Laboratorium analyses op darmbiopten in kader van de diagnostiek voor “refractaire coeliakie type I of II”

CRITICALLY APPRAISED TOPICS: 07 JUNI 2022 DR ANTOINE MAIRESSE

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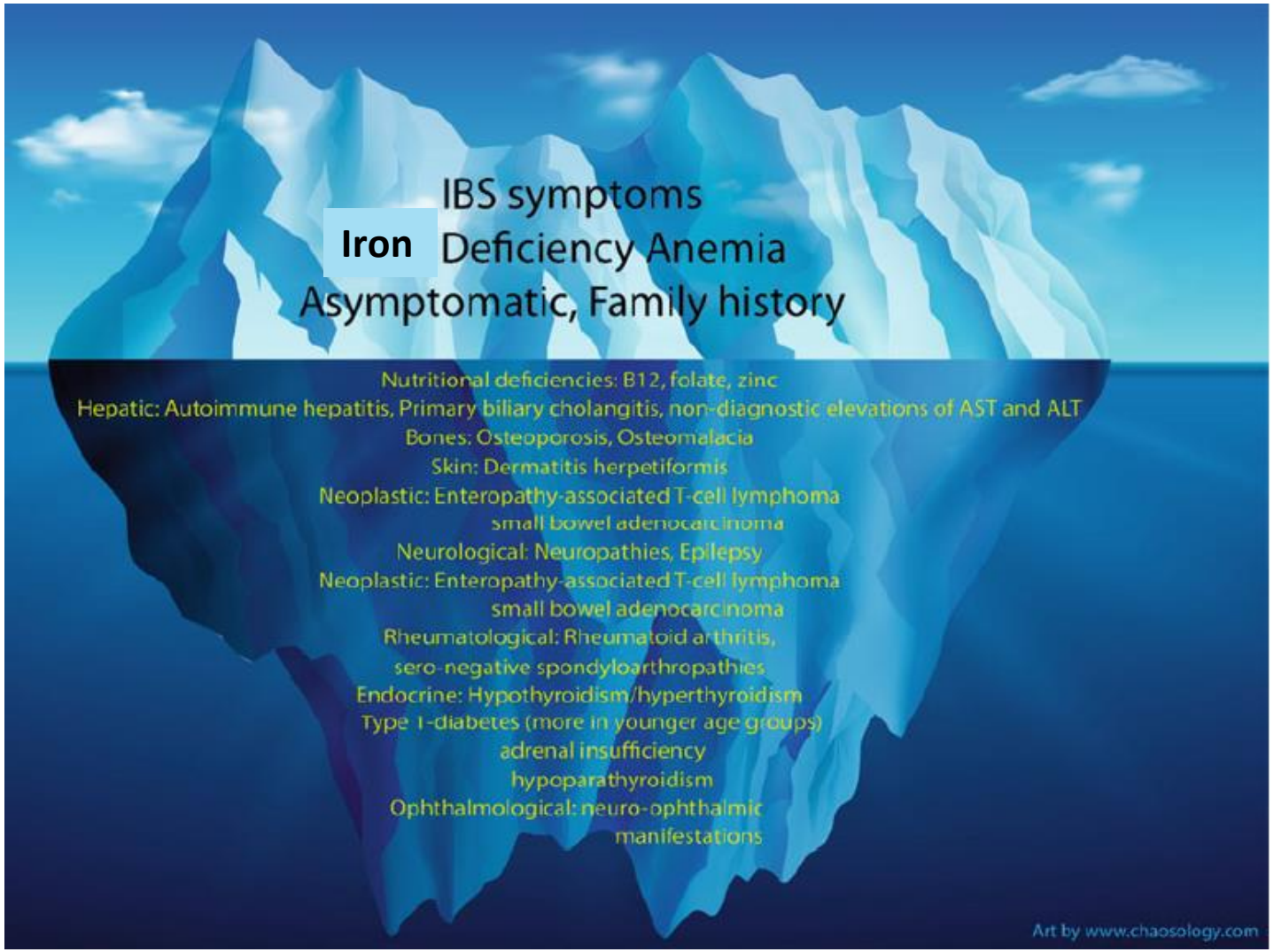
Introduction: celiac disease

"a chronic immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals (alleles encoding HLA DQ2 or DQ8)"

Worldwide prevalence of 0,6-1%

Underdiagnosed



Pitchumoni CS. Celiac Disease. 2020

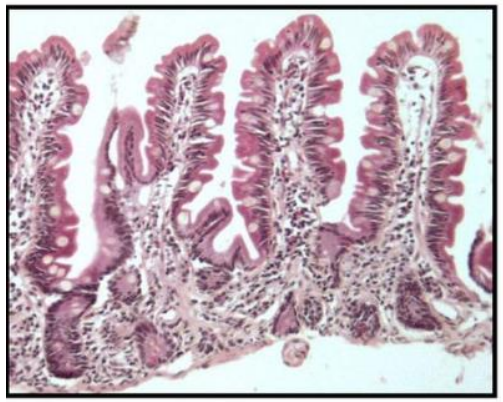
Celiac disease: diagnosis

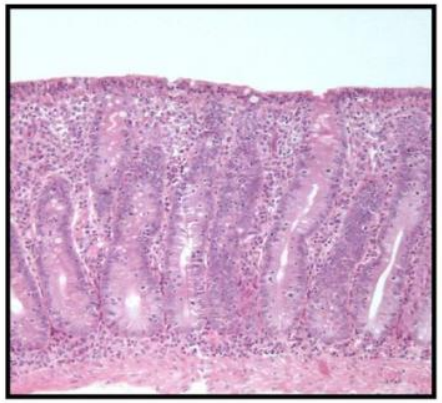
SEROLOGY

- IgA-TG2 antibody is preferred single test
- Total IgA level needs to be measured concurently with serology testing
- In patient with selective total IgA-deficiency, IgG-based testing (IgG-DGPs or IgG-TG2) should be performed
- All diagnostic serology testing should be done while patients on a gluten-containing diet

DUODENAL BIOPSIES

- Atrophic villi
- Crypt hyperplasia
- Increase in number of intra-epithelial lymphocytes (IELs)





Al-Toma A *et al.* European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. 2019

Celiac disease: treatment

Strict gluten-free diet

- Avoiding
  - Wheat (Tarwe – Blé)
  - Ry (Rogge – Seigle)
  - Barley (Gerst – Orge)
- Lifelong diet (expensive, socially isolating)
- Reduce symptoms, mortality and risk for malignancy
- Normalisation of histopathology
- Disappaearance of antibodies

Refractory Celiac Disease (RCD)

- Persisting or recurring **symptoms** despite strict adherence to gluten-free diet (>12 months) AND in absence of other causes
    - diarrhea, abdominal pain, involuntary weight loss,…
    - severe malnutrition, protein-losing enteropathy, ulcerative jejunitis,…
- Prevalence: <1% of CD patients, but significant morbidity and mortality
- Subdivised into
- RCD type I
- RCD type II

Refractory Celiac Disease (RCD)

TABLE 1 | Clinical and Immuno-phenotypic features of Refractory Celiac Disease (RCD) type I and II.

Features	RCD I	RCD II
Female predominance	−	+
Hypoalbuminemia	−	+
Low BMI	±	+
Anemia	+	+
Lymphocytic gastritis	±	+
Lymphocytic colitis	±	±
Extraintestinal Manifestation	−	+
Ulcerative jejunoileitis	−	+
Intra epithelial lymphocytes (IELs)	Normal	Aberrant T-cell IELs Clonal
Surface CD3	+	−
Surface CD8	+	−
Intracellular CD3		+
Trisomy 1q		+

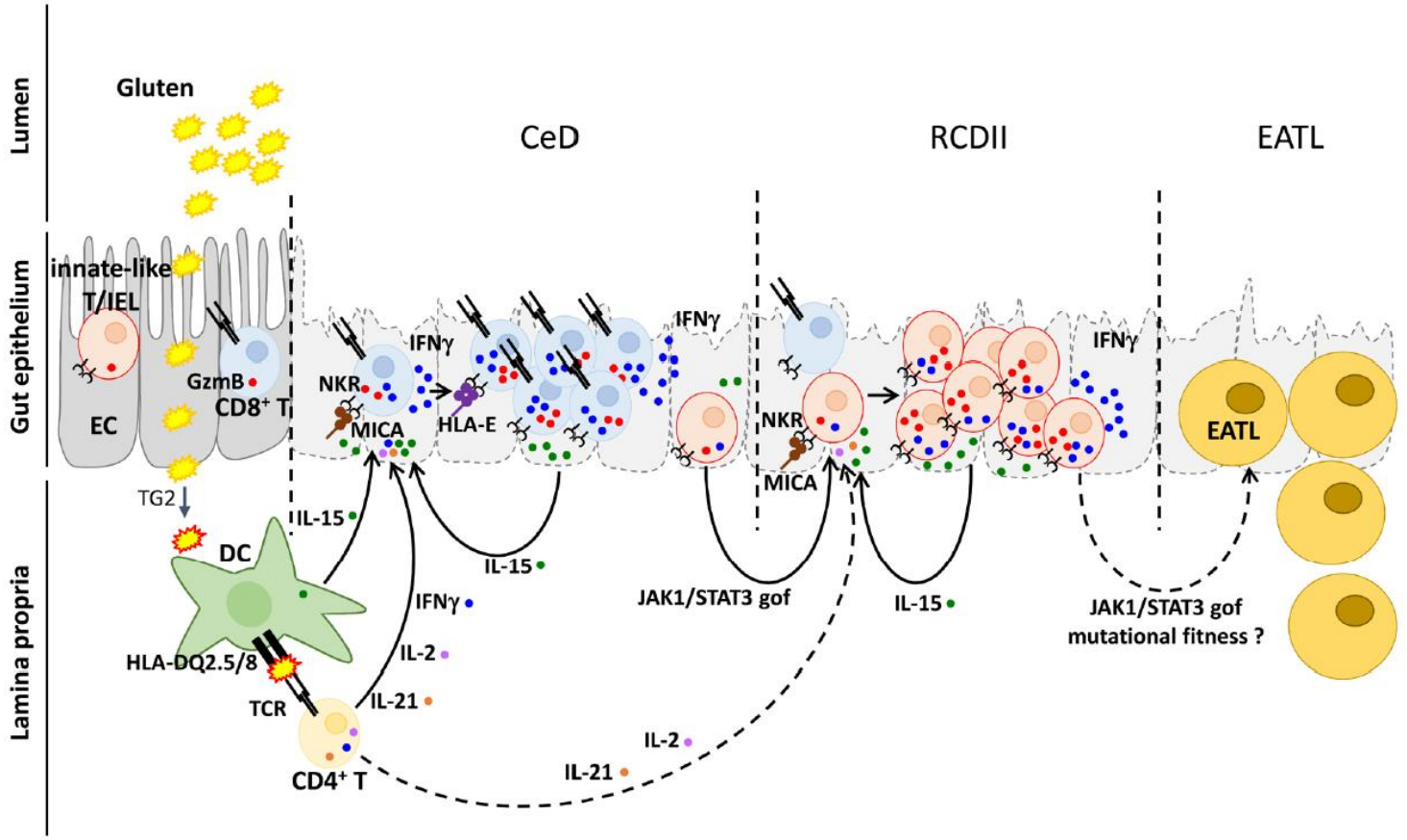
RCD type II

- high risk of developping EATL (40 – 50% within 5 years)
- Poor 5-year survival (50%)
- Pre-malignant (indolent lymphoma), requires cytotoxic chemotherapeutic therapy, eg. 2-CDA

Chibbar R. et al. *Front Med (Lausanne)*. 2020.

Refractory celiac diseases

Physiopathology



Malamut G et al. *F1000Res*. 2019.

Questions

Between 2015-2021, **16 patients** with a **possible** diagnosis of RCD were referred to our laboratory for FCM analysis of their duodenal biopsies.

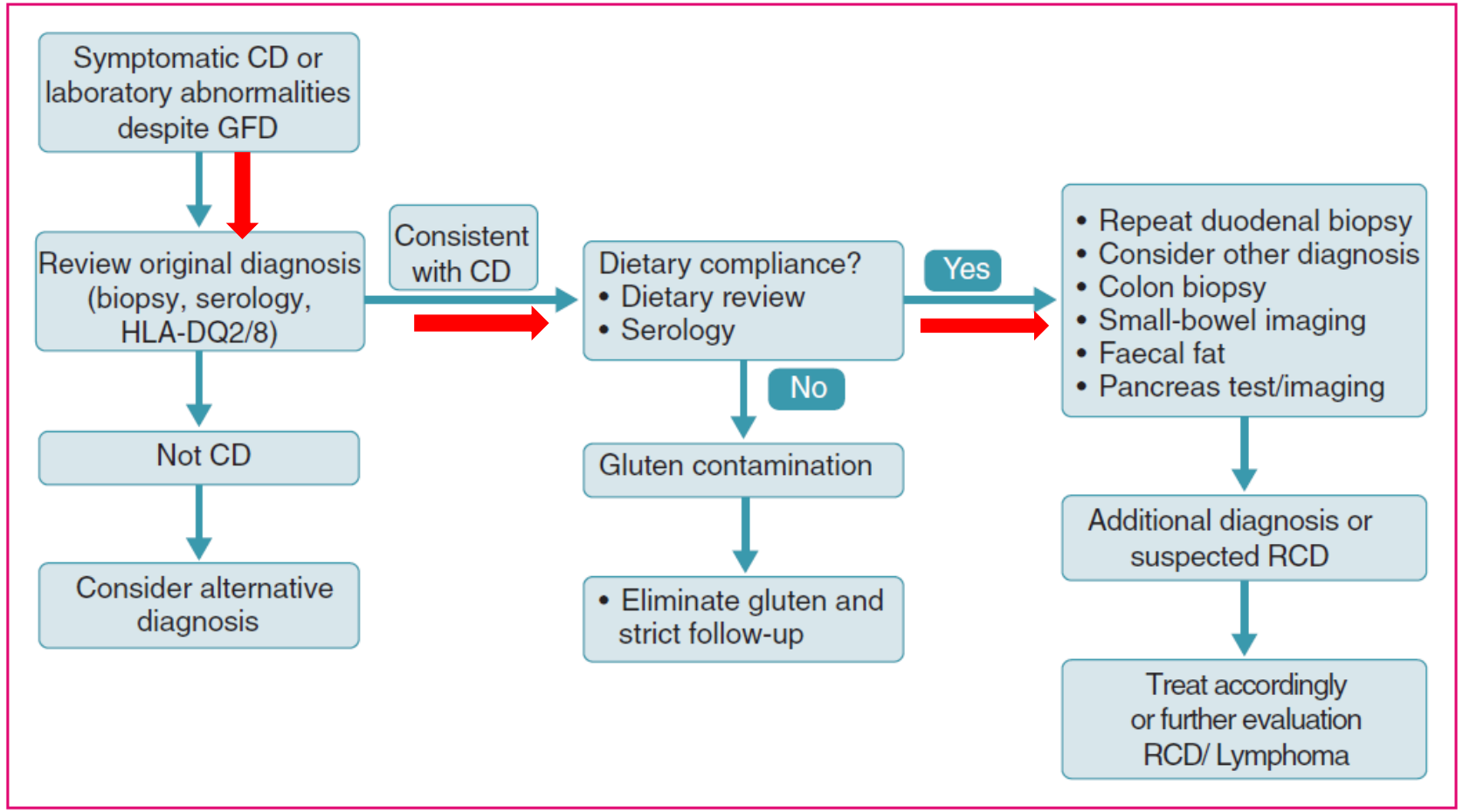
I. Determine which laboratory analytical techniques are used to diagnose RCD.

II. Determine which analytical laboratory techniques (FCM analysis, molecular analysis, pathology) are used to make the diagnosis of RCD type I versus RCD type II.

III. Determine the contribution of each analytical laboratory technique (FCM analysis, molecular analysis, pathology) to the final diagnosis of RCD type I/II (concordances/discordances)?

IV. Discuss the clinical course of patients diagnosed with RCD type I and RCD type II.

I. Diagnosis of RCD



Al-Toma A *et al.* European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. 2019

I. Diagnosis of RCD

16 patients referred for flow cytometry analysis

Review of CD diagnose:

- 62% clear
- 12% unclear
- 26% unconfirmed

Dietary compliance:
5 patients with poor compliance

6 patients with suspected RCD

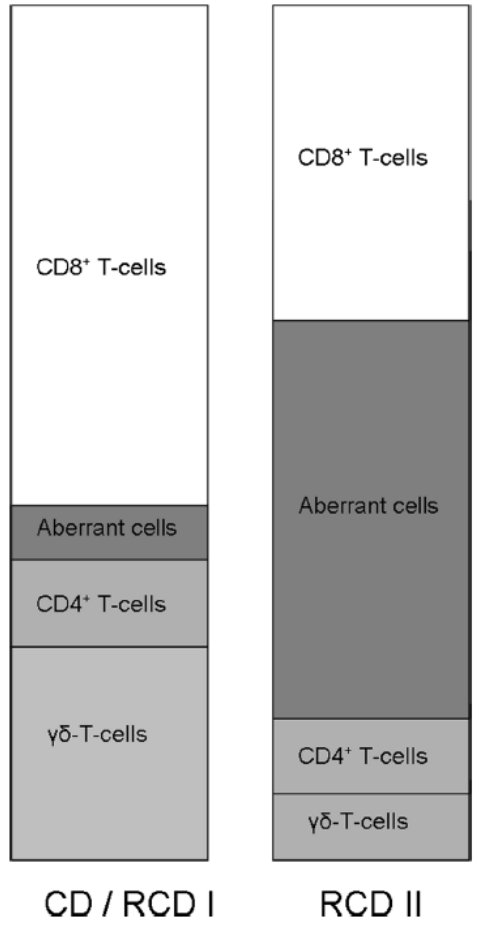
II. Diagnosis of RCD type I versus RCD type II.

Identify aberrant IELs

I. Flowcytometric immunophenotying

II. TCR gene rearrangement analysis

III. Immunohistochemistry: CD3 and CD8 staining



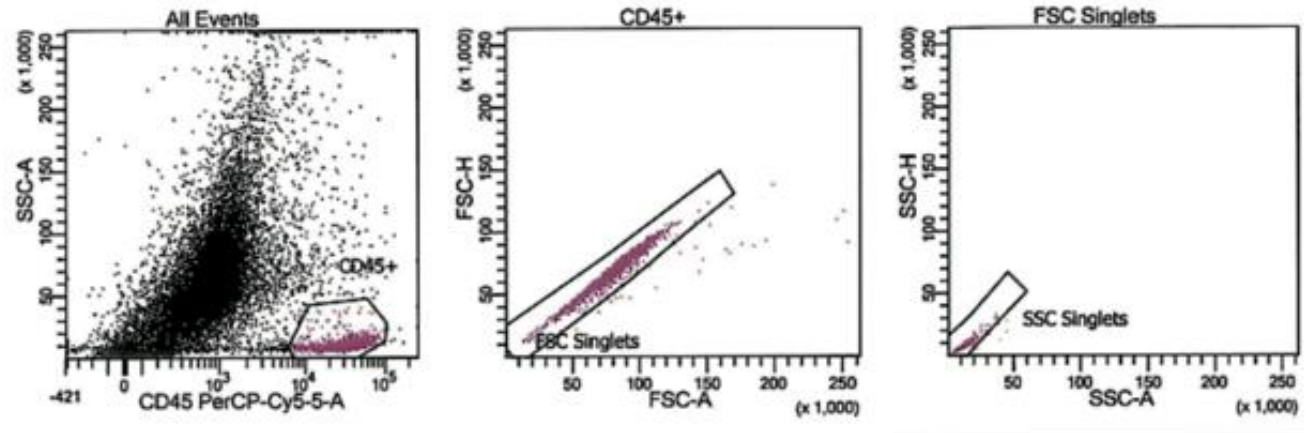
van Wanrooij et al. J Clin Immunol.2014

II. Diagnosis of RCD type I versus RCD type II.

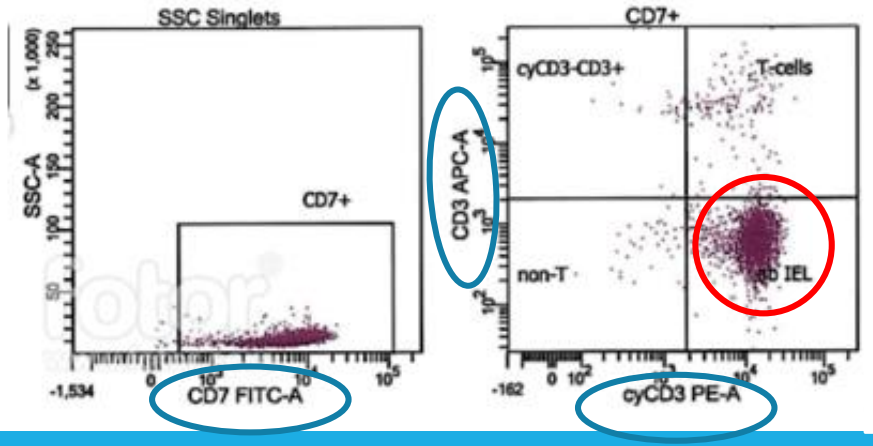
Flow Cytometry

Clonal expansion **cut-off:**

- RCD type I: <20% aberrant IELs
- RCD type II: 20-100% aberrant IELs



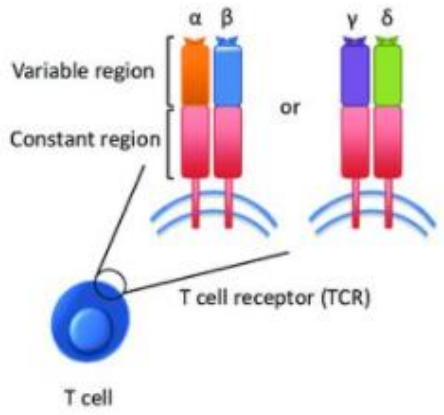
GATING STRATEGY

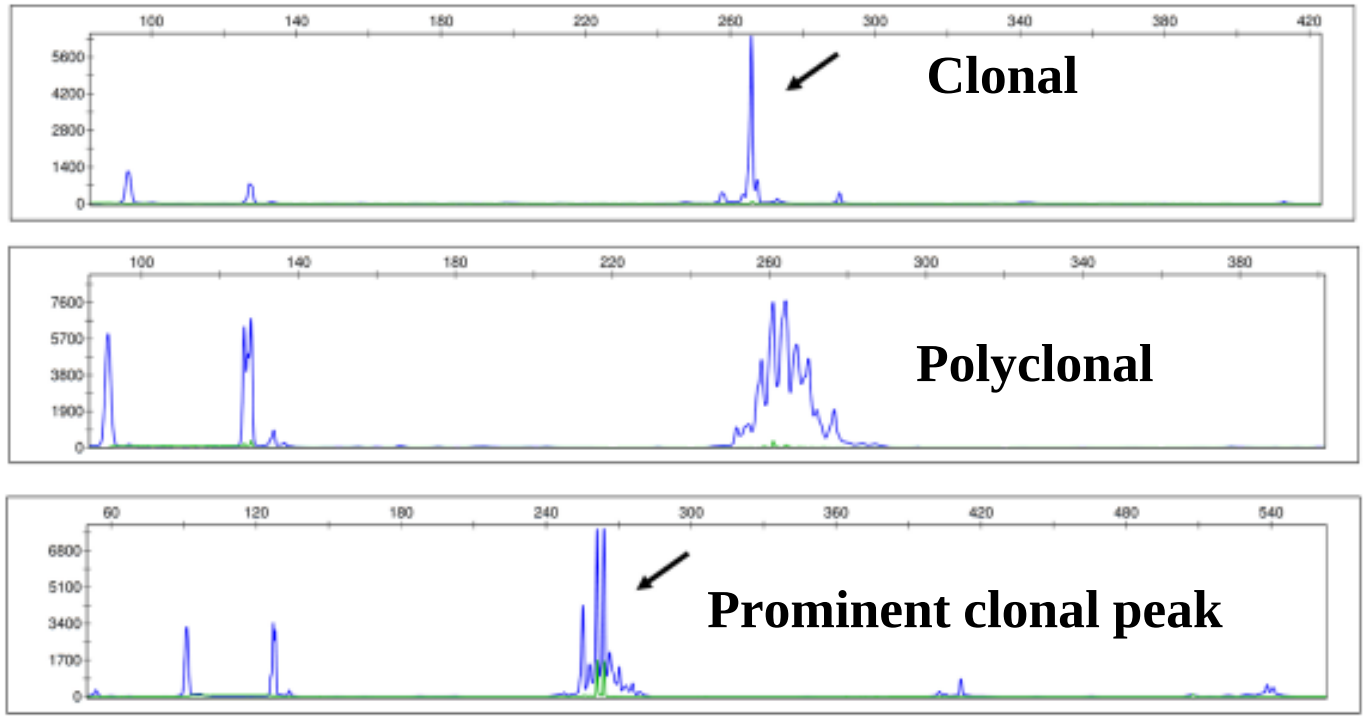


van Wanrooij et al. J Clin Immunol.2014

II. Diagnosis of RCD type I versus RCD type II.

TCR rearrangement analysis

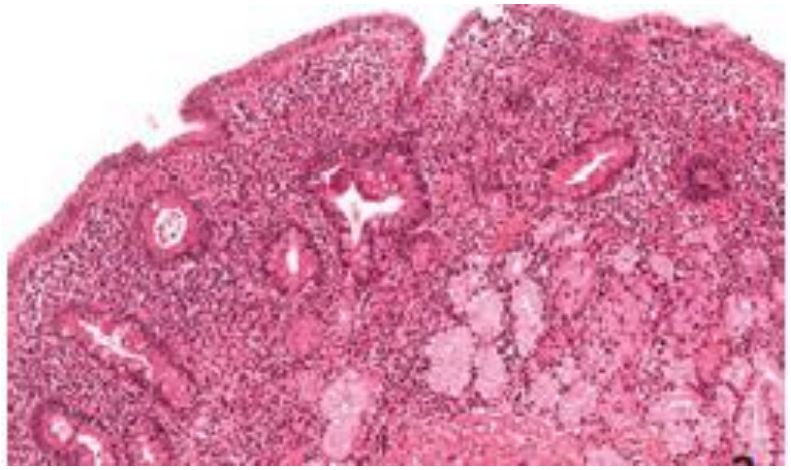




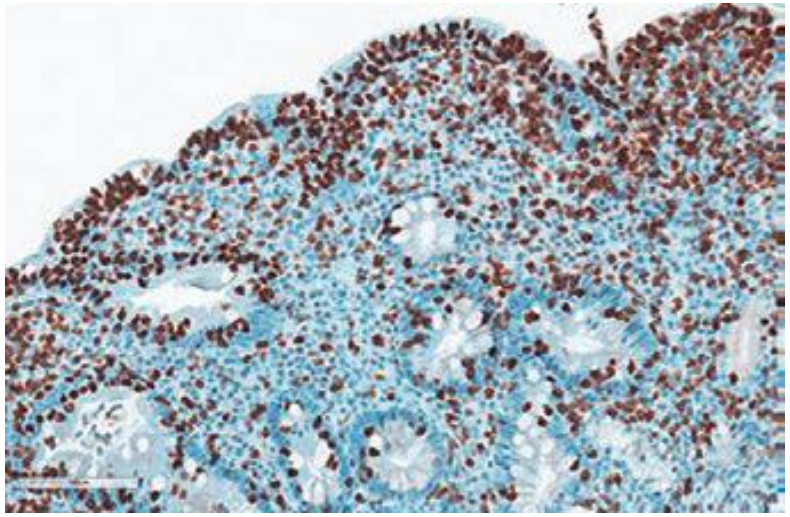
Hussein, S. et al. *Journal of clinical pathology*. 2018

II. Diagnosis of RCD type I versus RCD type II.

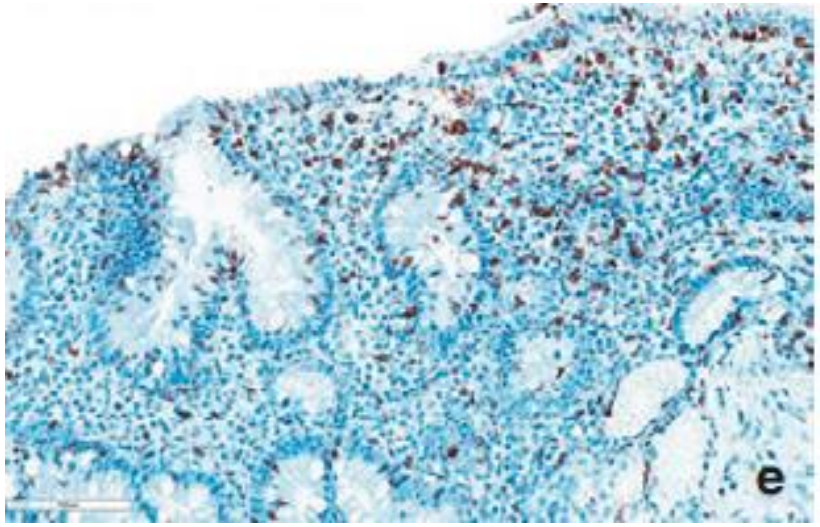
Immunohistochemistry



Villous atrophy (Marsh type 3)
Intraepithelial lymphocytosis



CD3+ IELs are increased in the surface epithelium



Less than 50% of IELs express CD8 indicating an aberrant abnormal phenotype

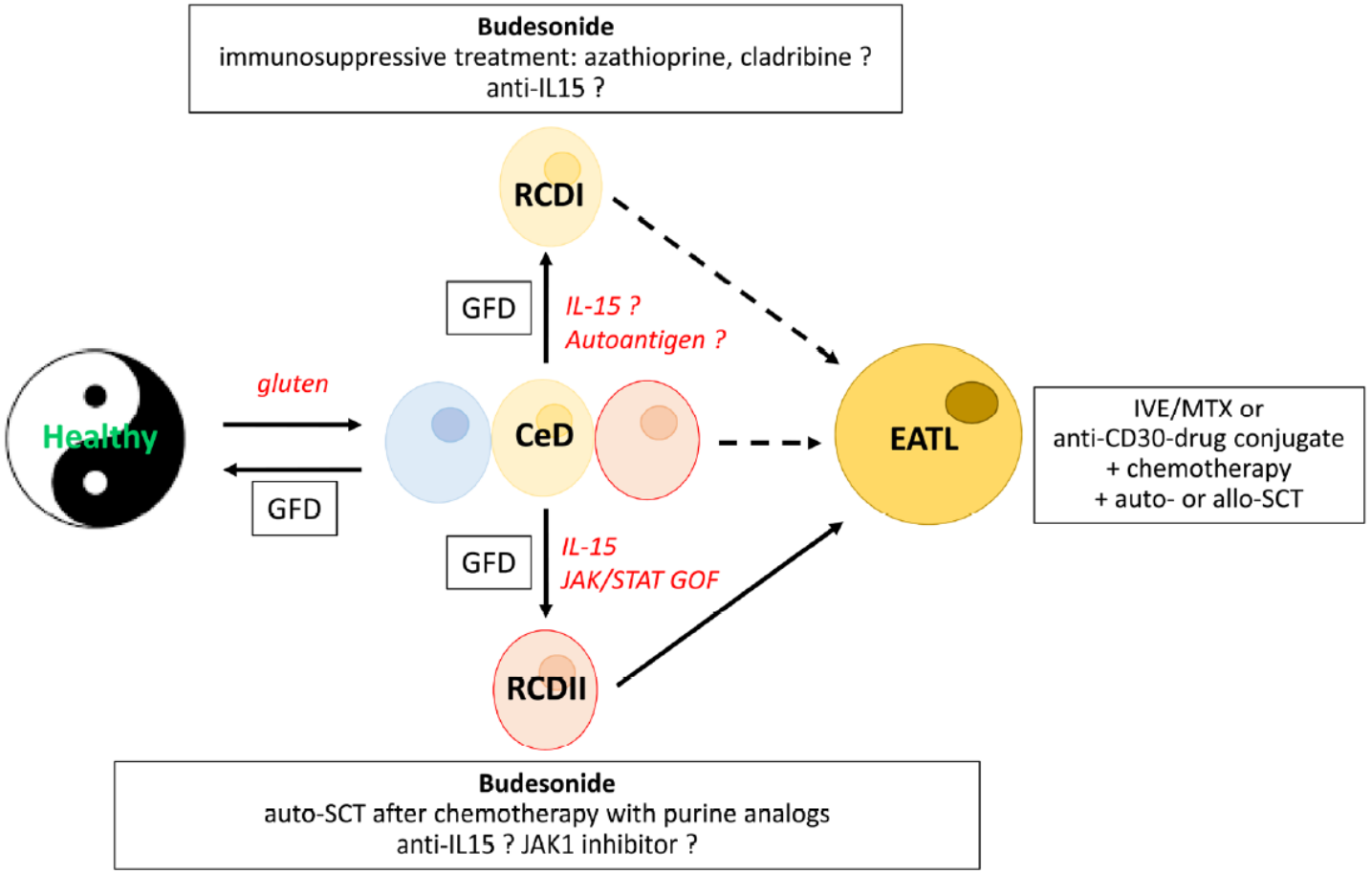
III. Contribution of each technique to the final diagnosis

Figure 2: Results of the FCM analysis (16 patients), TCR rearrangement analysis (8 patients) and IHC (16 patients) on the duodenal biopsies of the 16 patients.

Table 2	Pathology (n=16)	TCR rearrangement analysis (n=8)	FCM, % aberrant IELs (n=16)
Patients	*iCD3+CD8- IELs>50%*		
1	no	Monoclonal	0,6
2	no	Monoclonal	1,2
3	yes	Monoclonal	0,8
4	no	Not done	1,2
5	no	Not done	0,12
6	yes	Monoclonal	1,5
7*	no	Not done	4,1
8*	no	Monoclonal	0,9
9*	no	Polyclonal	0,2
10	no	Not done	0,2
11	no	Not done	<0,2
12	no	Not done	0,3
13	no	Not done	0,1
14*	yes	Monoclonal	73
15*	yes	Monoclonal	96,5
16*	no	Not done	0,6

* Patients with confirmed RCD

IV. Clinical course of patients with RCD



Malamut G et al. *F1000Res*. 2019.

IV. Clinical course of patients with RCD

Patient	Treatment	Outcome	Follow-up time
7	Budesonide	Indicates alive	-
8	Budesonide	Indicates alive	No abnormalities on follow-up histology in 2018 after treatment
9	Budesonide/medrol	Indicates alive	No abnormalities on follow-up histology in 2020 after treatment
14	Cladribine	Dead not disease related	-
15	Cladribine + everolimus	Indicates alive	Histological remission at the consultation in 2021
16	CHOP[2]	Dead not disease related	-

- = data not found
1= mycophenolate mofetil
2= cyclophosphamide, doxorubicine, vincristine and prednisone

TAKE HOME MESSAGE

- Recognize CD patients who are susceptible to RCD
- Exclude alternative causes
- Review diet compliance
- RCD type II patients are at risk for development of EATL
- FCM => powerful tool in the diagnosis and follow-up of RCD-II

  * Enumeration of the sCD3– icCD3ε+ aberrant IEL >20%

- Other techniques available with advantages and disadvantages => multidisciplinary approach

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