On the work-up of (Refractory) Coeliac Disease

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2012

document version
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CHAPTER 11

General discussion and future perspectives

TOEKOMSTPERSPECTIEF

Een beeld schetsen
van de wereld om je heen
kan niet zonder
(gebruik te maken van)
perspectief.

Dat leidt tot (denk)beelden
die naast elkaar blijven bestaan
zonder kans op convergeren.

In toekomstperspectief gezien,
kan dit tot niets anders leiden
dan zonder inzicht te (ver)dwalen.

Pas na divergeren
is er weer (uit)zicht.
Een beter en breder
toekomstperspectief!

Jan Tack

GENERAL DISCUSSION

Growing insight into the clinical presentation of coeliac disease (CD) has resulted in novel diagnostic, prognostic and therapeutic dilemmas. **Chapter one** provides an overview of the latest trends in epidemiology, clinical presentation, diagnosis, complications and treatment with respect to the spectrum of CD. Since the recognition of its broad clinical spectrum, especially adult-onset CD and complications of CD are a matter of debate, in particular refractory coeliac disease (RCD) and enteropathy associated T-cell lymphoma (EATL), which are recognised as relevant clinical entities since more than two decades. As a subset of these patients has a poor prognosis, it is of utmost importance to accurately differentiate between uncomplicated and complicated forms of CD in order to enable early intervention. Despite increased understanding during recent years, the underlying pathophysiology is not completely understood, some aspects of the diagnostic work-up are still under debate, a standardised treatment protocol is lacking and parameters for predicting and monitoring these complicated stages of CD are not yet available. This thesis provides novel insight in the diagnostic aspects and therapeutic options in CD and its complicated stages.

PART I: DIAGNOSTIC ASPECTS

Serum parameters beyond standard antibody testing

To date, small intestinal biopsies obtained by gastro-duodenal endoscopy are still required in the diagnostic work-up and follow-up of uncomplicated as well as complicated CD. The histopathological observation of villous atrophy in florid CD is well appreciated. To date, small intestinal biopsies obtained by gastro-duodenal endoscopy are still required in the diagnostic work-up and follow-up of uncomplicated as well as complicated CD in adults. The recently published revised ESPGHAN (European Society for Paediatric Gastroenterology Hepatology and Nutrition) guidelines for the diagnosis of coeliac disease in children mention the option for omitting duodenal biopsies and histology if certain conditions are fulfilled.[1] But, the guidelines for adult patients have not been revised yet. The histopathological observation of villous atrophy in florid CD is well appreciated. However, the behaviour of the enterocyte itself in adult-onset CD has not yet been investigated. As mucosal villous atrophy is the hallmark of both active CD and refractory CD, histopathological classification according to the modified Marsh criteria[2] is of no use to distinguish between these stages of CD. Although CD associated antibodies are valuable for diagnosing active CD and monitoring response to a gluten-free diet (GFD), these antibodies are within their reference range in complicated CD and therefore of no use in predicting as well as monitoring RCD and EATL.

Chapter two shows that enterocyte destruction determined by serum intestinal fatty-acid binding protein (I-FABP) levels is evidently increased in adult CD patients at time of diagnosis as compared to healthy controls. I-FABP levels correlate with both villous atrophy and IgA-tTG titres and decline after initiation of a GFD. In contrast to serum I-FABP levels in

childhood CD that rapidly decrease after initiation of a GFD[3], low grade enterocyte damage, detected by serum I-FABP levels, persists in adults upon long-term adherence to a GFD, despite the lack of villous atrophy and detectable IgA-tTGA levels in the majority of cases. These findings are in keeping with the slow and incomplete mucosal recovery observed in a substantial number of adult-onset CD patients upon a GFD with subsequent normalised serology and disappearance of symptoms.[4-6] Moreover, serum I-FABP levels correlate clearly with the degree of villous atrophy observed in patients adhering to a GFD. So far, however, it is unknown whether the observed persisting enterocyte damage in adults may indicate non-compliance or unintentional gluten intake, despite evaluation by a specialised dietitian, or a manifestation of the autoimmune disease itself. Taken together, our findings support the currently changing opinion towards the clinical aspects of adult CD. Although the diagnosis of CD in adult patients proceeds along the same lines as that of younger patients, it appears to be more complex due to its initial presentation with less prominent symptoms of impaired intestinal absorption, with signs of micronutrient deficiencies, other autoimmune diseases or even with malignancies.[7] Furthermore, as mucosal recovery may be slow in adult patients diagnosed with CD, closely monitoring clinical response and possible complications, including implications of long-lasting malabsorption, development of autoimmune diseases, refractory CD and (intestinal) malignancies, appears to be important. I-FABP clearly represents a potential marker for monitoring a GFD to detect ongoing mucosal abnormalities without the need to perform a gastro-duodenal endoscopy. However, larger prospective studies are mandatory to elucidate the specific value of serum I-FABP in diagnosis and follow-up. Currently, serum I-FABP levels in the complicated forms of CD are under investigation. Preliminary data show no differences in the severity of enterocyte damage between active CD and RCD I, II as well as EATL. In addition, it might be of interest to investigate the presence of IgA-tTG deposits in the small intestine and other (serum) markers indicating intestinal integrity in complicated CD.

Chapter three provides further insight in the ability of several immunological and biochemical markers in the peripheral blood to distinguish the various CD subsets at time of diagnosis. The gluten-induced inflammation in active CD and the gluten-independent inflammation in RCD I/II show resemblance based on the serum levels of IL-8, IL-17, IL-22 and sCD25 as well as the biochemical inflammatory parameters CRP, ESR and leukocyte count. RCD II differentiates itself by elevated serum IL-6 and granzyme-B concentrations. Furthermore, the clearly elevated levels of IL-8 and sCD25 found in both RCD subsets over the GFD group, suggest that these parameters might be helpful in monitoring the inflammatory disease status and in differentiating between these groups in ongoing research and daily clinical practice. In addition, serum IL-6 and albumin levels might be potentially useful to identify patients with complicated CD, as elevated IL-6 levels are a distinctive feature of both RCD II and EATL, and lower levels of albumin were observed in RCD I/II and EATL.

Whether RCD type I and II are two related disease entities or two independent conditions is still under debate. Our results show that both types share similar inflammatory characteristics and T-cell activation status. In addition, progression of RCD I into both RCD II[8] and EATL[9] has been observed, although only sporadically. However, a substantial aberrant IEL population[10] displaying a cytotoxic profile is the specific hallmark of RCD II. Increased serum levels of soluble granzyme-B and the high expression of intracellular granzyme-B in aberrant IELs (*chapter four*) were observed. Although group-wise trends can be appreciated, at least at the single patient level, so far no serum parameter appears sufficiently sensitive and specific for differentiating RCD type I and II or predicting transition into EATL, including those analysed in the present thesis. Hence, it follows that this is an area that needs further study.

Origin and immunophenotype of aberrant IELs in RCD II

The aberrant IEL population observed in RCD II lacks expression of the T-cell lineage-specific surface CD3-TCR-complex, but expresses the cytoplasmatic CD3 antigens and displays T-cell receptor rearrangements[10, 11] indicative of T-cell lineage commitment. These cells are considered a premalignant cell population from which aggressive EATL evolves in half of the cases.[11, 12] However, currently the cellular origin of these cells is unclear and there are no histopathological or immunophenotypic features identified that have a prognostic value in the evolution of aberrant IELs into an EATL. It has been hypothesised that they derive from mature TCR+ IELs, in particular gamma-delta T-lymphocytes[11, 13, 14], or from CD3-CD7+ NK/T-cell precursor cells found in the small intestine of healthy individuals possibly in consequence of the ongoing extrathymic maturation of T-cells in the intestinal mucosa throughout life.[15, 16] Following this, **chapter four** reveals a heterogeneous TCR-gene rearrangement pattern and a lack of expression of the early T-cell development-associated markers TdT, CD1alpha and CD34 in RCD II duodenal biopsies. This strongly suggests that the aberrant IEL population originates from a monoclonal expansion of partly matured T-cells that have deranged in their development before reaching full maturity. In contrast, Schmitz et al.[17] showed that three RCD II cell lines expressed several activation receptors and NK-associated genes, including KIRs, granzyme-H and NKG2D. Interestingly, in our study only those patients harbouring the most mature aberrant IEL population, based on an almost completed TCR rearrangement, developed an EATL. It is tempting to speculate that the presence of aberrant IEL populations with different levels of maturity is, at least in part, an explanation for the clinical observation that only half of the RCD II patients eventually develops an EATL. The observed decreased PCNA expression in aberrant IELs might indicate an impaired DNA-mismatch repair system[18, 19] and may consequently facilitate further transformation of these aberrant cells. Further research addressing the cause and consequences of this abnormal IEL development is definitely warranted.

As some RCD II patients display polyclonal TCR-gamma, yet clonal TCR-beta or TCR-delta rearrangements, we suggest TCR-beta gene rearrangement analysis as the most valuable of available rearrangement analyses in the diagnostic work-up of RCD. This recommendation is supported by a recent publication by Perfetti et al.[20] In addition, these results confirmed our previous findings that clonality analysis of only the TCR-gamma gene rearrangement misses monoclonal populations at risk for EATL. In contrast to immunophenotypical identification of aberrant populations.[10] Furthermore, as aberrant IELs are not strictly confined to the small intestine but are also observed in other, extra-intestinal organs[21], it is of interest to clarify their dissemination pattern in order to find a lead for novel therapeutic options.

Phenotypic and genomic characteristics of EATL

Currently, the immunophenotype of EATL determined by immunohistochemistry plays a pivotal role in its diagnostic work-up, showing expression of CD3, CD7 and CD103, but lack of expression of CD4, CD5 and CD8 in the majority of cases. Furthermore, type 1 EATL usually exhibits a cytotoxic immunophenotype, characterised by expression of CD30, TIA-1, perforin and granzyme-B.[22-24] **Chapter five** reports on an in-depth flow cytometric analysis of the immunophenotype and analysis of genomic alterations of EATL cells primarily presenting as leukemic ascites. The immunophenotype of this EATL is substantially different from the corresponding aberrant T-lymphocytes found in RCD II. First, the absence of the T-cell lineage associated markers CD2, CD7, CD52 and CD103, indicating further transformation. Second, the lack of CD103 and LFA-1 expression that may have contributed to migration of these cells outside the gastro-intestinal tract. Importantly, lack of CD7 and CD103 expression on EATL has not been reported so far and is not included in the current WHO classification of haematological malignancies.[24] Third, compared to aberrant IELs in RCDII *(chapter four)* an extensive proliferative activity indicated by high expression levels of Ki-67 and PCNA was found. Furthermore, the observed expression of CD25 on the EATL cells was in agreement with previous work indicating that sCD25 is a sensitive marker for tumour burden in some lymphomas.[25] However this could not be confirmed by the previously described study in *chapter three* (the serum levels of sCD25 did not significantly differ between EATL and RCD). Moreover, in keeping with the evidently increased serum levels of IL-6 in EATL as compared RCD I/II *(chapter three)*, IL-6 was clearly detected in the leukemic ascites, illustrative for the local pro-inflammatory environment.

In agreement with limited studies on the karyotype of aberrant IELs in RCD, we observed a gain in chromosome 9q and trisomy of chromosome 1, previously suggested as an early and late event of EATL transformation, respectively.[26] The current WHO classification postulates a 9q gain or a 16q loss responsible for inducing transition from RCD into EATL, and gains of 1q/5q and 8q distinguishing between most cases of type 1 and 2 EATL, respectively.[24] In contrast, we found both 1q and 8q gains, suggesting an increased level of chromosomal instability. Further insight into the mechanisms responsible for the development of EATL

may open new doors for early recognition and treatment of EATL. Extended genetic analysis including karyotyping and array CGH as well as functional and expression profiling of small intestinal aberrant IELs as compared to their normal counter parts, could be supportive in the search for new, more sensitive markers to predict the occurrence of EATL in an early stage.

PART II: THERAPEUTIC OPTIONS

Coeliac disease: AN-PEP

The only currently available treatment for CD consists of life-long dietary exclusion of gluten[27], perceived as a substantial burden particularly due to high costs, dietary restriction, reduced social activity, and increased health worries.[28] Alternative treatment modalities that reduce the need of dieting focus on modification of dietary components, enzymatic degradation of gluten, inhibition of intestinal permeability and modulation of the immune response.[29] In line with this, **chapter six** of this thesis describes a randomised double-blind placebo-controlled pilot-study to the gluten-degrading *Aspergillus niger-derived prolyl endoprotease* (AN-PEP) in CD. Although AN-PEP appears to be well tolerated in the majority of coeliac patients, a two week gluten challenge (with placebo) seems to be insufficient to induce a clear clinical response, based on Marsh grade and CD associated antibodies. Consequently, no treatment effects of AN-PEP could be detected. Therefore, further studies with modified study design including a high dose of gluten consumption for an extended period of time with many more patients are warranted to support the potential of AN-PEP in degrading gluten. It might also be interesting to perform a study designed the other way round: small amounts of gluten with AN-PEP or placebo for a much longer period of time. Moreover, in addition to a dietitian inquiry and the CD associated antibodies IgA-tTG, IgA-EMA, and IgA/G-DGA, it might be of interest to search for more sensitive makers to evaluate diet compliance and/or unintentional gluten intake. Recently, Biagi et al[30] presented a simple and rapid questionnaire that verifies compliance to a GFD, however, education by an experienced dietitian remains very important. As suggested previously *(chapter two)*, serum I-FABP levels and IgA-tTG deposits in the small intestine might be of potential use.

RCD I: Tioguanine

Although the prognosis of RCD I is much more favourable than that of RCD II, as reflected in 5-year survival rates of around 90% and 44-58%, respectively[9, 12, 31], treatment is believed to be important in preventing complications of longstanding malabsorption. Apart from a GFD and nutritional support, corticosteroids are the mainstay of treatment in RCD I. Unfortunately there are also snags attached to this therapy, including systemic side effects and corticosteroid dependency in the majority of cases.[9] To reduce corticosteroid dependency and in case of corticosteroid refractoriness, azathioprine has been advocated

with good clinical response rates.[32] Currently, treatment is largely empiric based on small series, showing good clinical response rates, yet histological response is lagging behind. **Chapter seven** reports on the non-conventional thiopurine derivative tioguanine in RCD I showing a good safety profile and clinical and histological response in the majority of cases. Therefore, tioguanine might be a suitable alternative treatment option for RCD I, either as first-line treatment, second-line treatment in patients unresponsive to corticosteroids and/or azathioprine, or as a steroid-sparing drug. In addition, further research is also warranted to establish the optimal treatment duration of tioguanine. In consequence of its rare incidence large multicentre international randomised trials including azathioprine, tioguanine, budesonide and placebo should be performed to establish the validity of each and any treatment option in RCD I.

RCD II: Cladribine and au-SCT

In the past decade several conventional and more experimental therapies have been evaluated in small series of RCD II[9, 31-39], yet, a validated standard treatment of RCD II is lacking. The high risk of progression to EATL with dismal prognosis highlights the need for new treatment strategies. Since 2005 cladribine is the drug of first choice in our medical centre, but patients not responding to cladribine receive high dose chemotherapy with subsequent autologous haematopoietic stem-cell transplantation (au-SCT). **Chapter eight** and **nine** show that our suggested treatment protocol seems to hold promise. Both cladribine therapy and high dose chemotherapy followed by au-SCT were well tolerated. Approximately half of the patients responded well to cladribine, having a significantly higher survival rate compared to those who were unresponsive. The observed 80% clinical response rate was consistent with previous studies[9, 31], hence, the potential advantage of cladribine seemed to be a better histological response, besides its steroid-sparing effect. The median overall survival and 2-year histological response rate of patients treated with up-front cladribine appeared to be superior to patients unresponsive to thiopurines and/or prednisone and subsequently treated with cladribine. Unfortunately progression into EATL could not be fully prevented, but, compared to previous reports showing EATL in 33–52% within 5 years[9, 12], cladribine therapy appeared to result in a lower rate of lymphomagenesis (17%), yet approximately half of the patients had a 5-year follow-up.

Although the overall clinical response rate of cladribine was actually good, with regard to our response criteria (defined as clinical, and complete histological and/or immunological response), for unknown reasons half of RCD II patients were unresponsive. Possibly a higher dose and/or a prolonged treatment schedule might result in a higher response rate. Therefore dose-finding studies need to be conducted. This specific group of unresponsive patients has a worse prognosis. If they manage to proceed high dose chemotherapy followed by au-SCT the observed 4-year survival of 66% seems promising. This is supported by an impressive clinical improvement and enhanced quality of life in almost all patients after transplantation. Approximately half of them showed a significant recovery

of the architectural abnormalities of the small intestinal mucosa. As EATL was observed only after four years of follow-up in one transplanted patient, chemotherapy followed by au-SCT might possibly delay the development of this type of lymphoma. Whether progression into EATL is prevented or delayed must be elucidated by more prolonged follow-up.

Overall, multicentre randomised trials comparing thiopurines and cladribine, and if unresponsive followed by au-SCT, to further explore these treatment strategies is warranted. Ongoing efforts to investigate other new treatment options such as IL-15 blocking antibodies (as IL-15 has a key role in the pathogenesis of RCD) are recommended in order to further decrease morbidity and mortality in this patient group.

EATL: Allo-SCT

EATL is a virtually lethal condition with an overall 5-year survival of less than 20%.[12, 40] To date, treatment of EATL is rather disappointing with a 2-year survival of 28% upon the classic CHOP (cyclophosphamide, doxorubine, vincristine and prednisone) protocol[41] and an estimated median survival of 7.5 months upon CHOP-like or other new chemotherapy regimens.[40, 42, 43] Approximately half of the cases is eligible for chemotherapy due to an advantaged stage at initial presentation, a poor performance status, multifocal involvement of the small intestine and/or complications. In addition, only half of them completes their scheduled chemotherapy.[41, 44] In small series dealing with au-SCT preceded by different induction and conditioning chemotherapy regimens, variable results were reported.[44-47] Patients with an early stage of disease and use of a more aggressive conditioning regimen seemed to benefit most. Following a prospective study of 14 patients, a single course of CHOP with subsequent aggressive regimen IVE/MTX (ifosfamide, etoposide, epirubicin/ methotrexate) followed by au-SCT seemed promising, with a 5-year survival of 60%.[44] An alternative treatment option evaluated in case-reports is Alemtuzumab (anti-CD52) plus chemotherapy, however, so far relapse could not be prevented[48, 49]. We are awaiting the results of a randomised phase III study in The Netherlands to evaluate the effectiveness of Alemtuzumab with 2-weekly CHOP versus 2-weekly CHOP alone and consolidated by au-SCT in patients <60 years of age (www.hovon.nl). As previous reports indicated lower relapse rates in allogenic haematopoietic stem-cell transplantation (allo-SCT) as compared to au-SCT in patients with indolent and high-grade non-Hodgkin lymphomas[50, 51], **chapter ten** reports the first two patients with a type 1 EATL in whom allo-RIC-SCT with a HLA-identical sibling donor was performed. Although both patients were chemosensitive and in clinical remission before transplantation, a relapse of EATL occurred within a few weeks after transplantation. As a graft versus lymphoma effect could not have occurred, the conditioning (fludarabine, cyclophosphamide) probably was not sufficiently potent to buy time. Possibly more intensive consolidation is necessary to improve current results in this highly aggressive lymphoma. Introducing graft versus lymphoma in an earlier stage, established by more rapid exclusion of immunosuppressive medication, might improve results.

FUTURE PERSPECTIVES

This thesis "On the work-up of (refractory) coeliac disease" reveals novel insight into clinical aspects and treatment of the spectrum of CD, yet many areas require further study.

First, the majority of coeliac patients respond well to a GFD, however it still has to be unravelled for what reason some, in particular adult patients, are refractory to a GFD ensuing RCD and/or EATL. In addition, the controversial role of strict adherence to a GFD in the development of RCD and EATL[52-54] has to be further addressed in ongoing research. Following this, further identification of novel serological, immunophenotypic, genetic and/or molecular prognostic parameters for these complicated forms of CD is of utmost importance to decrease morbidity and mortality. To differentiate between RCD II patients who will develop an EATL and those who will not, is the ultimate target. Apart from serum I-FABP levels, other parameters representing intestinal permeability, such as antibodies against food antigens and zonulin levels are included in current investigation. In addition to IL-6, IL-8 and granzyme-B levels *(chapter three),* cytokine production profiles in the peripheral blood or by intestinal aberrant IELs may be topic of further research. Ongoing immunophenotypic analysis of aberrant IELs as compared to their normal counter parts *(chapter four and five)* involving additional markers representing the NK- or T-cell lineage, dendritic cells, DNA mitotic cell cycle profile, homing receptors and apoptosis are recommended. At the molecular level, TCR-beta gene rearrangement analysis is strongly suggested as an additional marker in the current diagnostic work-up of RCD (*chapter four*), hence larger series are mandatory to validate these data. Investigation of TCR-alpha chain rearrangements may provide further information on the origin of aberrant IELs. Furthermore, it is likely that in addition to MYO9B[55], HLA-DQ2 homozygosity[56] and chromosomal gains of 1q, 9q and 5q[26] *(chapter 5)* or deletion in 16q[24], which are not routinely detected in the diagnostic work-up, other genes are associated with the development of RCD and EATL. International genome-wide association studies, and functional and expression profiling of small intestinal aberrant IELs in complicated CD may shed new lights on this matter.

Second, RCD II is a commonly accepted clinical disease entity with specific characteristics, whereas the role of RCD I within the spectrum of CD sill remains under debate. Do RCD I and II refer to a sliding scale within the CD spectrum, or does RCD I resemble florid CD in consequence of long-term unintentional gluten intake which could not be detected by currently available methods? Or does it represent an independent disease entity showing great similarity to CD?

Third, as aberrant IEls are not strictly confined to the small intestine[21] and can even be found outside the gastro-intestinal tract at initial presentation *(chapter five),* analysis of homing receptors and genetic profiling will largely contribute to our understanding of their dissemination pattern and may provide a lead for novel therapeutic options. In addition, it might be interesting to investigate aberrant IELs in the stomach and colon as well.

Fourth, a GFD remains the only effective treatment for CD despite alternative treatment modalities, including enzymatic degradation of gluten by AN-PEP *(chapter six)*, therefore new clinical trials are warranted. Several therapeutic options for RCD and EATL have been investigated. However treatment remains challenging due to the fact that EATL development could not be fully prevented in RCD II *(chapter eight and nine)* and EATL itself still has a dismal prognosis despite treatment *(chapter ten)*. In addition to novel treatment options such as blocking IL-15 or CD30 based on their involvement in the pathogenesis of RCD and EATL, respectively, evaluation of the most commonly used therapies in these complicated forms of CD in large randomised trials is mandatory (i.e. azathioprine, budesonide and tioguanine in RCD I; azathioprine and cladribine in RCD II; CHOP with alemtuzumab in EATL). As the low incidence of RCD and EATL hampers the development of such trials, improvement of therapeutic strategies requires international collaboration with consensus about diagnostic criteria, and clinical and histologic response definition during follow-up.

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