

## **SEROLOGICAL MARKERS OF ENTEROCYTE DAMAGE AND APOPTOSIS IN PATIENTS WITH CELIAC DISEASE, AUTOIMMUNE DIABETES MELLITUS AND DIABETES MELLITUS TYPE 2**

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## **SUMMARY**

Impairment of mucosal barrier integrity of small intestine might be causative in immune-mediated gastrointestinal diseases. We tested the markers of epithelial apoptosis – cytokeratin 18 caspase-cleaved fragment (cCK-18), and enterocyte damage – intestinal fatty acid-binding protein (I-FABP) and soluble CD14 (sCD14) in sera of patients with untreated celiac disease (CLD), those on gluten-free diet (CLD-GFD), patients with autoimmune diabetes mellitus (T1D), T1D with insulinitis (T1D/INS), and diabetes mellitus type 2 (T2D). We found elevated levels of cCK-18 ( $P<0.001$ ), I-FABP ( $P<0.01$ ) and sCD14 ( $P<0.05$ ) in CLD when compared to healthy controls. However, the levels of cCK-18 ( $P<0.01$ ) and I-FABP ( $P<0.01$ ) in CLD-GFD were higher when compared with controls. Interestingly, elevated levels of cCK-18 and I-FABP were found in T2D and T1D ( $P<0.001$ ), and T1D/INS ( $P<0.01$ ,  $P<0.001$ ). Twenty-two out of 43 CLD patients were seropositive for cCK-18, 19/43 for I-FABP and 11/43 for sCD14; 9/30 of T2D patients were positive for cCK-18 and 5/20 of T1D/INS for sCD14, while in controls only 3/41 were positive for cCK-18, 3/41 for I-FABP and 1/41 for sCD14. We documented for the first time seropositivity for sCD14 in CLD and potential usefulness of serum cCK-18 and I-FABP as markers of gut damage in CLD, CLD-GFD, and diabetes.

## **KEY WORDS**

cytokeratin 18 caspase-cleaved fragment, intestinal fatty acid-binding protein, soluble CD14, celiac disease, diabetes mellitus

## 1. INTRODUCTION

Chronic impairment of gut status and intestinal mucosal barrier integrity associated with increased enterocyte apoptosis and damage might be causative in the development of inflammatory and immune-mediated gastrointestinal diseases (Fasano and Shea-Donohue 2005). Celiac disease (CLD) represents a specific kind of immune-mediated intolerance of dietary wheat gluten with autoimmune features. Ingestion of water-insoluble fraction of gluten – gliadin triggers the enteropathy and damage of the small-gut mucosal barrier in genetically susceptible individuals. Life-long adherence to gluten-free diet (GFD) enables the regeneration of small-gut mucosa in CLD patients and prevents more severe forms of CLD and the development of autoimmune complications targeting various organs. The relevance of precise, early and non-invasive diagnostics of CLD and reliable markers for monitoring the disease course are essential since this disease is frequently associated with severe autoimmune endocrine, liver and rheumatologic disorders, T-cell lymphoma and reproductive insufficiency, as well as with a high population frequency of CLD estimated at 1:250 – 500 (Trier 1991, Husby *et al.* 2012).

The relationship between CLD and autoimmune diabetes mellitus (T1D) is intensively studied due to putatively associated etiopathogenesis (Lettre and Rioux 2008). The common features of CLD and T1D documented by genome-wide association study (GWAS) suggested the role of impaired mucosal barrier function in etiopathogenesis of both diseases (Zhernakova *et al.* 2013). The prevalence of CLD among patients with T1D has been estimated at approximately 4 % (range from 2 % to 11 %). The risk of co-morbidity with CLD is high at the onset of T1D, in children younger than 4 years, and increases with the duration of diabetes (Cerutti *et al.* 2004, Salardi *et al.* 2008, Pham-Short *et al.* 2012). In our previous study, 10.3 % of Libyan

children with T1D had biopsy-based clear histological evidence of CLD (Ashabani *et al.* 2003). The hypothesis linking the pathogenesis of the two diseases is supported also by partially shared HLA haplotypes – HLA-DQ2 and HLA-DQ8 in CLD and some T1D patients (Rewers *et al.* 2004, Rewers and Eisenbarth 2011, Hummel *et al.* 2011). It is assumed that the penetration of luminal components or pathogens from “leaky” gut in untreated CLD patients may alter the function of  $\beta$ -cells or induce insulinitis (Yajima *et al.* 2009, Kuo *et al.* 2010, Wang *et al.* 2010, Peng and Hagopian 2006). Increased translocation of food and microbial antigens from gut lumen into circulation in untreated CLD patients but also in individuals with impaired intestinal barrier function has long been presumed to activate immune response and to trigger and/or potentiate  $\beta$ -cell inflammatory destruction preceding clinical manifestation of T1D (Peng and Hagopian 2006, Knip *et al.* 2005, Knip and Simell 2012, Tlaskalová-Hogenová *et al.* 2011, Funda *et al.* 2001, Osterbye *et al.* 2010, Kučera *et al.* 2003). The contribution of immune system to diabetes pathogenesis is documented by analysis of enterobiopsies in T1D patients in whom elevated levels of IFN- $\gamma$  and TNF- $\alpha$  were found indicating pro-inflammatory conditions in the gut mucosa. In these patients, the production of high levels of IL-17 by peripheral CD45RA<sup>-</sup>CD25<sup>int</sup>CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells after activation with anti-CD3/CD28 antibodies was detected (Bruewer *et al.* 2005, Li *et al.* 2008, Westerholm-Ormio *et al.* 2003). The increasing intestinal permeability measured by lactulose/mannitol significantly correlated with luminal levels of zonulin i.e. protein modulating epithelial tight junctions (Vaarala 2008). On the other hand, it is known that only the proportion of saturated fatty acids and polyunsaturated fatty acids in dietary intake is sufficient for affecting the systemic inflammation (Králová Lesná *et al.* 2013). Interestingly, also diabetes mellitus type 2

(T2D) is associated with increased inflammatory response, which is modulated by intestinal microbiota and nutritional composition (Musso *et al.* 2010).

In this study we analyzed and compared the serum levels of cytokeratin 18 caspase-cleaved fragment (cCK-18) as a marker of epithelial apoptosis and intestinal fatty acid-binding protein (I-FABP) as indicator of enterocyte damage (Derikx *et al.* 2008, Adriaanse *et al.* 2013) in patients with newly diagnosed (untreated) CLD, CLD treated with GFD (CLD-GFD), T1D with insulinitis (T1D/INS), T1D with fading insulinitis (T1D), T2D, and healthy controls. Moreover, the testing of soluble CD14 (sCD14) was used as an indicator of activation of innate immunity cells in response to mucosal translocation of gram-negative bacteria or their components in these patients (Ancuta *et al.* 2008).

## **2. MATERIALS & METHODS**

### **2.1. Patients and healthy donors**

Table 1 summarizes baseline characteristics of tested patients with CLD, CLD-GFD, T1D/INS, T1D, T2D, and healthy controls. The CLD patients were diagnosed in accordance with modified ESPGHAN criteria (Husby *et al.* 2012), i.e. they were seropositive for IgA anti-tissue transglutaminase (anti-tTG) antibodies (Ab) and IgA and IgG anti-endomysial Ab (EMA), and pathological changes of their small bowel mucosa were classified as Marsh IIIA-C. Sera of CLD-GFD patients – collected after at least 12 months of adherence to GFD – were negative for EMA and anti-tTG Ab; all of these patients were free of symptoms of CLD. The Ab to tyrosine phosphatase-like insulinoma antigen 2 (anti-IA2) and Ab to glutamic acid decarboxylase (anti-GAD) were used for discriminating between T1D/INS and T1D (Wenzlau *et al.* 2007, Nokoff *et al.* 2012). The cohort of healthy controls represents blood donors.

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The serological testing and histological analysis were routinely performed in Department of Laboratory Medicine and Department of Pathology, Faculty Hospital Královské Vinohrady in Prague. ELISA kit IgA (Biosystems SA, Barcelona, Spain) for the estimation of levels of serum anti-tTG Ab, indirect immunofluorescence - monkey oesophagus (AEA - Anti Endomysium antibodies, Biosystems SA, Barcelona, Spain) for IgA, IgG EMA, Medizym Anti-GAD ELISA kit for anti-GAD Ab and Medizin Anti-IA2 ELISA kit (MEDIPAN GMBH, Dahlewitz/Berlin, Germany) for anti-IA2 Ab, Immulite 2000, Siemens Healthcare Diagnostics and ADVIA Centaur XP, Siemens Healthcare Diagnostics for anti-thyroid peroxidase Ab and anti-human thyroglobulin Ab was used. The serum levels of immunoglobulins were measured by employing nephelometry (BNII analyzer, Dade Behring – Siemens), albumin using a colorimetric assay with bromocresol green and ADVIA 1800 and Cobas 8000 and Cobas 6000 analyzer (Siemens Healthcare Diagnostics, and Roche Diagnostics), and the levels of C-reactive protein (CRP) by immunoturbidimetry (Siemens Healthcare Diagnostics, and Roche Diagnostics). The data are expressed as mean  $\pm$  standard error. The ALT and AST serum levels were tested using IFCC with pyridoxal-5-phosphate and ADVIA 1800 (Siemens Healthcare Diagnostics). Liver steatosis was evaluated by abdominal ultrasonography (data not shown).

None of the individuals from control group had autoimmune, inflammatory, malignant or infectious disease at the time of blood taking. The patients with T1D/INS, T1D and T2D associated with severe complications were excluded in the study, as well as patients having other chronic diseases, malignant disease, IgA-deficiency or abnormal levels of liver enzymes (ALT, AST). None of healthy controls and patients with diabetes was positive for anti-tTG Ab and EMA in this study. The study was reviewed and approved by the Local Ethics Committee from the Faculty

Hospital Královské Vinohrady in Prague (Czech Republic). Written informed consent was obtained from each participant in the study.

## **2.2. cCK-18, I-FABP and sCD14 ELISA**

The sera of patient and control groups were tested for cCK-18 using M30-Apoptosense ELISA (Peviva, Sweden), for I-FABP by Human, I-FABP ELISA Kit (Hycult Biotech Inc., US), and for sCD14 by Human sCD14 ELISA Kit, (Hycult Biotech Inc., US) according to the manufacturer's instructions.

## **2.3. Statistical analysis**

The cut-off values for cCK-18, I-FABP and sCD14 were calculated as the mean value plus 2 standard deviations (SD) of serum levels of individual markers tested in 41 healthy controls. The Mann-Whitney U-test was used to compare the serum levels of tested markers among groups of patients and controls.

## **3. RESULTS**

The occurrence of the markers cCK-18, I-FABP and sCD14 was tested in sera from 166 individuals including five groups of patients with: CLD, CLD-GFD, T1D/INS, T1D, T2D, and healthy controls. Figure 1 a, b, c documents individual distribution of serum levels of the tested markers. Table 2 summarizes the serum levels of tested markers in patient and control groups and statistical evaluation. All serological markers were significantly increased in patients with untreated CLD: cCK-18 ( $P < 0.001$ ), I-FABP ( $P < 0.01$ ) and sCD14 ( $P < 0.05$ ) in contrast to healthy controls. In these patients the mean levels of the tested markers reached the highest values: cCK-18 ( $339.3 \pm 178.4$  pM; mean  $\pm$  SD), I-FABP ( $1.7 \pm 1.4$  ng.ml<sup>-1</sup>), sCD14 ( $3.2 \pm 2.5$

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$\mu\text{g.ml}^{-1}$ ), whereas the values in healthy controls were  $137.2 \pm 86.3$  pM (cCK-18),  $0.8 \pm 0.7$  ng.ml<sup>-1</sup> (I-FABP), and  $1.8 \pm 1.2$   $\mu\text{g.ml}^{-1}$  (sCD14). The serum levels of tested markers were substantially diminished in patients with CLD-GFD compared with untreated CLD. However, slightly elevated levels of cCK-18 ( $P < 0.01$ ) and I-FABP ( $P < 0.01$ ), in contrast to healthy controls, persisted even if these patients strictly adhered to GFD. Table 3 presents the fractions of seropositive individuals who exceeded cut-off values for cCK-18, I-FABP and sCD14 in tested groups. The values above cut-off (which equals 310 pM for cCK-18, 2.2 ng.ml<sup>-1</sup> for I-FABP and 4.2  $\mu\text{g.ml}^{-1}$  for sCD14) are considered positive. While the fraction of seropositive patients with active CLD was 22 out of 43 for cCK-18, 19/43 for I-FABP and 11/43 for sCD14, none out of 12 patients with CLD-GFD was seropositive for cCK-18 and only 2 patients from this group for I-FABP and one patient for sCD14. Among patients groups, significantly increased serum levels of sCD14 were found only in cohort of untreated CLD patients, yet 5 out of 20 patients with T1D/INS were also seropositive for this marker. In healthy controls group, only 3 out of 41 were positive for cCK-18, 3/41 for I-FABP and 1/41 for sCD14.

We found statistically significantly elevated levels of cCK-18 and I-FABP also in patients with T2D ( $P < 0.001$ ,  $P < 0.001$ ), T1D ( $P < 0.001$ ,  $P < 0.001$ ), and T1D/INS ( $P < 0.01$ ,  $P < 0.001$ ) in comparison with healthy controls. Interestingly, in patients with T2D the serum levels of cCK-18 were high ( $355.4 \pm 287.5$  pM) and approximately equal to the values in untreated CLD patients. The fraction of 9 out of 30 patients with T2D exceeded the cut-off value for this marker. Moreover, 26 out of 30 T2D patients exhibited various degrees of liver steatosis (all of 9 patients seropositive for cCK-18 had a high degree of liver steatosis), none of untreated CLD patients manifested liver steatosis. In comparison with untreated CLD and T2D, the level of



cCK-18 in T1D and T1D/INS cohorts was lower:  $200.5 \pm 84.3$  pM and  $204.1 \pm 109.5$  pM. The frequency of patients seropositive for individual markers in the remaining group was low: 3 out of 20 T1D/INS and 3 out of 20 T1D for cCK-18, 5 out of 20 T1D and 2 out of 20 T1D/INS for I-FABP and none of 30 T2D for I-FABP. We did not find statistically significant differences among the mean levels of cCK-18, I-FABP, and sCD14 in T1D and T1D/INS patients.

#### 4. DISCUSSION

In our study, significantly elevated levels of serological markers of epithelial apoptosis (cCK-18) and enterocyte damage (I-FABP) were present in all patient cohorts, i.e. CLD, T1D, T1D/INS and T2D, indicating more extensive epithelium and enterocyte damage in these patients relative to healthy individuals. Our results document that serum levels of cCK-18 and I-FABP are lower in CLD-GFD patients compared with newly diagnosed (untreated) CLD, but slightly elevated levels of these markers persist in CLD-GFD patients. Adriaanse *et al.* (2013) documented significantly elevated serum levels of I-FABP in patients with active CLD, which decreased after introduction of GFD; however, the reduced values were not within the range observed in healthy controls despite the normalization of IgA anti-tTG Ab. Unfortunately, histological recovery in CLD patients on GFD is often incomplete or absent in a substantial subgroup of patients (Wahab *et al.* 2002). However, the absence of clinical symptoms and serological markers of CLD, despite their seropositivity for cCK-18 and I-FABP, did not allow us to perform small intestinal biopsy in these patients on GFD. A conclusive evidence of increased enterocyte apoptosis and damaged gut mucosal barrier was found only in untreated CLD patients (Shalimar *et al.* 2013).

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We found for the first time significantly elevated levels of serum sCD14 in the cohort of untreated CLD patients. The increasing sCD14 serum levels could be the consequence of involvement of innate immunity cells after translocation of commensal bacteria; alternatively, the sCD14 could originate in enterocytes (Funda *et al.* 2001). Interestingly, the serum levels of sCD14 were low in patients with T2D who were not treated with rosiglitazone, which is known to reduce CD14 leukocyte expression in these patients (Štulc *et al.* 2014).

The association of etiopathogenesis of T1D and T2D with impairment of intestinal physiological functions is generally accepted (Vaarala 2008, Baggio and Drucker 2002). Even though T1D and T2D differ in pathogenesis and manifestation clear association between the pathogenesis of these diseases exists and alteration of intestinal mucosa, as well as the assumption concerning the induction of diabetes as a consequence of alteration in the barrier function of small gut mucosa, was suggested (Westerholm-Ormio *et al.* 2003). The morphological and functional alterations of small intestinal mucosa accompanied by increasing number of goblet cells or depletion of Cajal cells occur in both diseases (Rayner and Horowitz 2006, Zhao *et al.* 2006). The T1D and T2D are also associated with intestinal motility impairment induced by hyperglycaemia-related diabetic autonomic neuropathy leading to chymus retention, dysfunction of intestinal barrier, leaky gut and dysbiosis manifested by constipation or diarrhoea (Virally-Monod *et al.* 1998, Damci *et al.* 2003, Shakil *et al.* 2008). Although acute hyperglycaemia has no effect on duodenal pressure waves and flow events, long-lasting hyperglycaemia may lead to disruption of the barrier function of the intestine and penetration of luminal food and bacterial antigens into the lymphatic system and blood circulation Kuo *et al.* 2010. The possibility of damage of gut mucosa in T2D patients and obese patients suggests

pro-inflammatory status documented by production of TNF- $\alpha$ , CRP and polarizing cytokine for TH1 response – IL-12 (Hotamisligil and Erbay 2008). In the light of these facts the increased levels of serum markers cCK-18 and I-FABP in diabetic patients could reflect increased enterocyte apoptosis and damage in these patients. On the other hand, several authors documented that elevated levels of cCK-18 could be also the consequence of associated hepatopathy – autoimmune hepatopathy in CLD or liver steatosis in T2D (Alavi Moghaddam *et al.* 2013, Singh *et al.* 2013, Drastich *et al.* 2012, Bantel *et al.* 2001). These observations correspond to our findings of a high prevalence of liver steatosis in the cohort of T2D patients seropositive for cCK-18 marker. The testing of serum cCK-18 is used as prognostic and pharmacodynamic biomarker in patients with colorectal cancer (Greystoke *et al.* 2012) as well as non-invasive prediction marker of development of liver disorders in T2D (Morling *et al.* 2014). Interestingly, increased levels of I-FABP were found also in obese humans with chronic hyperglycaemia (Verdam *et al.* 2011). Moreover, I-FABP gene polymorphism (Thr54) is associated with insulin resistance after high monounsaturated fat diet in obese non-diabetic patients (De Luis *et al.* 2013).

On the basis of our results we can conclude that cCK-18 and I-FABP represent sensitive non-invasive serological markers of epithelial apoptosis and damage. For this reason, the detection of serum levels of these molecules and sCD14 could be beneficial for monitoring CLD patients' course after introduction of GFD or as complementary markers for atypical, seronegative forms of CLD. The elevation of cCK-18 and I-FABP in some patients with T2D, T1D and T1D/INS supports the hypothesis that also intestinal damage could be involved in the pathology of diabetes. However, the clinical usefulness of testing cCK-18 and I-FABP in diabetes remains to be confirmed in large-scale studies.

## 5. ACKNOWLEDGMENTS

The work was supported by projects 13-14608S and P304/11/1252 of the Czech Science Foundation, NT/13483 of Internal Grant Agency of the Ministry of Health of the Czech Republic, TA01010737 and TA04010762 of Technology Agency of the Czech Republic, and Institutional Research Concept RVO: 61388971.

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## Serological markers of enterocyte damage and apoptosis

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**TABLES**
**Table 1**

Baseline characteristics of patients and controls

Cohorts	C	CLD	CLD-GFD	T1D/INS	T1D	T2D
Number	41	43	12	20	20	30
Mean age/range	39.4 (18 - 81)	30.8 (18 - 46)	41.2 (22 - 77)	53.5 (20 - 87)	47.1 (20 - 78)	66.3 (41 - 84)
Gender ratio (F/M)	19/22	27/16	8/4	7/13	9/11	12/18
Histology: Marsh	N.D.	IIIA-C	N.D.	N.D.	N.D.	N.D.
Antibodies						
anti-tTG IgA	0.6 ± 0.3	105.9 ± 22.75	2.7 ± 0.69	1.0 ± 0.24	3.0 ± 1.3	1.4 ± 0.3
EMA	negative	positive	negative	negative	negative	negative
Anti-GAD	2.7 ± 2.1	1.4 ± 0.58	1.4 ± 1.1	120.8 ± 23.4	1.8 ± 0.5	1.2 ± 0.5
Anti-IA-2	2.0 ± 0.4	3.6 ± 1.5	0.9 ± 0.6	40.3 ± 20.1	0.7 ± 0.4	0.5 ± 0.5
Anti-TPO (F/M)	negative	2 (1/1)	negative	5 (1/4)	5 (3/2)	1 (0/1)
Anti-hTG (F/M)	negative	2 (1/1)	negative	2 (0/2)	2 (2/0)	1 (0/1)
Total serum levels						
Albumin	43.2 ± 0.3	42.4 ± 0.79	43 ± 1.1	36.9 ± 0.9	37.0 ± 1.0	39.2 ± 0.8
CRP	3.1 ± 0.6	5.0 ± 1.7	4.1 ± 1.1	15.7 ± 4.9	5.7 ± 2.0	5.1 ± 0.9
IgA	2.1 ± 0.1	2.4 ± 0.2	1.8 ± 0.2	2.6 ± 0.3	2.5 ± 0.2	3.1 ± 0.3
IgG	10.9 ± 0.3	11.6 ± 0.74	10.3 ± 0.9	11.4 ± 1.0	9.8 ± 0.9	9.4 ± 0.5
IgM	1.1 ± 0.1	1.4 ± 0.2	1.5 ± 0.3	1.1 ± 0.2	1.1 ± 0.1	0.9 ± 0.1
IgE	63.8 ± 8.7	76.5 ± 35.9	131.0 ± 98.5	57.2 ± 15.9	106.6 ± 36.0	127.4 ± 49.9

C, healthy controls; CLD, active celiac disease; CLD-GFD, celiac disease on gluten-free diet; T1D/INS, autoimmune diabetes mellitus with insulinitis; T1D, autoimmune diabetes mellitus with fading insulinitis; T2D, diabetes mellitus type 2; F, female; M, male; tTG, tissue transglutaminase; EMA, endomysial antibodies; Marsh (Marsh classification); GAD, glutamic acid decarboxylase; IA-2, islet antigen-2; TPO, thyroid peroxidase; hTG, human thyroglobulin; CRP, C-reactive protein. Physiological values: IgA anti-tTG ( $\text{Ab} < 12.00 \text{ IU.ml}^{-1}$ ), Anti-GAD ( $< 5.00 \text{ IU.ml}^{-1}$ ), anti-IA-2 ( $< 10.00 \text{ IU.ml}^{-1}$ ), anti-TPO ( $0-35 \text{ kU.l}^{-1}$ ), anti-hTG ( $0-40 \text{ kU.l}^{-1}$ ) total serum levels of IgG ( $6.80-14.45 \text{ g.l}^{-1}$ ), IgA ( $0.71-3.74 \text{ g.l}^{-1}$ ), IgM ( $0.40-2.48 \text{ g.l}^{-1}$ ), IgE ( $< 100.0 \text{ IU.ml}^{-1}$ ), albumin ( $34.0-50.0 \text{ g.l}^{-1}$ ), CRP ( $< 5.0 \text{ mg.l}^{-1}$ ). The data are expressed as mean  $\pm$  standard error.

**Table 2**

Comparison of cCK-18, I-FABP and sCD14 levels in sera of adult patients with untreated celiac disease, those on gluten-free diet, autoimmune diabetes mellitus with insulinitis and those with fading insulinitis and diabetes mellitus type 2 (Mann-Whitney test)

Markers	C	CLD	CLD-GFD	T1D/INS	T1D	T2D
cCK-18	137.2 ± 86.3	339.3 ± 178.4	189.3 ± 57.1	204.1 ± 109.5	200.5 ± 84.3	355.4 ± 287.5
	-	P<0.001	P<0.01	P<0.01	P<0.001	P<0.001
I-FABP	0.8 ± 0.7	1.7 ± 1.4	1.3 ± 0.7	1.4 ± 0.7	1.5 ± 0.8	1.1 ± 0.4
	-	P<0.01	P<0.01	P<0.001	P<0.001	P<0.001
sCD14	1.8 ± 1.2	3.2 ± 2.5	2.5 ± 1.5	2.6 ± 2.4	3.1 ± 3.0	2.1 ± 1.6
	-	P<0.05	NS	NS	NS	NS

C, healthy controls; CLD, celiac disease; CLD-GFD, celiac disease on gluten-free diet; T1D/INS autoimmune diabetes mellitus with insulinitis; T1D, autoimmune diabetes mellitus with fading insulinitis; T2D, diabetes mellitus type 2; cCK-18, cytokeratin 18 caspase-cleaved fragment (pM); I-FABP, intestinal fatty acid-binding protein (ng.ml<sup>-1</sup>); sCD14, soluble CD14 (µg.ml<sup>-1</sup>). The data are expressed as mean ± standard deviation.

**Table 3**

The frequency of seropositivity for cCK-18, I-FABP and sCD14 in adult patients with untreated celiac disease, those on gluten-free diet, autoimmune diabetes mellitus with insulinitis and those with fading insulinitis and diabetes mellitus type 2

Markers	C	CLD	CLD-GFD	T1D/INS	T1D	T2D
cCK-18	3/41 (7.3 %)	22/43 (51.1 %)	0/12 (0 %)	3/20 (15 %)	3/20 (15 %)	9/30 (30 %)
I-FABP	3/41 (7.3 %)	19/43 (44.2 %)	2/12 (16.7 %)	2/20 (10 %)	5/20 (25 %)	0/30 (0 %)
sCD14	1/41 (2.4 %)	11/43 (25.6 %)	1/12 (8.3 %)	5/20 (25 %)	4/20 (20 %)	1/30 (3.3 %)

C, healthy controls; CLD, celiac disease; CLD-GFD, celiac disease on gluten-free diet; T1D/INS, autoimmune diabetes mellitus with insulinitis; T1D, autoimmune diabetes mellitus with fading insulinitis; T2D, diabetes mellitus type 2; cCK-18, cytokeratin 18 caspase-cleaved fragment; I-FABP, Intestinal fatty acid-binding protein; sCD14, soluble CD14. The cut-off values were calculated as the mean values plus 2 standard deviations of serum levels of individual markers tested in 41 healthy controls.

**FIGURE LEGEND**

Figure 1. Individual distribution of cCK-18, cytokeratin 18 caspase-cleaved fragment (a), I-FABP, intestinal fatty acid-binding protein (b), sCD14, soluble CD14 (c) in patients with untreated celiac disease (CLD), celiac disease patients on gluten-free diet (CLD-GFD), autoimmune diabetes mellitus with insulinitis (T1D/INS), autoimmune diabetes mellitus with fading insulinitis (T1D), diabetes mellitus type 2 (T2D) and healthy controls (C). Solid lines represent the mean value; „n“, number of patients and controls; \*\*\* (P<0.001), \*\* (P<0.01), \* (P<0.05); NS, not significant. The values above cut-off (which equals 310 pM for cCK-18, 2.2 ng.ml<sup>-1</sup> for I-FABP and 4.2 µg.ml<sup>-1</sup> for sCD14) are considered positive.

