

BULGARIAN ASSOCIATION OF CLINICAL IMMUNOLOGY



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- Specific IgE to natural rubber latex in nurses
- Clinical case of a patient with common variable immunodeficiency associated with membranous nephropathy
- Uric acid – friend or foe? A question with many answers
- Extrahepatic manifestations of hepatitis E
- Combining chemotherapy with immunotherapy in oncology-hopes and benefits
- BD Biosciences CEE





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CONTENTS

ORIGINAL ARTICLE

<i>M. Baleva</i> . Specific IgE to natural rubber latex in nurses.....	3
--	---

CASE REPORT

<i>S. Lesichkova, G. Mihneva-Kirilova, G. Zlatanova-Rashkova, M. Gaydarova, E. Naumova</i> . Clinical case of a patient with common variable immunodeficiency associated with membranous nephropathy	8
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SCIENTIFIC REVIEWS

<i>M. Nikolova, C. Marinova, Y. Vlahov</i> . Uric acid – friend or foe? A question with many answers	14
<i>M. Nikolova, Y. Vlahov</i> . Extrahepatic manifestations of hepatitis E	19

SHORT COMMUNICATIONS

<i>M. Milev</i> . Combining chemotherapy with immunotherapy in oncology-hopes and benefits	24
<i>R. Socha</i> . BD Biosciences CEE.....	24

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ORIGINAL ARTICLE

SPECIFIC IgE TO NATURAL RUBBER LATEX IN NURSES

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Abstract. 3/32 (9.37%) of nurses working in non-surgical departments have high levels of specific IgE to natural rubber latex (sIgE NRL) and total IgE within reference limits. Two of them have accompanying diseases – one systemic lupus erythematosus (SLE), the other – psoriasis. The patient with psoriasis had itching and urticaria, but the patient with SLE had no allergy symptoms. The third nurse had data for anaphylactic shock after contact with natural rubber latex (NRL) in the past, but at the time of examination had no manifestations for NRL allergy. At the time of examination she did not use latex gloves. After the detection of elevated sIgE NRL levels, the first two women stopped using latex gloves and in the following months the level of antibodies is normalized. Possible links between NRL allergy (NRLA) and psoriasis and SLE are discussed. The importance of the determination of sIgE NRL as a simple and convenient laboratory test for NRLA is emphasized.

Key words: latex allergy, specific IgE to latex, nurses

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INTRODUCTION

Allergic reactions to natural rubber latex (NRLA) are found in about 1% of the general population [1]. In 1927 G. Stern described urticaria and edema of the larynx after dental prosthesis made of caoutchouc [2]. In the same year A. Grimm described asthma attack caused by an electric cable covered with natural rubber latex (NRL), “which warmed up when the wire was electrified” [3]. The subsequent messages described various side effects after contact with NRL [4, 5] and the cases became more frequent in the eighties and nineties of the twentieth century [1] after the introduction of stricter requirements in the examination and treatment of patients with HIV virus [6, 7].

For first time A. F. Nutter in 1979 proved with patch test and skin prick test (SPT) a certain immediate type allergic reaction caused by NRL [4]. In 1980

L. Förström [8] described a nurse with a history of atopic dermatitis and allergic rhinitis who developed symptoms of acute urticaria, rhinitis and eyelids edema every time she worked with latex gloves. In 1984 K. Turjanmaa et al. [9] reported systemic anaphylactic reactions to latex gloves in two pregnant women during childbirth. In 1987 K. Turjanmaa published the first study of immediate type allergic reaction to latex in hospital employees [10].

MATERIALS AND METHODS

The concentrations of total IgE and specific IgE to NRL (sIgE NRL) was investigated in the sera of 32 working in the non-surgical departments' nurses (average age of 44.65 ± 2.75 years). Two of them have accompanying diseases – one systemic lupus erythematosus (SLE), the other – psoriasis. The patient with psoriasis had itching and urticaria, but the patient with SLE had no allergy symptoms. The third

nurse had data for anaphylactic shock after contact with natural rubber latex (NRL) in the past, but at the time of examination had no manifestations for NRL allergy. At the time of examination, she did not use latex gloves. The total IgE was determined with Ridascreen immunoenzyme method and sIgE NRL – with UniCAP 100 fluorescence enzyme immunoassay (CAP FEIA) system (Thermo Fisher). We used the following scale to determine the levels of sIgE NRL: grade 0-till 0.31 kUa/l-very low; grade I-0.31-0.55 kUa/l-low; grade II-0.55-1.4 kUa/l-moderate; grade III-1.4-3.9 kUa/l-high; grade IV-3.9-19 kUa/l-very high; grade V-19-100 kUa/l; grade VI >100 kUa/l – very high. For reference limits of total IgE in adults we accepted values up to 100 UI/ml [11].

RESULTS

The mean value of total IgE was 63.78 ± 58.43 UI/ml, i.e. below the referent level under 100 UI/ml. Four of nurses had high IgE and three (9.37%) – high sIgE NRL, but their total IgE values were normal (table 1).

Table 1. High levels of total IgE and sIgE NRL

age	total IgE (UI/ml)	sIgE NRL (kUa/l)	allergic disease	other disease
46	10	2.93	itching, urticaria	psoriasis
55	31.2	0.35	no	SLE, breast cancer
22	27	1.2	anaphylactic shock	no
68	127.6	(-)	no	no
45	420	(-)	no	no
65	400	(-)	no	breast cancer
28	200	(-)	no	no

DISCUSSION

NRL reactions have 3 forms: 1. Irritant contact dermatitis with dryness, scaling, cracing and erythema. These reactions are mainly localized; 2. Type I immediate hypersensitivity, which is connected with IgE and characterized by angioedema, bronchospasm, urticaria, pruritus, anaphylaxis. Reactions are acute (in 30 minutes) and local or generalized; 3. Type IV delayed hypersensitivity reactions, which involve T lymphocytes with erythema, scaling, vesicular lesions, eczema. Reactions are mainly local and onset is usually in 24-48 hours after exposure. In type I and type IV reactions usually previously sensitization present. Predisposing factors for NRLA are: hay fever, asthma, hand eczema, allergic rhinitis, urticarial, dust allergy, allergy to some fruits

and vegetables-banana, avocado, peanut, pineapple, grape, papaya, tomatoes, cherry, peach, fig, watermelon, mango [12, 13, 14].

We found that three from 32 (9.37%) nurses in non-surgical departments had elevated levels of sIgE NRL. The prevalence of high sIgE NRL in medical staff and nurses in particular, varies widely from 0% [15] to 38.3% [16]. According R. G. Kaczmarek et al. [17] in 6% of medical staff values vary from 0.7 ng/ml to 222.3 ng/ml (mean 1.8 ng/ml). Y. Levy et al [15] find sIgE NRL in 1.66% of medical staff within limits 0.87 UI/ml – 34.25 UI/ml. According F. L. Fallon and G. Radman [13] 6% of medical workers are SPT positive and 50% of them have high values of sIgE NRL. M. Grzybowski et al. [12] demonstrate that 8.9% of nurses have elevated sIgE NRL regardless workplace, but S. Köse et al. [16] show that this percent higher – is 38.3. N. Verna et al. [18] find elevated values of sIgE NRL in 3/42 (7.14%) of operating room nurses and in 5/33 (15%) in intensive care units. According S. R. Mace et al 6% of nurses in operating rooms have high sIgE NRL [19]. Y. Levy et al. do not find sIgE NRL in nurses working in pediatric units [15].

Many authors match the data between IgE and sIgE NRL. Some of them prove parallelism [17, 20, 21], others do not find relations [22] and third consider that parallelism exists at values above 0.5 UI/ml sIgE NRL [23]. Only in 22% of the studied 44 medical workers with high levels of sIgE NRL E. Nowakowska-Swirta et al. [24] found total serum IgE levels above 100 kU/l.

In 7 of the 32 nurses examined by us, we found high levels of either total serum IgE or sIgE NRL, but in none of the cases we found any correlations between these 2 parameters. Three nurses had increased values of sIgE NRL and IgE levels were in normal limits. Two of them had allergic symptoms: the patient with psoriasis suffer from itching and urticarial and second patient had anaphylactic shock in the past. The third patient had SLE and breast cancer, but without of clinical symptoms for latex allergy. The rest 4 nurses had high IgE levels, normal sIgE NRL levels and absence of allergic symptoms (table 1).

Immunological reactions in psoriasis are cell-mediated (type IV hypersensitivity), but there are data on the involvement of type III hypersensitivity [25, 26] and type I hypersensitivity [27, 28, 29]. Latex allergy is type I [30] or type IV [31].

Many authors consider that psoriasis and contact allergy rarely seen together [32, 33, 34, 35] but others have opposite opinion [36, 37]. In our patient with an well-controlled skin disease of psoriasis, contact with latex gloves (till this moment she had used only vinyl or nitrile gloves), provoked the appearance of another highly pruritic rash, diagnosed as latex contact allergy. The elevated sIgE NRL levels support this diagnosis. She stopped the use of latex gloves and she was treated with local corticosteroids and low doses systemic corticosteroids. After one month the allergic symptoms disappeared. We think that this is a first case of psoriasis patient with latex allergy [38].

IgE antibodies to nuclear antigens are described in SLE patient [39, 40]. B. Ponvilawan's meta-analysis [41] shows a higher chance of SLE appearance in patients with atopic dermatitis. In atopic dermatitis patients lupus is more severe and with poorer prognosis [42, 43, 44]. In only one report authors discuss the absence of sIgE NRL in patients with juvenile lupus [44]. We think that our description of high sIgE NRL in a patient is the first at this time [45]. It should be noted that this female patient had no clinical evidence of an allergic disease. Another important point in the history of this patient is the connection between SLE and breast cancer. According to some authors SLE represents a significant high risk of the development of oncological diseases [46, 47, 48], but breast cancer is not the most common tumor in SLE [48, 49, 50]. It is more common in patients with incomplete lupus [51], such as our patient. She fulfills only 3 criteria for SLE: arthralgia with swelling, positive ANA (1:1280) and positive DNA antibodies [52, 53, 54]. The diagnosis incomplete SLE was made several months after surgery intervention and subsequent chemotherapy for ductal carcinoma, but 5 years before the carcinoma was discovered, the patient had morning stiffness and joint swelling responding to therapy with chloroquine and corticosteroids. At that time no antibodies were detected. The joint complaints of the patient described by us disappeared and the antibody titer decreased after the removal of the tumor and after chemotherapy.

In most cases, lupus precedes the appearance of the malignant disease [55], less often neoplasia appears first [55, 56]. According to third authors, the two diseases develop independently rather than

influencing each other [46, 57, 58]. In our patient joints complaints in the absence of serological changes, preceded the onset of breast cancer. In our opinion the reduction of the ANA titer and the absence of pain syndrome after the removal of the tumor speaks in favor of the diagnosis of paraneoplastic syndrome. The absence of allergic symptoms at high levels of sIgE NRL may be explained by continued immunosuppressive therapy. Several months later sIgE NRL were absent, but the patient had worked only with non-latex gloves. Discussing the relationships between allergic diseases and tumor pathology, we should also note the other 65-year-old patient with breast cancer in whom we did not detect sIgE NRL, but total IgE levels were significantly elevated – 400 UI/l.

The third patient with high sIgE NRL had anaphylactic shock during surgery several years ago. Then allergy to latex was proven. She did not use latex gloves, but their sIgE NRL were elevated permanently.

CONCLUSION

We found high sIgE NRL in 9.37% of nurses working in non-surgical departments. We think that anamnestic data for symptoms to exposure and contact with latex objects, data on atopy and food allergies are very important for the diagnosis of latex allergy. In many cases, additionally performed skin tests – SPT, scratch test or patch test, support this diagnosis, but they cannot always be done due to another disease with skin symptoms, autoimmune disease or risk of systemic reactions. The proof of sIgE NRL is a reliable method to confirm the diagnosis of latex allergy.

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CASE REPORT**CLINICAL CASE OF A PATIENT WITH COMMON VARIABLE IMMUNODEFICIENCY ASSOCIATED WITH MEMBRANOUS NEPHROPATHY**

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Abstract. Background: Common variable immunodeficiency (CVID) is one of the most common Human inborn errors of immunity (IEIs). Renal involvement in CVID has been described in isolated and sporadic case reports. Membranous nephropathy (MN) is the predominant pathologic findings in patients with CVID. The hypogammaglobulinemia may contribute to immune dysregulation in some patients with membranous nephropathy. Identification of MN in patients with CVID is important as nephrotic range proteinuria results in increased renal losses of immunoglobulins, worsening their immunocompromised state and increasing susceptibility to infections. **Case presentation:** We report a case of a patient with Common Variable immunodeficiency and secondary membranous nephropathy, presenting at the age of 14 years old. He presented with a history of frequent infections, hypogammaglobulinemia, microscopic hematuria, hypertension, nephrotic range proteinuria. The immunoglobulin replacement therapy leads to remission of both diseases and contributes to controlling the clinical course of MN as well as CVID and pulmonary infections. **Conclusion:** CVID can be considered as a potential cause of secondary membranous glomerulopathy in young patients with a history of recurrent infections.

Key words: Membranous glomerulopathy, Common variable immune deficiency, immunoglobulin replacement therapy

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INTRODUCTION

Common variable immunodeficiency (CVID) is a primary immune deficiency (PID) involving a group of heterogeneous conditions united by impaired production of immunoglobulins and protective antibodies (defective antibody response after vaccination with protein and polysaccharide vaccines) and a variable immunological and clinical phenotype, with various renal changes described,

the most common being membranous nephropathy that could further worsen the immunosuppression in these patients [1, 2, 3]. The diagnosis of CVID is based on the decrease in IgG and IgA levels (below 2SD for age with and without IgM reduction), while excluding other clearly defined causes of hypogammaglobulinemia. Additional diagnostic criteria include low or missing specific antibody responses after vaccination and/or lack of isohemagglutinins [4].

Patients with CVID have a variety of disease manifestations, including recurrent bacterial infections, autoimmunity, chronic lung disease, gastrointestinal disease, lymphoid hyperplasia, granulomatous disease, and an increased risk of malignancy [5]. Persistent antigenic stimulation due to a defective immune system is the main cause of the development of autoimmunity in patients with primary immunodeficiency states.

Renal failure is a rare complication, affecting approximately 2% of CVID patients. Membranous nephropathy is an organ-specific autoimmune disease of the kidneys, which could manifest from asymptomatic proteinuria to severe nephrotic syndrome. It is the most common cause of nephrotic syndrome in adults; however, in children, it is very rare and contributes to < 5% of cases [6-9] it is a relatively rare entity in the pediatric population and there is a paucity of data about the incidence, prognosis, and optimal treatment of IMN in children and adolescents. We conducted this study to evaluate pediatric patients with IMN in order to clarify the presentation, response to therapy, and clinical outcome. Methods. A retrospective chart review was performed on patients identified with biopsy-proven IMN between 1988-2005. Patients with systemic lupus erythematosus or hepatitis-related lesions were excluded. The following data were tabulated: age, gender, ethnicity, presenting clinical and laboratory findings, proteinuria in a first morning urine specimen, estimated glomerular filtration rate (GFR_e). In the pediatric population, the underlying cause is mostly secondary due to hepatitis B or other infections, systemic lupus erythematosus, immune deficiency, malignancy, different medications or even dietary antigens [9] from infants to the elderly. While systemic disorders such as hepatitis B infection or lupus may more frequently cause secondary MN in the younger population, primary or "idiopathic" MN has generally been considered a disease of adults. Recent progress in our understanding of primary disease was recently made when the target antigen in primary MN was identified as the M-type phospholipase A2 receptor (PLA2R). The disease is defined by the presence of subepithelial immune deposits (between the GBM and the podocyte), with subsequent expansion of the GBM resulting in a membrane-like (membranous) thickening of the glomerular capillary wall [9]. The diagnosis is often made after kidney biopsy [10]. There are five morphologic stages of MN described,

but the most common lesion seen in children is stage II, followed by stage III and the mixed stage [9]. In pediatric patients with MN microscopic hematuria is more common than in adults [6, 8, 10] from infants to the elderly. While systemic disorders such as hepatitis B infection or lupus may more frequently cause secondary MN in the younger population, primary or "idiopathic" MN has generally been considered a disease of adults. Recent progress in our understanding of primary disease was recently made when the target antigen in primary MN was identified as the M-type phospholipase A2 receptor (PLA2R). Arterial hypertension and mild renal dysfunction may be present at clinical onset in a variable number of patients [6, 10]. Treatment of the underlying disease often leads to remission of glomerulonephritis.

Here, we describe a clinical case of CVID and MN and discuss whether the clinical course of the patient suggests that CVID should be considered an ultra-rare etiology of secondary MN.

CASE REPORT

We present a 20-years-old male patient with normal development and family history. At the age of 14, he was first admitted to the Department of pediatric nephrology due to persistent microscopic hematuria, proteinuria, and arterial hypertension. He has a history of recurrent upper and lower respiratory tract infections that began in early childhood. Asthma diagnosis is excluded. At the age of seven, he suffered from varicella infection with complicated skin lesions that required surgical intervention. Then, at the age of ten, he underwent emergency surgery for peritonitis caused by purulent appendicitis. At the age of 13, he was admitted to the hospital five times due to pneumonia combined with hemoptysis.

Physical examination: At presentation, we observed obesity with acanthosis nigricans and striae albae of the skin, without edema. His arterial blood pressure was elevated despite of the antihypertensive treatment (RR 145/95 mm Hg).

Laboratory findings: Urinalysis revealed nephrotic-range proteinuria (3.7 g at 24-hour collection) and hematuria without dysmorphic erythrocytes. The estimated glomerular filtration rate (eGFR) was 198 ml/min/1.73 m². His serum electrolytes, lipids, serum proteins, and complement factors C3 and C4 were within the normal range. Only high uric acid levels were detected.

Renal ultrasound revealed pathologically hyperechogenic renal cortex (Fig. 1).

Immunological tests revealed low IgG (4.53 g/l) and IgA levels (0.29 g/l), and normal IgM levels (0.22 g/l) corresponding to hypogammaglobulinemia. After referral to the Clinical Immunology Department, he was diagnosed with CVID based on additional specific immunological tests. Progressive reductions in IgG (3.273 g/l), IgA (<0.02 g/l) and

IgM (0.1 g/l) levels and low IgG1 (2.41 g/l), IgG2 (0.333 g/l), IgG3 (0.581 g/l) and IgG4 (<0.003 g/l) subclasses were found. There was no protective postvaccine immunity against tetanus toxoid (0.07 U/ml) or diphtheria toxoid (0.004 U/ml). Natural isohemagglutinins of the IgG and IgM classes are missing. Investigation of cellular immunity (Table 1) revealed pronouncedly impaired immunobiological balance due to decreased helper/inducer CD4+

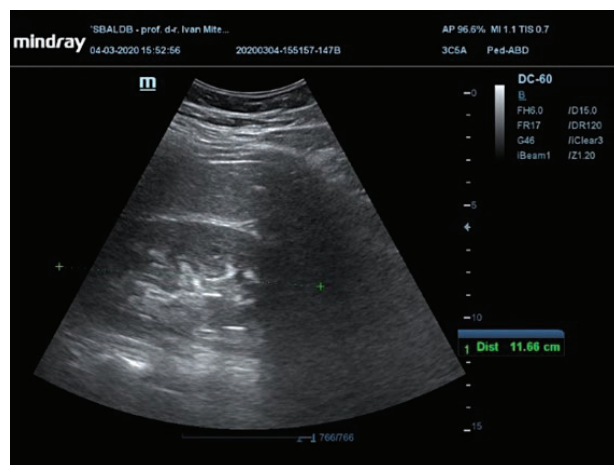
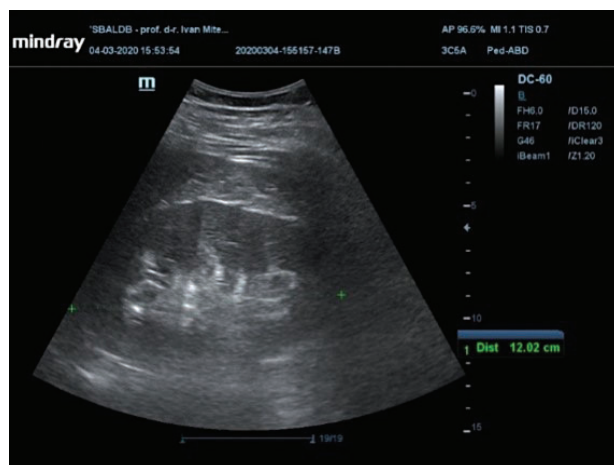


Fig. 1. Renal ultrasound shows normal size and location of the kidneys. The hyperechogenic renal cortex was bilateral without dilatation of the renal collecting system

Table 1. Results from the T- and B-cell immunity analyses

Cells populations	RESULTS 14.01.2019		RESULTS 15.07.2020		Reference range	
	%	cells/ μ l	%	cells/ μ l	%	cells/ μ l
Total T Ly	84	2075	88	2640	64 $\div$ 83	1050 $\div$ 1980
Activated T Ly (CD3+DR+)	35	865	42	1260	4 $\div$ 13	90 $\div$ 260
Th Ly (CD3+CD4+)	17	420	15	450	34 $\div$ 58	590 $\div$ 1330
CD4+ T Ly						
• Naïve (CD45RA+CD62L+)	5.2	-	12.2	-	37 $\div$ 97	-
• Central-memory (CD45RA+CD62L+)	80.2	-	71.4	-	13 $\div$ 76	-
• Effector-memory (CD45RA+CD62L-)	13.9	-	15.2	-	0.5 $\div$ 25	-
• Effector (CD45RA+CD62L-)	0.7	-	1.2	-	< 5.8	-
Tc Ly (CD3+CD8+)	63	1556	71	2130	22 $\div$ 33	440 $\div$ 770
CD8+ T Ly						
• Naïve (CD45RA+CD62L)	29.5	-	12.6	-	20 $\div$ 95	-
• Central-memory (CD45RA+CD62L+)	35.3	-	34.7	-	0.42 $\div$ 18	-
• Effector-memory (CD45RA+CD62L-)	21.7	-	36.6	-	4 $\div$ 100	-
• Effector (CD45RA+CD62L-)	13.6	-	16.2	-	9 $\div$ 65	-
CD4/CD8 ratio	0.27		0.2		1.0 $\div$ 1.6	
B- Ly (CD19+)	2.5	62	1 30	6 $\div$ 13		110 $\div$ 350
• CD19+ B Ly	76.8		-		51.3 $\div$ 81.5	
• CD27-IgD+ naive	16		-		4.6 $\div$ 18.2	
• CD27+IgD+ non switched memory	1.7		-		8.7 $\div$ 25.6	
• CD27+IgD+ switched memory						
NK cells (CD3-CD16&56+)	9	222	10	300	8.0 $\div$ 24	130 $\div$ 540
NKT cells (CD3+CD16&56+)	7	173	20	600	-	-
T or NK (CD57+CD8-)	1	25	1	30	3 $\div$ 13	44 $\div$ 356
T or NK (CD57+CD8+)	20	494	32	960	5 $\div$ 11	91 $\div$ 257
CD8+11b+ cells	47	1161	62	1860	6.5 $\div$ 16.5	34 $\div$ 304
Cytotoxic(CD8+11b-)cells	20	496	15	450	14 $\div$ 26	340 $\div$ 811

T cells and increased suppressor/cytotoxic CD8⁺ T-cell subsets (CD4/CD8 ratio of 0.28). A significantly reduced percentage of naive CD4⁺ T cells was also observed. Activated T lymphocytes (CD3+HLA-DR⁺), central memory CD8⁺ T cells and terminally differentiated effector T cells were increased. The number of B lymphocytes was severely reduced at the expense of the class-switched memory subpopulation. Evaluation of T-cell function revealed normal T-cell activation (normal expression of CD69 on T lymphocytes after in vitro PHA or anti-CD3/CD28 stimulation). The Th2 cytokine profile predominates.

The genetic study conducted at the Veritas Spain laboratory did not show the presence of pathogenic variants for CVID.

Renal biopsy revealed membranous nephropathy II-III stage (Fig. 2). Thickening of the GBM and the tubular apparatus displayed degenerative changes with atrophic tubules. Immunofluorescence revealed subepithelial granular deposits that stained 3-4+ for IgG, 3+ for IgM and C3, and 2-3+ for C1q.

Common viral infections or systemic diseases, such as other secondary causes were excluded: negative for anti-nuclear, anti-neutrophil cytoplasmic and anti-glomerular basement membrane antibodies; normal CH50; negative PCR for HBV-DNA, PCR for HCV-RNA, PCR for CMV-DNA, and PCR for EBV-DNA. A chest X-ray showed peribronchitis and vasal congestion and infiltrative changes in the retrocardiac and right lower lung fields. The patient

was diagnosed with latent tuberculosis (high positive T-SPOT-TB test) and long-term therapy with rifampicin, isoniazid and pyrazinamide was started. We also initiated treatment with corticosteroids because of increased proteinuria and intravenous immunoglobulin G replacement therapy (400 mg/kg/bw) for the treatment of hypogammaglobulinemia for three months. After that the replacement therapy was switched to subcutaneous immunoglobulin in a dose 143mg/kg per week administered at home. After six months of follow-up, our patient had no infections requiring hospitalization. The proteinuria level during the 24-hour collection decreased to 0.6 g, and the IgG concentration increased to 10.64 g/l. The T-SPOT-TB test was negative. The corticosteroid therapy was stopped. The patient continued to monitor his weight and blood pressure with Jardiance (sodium- glucose cotransporter 2 (SGLT2) blocker. Subcutaneous immunoglobulin therapy was continued, but without good patient compliance. Over a period of three years, his IgG level was suboptimal, around 5 g/l, and he had two episodes of immune thrombocytopenia, which required the introduction of a thrombopoietin agonist Revolade 25 mg.

DISCUSSION

We present the first case of a patient with CVID and membranous nephropathy in the Bulgarian registry for Primary immune deficiencies. CVID is a primary immunodeficiency disorder characterized

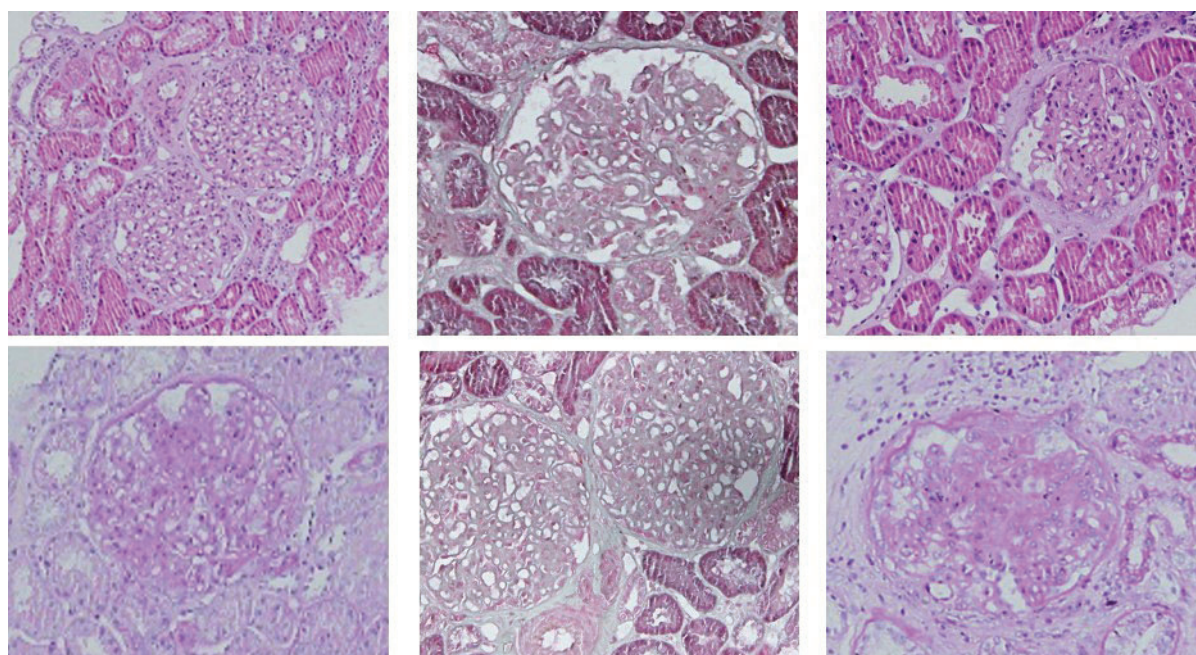


Fig. 2. Histology findings of patients with stage II-III membranous nephropathy

by hypogammaglobulinemia, recurrent infections, and autoimmune complications. A majority of patients with CVID do not display renal manifestations, but those who do show a predominance of MN. Membranous nephropathy in children poses many challenges for pediatric nephrologists due to its sporadic occurrence, nonspecific clinical presentation, and unpredictable treatment response. In most cases, the etiology is unknown, which is why practitioners should also consider secondary causes of the disease in every patient. To our knowledge, only two cases of secondary MN have been reported: a 13-year-old child [11] and a 36-year-old woman with the same etiology as our patient [12]. The child was successfully treated with corticosteroids and cyclosporine, but the woman developed acute kidney injury requiring dialysis and was treated successfully with rituximab and corticosteroids. In our patient, the diagnosis of both diseases (MN and CVID) was made at the same time, immediately after the proteinuria was detected. Renal biopsy revealed membranous nephropathy stage II-III. We started treatment with corticosteroids and replacement therapy for immune deficiency, which led to good results, and the patient is in remission of membranous nephropathy without immunosuppression. Monitoring of patient after adulthood shows mild to moderate proteinuria (0.5 g/L) with preserved renal function (eGFR CKD EPI 130 ml/min/1.73 m²).

Autoimmune phenomena occur frequently and are the second most common clinical manifestation of many forms of PIDs, surpassed only by infections. Hypogammaglobulinemia may be an important but largely unrecognized factor in the pathogenesis and progression of MN in some patients. This is the first reported case of a child with CVID and MN in Bulgaria. He was diagnosed with CVID based on the clinical presentation of frequent infections beginning in early childhood, hypogammaglobulinemia, poor anti-vaccine response and lack of isohemagglutinins. The evidence that PIDs are often associated with different autoimmune manifestations suggests that they could share common pathogenetic mechanisms, which result in broad immune dysregulation. The presumed mechanisms of CVID include the proliferation of autoreactive B cells due to impaired BCR-TCR interactions, loss of FcγRIIB inhibitory signaling or altered BAFF/APRIL survival signaling, and a decreased Treg population due to cytokine

aberrancies or impaired TCR signaling [13]. In this context, specific lymphocyte subset abnormalities were observed in our patient affecting B- and T-cell compartment: low count of CD4 and elevated CD8 T-cell populations resulting in inverted CD4:CD8 ratio; a reduced naïve CD4⁺ cell subset; increased activated T cells, central memory CD8 T-cell subset and terminally differentiated effector cells; and decreased total and class-switched memory B cells. Similar changes observed in pediatric [14] and adult CVID patients [14, unpublished our data] are in line with the immune dysregulation theory, based on exhausted immune system. It should also be mentioned that the replacement therapy administered to our patient, in addition to supplying immunoglobulins, may have further beneficial effects by modulating the immune response [15].

In conclusion, autoimmunity and immunodeficiency are two opposite conditions that may coexist in the context of general immune dysregulation. Given this strong association, underlying immune deficiency should always be ruled out, especially in the presence of early onset or multiple autoimmune manifestations. Current data on MN in infancy are sporadic and limited. It is essential to distinguish primary from secondary forms of MN, as many of the underlying conditions are successfully treated.

The rare combination of MN and CVID illustrates the complex nature of the diagnosis. This case emphasizes on the crucial importance of the comprehensive review of the patient's clinical and laboratory data and immune phenotype, including renal presentations, for the precise diagnosis and personalized management of CVID.

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SCIENTIFIC REVIEW

URIC ACID – FRIEND OR FOE? A QUESTION WITH MANY ANSWERS

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Abstract. In humans, uric acid (UA) is the end-product of purine metabolism. It can be a product of nuclear acid degradation or of de novo synthesis from fructose and other exogenous sources. UA increases substantially in dehydration, increased catabolism and cell degradation, genetic defects in synthesis, and in stated of generalized endothelial dysfunction. In normal serum levels, UA is a natural antioxidant and protects endothelium, it has cognitive function and regulates the immune response. Nevertheless, in pathological conditions, when UA serum levels are increased, it ats as prooxidant molecule and increases the formation of pro-inflammatory cytokines, insulin resistance and impairs bone metabolism. This review presents the current knowledge on the beneficial and the harmful effects of UA in humans.

Key words: uric acid, endothelial dysfunction, inflammation, metabolic syndrome, chronic renal disease

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INTRODUCTION

Uric acid (UA) is a weak organic acid, in humans being the end product of purine (adenine and guanine) metabolism due to the loss of the enzyme uricase that transforms it into the soluble allantoin [1, 2, 3, 4]. It is derived from three major pathways [1, 2, 3, 4]:

- Cell turnover – from damaged, dying or dead cells,
- De novo synthesis, and
- Food – exogenous purines, fructose and sucrose.

In mammals, purines are metabolized to xanthine which is further converted to uric acid via the xanthine oxidase pathway. As already mentioned, in many mammals UA is further metabolized to the soluble and non-toxic allantoin, but humans lack the

enzyme uricase (urate oxidase) and therefore, UA is the end product of this metabolic pathway. UA can crystallize in the form of monosodium urate (MSU) in the tissues when the solubility threshold is exceeded and/or factors increasing precipitation are present (plasma oversaturation, decreased local blood flow / stasis, hypoxia, acidosis, low temperature, local inflammation/trauma, etc.) [5]. MSU crystals can deposit in the articular and periarticular structures and soft tissues (causing gout and tophi formation), and the kidneys in the form intratubular precipitates, renal stones or extracellular deposits in the interstitial spaces, and cause local inflammatory reaction with the development of granulomatous structures that can perpetuate low-grade inflammation that further increase cardiovascular risk and affect immune response and insulin sensitivity [6, 7, 8].

UA was first isolated in 1776 by Carl Wilhelm Scheele from human urine and renal stones and 100 years later, in 1876, Alfred Garrod first demonstrated the link between gout and UA and proposed a diet aimed at gout control [3, 4, 9, 10, 11]. In the 1870s, Frederic Mohamed suspected the link between hypertension and hyperuricemia and his investigations revealed two major questions: whether hyperuricemia is an independent cardiovascular risk factor and if it is, how exactly UA increases cardiovascular risk [3, 4]. Moreover, the results of population studies and multiple meta-analyses reveal that both very low and very high UA serum levels are related to unfavorable cardiovascular prognosis [12], i.e., in physiological levels uric acid has protective role in humans and in sub- and supraphysiological levels it appears to be harmful. This review summarizes the current knowledge on the beneficial and harmful effects of UA – a natural substance that can be both a friend and a foe for humans.

SOURCES AND EXCRETION OF URIC ACID

In human body, UA can be derived either from the degradation of purines (endogenous and exogenous adenine and guanine) or from de novo synthesis from fructose, glucose, ethanol or other non-purine substances [3, 4, 13]. The de novo synthesis is stimulated by steroid hormones, growth factors, inflammatory cytokines, and insulin [14]. The uric acid pool of human body is up to 1200 mg. The endogenous UA synthesis is relatively constant – approximately 400 mg (300-600) a day, accounting for approximately half of the total amount of uric acid in human body, with the other half originating from food sources (including purine-rich and fructose-containing food) [14]. UA is synthesized mainly in the liver, the intestines, vascular endothelial cells, kidneys and striate muscles [14] and is excreted via specific transporter systems by the kidneys and the intestines [2, 14, 15, 16, 17]. UA excretion is approximately 600-800 mg a day (about two thirds by the kidneys and about two thirds by the intestines). Renal excretion follows four steps (glomerular filtration, tubular reabsorption, tubular secretion and repeated reabsorption) and intestinal excretion is mediated by enteric secretion and further bacterial uricolysis [2, 15, 16, 17]. Renal tubular excretion accounts for 60-70% of UA clearance: reabsorption uses several transporters, including URAT1, OAT4,

OAT10, and GLUT9, tubular secretion uses mainly MRP4, ABCG2, UAT, OAT1 and OAT3 [14]. Intestinal secretion accounts for 30-40% of UA clearance and uses mainly ABCG2 transport system. Bacterial uricolysis also plays important role in UA excretion and UA can impair gut microbiota and lead to the development of dysbacteriosis with further metabolic disturbances, including impairment of insulin sensitivity.

PHYSIOLOGICAL ROLE OF URIC ACID – THE FRIEND

In physiological levels, UA has several protective functions in humans [16, 18, 19, 20, 21]:

- *Natural antioxidant*, especially in the site of radical oxygen species generation – vascular endothelium, liver, mucous membranes of the respiratory and gastrointestinal tract. UA can also suppress lipoxygenases and serve as a substrate for the cyclooxygenase. Nevertheless, these antioxidant properties are observed only in normal levels of uricemia, and in hyperuricemia, UA can have prooxidant effect.

- *Tissue repair and protection of endothelial function*: UA has antioxidant properties and serves as a “danger signal” (cell degradation) and stimulates progenitor endothelial cells [20, 21]. Moreover, the very low UA levels are associated with endothelial dysfunction [20, 21], as are the very high UA levels, especially in patients with hyperuricemia and multiple components of metabolic syndrome [5] and in preeclampsia [22].

- *Danger signal*, important for scavenging of free radicals (reactive oxygen species) and cellular remnants. UA is known to induce type 2 immune response due to stimulation of inflammatory monocyte-derived dendritic cells [23, 24] as a protective mechanism. Moreover, UA is a major initiator and amplifier of allergic inflammation due to direct activation of spleen tyrosine kinase (Syk) and the phosphoinositol 3 (PI3)-kinase [25]. As many cysteine proteases and cysteine peptidases can stimulate barrier epithelial cells to produce type 2 cytokines and redirect the immune response towards type 2 response and hypersensitivity reactions, UA can serve as an important “danger signal” for cellular damage or death [24, 25, 26, 27], as a part of the “fight or flight reactions” that are crucial for the survival of the human body, and for the response to vaccines.

- *Defense against parasites* (*S. mansonii*, *S. haematobium*, *F. hepatica*) via interference with the

synthesis of arachidonic acid that can mediate the damage of parasite cells. This has been proven in experimental studies [28, 29].

– *Protective role against autoimmune diseases:* as a natural antioxidant [16, 19, 20] and a regulator of the immune response [23, 24].

– *Neuroprotection:* as a stimulator of cognitive function and a natural antioxidant under physiological conditions and levels, it blocks of peroxynitrites and radical oxygen species, regulates brain calcium homeostasis, mitochondrial function and counteracts glutamate toxicity on neuronal cells, regulation of the immune response [19, 23, 24, 25, 30]. Therefore, both very low and very high levels of UA are associated with cognitive dysfunction [31].

PATHOLOGICAL EFFECTS OF UA – THE FOE

It has been shown that UA is a major risk factor increased cardiovascular factor, especially in patients with metabolic syndrome [5, 7, 8, 10, 13, 18, 19]. Hyperuricemia is also associated with poorer prognosis in chronic kidney disease [7, 18]. In normouricemia, UA has antioxidant and immune response modulating properties but the high UA levels are associated with pro-oxidant state due to formation of reactive oxygen species (ROS), increasing lipid oxidation and protein nitrosylation, stimulation of NADH oxidase-dependent ROS formation, generation of ROS via xanthine oxidoreductase activation, activation of MAPK p38, ERK 1/2, decreased nitric oxide (NO) synthesis and bioavailability [19]. Moreover, UA can modulate immune response and in both very low and very high levels can induce pro-oxidant and pro-inflammatory reactions, i.e., hyperuricemia can serve as pro-inflammatory and vascular risk factor.

Hyperuricemia is known to cause *microvascular injury* via stimulation of the rennin-angiotensin-aldosterone system (RAAS), increased expression end response to rennin, endothelial dysfunction with *inhibition of NO synthesis and decreased expression of NO-synthase-1 in renal macula, stimulation of smooth-muscle cell proliferation* [19, 32]. This effect on endothelial function and NO synthesis is thought to relate UA with the pathogenesis of preeclampsia [22].

In the state of UA excess (hyperuricemia), UA can act as *pro-inflammatory molecule*: it can activate monocytes and macrophages and induce the genera-

tion of NRLP3 inflammasome, expression IL-1 β and IL-18 *via* caspase-1 pathway, cytokine secretion and both local and systemic inflammatory response. The pro-inflammatory effects are mediated by HMGM1 (high mobility group box chromosomal protein 1) and NF- κ B (nuclear factor kappa-B) and by the changes in gut microbiota, related to increased intestinal excretion of UA.

UA has *unfavorable effect on metabolic syndrome* (MetS) components, and the relations between UA and MetS seem to be bidirectional, closing a vicious circle, leading to endothelial injury and increased cardiovascular risk [33, 34]. In hyperuricemic state, UA leads to [33, 34, 35, 36, 37]:

- stimulation of pro-oxidant pathways and decreased NO synthesis,
- secretion of pro-oxidant and vasoconstrictor substances (IL-1, IL-6, tumor necrosis factor alpha (TNF α), endothelin 1 (ET1), thromboxane A2),
- changes in DNA methylation with the subsequent development of pro-inflammatory state,
- RAAS activation,
- insulin resistance due to increased expression of pro-inflammatory cytokines and change in gut microbiota,
- decreased activation of vitamin D by 1 α -hydroxylase with subsequent increase in parathyroid hormone levels and impairment of bone metabolism,
- direct stimulation of osteoclasts and increase in oxidative stress and inflammation.

Moreover, hyperinsulinemia in insulin resistance leads to further increase in UA levels, that worsens the insulin resistance itself [33, 34, 35, 36].

In supraphysiological concentrations, uric acid is highly nephrotoxic, leading to renal tubular obstruction, development of kidney stones, tubulointerstitial nephritis with tubular atrophy and interstitial scarring, nephrocalcinosis, granulomatous tubulointerstitial nephritis and rarely – glomerular involvement in the form of mesangioproliferative or sclerosing glomerulonephritis [19, 32, 38]. Additional mechanisms of renal injury can be the intake of non-steroid anti-inflammatory medications in gout and the comorbidities, related to gout, including MetS components.

In *hypouricemic state* the protective functions of UA are also lost and following abnormalities are observed [18, 33]:

- pro-oxidant state,

- endothelial dysfunction with decreased NO synthesis,
- cognitive dysfunction and dementia,
- increased autoimmunity.

Therefore, depending on its serum levels, UA can act as “the good, the bad and the ugly” player in the inflammatory process, endothelial function and insulin sensitivity (table 1).

In conclusion, in physiological levels, uric acid is an important defense molecule in humans, crucial for the normal endothelial function, antioxidant defense and regulation of immune response, tissue

reparation and normal metabolic and cognitive processes, and it is a friend to normal adaptive mechanisms of human body. Yet, the very low and very high levels of UA can act as a foe – it could have detrimental effects on the normal endothelial, immune, metabolic and cognitive functions. Therefore, the adequate choice of dietary regimen and treatment is crucial for the prevention of vascular and cognitive impairment. Uric acid serum levels should be assessed as a part of the routine prophylactic studies at population levels and all abnormalities should be addressed using dietary and therapeutic interventions.

Table 1. The positive and the negative effects of UA in humans, depending mainly on its serum levels – the good, the bad and the ugly role of UA in norm and pathology

The good	The bad	The ugly
– Antioxidant/free radical scavenger – Protection and preservation of endothelial function, mobilization of progenitor endothelial cells – Danger signal – Stimulation of cognitive functions, neuro-protection – Stimulation of tissue repair – Stimulation of type 2 immune response – Protection against autoimmune response – Defence against parasites.	– Gout, tophi – Nephrolithiasis, nephrocalcinosis – Granulomatous tubulointerstitial nephritis – Glomerular injury – Persistent low-grade inflammation – Epigenetic changes (DNA methylation) leading to pro-inflammatory state – Changes in gut microbiota with subsequent increase in insulin resistance and pro-inflammatory state – Decreased vitamin D activation and changes in bone turn-over.	– Chronic renal disease/chronic renal failure – Endothelial dysfunction – Pro-inflammatory state – Increased cardio-vascular risk – Preeclampsia – Development and progression of the MetS.

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SCIENTIFIC REVIEW

EXTRAHEPATIC MANIFESTATIONS OF HEPATITIS E

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Abstract. Hepatitis E virus (HEV) is one of the most common yet underestimated and underdiagnosed causes of acute viral hepatitis worldwide. The infection is detected in all parts of the world with certain epidemiological variations, depending on the geographic latitude and the socio-economic status of the population. HEV infection is generally a self-limiting disease with rare fulminant and chronic cases, especially in immunocompromised individuals. Patients frequently develop extrahepatic manifestations, particularly neurological and renal. We present the current knowledge on the epidemiological and clinical characteristics of HEV infection and the extrahepatic manifestations of HEV.

Key words: hepatitis E, clinical manifestations, extrahepatic involvement, diagnosis, treatment

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INTRODUCTION

Hepatitis E virus (HEV) represents a major health problem worldwide with approximately 20 million people infected every year [1, 2, 3]. This is one of the most common and usually underdiagnosed and underestimated causes of acute viral hepatitis [1]. HEV infection is detected in all parts of the world with, most common in Sub-Saharan Africa, East and South Asia, with certain epidemiological variations, depending on the geographic latitude and the socio-economic status of the population [2]. The virus can affect not only liver cells, but other epithelial and immune system cells and can cause severe extrahepatic manifestations, including neurological, renal, endocrine, etc. [1, 2]. This review is focused upon the clinical characteristics of HEV infection and its extrahepatic manifestations.

VIROLOGICAL DATA

HEV is an icosahedral RNA virus, belonging to the family of *Hepeviridae*. It was first discovered in

1983 [4] and its RNA was cloned in 1990 [1, 2, 3]. The virus affects both animals and humans and has been detected in many animal species, such as pigs, zoonotic pathogen with a wide range of reservoirs (rabbits, ferret, deer, wild boars, camels, bats and rats) [1, 2, 3, 5, 6]. At least 8 main HEV genotypes have been described, and genotypes 1 to 4 are known to be pathogenic in humans (HEV1, HEV2, HEV3 и HEV4) [1, 2, 3, 5, 6, 7] (Table 1). Genotypes 1 and 2 are zoonotic and genotypes 3 and 4 are anthroponotic [7]. It remains unclear whether genotype 8 is pathogenic to humans, and genotype 7 has been found pathogenic in immunocompromised individuals, especially in transplant recipients [1, 2, 3, 5, 6, 7]. The predominant route of transmission is fecal-oral and via oral intake of infected body fluids, milk, meat and secretions, the virus can also be transmitted via blood transfusion. The natural reservoir of genotypes 1 and 2 are probably infected humans with chronic and subclinical course of HEV infection, and in genotypes 3 and 4 – pigs and small mammals (rabbits, rats, ferrets, shrews) [6, 7]. The virus

Table 1. Main HEV genotypes [5, 6, 7]

Genotype	Affected species	Route of transmission
HEV1	Humans – mainly in the tropical and subtropical areas of Asia and Africa	<i>Anthroponosis</i> : fecal-oral route, blood transfusions, vertical (mother – fetus).
HEV2	Humans – Mexico, Chad, Nigeria	
HEV3	Humans, pigs, wild boars, mongoose, deer, rabbits, horses, clams – Europe, Oceania, Americas)	<i>Zoonanthroponosis</i> : transmission via consumption of infected meat and milk, contaminated water/food; from human to human - close human-human contact, blood transfusion, vertical (mother – fetus).
HEV4	Human, pigs, wild boars, deer, cattle – Asia, Europe.	
HEV5	Wild boars	<i>Zoonosis</i> : animal to animal transmission (fecal-oral, consumption of infected tissues and secretion, contaminated water/food), not detected in humans until now.
HEV6		
HEV7	Camels, genotype 7 can infect humans (rare, usually transplant recipients)	
HEV8		
Other animal HEV	Ferrets, bats, rats, birds, trouts	

cycle of HEV is still being studied, but it is known that HEV enters human cells via binding of its viral protein (ORF2) to a heat-shock protein (HSC70).

HEV is hepatotropic but not cytopathogenic – i.e., the major factor for hepatocyte destruction is *not the virus itself but the host immune response*. It can affect other cells and tissues, including nerve cells, myocardium, kidney, thyroid gland, gut, pancreas, bone marrow, lymphocytes, etc. [1, 2, 3, 5, 6, 7].

Antibodies against HEV have been detected in asymptomatic blood donors – in 6-11%, and in hyperendemic areas (i.e., Toulouse, France) up to 52% of asymptomatic blood donors tested positive for anti-HEV antibodies when using high-sensitive tests [8]. It remains unclear why in asymptomatic hemodialysis patients the prevalence of positive anti-HEV antibodies is particularly high [9].

EPIDEMIOLOGICAL DATA

According to WHO data, approximately 20 million people around the world get infected with HEV every year [10] and this results in approximately 3 million cases of acute viral hepatitis and more than 50 000 deaths. Epidemic outbursts of genotype 1 and 2 are common in the developing countries, due to the lack of fresh water and poor sanitary conditions. Genotypes 1 and 2 show seasonal variations, associated with monsoons and floods. HEV infection in pregnant women represent a major health problem due to the high mortality in this population. In the developed countries type 3 and 4 are the leading genotypes, transmitted usually via consumption of infected meat, milk and subproducts of infected animals (pigs, wild boars, rabbits, deer, etc.), with no clear seasonal variations and comparable mortality I during pregnancy. In certain regions (i.e., France,

Holland, Scotland, Germany, Italy, Czech Republic, etc.) the prevalence of genotype 3 is particularly high, probably due to the zoonanthroponotic character of the disease. The natural reservoirs of genotypes 1 and 2 are humans, and of genotypes 3 and 4 – the animal species described above. The epidemiological characteristics of the different genotypes of HEV are presented in table 2.

CLINICAL MANIFESTATIONS

The clinical manifestations of HEV infection are non-specific, as in all types of acute viral hepatitis [1, 2, 5, 7, 10, 11]. It is a little common in men than in women with equal prevalence across all age groups. Genotypes 1 and 2 are a little more common among younger and genotypes 3 and 4 – among a little older individuals. The incubation period is approximately 2-10 weeks, followed by non-specific constitutional symptoms (mild fever, loss of appetite, nausea, vomiting, weight loss) lasting several days. Rarely abdominal pain and discomfort, skin pruritus, rashes (usually urticarial), joint pain and diarrhoea are reported. In 5-30% of the infected individuals jaundice and darkening of the urine with acholic stools and hepatomegaly may develop [11]. These clinical manifestations last between 1 and 6 weeks and resolve spontaneously. Sometimes the clinical manifestations in genotypes 3 and 4 are difficult to differentiate from drug-induced liver injury, especially if the patient has taken non-steroid anti-inflammatory drugs for the alleviation of the non-specific constitutional symptoms. Chronic HEV infection (lasting more than 6 months) has been described only in genotypes 3 and 4, in which also progression to liver cirrhosis and fulminant cases have been reported. Genotypes 1 and 2 may have malig-

Table 2. Epidemiological and clinical characteristics of HEV infection in the developing and developed (industrial) countries [1, 2, 3, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17]

Characteristics / countries	Developing countries	Developed (industrial) countries
Epidemiological		
Predominant genotypes	HEV 1 & 2	HEV 3 & 4
Source	Infected humans (anthroponosis)	Infected animal products (zooanthroponosis)
Mechanism of transmission	Contaminated food and drinks (fecal-oral)	Consumption of products from infected animals
Transmission via blood products / transplantation	Yes	Yes
Perinatal transmission (vertical)	Yes	Yes
Transmission via breastfeeding	Unknown	Unknown
Prevalence	Different in different countries (dependent on community hygiene?)	Very high (up to 3% in highly endemic areas with high prevalence in animals)
Seasonal variation	Marked (monsoon season, floods)	He
Epidemic outbursts	Yes, thousands of infected individuals during an outburst	No – endemic distribution, sporadic cases, small clusters after consumption of infected animal products
Latent forms	Yes, in approximately half of the infected	Between 67% and 98% of the infected individuals remain asymptomatic
Travel-associated infection	Yes, in tourism to exotic destinations, including developing countries	Yes, especially in culinary tourism
Clinical		
Male:female ratio	2:1	Над 3:1
Age	Mainly young (15-30 years)	Usually > 50 years
Clinical manifestations	Usually, self-limiting acute viral hepatitis, with jaundice	Usually, self-limiting acute viral hepatitis, without jaundice (ANICTERIC), possible chronic course (esp., genotype 3)
Increased mortality during pregnancy	Yes (up to 25% during the third trimester!)	No
Chronic course	No	Yes (genotype 3, rarely genotype 4)
Extrahepatic manifestations	Rare	Common (esp., genotype 3)

nant course with fatal outcome during pregnancy, especially in the third trimester [12, 13]. Chronic and fulminant cases have been also reported in immunocompromised patients and in individuals with other underlying liver disease. One should not forget that the natural reservoir, mechanism of transmission, clinical course and evolution of genotypes 1 and 2 (observed mainly in the developing countries) and genotypes 3 and 4 (observed mainly in the industrial world) show some differences, presented in table 2.

HEV can affect extrahepatic structures, including the nervous and lymphoid tissue and the kidney and could lead to the development of clinical manifestations beyond the liver [1, 2, 5, 7, 10, 11, 12, 13, 14, 15, 16, 17]. The extrahepatic manifestations of HEV infection are mediated by the activation of T- and NK-cells, changes in macrophageal activation and Toll-like receptor mediated immune cell signaling, increased expression of pro-inflammatory cytokines TNF- α , IL-6, IFN- γ , TGF- β 1) and the production of cryoglobulins with subsequent development of small-vessel vasculitis [1, 2, 5, 7, 10, 11, 14, 15, 16, 17]. Extrahepatic manifestations are more com-

mon in genotype 3 infection and in individuals with impaired immune response, including organ transplant recipients [14, 15, 16], and are summarized in table 3.

In pregnant women, the clinical course of HEV (esp. genotypes 1 and 2) can be more severe and lead to the development of acute liver failure and fatal outcome in up to 15-25% of the cases [13]. The underlying mechanisms of the more severe course during pregnancy, especially during the third trimester, is unclear, but altered expression of Toll-like receptors (TLR3 and 7) has been suspected to play a role, and probably the lack of access to healthcare in the developing countries [12, 15].

In immunocompromised patients, underlying chronic liver or neoplastic diseases and malnutrition, HEV infection can be associated with delayed seroconversion, chronic course and re-activation of the virus at the background of suppressed immune response [19, 20]. In kidney transplant patients acute (usually pre-renal) renal failure, glomerular and tubule-interstitial changes and increased rejection risk have been described during the course of HEV infec-

Table 3. Extrahepatic manifestations of HEV infection [1, 2, 5, 7, 10, 11, 14, 15, 16, 17, 18, 19]

Extrahepatic involvement	Manifestations
Neurological	Guillain-Barre syndrome / polyradiculoneuropathy Encephalitis / myelitis Seizures Cerebral pseudotumor Neuralgic amyotrophy / Neuritis (frequent involvement of the vestibular, facial and oculomotor nerve) Mononeuritis multiplex & peripheral neuropathy Myasthenia Myositis
Hematological	Cytopeny (usually thrombocytopeny), hemolytic anemia (esp. in glucose-6-phosphate dehydrogenase [G6PD] deficiency and in ribavirin intake), aplastic anemia and leuko/lymphopenia (immune-mediated) Gammopathy Hemophagocytic syndrome CD30-positive lymphoproliferative disorder
Renal	Cryoglobulinemia Immune-mediated glomerulonephritis: IgA (new onset or exacerbation of existing), membranous, membranoproliferative, rarely – podocytopathy and nephroangiosclerosis Tubulo-interstitial nephritis (esp., drug-induced) Toxic renal damage (tubule-interstitial changes): drug-induced, hemoglobin precipitations in hemolysis due to G6PD-deficiency or ribavirin and bilirubin precipitations in severe liver damage within the tubular lumina Acute and chronic renal failure
Pancreatic	Acute pancreatitis New onset type 1 diabetes mellitus (esp. in chronic hepatitis C patients)
Cardiac	Myocarditis
Rheumatological	Myositis Vasculitis – Henoch-Schoenlein purpura, cryoglobulinemic and necrotizing, thrombotic microangiopathy
Thyroiditis	Subacute (De Quervain's thyroiditis) Autoimmune

tion [16]. In liver transplant patients and individuals with underlying liver disease the course of HEV infection may be protracted and there is increased risk of fulminant hepatitis and transplant rejection [21].

DIAGNOSIS

The diagnosis of HEV is based on serological data (antiviral antibodies of IgM class for acute infection, and of IgG type for past infection, IgA testing is also available) and detection of molecular testing (detection of HEV RNA in blood or stools, possible evaluation of viral load), antigenic tests and immunochemistry tests (not always unequivocally traceable or detectable) are also available [2].

TREATMENT

The treatment of acute HEV infection consists mainly in supportive and general care and isolation of the patient with screening of the contact individuals [19, 20, 21]. Symptomatic treatment to alleviate pruritus and hepatoprotectors are also indicated, and in severe cases, infusions, transfusions of human albumin and plasma [21]. In severe forms ribavirin may be initiated, especially in genotype 3 [21]. In immunocompromised patients the dose of immune-suppressing drugs, if taken, should be decreased

for the period of 3-6 months and antiviral treatment should be initiated [11, 16, 19, 20, 21]. In chronic cases (usually in genotype 3 infection), 12-weeks ribavirin course is indicated or until stable virological response is achieved. One should bear in mind that re-infection is possible, especially in highly endemic areas [20, 21].

In poor ribavirin tolerance or if contraindications are present, a possible alternative of ribavirin are alpha-interferon and sofosbuvir, alone or in combination with ledipasvir or ribavirin [20, 21, 22]. Anti-HEV vaccine is also available, indicated for high-risk populations, especially pregnant women, individuals with underlying chronic liver disease, and children <16 years of age in highly endemic areas [23].

In conclusion, HEV is one of the most common yet underestimated and underdiagnosed causes of acute viral hepatitis worldwide. It is generally benign in course with self-limiting natural evolution, with rare fulminant and chronic cases, especially in immunocompromised individuals. Extrahepatic manifestations are frequent, particularly neurological and renal, and there are therapeutic options for both acute and chronic HEV infection. Therefore, all patients with immune-mediated neurological

and renal diseases of unclear origin and with hepatocytolysis of unexplained cause should be investigated for HEV.

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SHORT COMMUNICATIONS**COMBINING CHEMOTHERAPY WITH IMMUNOTHERAPY
IN ONCOLOGY-HOPES AND BENEFITS****M. Milev***Hospital – Sofia region*

Immunotherapy with checkpoint inhibitors has succeeded chemotherapy in the treatment of many oncological diseases. It is used in melanoma, non-small cell lung cancer, carcinoma of the bladder and urinary tract, head and neck carcinoma, renal cancer, triple negative breast carcinoma, and many other malignancies.

A new milestone in the treatment in the field of antineoplastic treatment is the combination of traditional chemotherapy with immunotherapy (checkpoint inhibitors).

Oncologists hope for an additive effect in the co-administration, because checkpoint inhibitors change the immune response, especially the natural antitumor effects of the immune system.

It is becoming clear that, for example, in T-lymphocytes, antigen presentation together with the absence of suppression allow lymphocyte activation. But theory ends here. Therefore, one might think, that the activated lymphocyte goes into battle with multiple tumor cells and their antigens alone.

In fact, the activated lymphocyte undergoes changes defined as blast transformation. In blast trans-

formation, lymphocytes revert to young forms – lymphoblasts – that actively divide and proliferate (blast transformation of the lymphocyte may be induced not only by antigen but also by nonspecific mitogens).

This mitotic activity of transformed lymphocytes can be examined with a blast transformation assay to assess the immune status of the patient.

Another method of testing- of mixed lymphocyte cultures (now of historical importance) serves to determine HLA – compatibility (when mixing T – lymphocytes from a donor and a potential recipient in compatibility there is no blast transformation, in the absence of compatibility there is). The above shows that blast transformation is something, which has been known for a long time and used in immunology.

But regarding the combination of chemotherapy with immunotherapy (checkpoint inhibitors) it is logical to assume that chemotherapy, with its effects on intensely dividing cells (including activated lymphocytes undergoing blast transformation) will suppress the effect of immunotherapy.

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