (range 2-15 years). Fifty two percent of the RCD II patients progressed to/developed EATL within 4-6 years after the diagnosis of RCD II. More aggressive therapies seem necessary in RCD II and EATL.

In conclusion, this thesis provides a comprehensive overview of the diagnostic and therapeutic possibilities in what is usually difficult to manage clinical situations. Recent advances in immunophenotyping of intraepithelial lymphocytes have made distinction between different forms of refractory coeliac disease possible. HLA-DQ2 homozygosity is associated with high a risk of development of refractory state and lymphoma. Recent advances in small bowel enteroscopy and radiology have provided more insight and better understanding of the pathology of both RCD and EATL.

RCD with aberrant T-cells is difficult to treat and usually refractory to steroids and immunosuppressives; however, high dose chemotherapy followed by stem cell transplantation might be regarded as the treatment of choice for those who are usually desperate and critically ill patients. The theoretical possibility of delaying or preventing EATL development using ASCT is an intriguing concept and worth of more explorative effort.

EATL remains difficult to treat despite cytoreductive therapy with chemotherapy and partial small bowel resection. Still more work need to be done to find the best preconditioning regimen and/ or studying the added benefit of immunotherapy with or without high dose chemotherapy.

Nederlandse samenvatting

Dit proefschrift geeft een gedetailleerd overzicht van het management van gecompliceerde vormen van coeliakie, met name refractaire coeliakie, met de huidige onderverdeling in twee categorieën (RCD type I en II), en enteropathie geassocieerd T-cel lymfoom (EATL). Het onderscheid tussen RCD I en II is momenteel gebaseerd op T-cel flow-cytometrische analyse van dunne darm biopten, ter detectie van een significant percentage aberrante T-cellen. RCD I patiënten hebben een siginificant lager tot onmeetbaar percentage aberrante T-cellen vergeleken met RCD II. Deze laatste wordt beschouwd als pre-lymfoom vanwege het aanzienlijke risico op EATL progressie.

Hoofdstuk 1 geeft een overzicht van de historische mijlpalen in de diagnose, classificatie en behandeling van RCD; als toevoeging op het herkennen van de connectie tussen coeliakie en EATL.

Hoofdstuk 2 geeft gedetailleerd het huidige management van RCD en EATL weer, inclusief alle recente vooruitgang in diagnostische technieken en therapeutische middelen. Met betrekking tot de diagnose, is speciaal aandacht besteed aan recente ontwikkelingen in dunne darm enteroscopie (zowel video capsule endoscopie als dubbele- ballon enteroscopie) en computed tomografie van de dunne darm. Therapeutisch wordt het gebruik van immuunsuppressiva aan de orde gesteld. Ook van belang is een introductie van ons 'pionier werk' in de behandeling van refractaire coeliakie type II, middels hoge dosis chemotherapie gevolgd door Autologe Stam Cel Transplantatie. De 2 begeleidende artikelen zijn respectievelijk: een algemene en een gedetailleerde review van de techniek en toepassing van dubbele-ballon enteroscopie in het eerdergenoemde artikel en in het tweede artikel worden de huidige toepassing en toekomstperspectieven van stamcel transplantatie in het veld van de gastroenterologie in het algemeen en RCD in het bijzonder beschreven.

Hoofdstuk 3 beschrijft de epidemiologie van EATL in Nederland. Een onderzoek is verricht in het Pathologisch Anatomisch Landelijk Geautomatiseerd Archief, een landelijk computer-netwerk van alle pathologie-laboratoria in Nederland en de verslagen. het landelijke systeem (afgekort als *PALGA*). De data van patiënten gediagnosticeerd met EATL van 2000 -2004 zijn geanalyseerd. EATL wordt gezien in patiënten ouder dan 50 jaar. In de meerderheid van de gevallen wordt het gevonden in de proximale dunne darm.

Hoofdstuks 4, 5 en 6 gaan over de ontwikkelingen in diagnostische benadering van RCD en EATL. *Hoofdstuk 4* beschrijft onze bevindingen van de relatie tussen HLA DQ2 haplotypes en het risico op het ontwikkelen van een vorm van gecompliceerde coeliakie. Moleculaire HLA-DQ typering werd verricht bij on 43 RCD I, 43 RCD II, en 30 EATL patiënten, en vergeleken met leeftijd-gematchte groepen van 121 patiënten met histopathologisch gedefinieerde ongecompliceerde coeliakie en 183 gezonde controles. HLA-DQ2 was aanwezig bij 79% van de RCD I patiënten, 97.7% van RCD II, en 96.6% van EATL patiënten. Homozygotie voor HLA-DQ2 werd geobserveerd in 25.5% van RCD I, 44.1% van RCD II, en 53.3% van EATL patiënten versus 20.7% van uncompliceerde coeliakie patiënten en 2.1% van controles. We concludeerden dat homozygotie voor HLA-DQ2 is geassocieerd met RCD II en EATL. Vroege identificatie van HLA-DQ2 homozygote coeliakie patiënten

zou kunnen helpen bij de herkenning van patiënten met een hoog risico op het ontwikkelen van ernstige complicaties. *Hoofdstuk 5 en Hoofdstuk 6* beschrijven, respectievelijk, de rol van de dubbele- ballon enteroscopie en computed tomografie in het stellen van de diagnose van RCD en EATL.

Hoofdstuk 7 verschaft een overzicht van onze resultaten van de cladribinebehandeling van RCD II patiënten.

Hoofdstuk 8 geeft een gedetailleerde beschrijving van het eerst beschreven cohort RCD II patiënten behandeld met autologe stam cel transplantation (ASCT) na hoge dosis chemotherapie. Dertien patiënten met RCD-II werden geëvalueerd. Zeven patiënten [4M, 3V, gemiddelde leeftijd 61.5 jaar (range 51-69 jaar)] werden getransplanteerd. Na conditionering met fludarabine en melphalan, werd ASCT verricht. Alle 7 patiënten voltooiden succesvol de mobilisatie en leucoferese procedures en ondergingen vervolgens conditionering en transplantatie. 'Engraftment' gebeurde in alle patiënten. Er werden geen grote non-hematologische toxiciteit of transplantatie-gerelateerde mortaliteit geobserveerd. Er was een significante reductie in de aberrante T-cellen in de duodenum biopten geassocieerd met verbetering in klinisch welzijn, en normalisatie van hematologische en biochemische markers (gemiddelde follow-up 15.5 maanden, range 7-30 maanden). Deze resultaten laten zien dat hoge-dosis chemotherapie gevolgd door ASCT haalbaar en veilig lijkt, en zou kunnen resulteren in lange termijn verbetering RCD II patiënten wiens conditie niet reageerde op direkt beschikbare medicatie. Het uiteindelijke doel van het 'resetten van de immuun respons' om de ontwikkeling van EATL te voorkomen of vertragen moet nog bewezen worden.

Hoofdstuk 9 beschrijft ASCT in EATL patiënten. Tussen 2001 en 2006, hebben we vier volwassen patiënten (2M:2V) met de diagnose van EATL behandeld met hoge-dosis chemotherapie gevolgd door ASCT. De gemiddelde leeftijd was 65 jaar (range 60-69 jaar). De eerste patient had complete remissie 30 maanden na transplantatie, terwijl de 3 andere patiënten overleden vanwege van een recidief enkele maanden na transplantatie. Het combineren van immunotherapie (adalumimab) met chemotherapie bij de preconditioning was ook niet succesvol bij de patiënt die deze combinatietherapie ontving. We concludeerden dat de huidige behandeling van patiënten met intestinale T-cel lymfomen onvoldoende zijn, met slechts enkele overlevenden op de lange termijn. Het eerder stellen van de diagnose, de ontwikkeling van effectievere behandelingen inclusief anti-CD52, betere preconditionering en mogelijk het gebruik van T-cel gedepleteerde grafts of allogene stam cel transplantatie (met of zonder primaire centraal zenuwstelsel prophylaxe) zijn vereist om de prognose van deze patiënten te verbeteren.

Hoofdstuk 10 geeft een uitgebreide analyse van de respons op beschikbare therapieën door de verschillende groepen gecompliceerde coeliakie en geeft inzicht in de lange termijn overleving van deze.

Concluderend geeft dit proefschrift een uitgebreid overzicht van de diagnostische en therapeutische mogelijkheden in wat gewoonlijk moeilijke klinische situaties zijn. Recente ontwikkelingen in immunofenotypering van de intraepitheliale lymfocyten hebben onderscheid mogelijk gemaakt tussen verschillende vormen van refractaire coeliakie.

HLA-DQ2 homozygotie is geassocieerd met een verhoogd risico op de ontwikkeling van refractaire coeliakie en een lymfoom. Recente ontwikkelingen in dunne darm enteroscopie en radiologie hebben meer inzicht in en beter begrip van de pathologie van zowel RCD als EATL gegeven.
RCD met aberrante T-cellen is moeilijk te behandelen en meestal steroid- en immuunsuppressiva-refractair; hoe dan ook, zoals aangetoond in ons pionier werk, zouden hoge dosis chemotherapie gevolgd door stam cel transplantatie beschouwd kunnen worden als de behandeling van keus voor de patiënten in kritieke toestand. De theoretische mogelijkheid om EATL ontwikkeling te vertragen of voorkomen door ASCT is een intrigerend concept en de moeite waard om verder te exploreren. EATL blijft moeilijk te behandelen ondanks debulking met partiële dunne darm resectie. Er moet nog steeds veel werk verricht worden om het beste preconditioneringsmiddel te vinden en om het additionele voordeel van immunotherapie met of zonder hoge dosis chemotherapie te onderzoeken.

الخلاصة

هذه الإطروحةِ تُزوّدُ نظرة عامّة و مفصلة حول سبل تشخيص وعلاجَ الأشكال المعقدةِ لمرض الزلاقي disease Coeliac. بصورة عامة ان مرض الزلاقي المعقد مقاوم لاغلب العلاجات المتوفرة. يمكن تصنيف مرض الزلاقي المعقد الي صنفين رئيسيين (النوعين الاول والثاني) ويرمز لهما اختصارا RCD I and II, اضافة الي اورام الغدد اللمفاوية من صنف T ويرمز لها اختصارا EATL.

يستند التفريق بين نوعي RCD على اجراء فحص عينة من الأمعاء الدقيقة بطريقة Flowcytometry حيث يتم البحث عن وجودِ خلايا غير طبيعية او شاذة.

يتناول **الفصل الاول** من هذه الإطروحةُ نظرة عامّة عن تاريخ التشخيص وتصنيفِ ومعالجةِ الانواع المختلفة من مرض الزلاقي واشكالة المعقدة ؛ بالأضافة إلى المراحل الزمنية لاكتشاف العلاقة بينَ مرض الزلاقي و اورام الغدد اللمفاوية.

اما **الفصل الثاني** فيقدم مراجعة تفصيلية حول سبل تشخيص و علاجَ الأشكال المعقدةِ لمرض الزلاقي .

في **الفصل الثالث** تم تناول الجوانب الاحصائية لاورام الغدد اللمفاوية من صنف T في هولنداوعلاقتها مع مرض الزلاقي.

الفصول **الرابع والخامس والسادس** تَتعاملُ مع التطورات الجديدةِ في طرق التشخيص لمرض الزلاقي المعقد.

الفصل الرابع يَصِفُ نتائج دراستنا عن العلاقةِ بين HLA haplotypes وخطر حدوث اشكال معقدة منَ مرض الزلاقي.

الفصلين الخامس والسادس يَتعاملان مع دور منظار الامعاء الدقيقة Double Balloon Enteroscopy و الفحوصات الشعاعية و بالاخص دور المفراس CT scan في تشخيص مرض الزلاقي و اورام الغدد اللمفاوية.

الفصول السابع والثامن والتاسع تتناول الجوانب العلاجية, حيث يتطرق الفصل السابع الي استعمال عقار Cladribine في علاج مرض الزلاقي المعقد النوع الثاني.

اما علاج مرض الزلاقي المعقد بواسطة العقارات الكيمياوية و زرع الخلايا السلالية المستخرجة من جسم الشخص نفسهِ Autologous Stem Cell Transplantation فيتم تناوله في **الفصل الثامن**. حيث لوحظ تحسن مهم في كافة الجوانب السريرية و المختبرية و الفحوصات النسيجية عند المرضي اللذين تم علاجهم بهذة الطريقةِ.

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ADDENDUM VI

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Curriculum vitae

The author was born on March 26, 1963 in Basrah, Iraq. He attended the college of Medicine in Basrah in 1981 and obtained his M.B.Ch.B. degree (cum laude) in 1987. He started his carriere in internal medicine at the department of internal medicine of *Baghdad medical city teaching hospital*. In the period from September 1990 until November 1994, he joined the postgraduate training program of the *Iraqi commission of Medical Specialisations*. During this period he finished his research entitled *Echocardiography in Iraqi Hypertensive Patients* and awarded the PhD degree. At the end of this training he gained his registration as a specialist in Internal Medicine (Internist) and worked as a staff member of the college of medicine of the University of Basrah. In July 2000 he awarded the membership of the Royal Colleges of Physicians (*MRCP -UK*). In October 2001 he obtained his license (artsenexamen) from the College of Medicine, University of Utrecht, the Netherlands. In September 2006 he finished his training in Gastroenterology at the Free University Medical Centre in Amsterdam (Prof. Dr. CJJ Mulder) and since then he is working as a Gastroenterologist in Antonius hospital in Nieuwegein, the Netherlands.
He is married with Amera Sabti. They have 2 daughters, Dania (1998) and Minen (2003).

List of abbreviations

ASCT	Autologous Stem Cell Transplantation
CD	Coeliac Disease
2-CDA	Cladribine (2-Chlorodeoxyadenosine)
CR	Complete remission
CT	Computed Tomography
DBE	Double-balloon enteroscopy
EATL	Enteropathy associated T cell Lymphoma
EMA	Anti-endomysium antibodies
GDS	Gastroduodenoscopy
GFD	Gluten Free Diet
HDT	High dose chemotherapy
HLA	Human Leucocyte Antigen
HSCT	Haematopoietic Stem cell transplantation
IEL	Intraepithelial Lymphocytes
MR enteroclysis	Magnetic resonance enteroclysis
PET	Positron Emission Tomography
RCD	Refractory Coeliac Disease
TBI	Total body irradiation
TCR	T-cell Receptor
TRM	Transplantation -related mortality
tTg	Tissue transglutaminase
VCE	Video Capsule Endoscopy