Thesis for the Degree of Doctor of Philosophy

(Ph.D)

PATHOLOGIC ANTIBODY RESPONSES IN CELIAC DISEASE: SPECIFICITY AND IMMUNOLOGICAL CORRELATIONS

Dr. Éva Nemes

Department of Pediatrics, Medical and Health Science Center, University of Debrecen

Debrecen, 2008

INTRODUCTION AND AIMS

Celiac disease (CD) or gluten sensitive enteropathy induced by the gluten fraction of cereals in genetically susceptible people is characterized by a variety of intestinal and extraintestinal symptoms, but it also may persist in a symptom-free form. In Europe, at least one percent of the population is affected. However, screenings show that 85-90% of the cases are not diagnosed. The disease is lifelong, but the symptoms may first present in childhood or adulthood.

There has been a dramatic change in the prevalence of CD and its symptoms in the last 30-40 years. Earlier, the majority of newly diagnosed patients were younger than two years old, and the disease was only suspected when the patient presented with typical gastrointestinal clinical symptoms of CD. The clinically identified cases, however only represent the tip of the "celiac iceberg": the frequency of clinically silent or latent forms is 3-20 times more. This means that the majority of CD patients are not clinically identifiable.

The diagnosis of the disease is based on the presence of villous atrophy of the small intestine and detection of autoantibodies characteristic of CD. Diagnostic small bowel biopsy is an invasive examination therefore non-invasive detection of CD-specific antibodies in serum samples is very important in the screening of patients with clinical symptoms, of risk groups and of the population. In diagnosing patients with few or no symptoms, use of the antibody tests is needed. The traditional endomysium antibody (EMA) test is highly specific, although it needs immunofluorescent equipments not everywhere available and requires experience in the evaluation. Tissue transglutaminase antibody (TGA) tests detect a homologous antibody, but the specificity and predictive value of commercial kits is not always sufficient. In addition, laboratory detection of TG2-based antibody is expensive, and it needs centralized, special laboratory background and the results are not immediately available. In contrast, quick

decisions are needed in clinical diagnosis, especially in the case of seriously sick CD patients. Therefore, rapid evaluation of the celiac antibodies is needed what can preferably be done by doctors on site during the clinical workup.

According to genetic studies presence of HLA-DQ2 or DQ8 alleles is mandatory for CD to develop. Gliadin peptides are presented to T lymphocytes by antigen presenting cells (APC) in conjunction with HLA-DQ2 or DQ8 molecules, and this induces the pathological immune reactions that cause tissue damage. Carrying HLA-DQ2 or DQ8 alleles also predisposes for other pathological conditions too, such as various autoimmune diseases and insufficient immune response to hepatitis B vaccination. It is unknown whether carrying HLA-DQ2 alone is causing in itself hepatitis B non-responder status. Neither is known whether environmental factors such as gluten intake also interfere with the immune response in CD patients.

CD is a multi-factorial disease where symptoms are induced by gluten ingestion. There are other environmental factors, too, that can contribute to its appearance e.g. the time of introducing gluten into the diet of genetically susceptible children. The possibility of a relation between the appearance of CD and microbial antigens in the lumen of the bowel was raised by new information in infectious immunology: the study of adaptive and innate immunity. Children are highly susceptible to gastrointestinal infections and in certain years, the seasonality of CD appearance was observed. CMV infection causes serious gastrointestinal symptoms in immune suppressed patients. The question arises whether CMV infection in infancy when the immune system is still immature can be related to CD with serious malabsorption.

New information on CD leads to further questions. By using a rapid test for detecting CD specific TGA, my aim was to investigate whether CD patients could be identified earlier. I also studied the efficacy of hepatitis B immunization in CD patients and the factors affecting seroconversion in immunized patients. In addition to detecting CD specific antibodies by the

rapid onsite test, I studied what other serological examinations can contribute to diagnosing and evaluating the clinical status and therapeutical effect of CD patients.

AIMS

1. The first aim of the present study was to evaluate results of onsite rapid detection of IgA type TGA by using endogenous transglutaminase antigen of the patients themselves and to compare this method with conventional serological tests (EMA, TGA detection). It was my aim to study the clinical benefit of having immediately these results.
2. The second aim was to investigate the seroconversion following hepatitis B immunization of CD patients and to see if there is a correlation between anti-HBs concentration following hepatitis B vaccination and the gluten-free diet. I also wanted to study whether haptoglobin polymorphism influences response to hepatitis B immunization in CD patients.
3. My third aim was to examine the lymphocyte subpopulations and the prevalence of non-organ specific autoantibodies in CD and to correlate autoantibody positivity with the gluten-free diet.
4. My next aim was to examine whether CMV infection can act as a specific infective agent in triggering CD, and whether CMV seroconversion occurs more frequently before CD symptoms appear.
5. My last aim was to examine the prevalence and the possible diagnostic value of certain microbial cell wall (anti-glycan) antibodies in newly diagnosed and treated CD patients.

2. PATIENTS AND METHODS

The subjects of the study were CD patients diagnosed in childhood or adulthood. The diagnosis of CD was verified by histological examination of the small bowel in all cases and was based on the presence of severe (Marsh III) villous atrophy. In studies of diagnostic efficacy, CD was excluded in the controls as well by small bowel biopsy.

2.1 Subjects

2.1.1 Onsite detection of CD autoantibodies by Biocard™ rapid test

First stored and thawed blood samples from 121 CD patients and 107 control subjects with normal villous structures were tested, than 211 subjects (89 patients and 122 relatives) were prospectively studied. The further evaluation of rapid antibody testing was done by screening 6-year-old children in Jász-Nagykun-Szolnok County in Hungary. Altogether 2676 children were screened with the help of 120 district nurses.

2.1.2 Immune response to hepatitis B immunization in CD

128 celiac children and 113 age matched healthy controls were studied. 22 CD patients (11 girls and 11 boys, median age 8,8 years, range 4-12,5 years) received their hepatitis B immunization prospectively, following establishing the diagnosis of CD and during a gluten-free diet. One month after the vaccination peripheral blood samples were taken to evaluate specific antibody response (anti-HBs). The vaccination was given at the age of 14 at school as part of obligatory Hungarian vaccination programme to 106 CD adolescents (67 girls and 39

boys, median age 16,7 years) and the controls (70 girls and 43 boys, median age 16,1 years), irrespective of their CD diagnosis or their dietary status. Serum samples were obtained for anti-HBs determination at various times after the immunization. At the same time EMA and TGA were also measured in order to evaluate dietary compliance.

If anti-HBs antibodies were negative, the patients were offered further booster vaccination, and anti-HBs titer was determined a month later. During that time, all the CD patients were following a strict gluten-free diet.

2.1.3 Lymphocyte subpopulations and non-organ specific autoantibodies in CD patients

The blood samples of 57 treated CD patients (39 girls and 18 boys, median age 11,9 years, range 1,7-32 years) and 45 EMA and TGA negative controls (20 girls and 25 boys, median age 12 years, range 2,8-20,6 years) were tested for cell surface antigens (CD3+, CD4+, CD8+, CD19+ és CD56+) and for marker autoantibodes of polysystemic autoimmune diseases (ENA, anti-DNA, ANF, Sm, Sm/RNP, SS-A, SS-B, Scl-70, Jo-1, centromere, cytoskeleton).

2.1.4 The prevalence of CMV infection in children between the ages of one and two years and having malabsorption

Between May 1st 1987 and January 31st 2003, the possible role of CMV infection in provoking CD was studied in 41 CD patients (32 girls and 9 boys, median age 1,5 years, range 0,9-2 years) presenting with malabsorption between the ages of 1-2 years and in 40 age-matched controls (16 girls and 24 boys, median age 1,5 years, range 0,9-1,9 years) who

also underwent small bowel biopsy because of symptoms of malabsorption. We chose the above age group because CMV infection tends to be chronic and the onset of CD cannot be determined exactly if it is diagnosed later. The onset of CD is never earlier than introduction of gluten into the diet. In the above groups malabsorption symptoms manifested within a couple of months. Antibodies of maternal origin must have completely disappeared by that age.

2.1.5 Antibodies directed against microbial cell wall components in CD

The blood samples of 42 CD patients (9 males, 33 females, median age 40,7 years, range 15-78 years) were studied at the time of diagnosing CD for antibodies against mannan epitope of *Saccharomyces cerevisiae* (gASCA IgG), mannobioside carbohydrate (AMCA IgG), chitobioside carbohydrate (ACCA IgA) and bacterial outer membrane proteins (OMP). The serum samples of 30 of them were re-evaluated during a long term, strict gluten-free diet. 100 healthy blood donors (47 males, 53 females, median age 36,6 years, range 22-43 years), who were negative for EMA and TGA antibodies were used as controls. The clinical diagnoses of non-celiac control subjects taking part in the studies are shown in the table.

	Biocard TGA	HBV immuni-sation	Non-organ specific autoantibody	CMV serology	Anti-glycan antibody
Verified CD, n	168*	128*	57	40	42
Controls, n	107**	113	45	41**	100
Gastro-esophageal reflux	15		8	1	
IBD	14		4		
Irritable bowel syndrome	2				
Nutritive allergy	8		3	6	
Postinfectious lactase deficiency	7		2	8	
Congen. sucrase-isomaltase deficiency	5				
Non-specific chronic diarrhoea	29		8	21	
Recurrent abdominal pain	2		12		
Intestinal lymphangiectasia	1			1	
Cystic fibrosis	1		1		
Shwachman-Diamond syndrome	1				
Familial adenomatous polyposis	4				
Retarded growth	6			1	
Eating disorder	2			3	
Unspecified anemia	7				
Familial CD	3		2		
Gilbert-syndrome			2		
Constipation			2		
Recurrent aphthosis			1		
Healthy		113			100

* Retrospective and prospective clinical studies
** Controls who had small bowel biopsy

2.2 Methods

2.2.1 Histological examination

Histological examinations were carried out by taking a sample from the distal part of the duodenum with upper endoscopy (using general anaesthesia in children) or from the duodeno-jejunal border with Watson capsule (administering metoclopramide and midazolam). The samples were evaluated in the Department of Pathology, Medical and Health Science Center,

University of Debrecen by routine histological examination. We also used morphometry and immune histochemical analysis of frozen non-embedded sections.

2.2.2 Laboratory tests

EMA IgG and IgA antibodies were detected in all cases by indirect immunofluorescent method. ELISA was used to detect IgA type TGA antibodies. Cut-off value for TGA positivity was 5 U/mL. In subjects with total serum IgA<0.2 g/L, IgG class EMA and TGA were investigated and interpreted instead of IgA class antibodies. With the help of Biocard Celiac Disease rapid test, anti-TGA autoantibodies (IgA type) were detected within five minutes from one drop of whole blood using endogenous transglutaminase antigens of the patients' own red blood cells.

HLA-DQ2 and DQ8 haplotypes were typed by PCR-based techniques with sequence specific primers with a low-resolution kit. All the examinations were performed according to the instructions of the manufacturer. In cases when this examination did not give unambiguous results, subtyping was performed with DQB1*02, DQB1*03 subtyping kits or DQA1 typing kit.

Lymphocyte subpopulations were studied by flow cytometry.

Anti-HBs and anti-CMV IgM and IgG antibodies were determined by ELISA using commercial kits following the manufacturer's instructions. ELISA was also used to examine anti-glycan antibodies and part of the marker autoantibodies characteristic of polysystemic autoimmune diseases (ENA, anti-DNA, Sm, Sm/RNP, SS-A, SS-B, Scl-70, Jo-1). Detection of ANF, centromere, cytoskeleton autoantibodies on Hep2 cells were performed by indirect immunofluorescence.

Sodium-dodecil-sulphat electroforesis and immunoblotting were used to phenotype haptoglobin from various serum samples.

2.2.3 Statistical analysis

Chi-square test was used to express the significant difference between the various groups ($p<0,05$). The confidence intervals related to the tests were determined with Wilson's methods at confidence level of 95%. The analysis was done with SISA program (http://home.clara.net/sisa/).

RESULTS

3.1 Onsite detection of CD autoantibodies with Biocard™ rapid test.

The Biocard rapid test identified CD patients from thawed blood samples with 97% sensitivity and 94% specificity. In the onsite tests, 55 patients proved to be Biocard positive. 47 of them had small bowel biopsy and 46 of them were shown to have serious villous atrophy. The results of the onsite rapid test were confirmed by laboratory EMA positivity in 97.2% of the cases and by TGA positivity in 96.7%. With the tests carried out from fresh blood, specificity was higher. Although prospectively studied patients had fewer examinations, almost half of CD patients underwent small bowel biopsy within 3 days of first presentation.

During of population screening of 6 year old children in Jász-Nagykun-Szolnok County, 78% of antibody positive children were identified on the spot. CD was newly diagnosed in 32 (1.2%) of the screened children, 24 girls and eight boys. According to the survey, the

prevalence of CD confirmed by small bowel biopsy was 1.38% and that of antibody positivity at age 6 was 1.79% in Hungary in 2005. Because of the large number of investigated patients this study is representative for the Hungarian population.

3.2 Immune response to hepatitis B immunization in CD

Our study found similar rates of seroconversion (95.5%) in healthy people and prospectively immunized CD patients on gluten-free diet following hepatitis B vaccination. However, the rate of responders was only 50.9% if immunization was done independently of diagnosis or dietary status. We found a surprisingly high rate (74.1%) of negative anti-HBs antibody levels in CD adolescents who had not been identified or treated at the time of vaccination. 97.3% of CD patients who had not been treated earlier produced protective immune response to booster vaccination after negative CD antibody (EMA, TGA) status had been reached.

No significant difference was found in the rate of seroconversion between HLA-DQ2 homozygotes and heterozygotes.

Haptoglobin polymorphism in CD was not related to the result of either hepatitis B immunization, the activity of the disease, or the dietary status of the patients.

3.3 Lymphocyte subpopulations and non-organ specific autoantibodies in CD patients

In CD patients the rates of CD3+ and CD4+ cells were significantly lower, while that of CD19+ cells was significantly higher than in controls. The rate of CD3+ lymphocytes was significantly higher in CD patients who were negative for non-organ specific autoantibodies. 47.4% of CD patients were positive for EMA and TGA indicating active disease. Their

prevalence of non-organ specific autoantibody positivity was significantly ($p<0.001$) higher than in controls. 56.1% of the patients were positive for at least one, and 19.3% for two or more of other autoantibodies. Anti-DNA positivity was the most common. In patients positive for non-organ specific autoantibodies non-compliance with the gluten-free diet was significantly higher.

3.4.1 The prevalence of CMV infection in malabsorption in children between the ages of one and two.

IgG type anti-CMV antibodies were found in 24.4% of CD patients without anti-CMV IgM positivity. In the non-celiac control group tested because of chronic diarrhoea, anti-CMV positivity was significantly more common than in CD patients. Furthermore, three of the above patients were anti-CMV IgM positive as well. Quantitative results of the activity of disaccharidase enzymes were available in 80% of control patients without villous atrophy. The rates of normal and lower disaccharidase enzyme activities were identical in CMV seropositive and seronegative subjects.

2.2.2 Antibodies directed against microbial cell wall components (anti-glycan) in CD

At the time of diagnosis, the prevalence of anti-glycan antibodies was significantly higher in CD patients than in healthy controls and 69.1% of CD patients were positive for at least one antibody type. When gluten was eliminated, the positivity of anti-glycan antibodies disappeared completely, and the concentration of all antibodies became significantly lower.

DISCUSSION

The immunological tests in the study examined the various immunological aspects of CD and the efficiency of the treatment of the patients (pathomechanism, diagnosis, survey of the clinical status, managing associated problems). These issues overlap and may facilitate both the better understanding of the disease and the better practical care of the patients. In addition to the main or most characteristic (anti-tissue transglutaminase) immune reaction, other significant changes were found, in which gluten itself may play a role and which have to be taken into account clinically if we want to achieve proper therapeutical results. Associated immune disorders were found to be related to the detectability of TGA and compliance with the gluten-free diet. Defective immune response to infective agents in CD patients may also reflect a primary disorder of the immune system, which can contribute to the emergence of gluten-sensitivity.

In our studies, we showed that CD can be discovered by a method based on the use of endogenous transglutaminase antigen in the patients' own red blood cells, which did not need either laboratory equipment or skills. The CD rapid test enables the doctor to get the results more quickly. Furthermore, it can be done by the patient himself at home. Whole blood fingertip sampling requires less blood and it is less stressful for small children than traditional venous blood sampling for serum examinations.

We found that the Biocard onsite rapid test makes it possible to select the subjects, who need further examination quickly and efficiently.

Screening by the onsite rapid test and biopsy intervention following a positive result need less time and diminish the number of other invasive tests. It also improves the dietary compliance and the status of the patients. Family members and the population can also be screened by a minimally invasive method in this way. Although a positive Biocard result is highly

predictive and it is practically equivalent with a positive EMA result, the patient should be referred to a gastroenterology centre and should not be put on a gluten-free diet based on that fact alone. The costs of the screening done by a simple onsite rapid test are expected to be much lower than those of traditional screening method. Furthermore, a rapid test can be used in places where blood samples cannot be stored. That examination should also be part of evaluation by general practitioners who could identify a high number of new patients and also could check their dietary status more properly.

HLA-DR3:DQ2 haplotypes that are specific to CD affect the activation of T lymphocytes and practically all aspects of the immune system related to cytokine production. In our study, we examined whether the immune response manifested in the production of protective antibodies to the protein-like HBsAg is diminished in CD. Earlier retrospective studies did not analyse the results of treated and untreated CD patients separately. Our study showed that CD patients on a strict gluten-free diet and immunized prospectively had seroconversion to the same degree as healthy subject. If, however, immunization was performed irrespective of diagnosis and dietary status, the rate of responders was only 50.9%. The correlation observed between successful primary and booster vaccinations during a controlled and strict gluten-free diet and the positivity of CD antibodies and non-responder status suggests that CD activity plays a primary role in the failure of hepatitis B vaccination. However, non-responder status is not permanently associated with CD. Consequently, we consider it necessary to determine the concentration of anti-HBs antibodies in newly diagnosed and previously immunized CD patients. When it is negative, revaccination should be started after CD specific autoantibodies (EMA, TGA) had been disappeared. On the other hand, it also should be regarded important to exclude CD in people with negative anti-HBs status. Park et al noted that susceptibility to hepatitis B infection may persist in spite of the fact that hepatitis B vaccination is universal. On the other hand, the adverse effect of gluten challenge for HBV immunisation may mean

that immune response to other antigens should also be studied in CD patients. Our results suggest that carrying HLA-DQ2 in itself does not cause insufficient humoral immune response to hepatitis B vaccination. No correlation was found between the immune response to the hepatitis B vaccination and haptoglobin polymorphism of the patients.

The use of serological tests that are non-specific to CD has long been disputed. Our study also evaluated the diagnostic value of non-organ specific autoantibodies and anti-glycan antibodies as well as how they are affected by the gluten-free diet. We found that their presence depends on the activity of CD. That is partly due to tissue damage and partly to the loss of tolerance to microbial antigens. In the absence of other autoimmune diseases, the above secondary antibodies disappear from blood when gluten is eliminated. Thus when they are accidentally found during other examinations, CD should be suspected.

Examination of the lymphocytes subpopulations of peripheral blood provides important information on autoimmune processes. The presence of the former was found to be related to intestinal T cell activation. Viruses are known to initiate autoimmune processes in the body either by molecular mimicry or by indirect effect on the immune regulation. The possible etiological role of CMV in the autoimmune mechanism of CD was raised by the high anti-DNA antibody positivity that we noted as well. The presence of homologous sequences in the N-terminal domain of tissue transglutaminase was another reason. In addition, Lunardi et al have shown that the antibodies in the sera of patients with multiple sclerosis may have cross reaction the late protein of CMV. Our studies did not confirm the etiological role of CMV we had postulated in CD. At the same time the significantly higher rate of infection in the control group suggests that CMV infection might be the cause of intractable diarrhoea, and therefore serological examinations should be performed even in immunocompetent patients in such cases.

The aim of my thesis was to present the different ways of using both tests specific to CD and other non-specific serological tests. They facilitate diagnosing CD, monitoring dietary intervention and prevention of contagious diseases. The role they play in the pathomechanism of CD needs further studies.

New findings

1. The detection of TGA by onsite rapid test improves the efficiency of diagnosis and makes it possible to screen family members and the population with a minimally invasive method. Monitoring CD patients on gluten-free diet by the rapid test improves dietary compliance and the intervention can be performed immediately.
2. Gluten intake plays a significantly negative role in the immune response following HBV vaccination in patients with active CD. Seroconversion in retrospectively immunized CD patients is similar to that of healthy controls. Revaccination with hepatitis B vaccine is recommended during a controlled gluten-free diet. Carrying HLA-DQ2 in itself dose not cause insufficient humoral immune response to hepatitis B vaccination. Anti-HBs seroconversion is not affected by haptoglobin phenotypes.
3. High prevalence of non-organ specific autoantibodies was found in CD patients. When non-organ specific autoantibodies are positive, CD screening should be performed.

4. CMV infection does not trigger the appearance of the development of CD. Acute CMV infection was diagnosed in immunocompetent patients with intractable diarrhoea.

5. Antibodies against microbial antigens are additional markers of CD. Following gluten elimination, they disappear from the blood stream.

LIST OF PUBLICATIONS

This thesis is based on the following original publications

1. Opre J, **Nemes É**, Korponay-Szabó I, Woolley N, Oláh É: A homozygote DR3;DQ2 family in celiac disease. Gyermekgyógyászat 2004; 55(4): 449-52 .
2. **Nemes É,** Opre J, Szűcs E, Aleksza M, Tumpek J, Sipka S, Korponay-Szabó I: Lymphocyte subpopulations and non-organ specific autoantibodies in celiac disease. Gyermekgyógyászat. 2005; 56(5): 251-7.
3. **Nemes É**, Lefler É, Kapitány A, Opre J, Tumpek J, Sipka S, Korponay-Szabó I. Immune response to hepatitis B vaccination in celiac disease. Gyermekgyógyászat 2006; 57: 338-43.
4. Korponay-Szabó I, Raivio T, **Nemes É**, B. Kovács J, Laurila K, Kaukinen K, Maki M. Detection of celiac disease antibodies by the Biocard rapid test. Gyermekgyógyászat 2006; 57: 351-7.
5. Raivio T, Kaukinen K, **Nemes E**, Laurila K, Collin P, Kovacs JB, Maki M, Korponay-Szabo IR. Self transglutaminase-based rapid coeliac disease antibody detection by a lateral flow method. Aliment Pharmacol Ther 2006 Jul 1; 24(1): 147-54. **IF:3,287**
6. Korponay-Szabó IR, Szabados K, Pusztai J, Uhrin K, Ludmány É, **Nemes É**, Kaukinen K, Kapitány A, Koskinen L, Sipka S, Imre A, Mäki M. Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study. Brit Med J 2007 Dec 15; 335(7632): 1244-7. **IF:9,245**
7. **Nemes É**, Lefler É, Szegedi L, Kapitány A, B.Kovács J, Balogh M, Szabados K, Tumpek J, Sipka S, Korponay-Szabó IR. Gluten intake interferes with the humoral immune response to recombinant hepatitis B vaccine in patients with celiac disease. Pediatrics, in press **IF:5,012**

8. Raivio T, Korponay-Szabó IR, Paajanen T, Ashorn M, Iltanen S, Colllin P, Laurila K, **Nemes É**, B.Kovács J, Carrard, G, Saramaki M, Maki M, Kaukinen K. Comparison of a novel whole blood transglutaminase-based ELISA with whole blood rapid antibody test and established conventional serological coeliac disease assays. J Pediatr Gastroenterol Nutr, in press **IF:2,067**

IF: 19,611

Other publications related to the thesis:

1. Korponay-Szabó IR, Vecsei Z, Király R, Dahlbom I, Chirdo F, **Nemes É**, Fésüs L, Mäki M. Deamidated gliadin peptides form epitopes that transglutaminase antibodies recognize. J Pediatr Gastroenterol Nutr 2008; 46: 253-61. **IF:2,067**
2. Papp M, Foldi I, **Nemes E**, Udvardy M, Harsfalvi J, Altorjay I, Mate I, Dinya T, Varvolgyi Cs, Barta Z, Veres G, Lakatos PL, Tumpek J, Toth L, Szathmari E, Kapitany A, Gyetvai A, Korponay-Szabo I. Haptoglobin polymorphism: a novel genetic risk factor for celiac disease development and its clinical manifestations, Clin Chem, 2008 doi:10.1373/clinchem.2007.098780 **IF:5,454**

IF: 7,521

Other publications

1. Lakatos L, Csathy L, **Nemes E.** „ Bloodless" treatment of a Jehovah's Witness infant with ABO hemolytic disease. J Perinatol 1999; 19 (7): 530-2. **IF:0,616**

2. Tar I, **Nemes E,** Nemes J, Alberth M, Keszthelyi G. The role of salivary immunoglobulins in caries prevalence and primary B-cell deficiency. Fogorvosi Szemle 1999; 92(11): 331-8.
3. **Nemes É**, Teichman F, Roos D, Maródi L. Activation of human granulocytes by intravenous immunoglobulin preparations is mediated by FcγRII and FcγRIII receptors. Ped Res 2000; 47: 357-61. **IF:2,794**
4. Marodi L. Kaposzta R, **Nemes E.** Survival of group B streptococcus type III in mononuclear phagocytes: differential regulation of bacterial killing in cord macrophages by human recombinant gamma interferon and granulocyte-macrophage colony-stimulating factor. Infect Immun 2000; 68(4): 2167-70. **IF:4,204**
5. Tarjan P, Sipka S, Marodi L, **Nemes E,** Lakos G**,** Gyimesi E, Kiss E, Ujj G, Szegedi G. No short-term immunological effects of Pneumococcus vaccination in patients with systemic lupus erythematosus. Scand J Rheumatol 2002; 31(4): 211-5. **IF:1,396**
6. **Nemes É.** Immunization in premature infants. Családorvosi Fórum 2004; 6:36-9.
7. **Nemes É.** Multivitamin supplementation and anti-oxidants in childhood. Gyermekorvos továbbképzés. 2006; 5(3): 201-5.
8. **Nemes É.** Prevention of old infectious diseases with new vaccines. Családorvosi Fórum 2006; 8: 31-4.
9. Derekas B, **Nemes É**, Korponay-Szabó IR. Automatic registration of upright and supine position helps evaluation oesophageal pH-monitoring in children. Gyermekgyógyászat 2007;58: 377-80.

IF: 9,01

Cumulative IF: 36,142

Abstracts

1. Korponay-Szabo IR, Raivio T, Laurila K, B.Kovács J, **Nemes É**, Kaukinen K, Mäki M. Rapid detection of coeliac autoantibodies in the office. 38th Annual Meeting of the ESPGHAN, 2005. J Pediatr Gastroenterol Nutr. 2005;40:619.
2. Korponay-Szabo IR, Kapitány A, B.Kovács J, Lőrincz M, Opre J, **Nemes É**, Tumpek J, Sipka S. Is coeliac disease a dominantly inherited disorder? 38th Annual Meeting of the ESPGHAN, 2005. J Pediatr Gastroenterol Nutr. 2005;40:638.
3. **Nemes É**, Lefler É, Kapitány A, B. Kovács J, Balogh M, Szabados K, Opre J, tumpek J, Sipka S, Korponay-Szabó IR. Gluten intake interferes with the immune response to hepatitis B vaccination in patients with coeliac disease. 40th Annual Meeting of the ESPGHAN, Barcelona, 2007. J Pediatr Gastroenterol Nutr, http://www.espghan2007.org/PH01-05.
4. Korponay-Szabó IR, B.Kovács J, Király R, **Nemes É**, Mäki M. High efficiency of gluten-dependent transglutaminase-specific intestinal IgA deposits as candidate diagnostic criteria in coeliac disease. 40th Annual Meeting of the ESPGHAN, Barcelona, 2007. J Pediatr Gastroenterol Nutr, http://www.espghan2007.org/PG01-09.
5. Korponay-Szabó IR, B.Kovács J, **Nemes É**, Dahlbom I, Mäki M. Coeliac antibody testing with deamidated gliadin peptides in difficult patient samples. 40th Annual Meeting of the ESPGHAN, Barcelona, 2007. J Pediatr Gastroenterol Nutr, http://www.espghan 2007.org /PG05-03.