Gluten in coeliac disease and 'non-coeliac gluten sensitivity'

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ORIGINAL ARTICLE

The Oslo definitions for coeliac disease and related terms

Jonas F Ludvigsson,^{1,2} Daniel A Leffler,³ Julio C Bai,⁴ Federico Biagi,⁵ Alessio Fasano,⁶ Peter H R Green,⁷ Marios Hadjivassiliou,⁸ Katri Kaukinen,⁹ Ciaran P Kelly,³ Jonathan N Leonard,¹⁰ Knut Erik Aslaksen Lundin,¹¹ Joseph A Murray,¹² David S Sanders,^{13,14} Marjorie M Walker,¹⁴ Fabiana Zingone,¹⁵ Carolina Ciacci¹⁶

Gluten intolerance

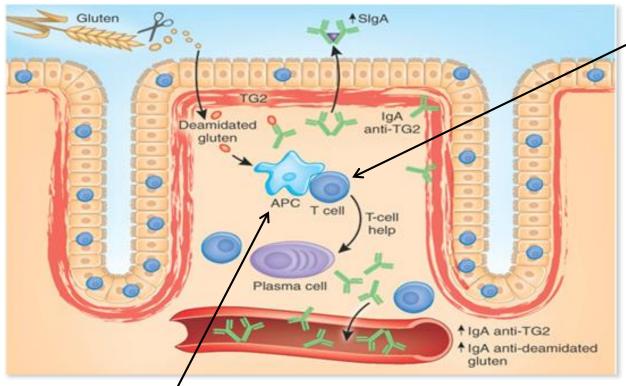
- the broadest term for all aspects of adverse reactions to gluten
- Coeliac disease
 - a small intestinal enteropathy, usually also typical serology (IgA-tissue transglutaminase)
- Wheat allergy a rapid, allergic response
- Non-coeliac gluten sensitivity (NCGS)
 - clinically quite like coeliac disease, but without enteropathy or serology





HLA-DQ2-gliadin tetramers and diagnosis

Modified gluten peptides bound to HLA-DQ2 in antigen presenting cells



Tetramer construct for detection of gluten specific T cells

T cells recognize

HLA-DQ2/DQ8-bound gluten peptides





Generation of T-cell epitopes in the gut

```
α2-gliadin (AJ133612)

1 MVRVPVPQLQ PQNPSQQQPQ EQVPLVQQQQ FPGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
61 PFPQPQLPYP QPQLPYPQPQ LPYPQPQFR PQQPYPQSQP QYSQPQQPIS QQQQQQQQQQ
121 QQKQQQQQQQ QILQQILQQQ LIPCRDVVLQ QHSIAYGSSQ VLQQSTYQLV QQLCCQQLWQ
181 IPEQSRCQAI HNVVHAIILH QQQQQQQQQQ QQPLSQVSFQ QPQQQYPSGQ GSFQPSQQNP
241 QAQGSVQPQQ LPQFEEIRNL ALETLPAMCN VYIPPYCTIA PVGIFGTNYR

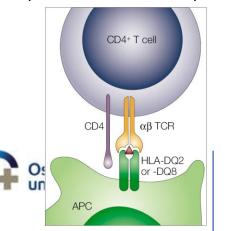
Transglutaminase (QXP)

after transglutaminase treatment
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61 PFPQPELPYP QPELPYPQPE LPYPQPQPF

peptide (33 amino acids)

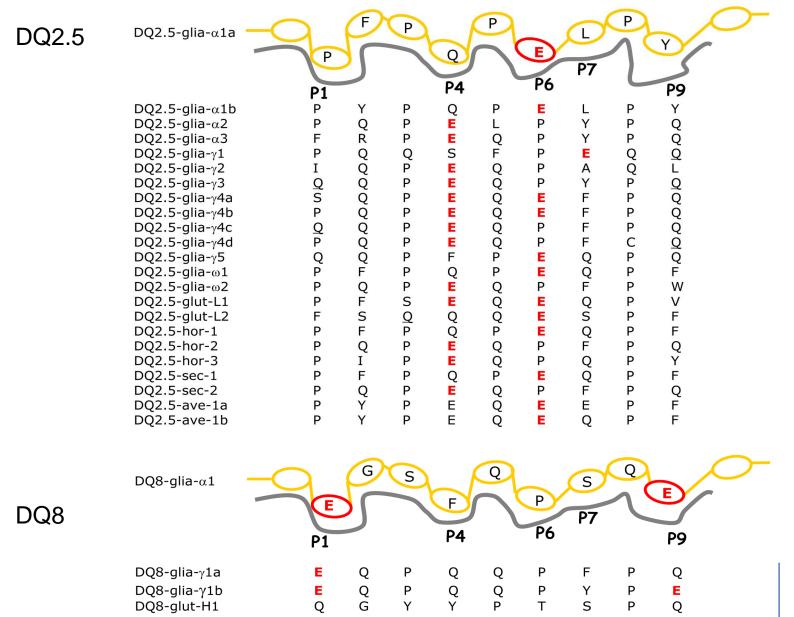
```
PFPQPELPY
PQPELPYPQ 6 copies of
PYP QPELPY T cell epitopes
P QPELPYPQ
PYPQPE LPY
PQPE LPYPQ
```





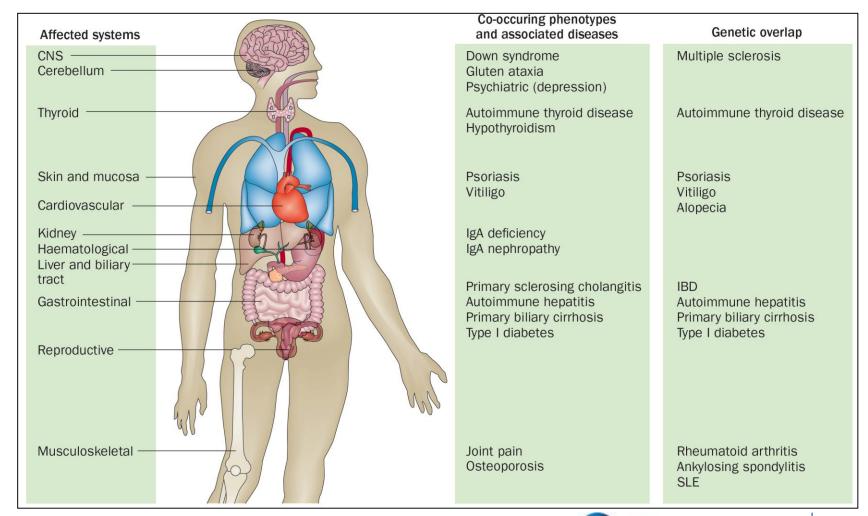
LQLQ

HLA-DQ2.5 and HLA-DQ8 restricted gluten T-cell epitopes





Genetic and clinical overlap







Coeliac disease

- An adaptive immunology, T cell mediated disease dependent on gluten intake
- Strongly associated to HLA-DQ2 and –DQ8
- Very much is understood
 - Restricted T cell receptor usage, long-lived T cells!
 - Restricted B cell receptor usage
 - Well defined gluten peptides
- But little is known why this develops!





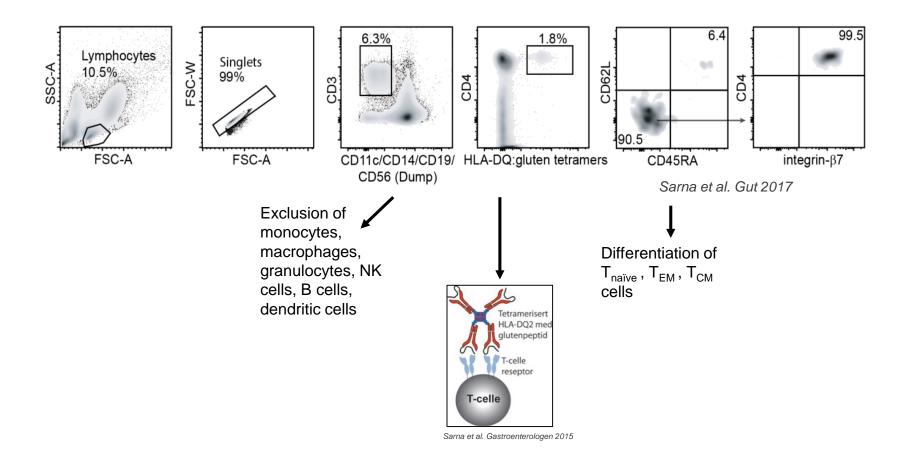
Experimental diagnosis of CD

Flow cytometry based





Flow cytometry



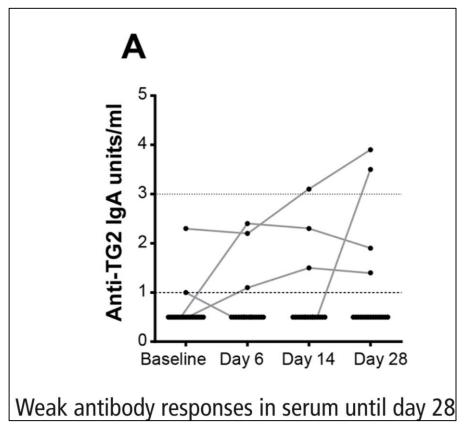


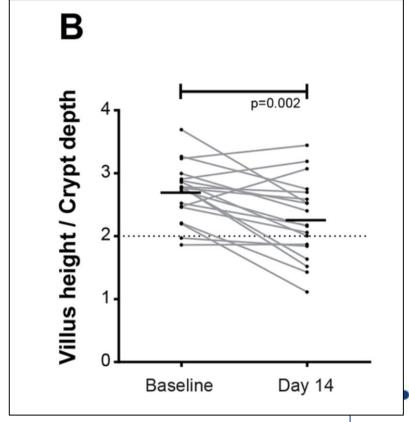


ORIGINAL ARTICLE

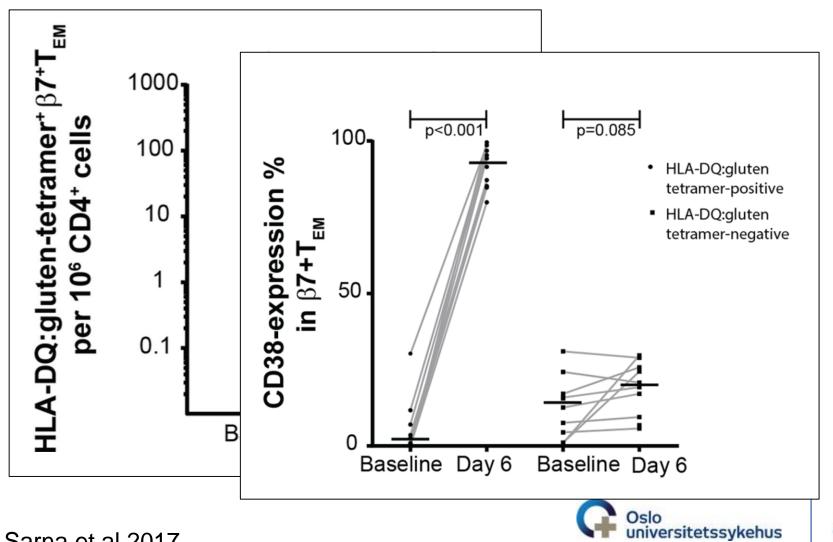
HLA-DQ:gluten tetramer test in blood gives better detection of coeliac patients than biopsy after 14-day gluten challenge Gut 2017

Vikas K Sarna, ^{1,2} Gry I Skodje, ^{2,3} Henrik M Reims, ⁴ Louise F Risnes, ^{1,5} Shiva Dahal-Koirala, ^{1,5} Ludvig M Sollid, ^{1,2,5} Knut E A Lundin ^{2,5,6}





Experimental read-out of gluten challenge







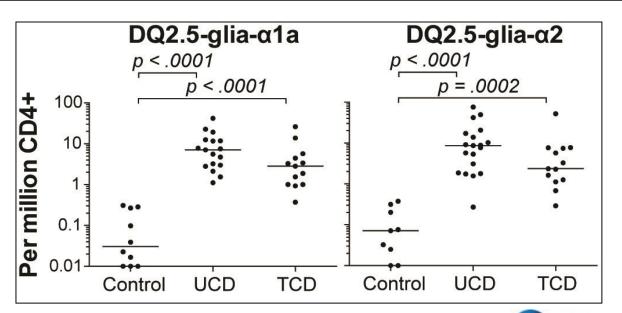
Tetramer-visualized gluten-specific CD4+ T cells in blood as a potential diagnostic marker for coeliac disease without oral gluten challenge

United European Gastroenterology Journal 0(0) 1-11

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Asbjørn Christophersen¹, Melinda Ráki¹, Elin Bergseng¹, Knut EA Lundin^{1,2}, Jørgen Jahnsen³, Ludvig M Sollid¹ and Shuo-Wang Qiao¹



<u>UCD</u> Untreated celiac disease

TCD Treated celiac disease

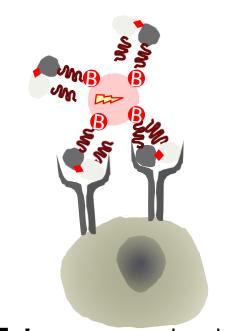




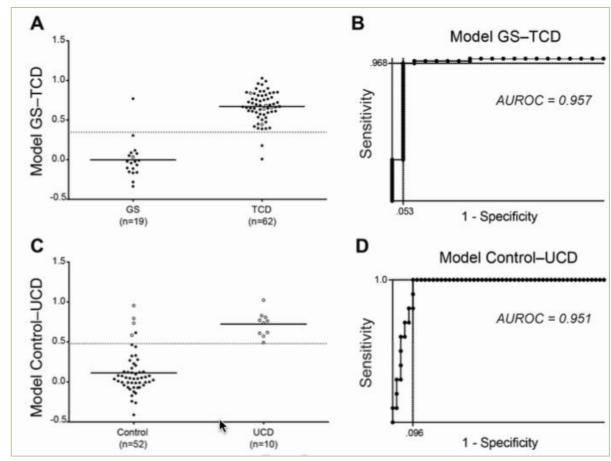


A blood test to sort out coeliac disease without gluten exposure!





Tetramer construct for detection of gluten specific T cells







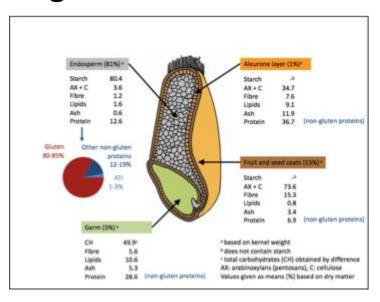
Then what about 'non-celiac gluten sensitivity?'





Opposing views

 NCGS is caused by a specific, innate immune reaction to proteins in "gluten"



- NCGS is caused by a reaction to "fermentable oligo-, di-, monocarbohydrates and polyols" (FODMAP)
- Fructan is one of several FODMAPS
- Bread is rich in fructan



NCGS – why bother?

- NCGS aka as
 - NCWS (Non-coeliac wheat sensitivity)
 - PWAG (People who avoid gluten)
- Mostly self instituted gluten free diet
- These people come to the health care
 - Do I have coeliac disease?
 - Do I have to stay on a gluten free diet? (they mostly answer themselves)
 - Can I have reimbursement?





Oslo work on NCGS – PhD Margit Brottveit

- Coeliac disease rare among NCGS "individuals" from general population
 - 130 responders, 35 were DQ2+, 3 with CD
 - Gastroscopy and "HLA-DQ2:gliadin peptide tetramer test"
 - Brottveit et al Am J Gastro 2011
- No signs of psychosomatic disorder
 - Three days challenge with bread
 - Brottveit et al Scand J Gastro 2012
- NCGS intestines with signs of immune activation
 - Increased levels of IEL, activation of IFN-γ after bread challenge
 - Brottveit et al Am J Gastro 2013



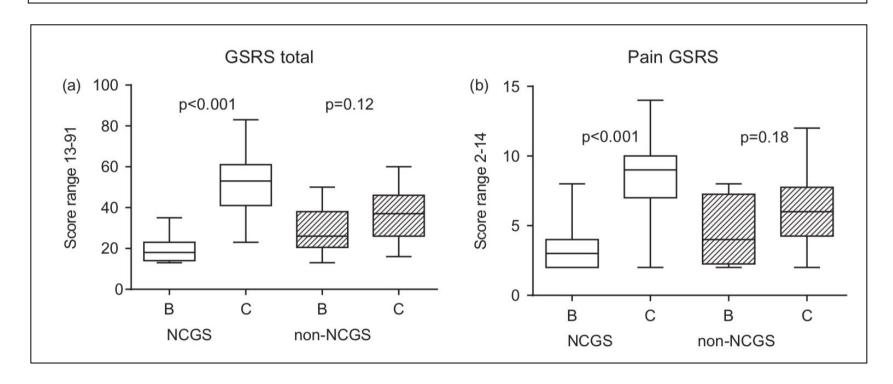




ORIGINAL ARTICLE

Wheat challenge in self-reported gluten sensitivity: a comparison of scoring methods

Gry I. Skodje^a, Christine Henriksen^b, Trude Salte^c, Thea Drivenes^c, Ieva Toleikyte^a, Astrid M. Lovik^a, Marit B. Veierød^{b,d} and Knut E.A. Lundin^{e,f,g}



Open challenge with regular bread (gluten and FODMAP)





Challenge – the placebo problem





- Supply all food?
- Traditional placebo
 - Capsules flour or placebo
 - Research tool
 - Lundin and Alaedini 2012
 - Catassi et al 2016
- Quinoa based müsli bars with and without "clean" gluten and FODMAP

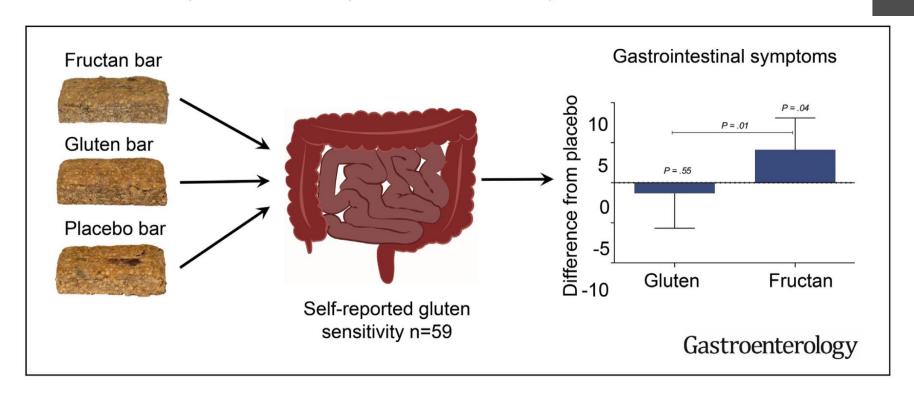




Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-Reported Non-Celiac Gluten Sensitivity



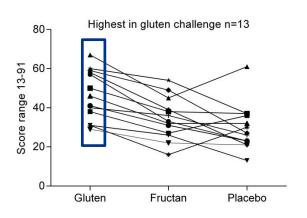
Gry I. Skodje, ^{1,2,4} Vikas K. Sarna, ^{2,3} Ingunn H. Minelle, ⁴ Kjersti L. Rolfsen, ⁴ Jane G. Muir, ⁵ Peter R. Gibson, ⁵ Marit B. Veierød, ^{4,6} Christine Henriksen, ^{2,4} and Knut E. A. Lundin ^{2,3,7,8}

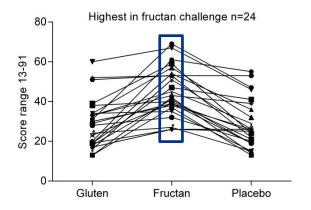


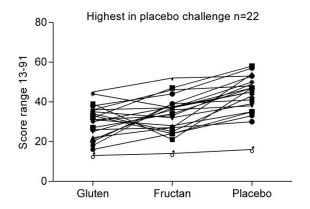


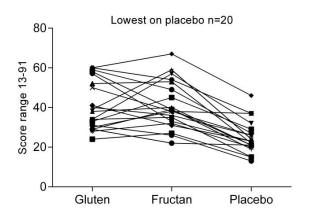


Individual courses





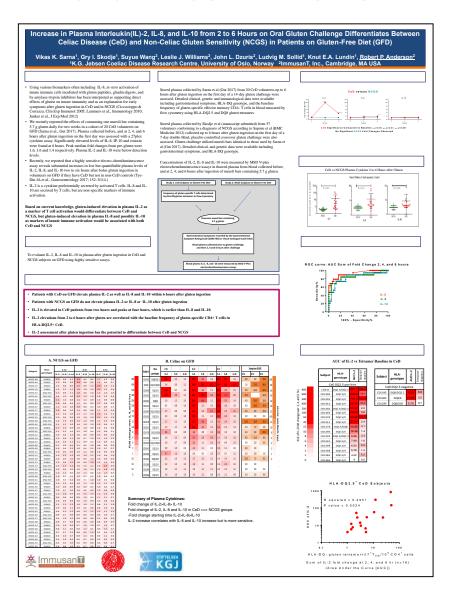


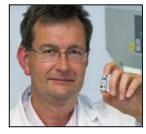




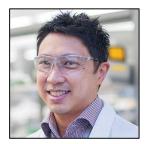


Cytokines after 4 h in CD and NCGS - ICDS 2017











An acute cytokine signature elicited by a bolus gluten challenge identifies patients following a gluten-free diet with celiac disease from those without

Jason A. Tye-Din¹, John L. Dzuris², Amy K. Russell¹, Suyue Wang², Kaela Goldstein², Leslie J. Williams², Robert P. Anderson² ¹Walter and Eliza Hall Institute, Parkville, Australia and ²ImmusanT, Inc., Cambridge, MA, USA

Introduction

- The accuracy of celiac disease (CeD) diagnosis
- Adoption of a gluten free diet (GFD) to manage perceived symptomatic sensitivity to gluten is common the community and obstructs formal CeD diagnosis.
- Dose-dependent elevations in IL-2 and IL-8 are observed 4h after a single injection of Nexvax2[7], a therapy targeting gluten-specific T cells in CeD1.
- We reasoned that if oral gluten challenge induces a similar, disease-specific cytokine response as Nexva this could be exploited as a novel diagnostic for CeD.

Assess changes in circulating levels of cytokines in CeD and non-CeD volunteers on GFD after consuming a single meal of gluten.

Study 1: Randomized, double-blind placebo-controlled food challenge (DBPCFC) Participants were HLA-DQ2.5+ CeD patients following a

•GFD adherence was based on a Celiac Dietary Adherence Test (CDAT) score < 13 and negative tTG-IgA and/or

ontaining gluten (5 gram Bob's Red Mill® Vital Wheat Gluten, estimated to contain 3 gram gluten?) or an

equivalent amount of gluten-free rice flour *Blood was collected via an intravenous catheter (hourly to 8h) then at +24h, and +6d and, in a later cohort, by separate venipuncture (0, +4h, +6h, +24h, and +6d).

Study 2: Open-label food challenge

Participants included CeD and non-CeD patients following

•Non-CeD patients had negative HLA-DQ2/8 genetics or ormal histology or serology while consuming gluten. •Challenged with 2.3 slices of white bread (~6 gram gluten)

·Blood was collected by venipuncture (0, +3h and +4h).

Cytokine/chemokines in serum and/or plasma by MSD® MULTI-SPOT Assay System; Chemokine Panel 1 (human) and Proinflammatory Panel 1 (human) kits.

outcome measure based on the CeD PRO3 ·Adverse events were recorded and graded by the study

nurse according to "The Common Terminology Criteria for Adverse Events (CTCAE) version 4.0" (FDA Guidance for Industry – Toxicity Grading Scale).

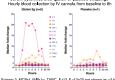
Study 1 (DBPCFC) results 56 (24-70)

- median fold change from baseline 12.6 vs placebo: 1.0 p=0.0001 2-tail Mann Whitney) and IL-8 (gluten: 2.4 fold vs placebo: 1.14, p=0.012) vs. placebo (Figs. 1 and 2).
- Elevations in IL-2 and IL-8 peaked within 2-4h after

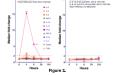
ledian age (range)

- Elevations in other cytokines and chemokines such as IL-10 and MCP-1 were more modest and less consistent than IL-2 or IL-8, and peaked later, ~ 3-6h after gluten ingestion
- IL-6 was increased after gluten and placebo but only
- Cytokine changes in serum and plasma were comparable (data not shown).

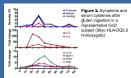




with optimized cut-offs	Elevated IL-2	Elevated IL-8					
Sensitivity % (95% CI)	91 (59-99.8)	82 (48-98)					
Specificity % (95% CI)	100 (63-100)	100 (63-100)					
Serum cytokines after gluten or placebo challenge Blood collection by separate venipunctures							



- gluten vs placebo (P <0.0001, Fisher's Exact test).
- Bloating, diarrhea and vomiting (but not other gastrointestinal symptoms, tiredness or headache) were statistically more likely after gluten.
- The rise in IL-2 typically preceded symptom onset in patients who were symptomatic (Figure 3)

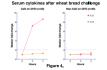


Study 2 (Open-label challenge) results

Participants	CeD (n=20)	Non-CeD* (n=2			
Sex	13F : 7M				
Median age (range)	51 (20-66)	52 (27-70)			
* All except for 2	followed a GFD	because of symp			
experienced after con-	cumina alutan conti	sining food			

- IL-2 and IL-8 did not increase in anyone without CeD (Figure 4).
- In the CeD cohort, the rise in IL-2, and particularly IL-8. was lower compared to the 3g gluten drink in Study 1.
- The non-CeD group were more likely than the CeD group to be symptomatic (95% vs 81%; P=0.004, Fisher's Exact test) and report abdominal cramping pain, bloating, constipation, diarrhea, gas (flatulence), nausea, headache and tiredness.

Serum cytokines after wheat bread challenge

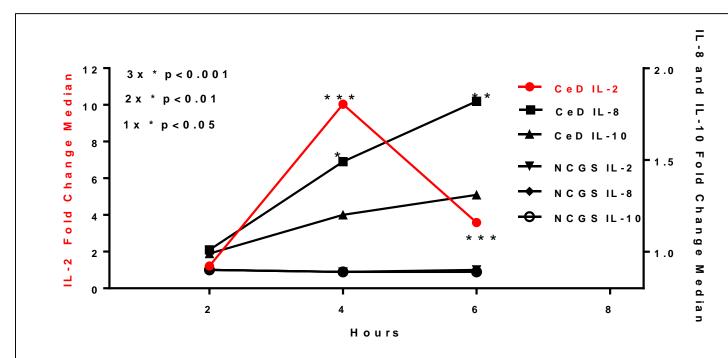


Diagnostic accuracy for CeD at optimal cut-off	Elevated IL-2	Elevated IL-8		
Sensitivity % (95% CI)	70 (46-88)	55 (31-77)		
Specificity % (95% CI)	95 (75-99.9)	100 (83-100)		
And the second second second				

- IL-2 rises within 2 hours of gluten ingestion in CeD patients but not those without CeD
- The cytokine signature frequently included IL-8 and less frequently other cytokines/chemokines
- qualitatively the same as that induced by a gluten-specific
- Blood collection and gluten delivery methods can impact the cytokine readout This novel approach can differentiate patients with CeD
- from those without and avoids the need for prolonged gluten exposure. Further studies are warranted.

Goel G et al. Lancet Gastroenterol Hepatol. 2017; 2. Hoppe C et al. Eur J Nutr 2015; 3. Leffler D et al. Gastroenterology 2015

4-hour test distinguishes CD and NCGS!



	IL-2			IL-8			IL-10		
	2 hr	4 hr	6 hr	2 hr	4 hr	6 hr	2 hr	4 hr	6 hr
Median for CeD	1.2	10.0	3.6	1.0	1.5	1.8	1.0	1.2	1.3
IQR (25-75)	(1.00-	(0.85-	(0.95-	(2.02-	(1.13-	(1.06-	(1.75-	(1.17-	(1.06-
IQR (25-75)	2.65)	1.18)	1.025)	25.58)	3.98)	1.58)	18.56)	2.71)	1.54)
Median for NCGS	1.0	0.9	1.0	1.0	0.9	0.9	1.0	0.9	0.9
IQR (25-75)	(0.97-	(0.81-	(0.88-	(0.96-	(0.78-	(0.79-	(0.96-	(0.84-	(0.70-
IQN (25-75)	1.00)	1.06)	1.09)	1.11)	1.05)	1.02)	1.18)	1.11)	0.92)



Wrap up

- 14 d gluten challenge in CD
 - Symptoms, slow serological response, but mucosal response and immune response
 - Efflux of IL-2, IL-8, IL-10 within 4 hours
- 7 d gluten challenge in NCGS
 - None of the above
 - Symptoms of FODMAP challenge
 - (But the participants are still on a GFD...)
- Where did the innate response to gluten go?





Thanks!

- UiO/OUS
 - Gry Skodje
 - Vikas Sarna
 - Christine Henriksen
 - Marit Veierød
 - Margit Brottveit
 - Ludvig M. Sollid
- Monash University
 - Jane Muir
 - Peter Gibson

- ImmusanT
 - Bob Anderson
 - Leslie Williams
 - John Dzuris
 - Gautam Goel

- Exstrafoundation
- Helse Sør-Øst

All the participants!







