ANALYSE VAN DARMBIOPTEN IN KADER VAN REFRACTAIRE COELIAKIE

NVC - SKML, 21-11-2018

N. BOECKX, MD., PHD.

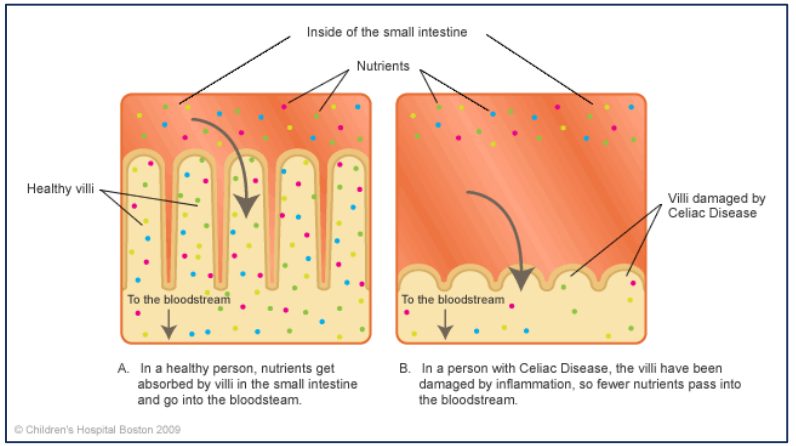
LABORATORIUMGENEESKUNDE, UZ LEUVEN

CELIAC DISEASE: WHAT?

- **Auto-immune** disorder that chronically affects **the small intestine**
- Induced by **dietary gluten** in genetically predisposed individuals (alleles encoding HLA-DQ2 or DQ8)
- Worldwide **prevalence ~1%**

CELIAC DISEASE: CLINICAL FEATURES

- **GASTRO-INTESTINAL** signs and symptoms
  - chronic diarrhea and abdominal pain
  - steatorrhea
  - weight loss, failure to thrive, growth failure, anorexia
  - bloating
  - vomiting, …



- **EXTRA-INTESTINAL** signs and symptoms
  - iron-deficiency anemia and other nutritional deficiencies (vitamin B12, vitamin D, folate, zinc, vitamin B6)
  - fatigue, …

- **ASSOCIATED (AUTOIMMUNE) CONDITIONS**
  - type I diabetes
  - autoimmune thyroid / liver disease
  - Sjögren syndrome, ….

} all associated with HLA risk alleles (HLA haplotypes DQ2 and/or DQ8)

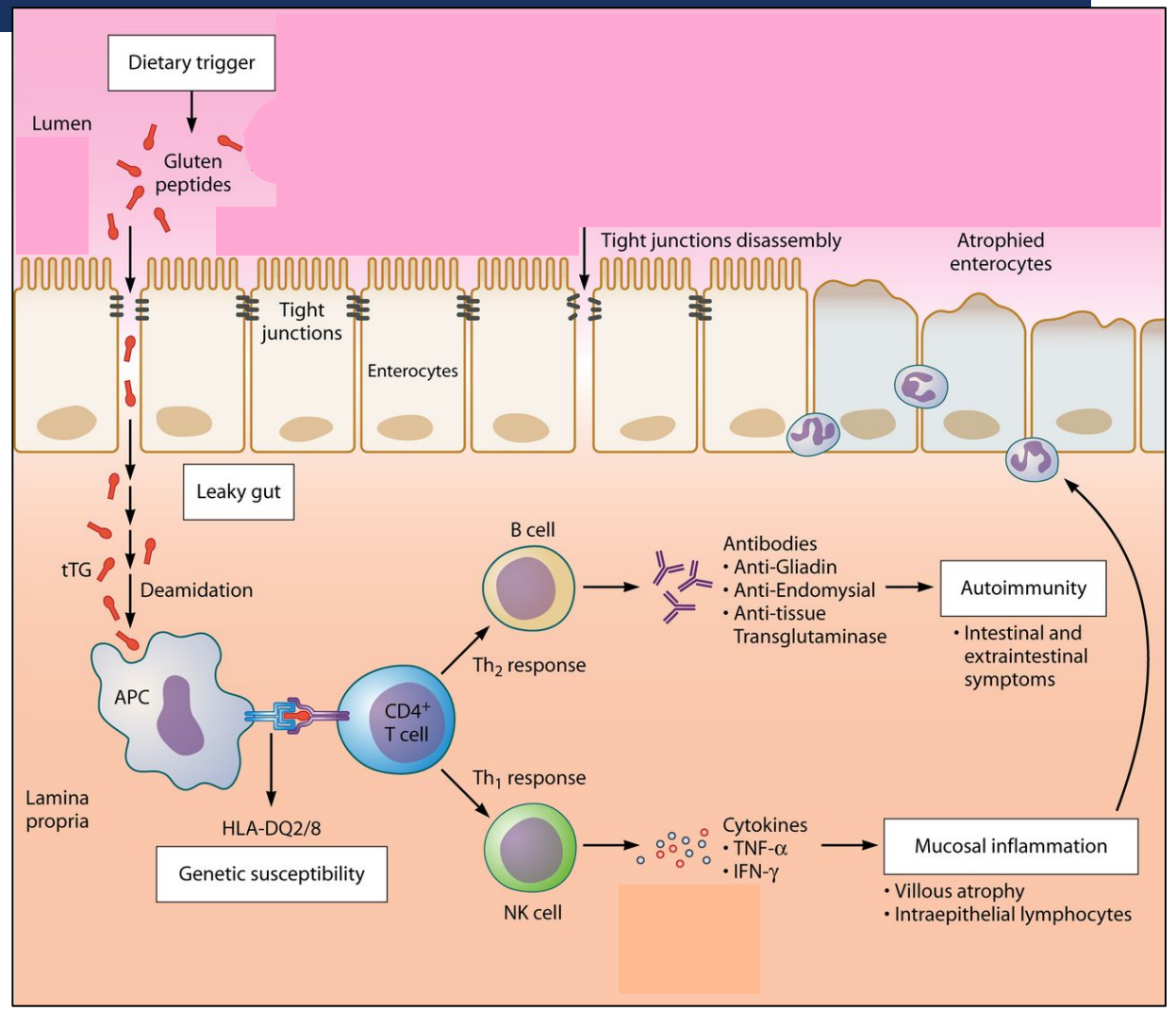
Kelly CP. *et al.* Gastroenterology 2015, 148:1175-86.

CELIAC DISEASE: DIAGNOSIS

1. **Serologic** markers of celiac disease
   - IgA/IgG against tissue transglutaminase (tTG)
   - IgA Endomysial antibody (EMA)
   - IgA/IgG against deamidated gliadin peptide (DGD)

2. Intestinal **biopsies**
   - Mucosal injury, more pronounced in proximal intestine, mild or absent distally
   - Microscopic findings: atrophic villi, crypt hyperplasia, increase in number of intra-epithelial lymphocytes (IEL) (not specific for CD)

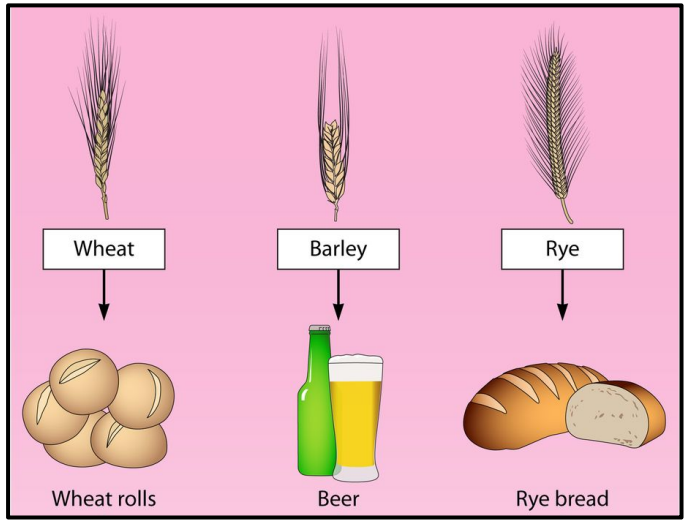
3. **Genetics**
   - Class II HLA DQ2 / DQ8 (in almost all CD patients, but also in 30-40% of Western Caucasian population; only 3% of individuals with these haplotypes develop CD)



Luís Fernando de Sousa Moraes et al. Clin. Microbiol. Rev. 2014

CELIAC DISEASE: TREATMENT

- the only treatment for celiac disease is a **strict gluten-free diet**
  - reduces symptoms, mortality and risk for malignancy
  - lifelong diet (expensive, socially isolating)
  - avoiding
    - wheat (‘*tarwe*’)
    - rye (‘*rogge*’)
    - barley (‘*gerst*’)



OBVIOUS SOURCES OF GLUTEN:

bread, bagels, cakes, cereal, cookies, pasta, noodles, pastries, pies, rolls

GLUTEN-FREE DIET

HLN

NIEUWS | SPORT | SHOWBIZZ | nina | REGIO | VIDEO

CELEBRITIES | FILM | ROYALTY | KUNST & LITERATUUR | JOEPIE | TV | MUZIEK

Showbizz > TV

Kobe Ilsen ontmoet koningin Mathilde in 'Over Eten': "De koning eet geen gluten"

DBJ | 21 november 2018 | 06u57 | Bron: NB

DEEL 3 5 REACTIES

© VRT - Koningin Mathilde en Kobe Ilsen

REFRACTORY CELIAC DISEASE (RCD)

- persisting or recurring symptoms **despite strict adherence to gluten-free diet**
  - diarrhea, abdominal pain, involuntary weight loss, …
  - severe malnutrition, protein-losing enteropathy, ulcerative jejunitis, ….
- patients are nearly always adults (50 years or thereafter)
- affects less than 1% of CD patients, but significant morbidity and mortality
- subdivided into 2 types of RCD
  - **RCD type I**
  - **RCD type II**

Higher proportion of woman are affected by both subtypes (up to 78% in RCD type I and 60% in RCD type II)

RCD TYPE I AND II

RCD type I (68-80% of RCD)	RCD type II
low risk (3-14%) for enteropathy-associated T-cell lymphoma (EATL)	increased risk (30-52%) to develop EATL

Revised WHO classification 2016

RCD TYPE I AND II

RCD type I (68-80% of RCD)	RCD type II
low risk (3-14%) for enteropathy-associated T-cell lymphoma (EATL)	increased risk (30-52%) to develop EATL
high 5-year survival (80-96%)	poor 5-year survival (44-58%)

Revised WHO classification 2016

RCD TYPE I AND II

RCD type I (68-80% of RCD)	RCD type II
low risk (3-14%) for enteropathy-associated T-cell lymphoma (EATL)	increased risk (30-52%) to develop EATL
high 5-year survival (80-96%)	poor 5-year survival (44-58%)
BENIGN => often responds to treatment with eg. topical steroids, immunosuppressive regimens	**PRE-MALIGNANT** (indolent lymphoma (pre-EATL)) => requires cytotoxic chemotherapeutic therapy, eg. 2-CDA, auto-SCTX (2 CDA-failure))

Revised WHO classification 2016

RCD TYPE I AND II

RCD type I (68-80% of RCD)	RCD type II
low risk (3-14%) for enteropathy-associated T-cell lymphoma (EATL)	increased risk (30-52%) to develop EATL
high 5-year survival (80-96%)	poor 5-year survival (44-58%)
BENIGN => often responds to treatment with eg. topical steroids, immunosuppressive regimens	**PRE-MALIGNANT** (indolent lymphoma (pre-EATL)) => requires cytotoxic chemotherapeutic therapy, eg. 2-CDA, auto-SCTX (2 CDA-failure))

Revised WHO classification 2016

RCD TYPE I AND II

RCD type I (68-80% of RCD)	RCD type II
low risk (3-14%) for enteropathy-associated T-cell lymphoma (EATL)	increased risk (30-52%) to develop EATL
high 5-year survival (80-96%)	poor 5-year survival (44-58%)
BENIGN => often responds to treatment with eg. topical steroids, immunosuppressive regimens	**PRE-MALIGNANT** (indolent lymphoma (pre-EATL)) => requires cytotoxic chemotherapeutic therapy, eg. 2-CDA, auto-SCTX (2 CDA-failure))
mainly intra-epithelial lymphocytes (IELs) with normal phenotype, only low numbers of aberrant IELs	high(er) numbers of aberrant IEL, which can clonally expand

Revised WHO classification 2016

PHENOTYPE OF IELs

Normal IELs

- Majority (>70%) of IELs are sCD3+ T-cells
  - TCRab (80%)
    - >85% CD8+
    - only ~10% CD4+
  - TCRgd (5-15%) with variable expression of CD8 (40-80%)
- 10-20% of IELs are CD3- cells

F. Leon. Journ of Immunol Methods 2011, 363: 177-186.

PHENOTYPE OF IELs

Normal IELs

- Majority (>70%) of IELs are sCD3+ T-cells
  - TCRab (80%)
    - >85% CD8+
    - only ~10% CD4+
  - TCRgd (5-15%) with variable expression of CD8 (40-80%)
- 10-20% of IELs are CD3- cells

Aberrant IELs

- T-cells
  - surface CD3-
  - surface CD8-
  - cytoplasmatic CD3+

F. Leon. Journ of Immunol Methods 2011, 363: 177-186.

METHODS TO IDENTIFY ABERRANT IELS

1. Immunohistochemistry : CD3 and CD8 staining

2. TCR gene rearrangement studies

3. Flowcytometric immunophenotyping

METHODS TO IDENTIFY ABERRANT IELS

		
Immunohistochemistry CD3 and CD8 staining	IHC and TCR-clonality studies: • reliable tools to identify dominant aberrant IEL populations • BUT fails to identify a moderate increase of these cells	no differentiation between cyCD3 and sCD3 lower sensitivity: high cut-off (>50% CD3+CD8- of CD3+ IELs) high interobserver variability
TCR gene rearrangement studies		fails to identify clonal IELs in patients with 20-25% aberrant IELs clonal GR: not specific for RCDII (also seen in RCDI (17%) and GFD (6%)

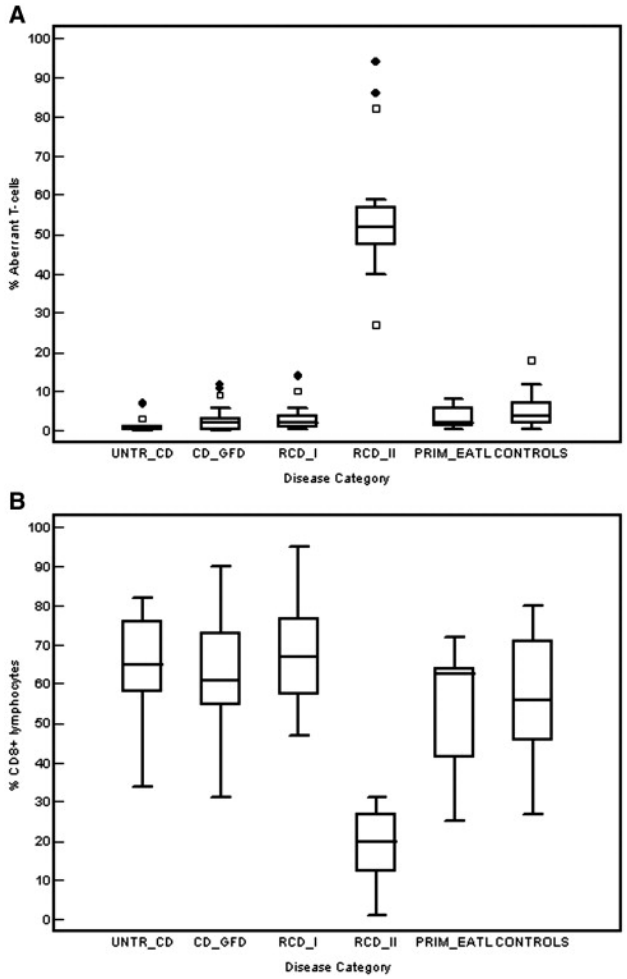
van Wanrooij RLJ. *et al.* J. Clin. Imm. 2014, 34: 828-35.
Verbeek WHM. *et al.* Clin. Imm. 2008, 126: 48-56.
Tack GJ. *et al.* Mol. Imm. 2012, 50: 262-70.
Hussein et al . Clin J. Path 2018.

METHODS TO IDENTIFY ABERRANT IELS

		
Immunohistochemistry CD3 and CD8 staining	• widely available	• no differentiation between cyCD3 and sCD3 • lower sensitivity: high cut-off (>50% CD3+CD8- of CD3+ IELs) • high interobserver variability
TCR gene rearrangement studies		• fails to identify clonal IELs in patients with 20-25% aberrant IELs • clonal GR: not specific for RCDII
Flowcytometric immunophenotyping ⬇ **GOLDEN STANDARD**	• can differentiate between cyCD3 and sCD3 • can also identify patients with only a moderate increase in aberrant IELs **(sCD3-CD8-CD7+cyCD3+)**	in 95% of non-refractory CD and control patients, the highest % aberrant T-cells in duodenal biopsy specimens is **20%**

van Wanrooij RLJ. *et al.* J. Clin. Imm. 2014, 34: 828-35.
Verbeek WHM. *et al.* Clin. Imm. 2008, 126: 48-56.

FCM LYMPHOCYTE SUBSETS IN DUODENAL BIOPSY SPECIMENS



⇒ Percentage aberrant T-cells (CD7+ surface CD3− cytoplasmic CD3+) in duodenal biopsy specimens of each disease category. There were **significantly more aberrant T-cells in the RCD II group** as compared to all other groups, in all cases $p < 0.0001$.

⇒ Percentage CD8+ lymphocytes in duodenal biopsy specimens of each disease category. There were **significantly less CD8+ T-cells in RCD II** as compared to all other groups, in all cases $p < 0.0001$.

Verbeek WHM. *et al.* Clin. Imm. 2008, 126: 48-56.

T-CELL CLONALITY ANALYSIS *VERSUS* FCM ANALYSIS

	RCD evolving to EATL, N = 10	RCD without EATL, N = 13
Detection of aberrant IELs		
>20% aberrant IELs	10	7
<20% aberrant IELs	0	6
T-cell clonality analysis		
Monoclonal	7*	7
Polyclonal	2	6

* Poor quality DNA, clonality analysis inconclusive

	FCM	Molecular
Sensitivity	100%	78%
Specificity	46%	46%
NPV	100%	75%
PPV	59%	50%

Verbeek WHM. *et al.* Clin. Imm. 2008, 126: 48-56.

FCM ANALYSIS UZL: PRE-ANALYTICAL CONDITIONS

- No external samples, only in-house taken biopsies
- Only after appointment with laboratory
- Recipient brought to endoscopy room by lab technician
- Biopsies are immediately brought to lab after gastro-duodenoscopy is finished

⇒ Time between endoscopy and arrival to lab: <1 hour

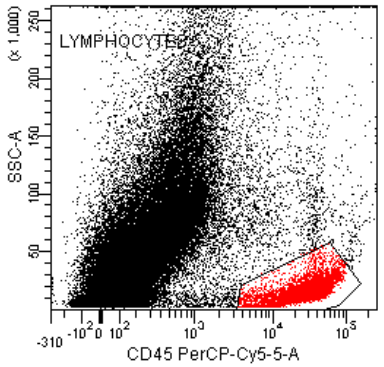
FCM ANALYSIS UZL: ISOLATION OF IELS

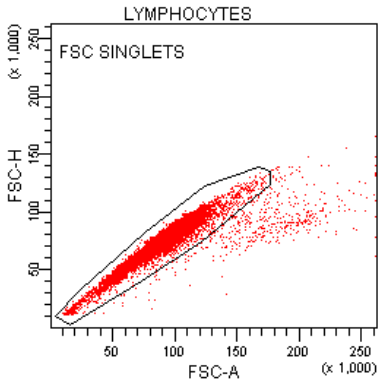
- 4 – 8 biopsies (stored in PBS at 0-4°C)
- isolation of IELs from intestinal biopsies
  - no chemical or enzymatic treatment
  - done by vigorous shaking : 60 min at 37°C (can also be done at room temperature)
    - calcium chelants (DTT, EDTA): induces the disassembly of inter-epithelial junctions and the release of epithelial cells and IELs
    - ~100.000 IELs per cubic millimeter small bowel biopsies (1 x 1 x 1 mm): enough for staining of IELs required for diagnosis and monitoring of CD (IELs will constitute ~5% (1-10% range) of the released cells)
    - **IELs in supernatant**

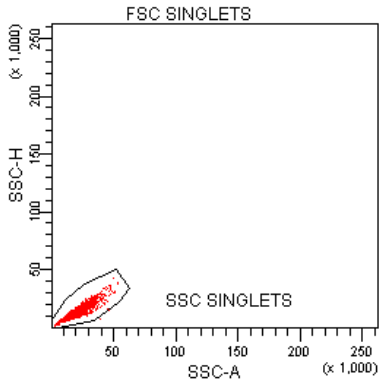
FCM ANALYSIS UZL: STAINING OF CELLS

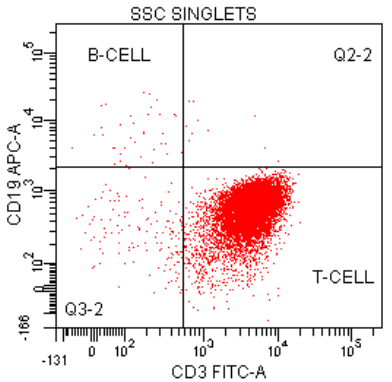
- Remove the biopsies from the solution, do not remove supernatant
- Supernatant: 2x wash step
- Surface staining
  - CD3 – CD16/56 – CD45 – CD19 – CD4 – CD8
- Intracellular staining
  - CD7 – cy isotype – CD45 – sCD3
  - CD7 – cy CD3 – CD45 – sCD3

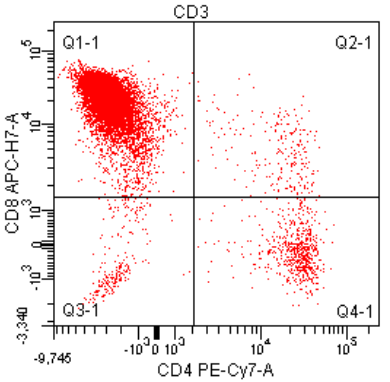
FCM ANALYSIS UZL: GATING STRATEGY

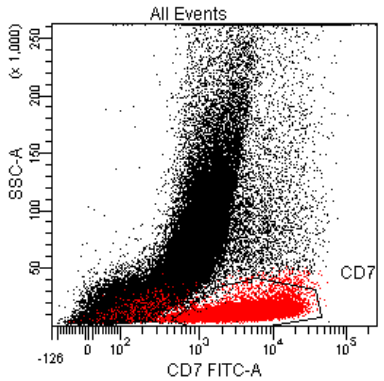


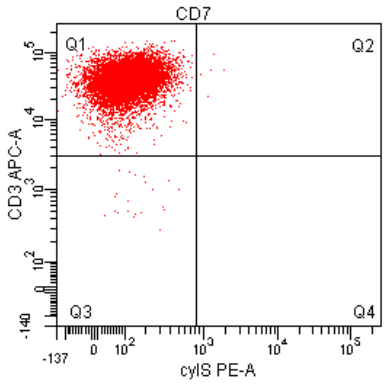


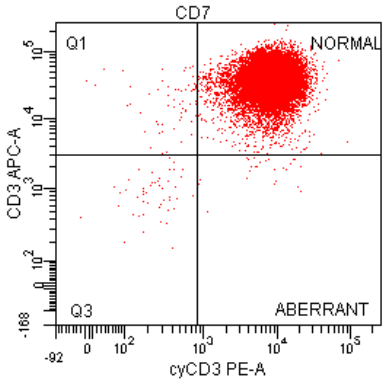






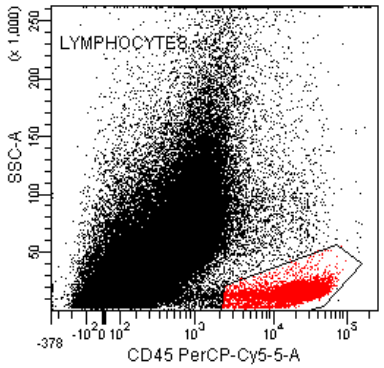


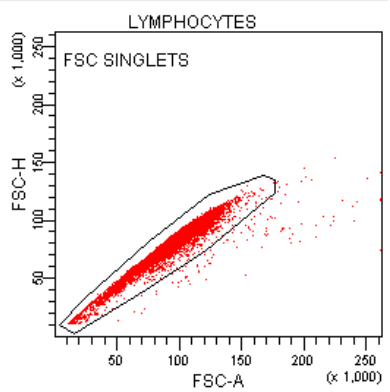


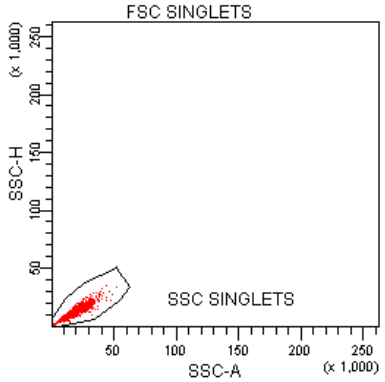


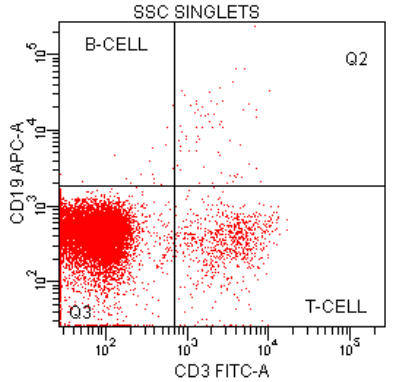
RCD type I

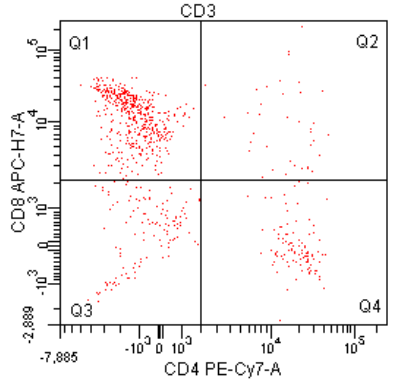
FCM ANALYSIS UZL: GATING STRATEGY

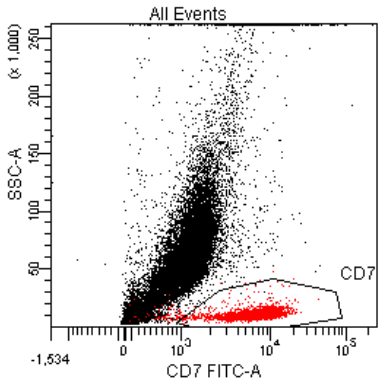


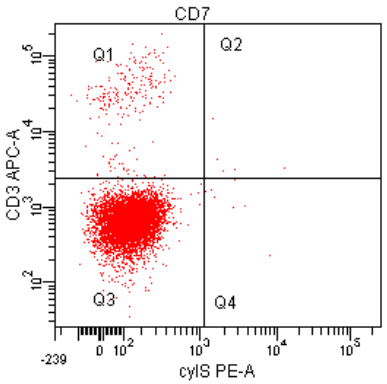


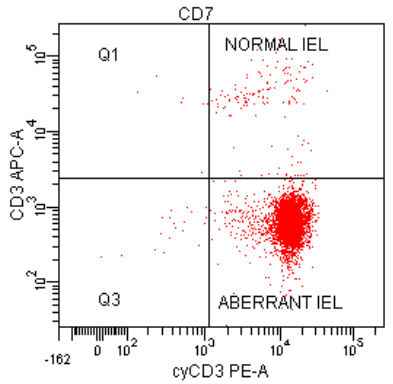












RCD type II

FCM ANALYSIS UZL: DIAGNOSIS OF RCD TYPE

Pat	Sex	Age	CD8 (APO)	Conclusion by pathologist	TCR clonality	FCM % IEL	RCD type
1	M	57	positive	CD	not done	0,6	I
2	M	58	negative	dysplasia of T-cells	monoclonal	96,5	II
3	M	78	negative	evolution to T-cell lymphoma?	monoclonal	73	II
4	M	63	positive	CD	not done	0,1	I
5	F	36	positive	CD	not done	0,3	I
6	F	63	positive	CD	not done	<0,1	I
7	F	52	positive	CD	not done	0,2	I
8	F	26	positive	CD	not done	0,2	I
9	F	26	not done	not done (referred from other hospital)	not done	0,9	I
10	F	80	not done	not done (referred from other hospital)	not done	4,1	I

RCD type I accounts for majority of cases

FCM ANALYSIS UZL: DIAGNOSIS OF RCD TYPE

Pat	Sex	Age	CD8 (APO)	Conclusion by pathologist	TCR clonality	FCM % IEL	RCD type
1	M	57	positive	CD	not done	0,6	I
2	M	58	negative	dysplasia of T-cells	monoclonal	96,5	II
3	M	78	negative	evolution to T-cell lymphoma?	monoclonal	73	II
4	M	63	positive	CD	not done	0,1	I
5	F	36	positive	CD	not done	0,3	I
6	F	63	positive	CD	not done	<0,1	I
7	F	52	positive	CD	not done	0,2	I
8	F	26	positive	CD	not done	0,2	I
9	F	26	not done	not done (referred from other hospital)	not done	0,9	I
10	F	80	not done	not done (referred from other hospital)	not done	4,1	I

RCD type I accounts for majority of cases

FCM ANALYSIS UZL: MONITORING OF RCD TYPE II

Pat	Sex	Age	Date biopsy	FCM % IEL
2	M	58	06-2015	**96,5**
			↓ cladribine & everolimus	
			03-2016	**93,6**
			↓	

Pat	Sex	Age	Date biopsy	FCM % IEL
3	M	78	01-2015	**73**
			↓ cladribine	
			03-2016	**8,7**
			↓ no specific R/	
			09-2017	**44,3**
			↓ cladribine	
			11-2017	**11,4**
			↓	

Cladribine therapy

- induces only a limited reduction of the % of aberrant IEL in 40% of cases
- majority still harbours a substantial aberrant population of IEL after treatment
- does not prevent EATL development in all treated patients

TAKE HOME MESSAGES

- RCD type II patients are at risk for development of EATL
- FCM is well suited for the identification of RCD type II patients
- A cut-off value off 20% aberrant IELs appears reliable for early risk stratification and targeted therapeutic options in RCD patients
- Quantification of aberrant IELs is useful for subsequent follow-up of treated RCD II patients

ACKNOWLEDGEMENTS

Dept. of Laboratory Medicine

S. Govers

N. Van den Panhuyzen

B. Timmermans

Dept. of Gastroenterology

M. Hiele

T. Vanuytsel

Dept. of Hematology

P. Vandenberghe

G. Verhoef

Dept. of Gastroenterology

K. Nys

J. Cremer

S. Vermeire