overestimated, leading to a lack of power of the study to detect a statistically significant difference in therapeutic efficacy between the two drugs.

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# Accurate classification of RCD requires flow cytometry

We read with interest the article of Liu *et al* in *Gut*, in which the authors emphasise the need for monitoring of clonality and intraepithelial lymphocyte (IEL) immunophenotype in the surveillance of refractory coeliac disease (RCD).[1]

The authors state there is no consensus on the cut-off of aberrant cells distinguishing between non-complicated coeliac disease (CD), RCD type I (RCDI) and RCD type II (RCDII). However, in 2000 it was shown that based on the number of aberrant T cells, CD can be distinguished from RCD by immunohistochemistry.[2] Furthermore, using flow cytometry, Verbeek defined a clinically well-validated cut-off of 20% IELs as being diagnostic for RCDII.[3]

In their paper, the authors describe that a high percentage of patients (80%) progress from RCDI to RCDII. This is somewhat surprising since studies so far have indicated that transition from RCDI to RCDII or EATL (enteropathy-associated T cell lymphoma) is a rare phenomenon. In fact, in our institute >100 patients with RCD have been analysed using flow cytometry with a follow-up of 10 years. So far, only one patient with RCDI transformed to RCDII (C J Mulder, personal communication in 2010). This rare transition is reflected by the favourable 5-year survival of patients with RCDI compared with those with RCDII.[4] If the occurrence of this transformation were as common as suggested, the 5-year survival would be expected to be significantly lower.

A potential explanation for these contradictory results may relate to the fact that the methodology used in this study has well-known limitations and potential pitfalls in the identification of aberrant T cells in the gut. The most important problem is that immunohistochemistry does not allow differentiation between surface CD3 and cytoplasmatic CD3, and consequently the identification of aberrant IELs is solely based on the absence of CD8 in CD3-positive cells. However, $CD3^+CD4^+$ T cells comprise a considerable percentage of the IEL population, both in normal duodenum and in patients with CD and RCD.[3] According to the criteria of Liu *et al*, these cells would have been classified as aberrant T cells. Furthermore, γ-δ lymphocytes express a similar $CD3^+CD8$ marker pattern to aberrant IELs and therefore these cells may also be erroneously classified as aberrant IELs. This is particularly relevant since γ-δ cells comprise up to 50% of the IEL compartment, with increased percentages in active CD, and drastically decreased numbers in RCDII.[3] Finally, we sometimes encounter patients with aberrant ($sCD3^-cytCD3^+$) T cells that do express sCD8, which would have been classified as normal cells using immunohistochemistry. Including $CD3^+CD4^+$ T cell and γ-δ lymphocyte populations in the enumeration of the aberrant T cell population using immunohistochemistry leads to a relatively high cut-off value as compared with flow cytometry. Consequently, it cannot be excluded that in the study of Liu *et al* a substantial number of patients already had increased baseline numbers of aberrant T cells that did not exceed the cut-off value of 40% and thus were initially misclassified as RCDI. In a later phase, when disease develops and the percentage of aberrant cells increases further, patients are diagnosed as RCDII. This would explain the relatively high number of transitions from RCDI to RCDII.

Whereas patients with RCDI have an excellent prognosis, up to 60% of patients with RCDII will progress to EATL with a very poor outcome. Cladribine treatment and/or autologous stem cell transplantation may delay or even prevent the development of EATL. A correct initial investigation is therefore of utmost importance. So far there are no head-to-head comparisons between immunohistochemistry and flow cytometric evaluation of aberrant T cells. Based on the aforementioned considerations we feel that flow cytometry is a validated, easy applicable methodology for the enumeration of aberrant T cells that is superior to combined T cell clonality analysis and immunohistochemistry.

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## CORRECTION

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**Williams L**, Armstrong M, Finan P, *et al*. The effect of faecal diversion on human ileum. *Gut* 2007;**56**:796–801. The correct author list should read Williams L, Armstrong MJ, Finan P, *et al*.