The role of genetic, immunological and environmental factors in the pathogenesis and outcome of hepatitis C vírus (HCV) infection

Hepatitis C virus (HCV) is a major global health problem: 170 million subjects are suffering from HCV infection worldwide. About 80 % of HCV-patients have chronic hepatitis and cirrhosis develops in 20% of them, representing the most frequent indication of liver transplantation. The *outcome of HCV infection is variable* from spontaneous viral clearance or symptomfree HCV carrier state to hepatitis, cirrhosis and hepatocellular carcinoma: while some individuals have liver disease of *rapid progression*, others remain *„healthy" HCV carriers*. It is not completely understood what may be the *exact cause of the different outcomes,* but presumably partly direct viral cytopathy, partly genetically determined immune mechanisms and environmental factors are responsible.

Since strong innnate and adaptive *immune responses, natural killer (NK) and T-cell reactions* are essential for both the clearance of HCV and the virus-induced tissue damage, *genetic factors* that govern immune reactions may be of importance in the pathogenesis. In addition, *environmental* factors are also capable of modifying the host's response.

We aimed to study some of these factors, in different forms of chronic HCV infection, investigating symptomfree HCV carriers with persistently normal alanine amino-transferase, patients with chronic hepatitis C, (among them responders and non-responders to anti-HCV interferon therapy) and patients with HCV cirrhosis. We wanted to elucidate the potential causes which may determine the differences between the above mentioned groups of HCV-infected individuals, therefore we examine simultaneously as follows 1) genetic polymorphism of certain cytokine genes, 2) features of peripheral blood natural killer (NK) and T cell functions and 3) role of viral co-infection, that is the frequency and effects of simultaneous hepatitis B virus (HBV), hepatitis G virus (HGC), TT virus (TTV) and SEN virus (SENV) infections.

Identifying correlation between *specific gene polymorphisms* and the *course of HCV infectio*n, then finding relationship between these and the *immune response,* may contribute to the better understanding of the pathogenesis of chronic hepatitis C, and improve our knowledge regarding the prognosis of disease, and can even be useful in development of appropriate therapeutic strategies to control HCV infection.

Genetic studies

Cytokine (IL-10R, IL-28B, LT-A) gene polymorphysms as pedictors of antiviral treatment in HCV infection. Preliminary studies.

Since the clearance of hepatitis C virus (HCV) infection depends on the cytokines which are under genetic control, we have studied genetic polymorphisms of two pro-inflammatory interleukin-28B (IL-28B) (also named as Interferonλ-3) and lymphotoxin A (LTA) as well as of one anti-inflammatory cytokine interleukin-10 (IL-10) genes in patients with HCV infection. We examined the allel frequencies of these genes in HCV patients as compared with healthy controls, and determined their association with sustained virological response (SVR) on PEG-IFN alfa-2a + ribavirin (RBV) (P/R) treatment, to assess the predictive value of these genetic variants. A total of 292 chronic HCV genotype 1 infected patients and 104 healthy controls have been studied. The samples were genotyped using PCR-RFLP and ABI Taqman genotyping assay.

Results: ***IL-28B*****:** the C/C genotype in HCV patients occurred with lower frequency than in healthy controls (28,11% vs 51.92%, p = 0.0001), suggesting a protective role of this variant. At the same time, P/R treated patients with this C/C genotype achieved SVR in higher rate, than those who have TT genotype (54.34% vs 29.16%, p= 0.0447)
LTA A252G: the frequency of *A/A* genotype did not differ between HCV patients and controls, but *G/G homozygosity* was found reduced rate in non-treated subgroup of HCV patients as compared to controls (2.91% vs 9.90%, p = 0.041) The G/G genotype seemed to be a predictor of SVR vs A/A genotype: SVR occurred in G/G pts 54,54% vs 44.94% in AA cases (not significant, NS).
IL-10R 1087: the *G/G* genotype in HCV patients occcurred with lower frequency than in controls (37.15% vs 52,74%, p =0.00957, OD 1.89). G/G harboring patients showed higher SVR than patients with A/A genotypes (41.26% vs 28.57%) (NS).
Conclusion*: we have found that IL-28B C/C genotype was a protective genetic variant and a predictor of SVR in chronic HCV infection. Furthermore, our data suggest that presumable predictors may also be both IL-10 1087 and LTA A252G gene polymorfisms.*

Both IL28B CC and IL10R -1087 GG polymorphisms are protective in chronic HCV1 infection: a nationwide multicentric study in Hungary.
Background: Since earlier we have found that not only IL28B but IL10R -1087 gene polymorphisms may also play a role in HCV infection, we have initiated further investigations with higher number of patients to support our preliminary results
Patients and Methods: A multicentric national study was initiated and performed, in which the allele frequencies of these genetic variants in HCV1 infection were compared to that of healthy controls, and the relationship between the polymorphisms and the response to P/R was examined. A total of 760 chronic HCV genotype 1 patients have been enrolled. Out of them 454 were treated with P/R for 24-72 weeks, and 195 patients (42.95%) achieved sustained virological response (SVR). One hundred and four healthy individuals served as controls. DNA was isolated from peripheral blood by standard desalting method, and the samples were genotyped using PCR-RFLP and ABI Taqman genotyping assay.
Results: IL28B (rs12979860) CC genotype in HCV1 patients occurred with lower frequency than in healthy controls (27.30% vs 51.92%, $p<0.05$, OR 0.36). P/R treated patients with the CC genotype achieved SVR in higher rate, than those who have TT alleles ($p< 0.05$). IL10R -1087 (rs1800896) GG genotype in HCV1 patients was found with significantly lower frequency than in controls ($p<0.05$) suggesting its favourable effect in HCV1 infection. Patients having both IL28B CC and IL10 -1087 GG genotypes achieved 78.12% SVR, raising a potential synergy between these genetic variants.
Conclusion: The results confirmed our original findings that similar to IL28B CC, IL10R -1087 GG gene may also be protective against chronic HCV1 infection.

Immunological studies

IL28B CC genotype is associated with increased LSP induced Th1 type cytokine production by peripheral blood mononuclear cells in chronic HCV-1 infection.
Aim: IL28B CC genotype is known to be the strongest pretreatment predictor of sustained virological response (SVR) in HCV genotype 1 patients treated with IFN. IL28B CC variants also associated with improved early viral kinetics and greater likelihood of rapid virological response (RVR) compared with CT or TT genotypes. We compared the IFN-γ, TNF-α, IL-2, IL-4, IL-6 and IL-10 cytokine production by peripheral blood monocytes (PBMC) and lymphocytes in HCV-1 patients with IL28B CC, CT and TT genotypes.

Methods: Fourty HCV-1 patients were genotyped as CC (n=12), CT (n=20) or TT (n=8) at polymorphic site of IL28B rs12979860. IFN-γ, TNF-α, IL-2, IL-4, IL-6 and IL-10 production of LPS stimulated PBMCs and PMA+ionomycine stimulated lymphocytes were determined by FACS-CBA assay in each genotype group.

Results: LPS induced TLR4 activation of the monocytes resulted in significantly higher TNF-α, production in patients with CC genotype compared to CT and TT variants (2,04 ng/ml vs. 0,76 and 0,59 ng/ml). In patients with CC genotype, increased Th1 type cytokine production of peripheral blood lymphocytes was found compared to non-CC genotype groups. Lymphocyte TNF-α, IL-2, IFN-γ production were significantly higher in CC patients compared to CT and TT groups (CC: TNF-α, 14,6 ng/ml, IL2: 156,9 ng/ml, IFN-γ, 225,7 ng/ml, CT: TNF-α, 7,1 ng/ml, IL2: 40,5 ng/ml, IFN-γ 94,8 ng/ml, TT: TNF-α 6,9 ng/ml, IL2: 37,9 ng/ml, IFN-γ 109,2 ng/ml $p<0,01$). IL-4, IL-6 and IL-10 production by PMNCs and lymphocytes did not differ between study groups.

Conclusion: *HCV-1 infected patients with IL28B CC genotype had significantly increased LPS induced TNF-α production by monocytes, and increased TNF-α, IL-2 and IFN-γ production by lymphocytes compared to patients with CT or TT variants. We suppose that in IL28CC variants the increased inducible antiviral Th1 type cytokine production may play a crucial role in the rapid immune control of infection.*

Rapid virological response to peginterferon plus ribavirin therapy is associated with increased baseline proinflammatory cytokine production in chronic hepatitis C

Background: Chronic hepatitis C (CHC) patients achieving rapid virological response (RVR) on PEG-IFN/ribavirin (P/R) therapy have high chance of sustained virological response (SVR). To analyze host immunological factors associated with RVR, viral kinetics, phenotype distribution and Th1/Th2 cytokine production by peripheral blood mononuclear cells (PBMC) were studied prior to and during P/R therapy.

Methods: TNF-α, IFN-γ, IL-2, IL-6, IL-4 and IL-10 production by PBMC were measured after Toll-like receptor 4 (TLR-4) or phorbol myristate acetate /Ionomycin stimulation in 20 healthy controls and in 50 CHC patients before receiving and during P/R therapy. RVR was achieved by 14, complete early virological response (cEVR) by 19 patients and 17 patients were null-responders (NR).

Results: Patients with RVR showed an increased baseline TNF-α and IL-6 production by TLR-4 activated monocytes and increased IFN-γ, decreased IL-4 and IL-10 production by lymphocytes compared to non-RVR patients. SVR was also associated with increased baseline TNF-α production and decreased IL-10 levels compared to patients who did not achieve SVR. Baseline IL-2 production was higher in cEVR compared to NR patients. Antiviral treatment increased TNF-α, IL-6 production by monocytes and IFN-γ secretion by lymphocytes and decreased IL-4 and IL-10 production by lymphocytes in cEVR compared to NR patients.

Conclusion*: RVR was associated with increased baseline proinflammatory cytokine production by TLR-4 stimulated monocytes and by activated lymphocytes. In null-responders and in patients who did not achieve SVR both TLR-4 sensing function and proinflammatory cytokine production were impaired, suggesting that modulation of TLR activity and controlled induction of inflammatory cytokine production may provide further therapeutic strategy for CHC patients non-responding to P/R treatment.*

Altered expression of inhibitory KIR2DL3, KIR3DL1 and activating CD160, NKG2C and NKG2D receptors and increased proinflammatory cytokine production of NK cells in chronic hepatitis C virus (HCV) infection

Background: As the role of natural killer (NK) cell activity in chronic HCV infection is controversial, we analysed different killer inhibitory and activatory receptor expression, spontaneous cytotoxic activity and cytokine production of activated NK and cytotoxic T cells in HCV patients. The effect of TGF-beta1 on NK cytotoxicity, KIR/KAR expression was also studied.

Methods: Twenty one patients with chronic hepatitis C (CHC), 11 HCV RNA positive patients with normal serum alanine aminotrasferase (ALT), 15 healthy controls were enrolled. The percentage of peripheral regulatory T, CD56dim+/CD56bright+ NK cells, CD8+ cells, KIR2DL3, ILT-2, KIR3DL1, CD160, NKG2D, NKG2C expression, spontaneous NK cytotoxicity were determined by FACS. IL-2, IL-4, IL-5, IL-10, TNF-alpha, IFN-gamma production of separated NK and T cells were determined by FACS-CBA assay and plasma TGF-beta1 levels by ELISA, respectively.

***Results*:** In chronic HCV hepatitis, NK cells showed increased KIR2DL3, NKG2C, decreased CD160, NKG2D, KIR3DL1 expression compared to controls. Decreased expression of CD160, NKG2D, NKG2C activatory receptors on CD8+ T cells was also found in CHC, but not in HCV+ patients with normal ALT. TGF-beta1 levels inversely correlated with NKG2D expression. In vitro TGF-beta1 inhibited NK cytotoxicity and NKG2D expression. While impaired spontaneuos NK cytotoxicity in CHC was associated with decreased percentage of peripheral CD56dim+ NK cells, in vitro activated NK cells IL-10, TNF-alpha and IFN-gamma production was enhanced in CHC patients. NK cell and CD8+ cell IL-4, IL-10, TNF-alpha production were significantly higher in HCV RNA+ patients with normal ALT compared to HCV hepatitis patients.

Conclusion: *We demonstrated complex dysregulation of killer activatory and inhibitory receptor expression on NK and cytotoxic T cells in HCV infection. In chronic HCV hepatitis NK cells proinflammatory cytokine production was significantly enhanced. Chronic HCV infection with normal ALT was associated with increased IL-4, TNF-alpha production of NK cells and increased IL-10 production of cytotoxic T cells, suggesting that the cytokine production of these cells might play important role in the pathogenesis of liver inflammation.*

Impaired STAT4 phosphorylation is associated with non-response to PEG-IFN plus ribavirin treatment in chronic HCV hepatitis patients

Aim: To investigate the underlying molecular mechanisms of non-response to PEG-IFN plus ribavirin (P/R) treatment we compared IFN-alfa-induced STAT4/STAT6 signaling pathways in responder and non responder HCV patients treated with P/R.

Methods*:* Peripheral blood mononuclear cells (PBMC) from HCV patients and controls were separated and treated with IFN-alfa2b in vitro. Lysates of PBMC were subjected to Western blotting to detect phosphorylated STAT4 and STAT6 transcription factors. Th1/Th2 cytokine production was also detected by CBA assay from the supernatants of the stimulated PBMC.

Results: the samples of responders showed strong STAT4 phosphorylation and increased Th1 cytokine production to in vitro IFN-alfa2b stimulation, as compared to controls and non responders. IFN-induced phosphorylation of STAT4 was reduced in non-responders compared to controls. STAT6 transcription factor activation did not differ between study groups.

Conclusion: *the differences of IFN induced signal transduction pathways between responders and non-responders to P/R treatment in HCV patients, may provide a rationale for the further design and use of new therapeutic approaches targeting the signal transduction pathways in HCV treatment.*

Increased cytotoxic potential of CD160 receptor positive natural immune cells in chronic HCV infection

Background/Aims: Since innate immune cells are uniquely enriched in the inflammatory cell infiltrate of HCV infected liver, the importance of these innate cells in the pathogenesis of liver inflammation emerges. Altered natural killer (NK) cell activity in chronic HCV infection is still controversial, since both impaired and intact NK cell function have been described. Activation of CD160 receptor causes strong cytotoxic activity and Th-1 type pro-inflammatory cytokines production in NK cells. Very little information is currently available about the expression pattern and function of CD160 receptor in innate immune cells during chronic HCV infection. In our study we analysed cytotoxic characters of different CD160 receptor positive (CD160+) natural immune cells during chronic HCV infection.

Methods: We investigated the expression of Fas molecule (a marker of susceptibility to apoptosis) and TIM3 (a marker of Th1 phenotype) and intracellular cytotoxic granule content (perforin, granzyme) of peripheral blood CD160+ gamma/delta T cells, NK cells and invariant NKT (iNKT) cells by Flow Cytometry in 10 patients with chronic HCV hepatitis and in 10 healthy controls.

Results: TIM3 expression and cytolytic granule content of CD160+ gamma/delta T cells were significanty enhanced in chronic HCV infection compred to controls. The CD160+ iNKT cells had also increased TIM3 expression but did not show elevated cytotoxic potential. The perforin expression of the CD160+ NK cells was also significantly higher in chronic HCV hepatitis compared to helathy controls. HCV infection was associated with elevated expression of Fas molecule on CD160+ iNKT lymphocytes, but the susceptibility to apoptosis of CD160+ gamma/delta T cells and CD160+ NK cells did not differ from control.

Conclusion*: Our results suggest that chronic HCV hepatitis is associated with increased cytotoxic granule content of CD160+ cells. These innate immune cells infiltrating the liver might induce inflammatory cytotoxic response and contribute to the pathogenesis of chronic HCV hepatitis. Therapeutic strategies modifying innate immune cell function may be of benefit for limiting intrahepatic inflammatory process and liver injury.*

Non invasive fibrosis assessement in chronic HCV infection

Non-invasive diagnosis of fibrosis in chronic hepatitis C: Wai's aspartate-aminotransferase to platelet ratio index (APRI), procollagen-III pepetide (P-III-P) serum hyaluronic acid (HA), plasma transforming growth factor beta (TGFb) level and liver stiffness (LS) measurement using transient elastography (TE).

Background: Although liver biopsy is the gold standard for the morphological diagnosis of the liver disease, recently noninvasive tests may also play a role in the evaluation of liver fibrosis. We have studied blood fibrosis markers and liver stiffness (LS) measurement to assess fibrosis in different forms of chronic hepatitis C virus (HCV) infection.

Patients and methods: out of 119 HCV-infected patients 75 had biopsy-proven chronic hepatitis C, 24 had HCV-cirrhosis, 20 individuals were symptomfree HCV-carriers with persistently normal alanine aminotransferase (PNA) and 30 healthy blood donors served as controls. Wai's aspartate-aminotransferase to platelet ratio index (APRI) was calculated from serum aspartate-aminotransferase (AST) and blood platelet number. Serum HCV-RNA, procollagen-III-peptide (P-III-P) and hyaluronic acid (HA), plasma transforming growth factor β-1 (TGFβ-1), Knodell's histological activity index (HAI) and METAVIR fibrosis score were determined, for LS measurement transient elastography (TE) (FibroScan) was applied.

Results: In chronic hepatitis C patients all fibrosis markers were significantly elevated compared with normal controls. HA, APRI and LS values were highest in HCV-cirrhosis and non-responders to PEG-IFN + ribavirin (P/R) therapy. High P-III-P values occurred only in advanced fibrosis. Serum HA correlated with fibrosis stage. Plasma TGFβ-1 was significantly higher in patients with chronic hepatitis C than in symptomfree HCV-carriers, and it correlated with HAI. After 3-6 months P/R therapy, both HA and TGFβ-1 levels significantly decreased even in virological non-responders. APRI was 0.21±0.05 in normal controls, 0.20±0.08 in symptomfree HCV-carriers, 0.70±0.40 in patients with chronic hepatitis C and 3.21± 0.30 in HCV-cirrhosis. LS values were 5.10±1.19 kPa in controls, 5.38±1.37 kPa in HCV-carriers with PNA, 9.67±4.11 kPa in chronic hepatitis C, 6.56±2.61 kPa in patients with sustained virological response, 18.55±11.65 kPa in non-responders to P/R and 37.40±16.85 kPa in HCV-cirrhosis. Using a novel sequential algorithm that comprises APRI and LS for assessment of fibrosis, 47% of our HCV patients did not need biopsy for diagnosing significant (F≥2) fibrosis.
Conclusion: both blood fibrosis markers and LS, particularly in combination, represent an advance in the noninvasive assessment of fibrosis in HCV infection.

Transient elastography reveals regression of fibrosis in chronic HCV patients with sustained virological response (SVR)
Background/Aim: Several data suggest that liver fibrosis is a dynamic process and fibrosis or even early stage of cirrhosis is not irreversible and cellular recovery is possible. Aim of our study was to investigate the effect of pegylated interferon alpha plus ribavirin (P/R) treatment on liver fibrosis progression in patients with chronic HCV hepatitis (CHC).
Patients and methods: We performed liver stiffness (LS) measurements by FibroScan (Echosens) on 30 CHC patients before and 24 weeks after the end of treatment (EOT +24). We also followed-up further 15 CHC patients who were not eligible for treatment (because of older age or contraindications), in these cases minimum 24 months elapsed between their first and second LS measurements.
Results: Patients who later achieved sustained virological response (SVR) had lower LS values at baseline compared to patients who became non-responders (9,44±0,8 kPa vs 15,1±2,56 kPa, p<0,01). P/R therapy caused significant reduction of LS at EOT+24, compared to baseline values in SVR group (6,86±0,5 kPa vs 9,44±0,8 kPa, p<0,01), but not in non-responders (13,5±2,47 kPa vs 15,1±2,46 kPa, NS). Out of patients with SVR 68 % (15/22) had at least one-point drop reduction in fibrosis score and 32 % (7/22) had at least two point drop reduction of fibrosis score at EOT+24 compared to baseline values. Fibrosis regression was less common in non-responders, 62,5 % (5/8) had no change, 25 % (2/8) had one point drop reduction of fibrosis score at EOT+24. In untreated HCV patients LS values increased during follow-up from 18,86 ±1,9 kPa to 25,87±2,8 kPa (p<0,05).
Conclusion*: Our data suggest that P/R therapy inhibits liver fibrosis in chronic HCV infection. SVR was associated with significant fibrosis regression and even cirrhosis regression assessed by transient elastography, thus, the achievement of viral clearance will reduce long term liver-related morbidity in these patients.*

Transient elastography as a predictor of oesophageal varices in chronic liver disease.
Background: As one of the most serious complications of liver cirrhosis is the variceal bleeding, the early recognition of the oesophageal varices is of primary importance in its bleeding. Endoscopy is the only means to directly visualize varices and measure their size, as one of the most important predictor of the risk of bleeding. During the course of cirrhosis even repeated oesophago-gastro-bulboscopy examinations are recommended.

However, these interventions are costly and often poorly accepted by patients. Thus, there is a need for non invasive methods able to predict the progression of portal hypertension as well as the presence of oesophageal varices. It was suggested that liver stiffness (LS) measured by transient elastograpy (TE), a novel non-invasive technology may reflect not only the fibrosis and portal pressure but even predict the presence or absence of large oesophageal varices.
Patients and Methods: We studied the diagnostic accuracy of TE using FibroScan for selecting patients at risk of bearing large (Paquet-grade ≥ II) oesophageal varices. We paralelly performed upper tract endoscopy and TE examination in 74 patients with chronic liver disease, 27 patients suffered from chronic hepatitis and 47 from cirrhosis. The correlation between the presence of oesophageal varices (Paquet-grade 0-IV) and the LS (kPa) and the blood hematological and biochemical parameters (INR, platelet count, ALT, AST, albumin, and aspartate aminotransferase / platelet ratio index, APRI score) was investigated. We analysed the predictive role of LS for selecting patients with varices and who are at high risk of variceal bleeding.
Results: LS values correlated to the grade of oesophageal varices (Paquet–grade) (r= 0,67, p<0,0001). The LS of **19.2 kPa value** was highly predictive of the presence of oesophageal varices and allowed to predict the high grade varices (P≥II) LS value <19,2 kPa was highly predicitive of the absence of large (P≥II) varices (sensitivity: 95%, specificity: 70%, PPV:54%, NPV: 97%), and when LS <19,2 kPa, the presence of large varices is not probable.
Conclusion: *TE may help to select patients who are at high risk of bearing large (P≥II) oesophageal varices and variceal bleeding and need endoscopic screening. LS of above 19,2 kPa indicates an oesophageal-gastro-buboscopy for the judgement of varices.*

Virological studies

Co-infections with torque teno viruses (TTV and SENV) and HBV in patients with chronic hepatitis C virus (HCV) infection
The potential role of co-infections with novel hepatitis viruses such as torque teno viruses (SENV and TTV) is not clear. We determined the prevalence and clinical significance of these agents in different forms of chronic HCV infection. In addition, we evaluated the frequency of serological markers of hepatitis B virus (HBV) infection as well.
Patients: A total of 365 HCV infected patients (pts) have been investigated. Out of them 33 were symptomfree HCV carriers with persistently normal alanine aminotransferase (ALT), 97 suffered from chronic hepatitis C not treated with interferon (IFN)-based therapy, 48 pts had chronic hepatitis C and have been treated with IFN + ribavirin (RBV) for one year, 76 pts previously treated with IFN+RBV who achieved SVR and 111 pts were non-responders to anti-HCV treatment. One hundred and eight healthy volunters served as controls for TTV studies and 40 for SENV investigations.
Methods: HBsAg, anti-HBc, anti-HBs and anti-HCV were detected using ELISA, HCV RNA TTV DNA, SENV-D DNA and SENV-H DNA by PCR method.
Results: 10.9 % of HCV patients had previous HBV hepatitis (based on anti-HBc + anti-HBs), 8.5% had been successfully vaccinated (anti-HBs) and 11.6 % might have occult HBV infection (anti-HBc). Prevalance of both SENV-D and SENV-H as well as TTV DNA was higher in HCV pts than in healthy controls. Both SENV and TTV carriers have lower serum ALT and higher HCV RNA levels than SENV negative and TTV negative patients suggesting an immune suppressive effect of the co-infection. IFN+RBV treated pts with SVR, after the therapy showed significantly lower frequency of SENV-D (10.5%) and TTV (3.9%) than not treated counterparts. SENV-D carrier HCV pts achieved lower SVR (33%) than SENV-D negative ones (45%).

Conclusion*: Torque teno viruses can be frequent "accidental tourists" in HCV infection. They may interfere with HCV, but their role in the liver injury is questionable. SENV-D and TTV may be IFN sensitive agents. Whether SENV-D is a negative predictor when treating HCV hepatitis with IFN/RBV needs further investigation. Co-infection with HBV may occur in about 20 % of HCV.*

Molecular epidemiology of hepatitis C virus genotypes and subtypes among injecting drug users in Hungary

We determined the distribution of hepatitis C virus genotypes/subtypes among people who inject drugs (PWID).

Patients and Methods: Of the 2,133 PWID tested, 509 proved to be positive for anti-HCV antibodies. Viral RNA was detected in 65% of 323 HCV antibody-positive samples that were available for PCR analysis.

Results: Among those 198 PWID, 74.2% were infected with genotype 1, 22.7% with genotype 3, and 3.0% with genotype 4. Of the HCV RNA-positive patients from the general population sample, 96.6%, 2.2% and 1.1% carried genotype 1, 3 or 4, respectively.

The prevalence of genotype 3 among PWID was significantly higher than in the general population ($p<0.001$). The frequency of genotype 1 was significantly lower ($p<0.001$) among PWID, while that of genotype 4 was similar in both groups. Subtype 1a was significantly more frequent among PWID than in the general population ($p<0.001$).

Genotype 3 was significantly more prevalent among those who had been injecting drug for a longer period of more than five years or belonged to older age groups (25–34 and >34 years), than among those who had started injecting more recently (less than five years before testing) or belonged to younger age groups.

After data were adjusted to age and geographical distribution, the difference in the prevalence of genotype 3 HCV remained significant between PWID and the general population ($p<0.001$). Multivariable logistic regression confirmed that among PWID, genotype 3 were significantly more prevalent in provincial towns than in Budapest after the data were adjusted to age (≤34 or >34 years) and duration of drug use ($p<0.001$). Phylogenetic analysis of the NS5B regions revealed that the HCV genotype 3 sequences of Hungarian PWID did not form a separate clade, but certain sequences were grouped together, forming at least three subclusters.

Conclusions: *When determining the distribution of HCV genotypes/subtypes among people who inject drugs (PWID) in Hungary, the prevalence of genotype 3 among PWID was found to be significantly higher than in the general population ($p<0.001$). The frequency of genotype 1 was significantly lower ($p<0.001$) among PWID, while that of genotype 4 was similar in both groups. The abundance of genotype 3 and subtype 1a among PWID was in accordance with data on PWID in industrialised nations worldwide. Since genotype 3 and subtype 1a are rare in the general Hungarian population, we assume that Hungary is involved in the worldwide epidemic of these genotypes among PWID.*

Summary

The knowledge of all these – though arbitrarily chosen, but interrelated - genetic, immunological and environmental factors may have of significance concerning the pathogenesis of HCV infection. The findings may improve our understanding of the differences in the outcome of HCV infection and can even help to identify those patients who are most likely to progress and who need antiviral treatment most urgently.

Közlemények

Papp M, Norman GL, Vitalis Zs, Tornai I, Altorjai I, Földi I, Udvardy M, Pár A, Lakatos L.: Presence of antimicrobial antibodies in liver cirrhosis - A tell-tale sign of compromised immunity? **Plos ONE 2010. 5, 1-9 e12957 doi: 10.1371.**

Altorjay I, Vitalis Zs, Tornai I, Palatka K, Pár A, Pár G. Papp M: Mannose-binding lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients with liver cirrhosis. **J. Hepatol. 2010. 53, 484-491.**

Pár A, Kisfali P, Melegh B, Tornai I, Gervain J, Szalay F, Hunyady B, Vincze Á, Pár G: Cytokine (IL-10, IL28B and LT-A) gene polymorphisms in chronic hepatitis C virus infection. **Clin. and Exp. Med. J.** 2010. 4. 1-11 Doi:10.11556/CEMED.4.2010.2.2

Pár A: Hepatitis C vírus (HCV) infekció - 20 év után. Orv. Hetil. 2010, 151 485-493.

Pár A. A hepatitis C- virus (HCV) infekció kezelésének új lehetőségei a horizonton: HCV-specifikus célzott antiviralis terápia. Orv. Hetil. 2010, 151, 2045-2056.

Pár A, Pár G: Nem invazív fibrosisdiagnosztika krónikus C-hepatitisben: aszpartát-aminotranszferáz/thrombocyta hányadosindex (APR) és tranziens elasztográfia (FibroScan). Orv. Hetil., 2010, 151, 1951-1955.

Pár A: Genetikai polimorfizmusok mint a terápiás válasz prediktorai hepatitis C vírus fertőzésben. Orv. Hetil. 2011, 152, 876-881.

Pár G, Vincze Á Berki T, Miseta A, Kereskai L, Pajor L, Oszter A, Jakab F, Szereday Z, Nagy Á, Hunyady B, Pár A: Serum fibrosis markers (procollagen-III-peptide, hyaluronic acid, transforming growth factor-béta-1), aspartate-aminotransferase to platelet ratio index (APRI) and transient elastography (FibroScan) in patients with chronic HCV infection. **Clin. and Exp. Med. J. 2011; 5: 101-118.**

Hunyady B, Gervain J, Horváth G, Makara M, Pár A, Szalay F, Tornai I, Telegdy L, Újhelyi E: C-vírus-hepatitis diagnosztikája, antiviralis kezelése, kezelés utáni gondozása. Szakmai konszenzusajánlás Magy. Belorv.Arch. 2012. 45: 214-234.

Pár G, Szereday L, Berki T, Palinkas L, Halász M, Miseta A, Hegedüs G, J. Szekeres-Bartho, Vincze A, Hunyady B, Pár A. Increased baseline proinflammatory cytokine production in chronic hepatitis C patients with rapid virological response to peginterferon plus ribavirin **PLos ONE 8 (7) e67770. doi:10.1371/journal.pone.0067770**

Pár A, Pár G, Tornai I, Szalay F, Várszegi D, Fráter E, Papp M, Lengyel G, Fehér J, Varga M, Gervain J, Schuller J, Nemes Zs, Péterfi Z, Tusnádi A, Hunyady B, Haragh A, Szinku Zs, Pálinkás L, Berki T, Vincze Á, Kisfali P, Melegh B: IL28B CC genotípus: védő tényező és az interferonválasz prediktora krónikus hepatitis C vírus infekcióban. Orv. Hetil 2013; 154: 1261-1268.

Tresó B, Takács M, Dencs Á, Dudás M, Pár A, Rusvai E: Molecular epidemiology of hepatitis C virus genotypes and subtypes among injecting drug users in Hungary **Eurosurveillance. 2013, 18** (47): pil=20639. **IF: 5.49**

Pár G., Trosits A, Pakodi F, Szabó I, Czimmer J, Illés A, Gódi Sz., Bajor J, Sarlós P, Kenyeres P, Miseta A.,Vincze Áron, Pár A.: Tranziens elasztográfia mint a nyelőcső-varicositás prediktora cirrhosisos betegekben. Orv. Hetil. 2014; 155: 269-76.

Pár A, Pár G, Tornai I, Szalay F, Várszegi D, Fráter E, Papp M, Lengyel G, Fehér J, Varga M, Gervain J, Schuller J, Nemes Zs, Péterfi Z, Tusnádi A, Hunyady B, Haragh A, Szinku Zs, Pálinkás L, Berki T, Vincze Á, Szereday L, Kisfali P, Melegh B: IL28B and Il10R -1087 polymorphisms are protective for chronic genotype 1 HCV infection and predictors of response to interferon-based therapy in an East-Central European cohort.
BMC Research Notes 2014, 7: 12

Tornai I, Bányai T, Gervain J, Horváth G, Makara M, Martyin T, Nemes Zs, Pár A, Pár G, Péterfi Z, Szalay F, Szinku Zs, Tóth T, Vincze Á, Hunyady B: Előrehaladott májbetegséggel járó krónikus C vírushepatitises betegek kezelésével nyert tapasztalataink a telaprevir korai hozzáférési program keretében: interim analizis. LAM. 2014; 24: 35-41.

Hunyady B., Gervain J, Horváth G, Makara M, Pár A, Szalay F, Telegdy L, Tornai I: A C-vírus hepatitis diagnosztikája, antiviralis kezelése, kezelés utáni gondozása. Magyar konszenzusajánlás. Orv. Hetil. 2014; 155: suppl. 2. 3-24.

Lengyel G, Gervain J, *Pár G,* Szalay F, Hunyady B, Schneider F, Nemesánszky E, Tusnádi A, Bányai T, Gyulay K, Tornai I. Real-world virologic response rates and prediction of outcomes with peginterferon alfa-a/ ribavirin in Hungarian HCV genotype 1 patients.
Gastroenterol Pancreatol Liver Disord. 2014. 1 (4): 1-8.

Hunyady B, Gerlei Zs,, Gervain J, Horváth G, Lengyel G, *Pár A,* Rókusz L, Szalay F, Telegdy L, Tornai I, Werling K, Makara M. Hepatitis C vírus fertőzés: diagnosztika, antivirális terápia, kezelés utáni gondozás. Magyar Konszenzusajánlás. Orv. Hetil. 2015; 156, (9) 343-351.

Idézhető absztraktok

Pár G, Vincze Á, Berki T, Miseta A, Pajor L, Nagy Á, Hunyady B, Pár A. Fibrosis markers (serum hyaluronic acid, procollagen-III-peptide, plasma tranforming factor-beta) and transient elastography in chronic HCV infection**.** 52-nd Annual Meeting of the Hungarian Society of Gastroenterology, June 12-15, 2010, Tihany. **Z. Gastroenterol. 2010, 48, 611.**

Pár A, Kisfali P, Melegh B, Miseta A, Tornai I, Hunyady B, Vincze Á, Pár G: Genetic polymorphisms in IL-10R, IL-28B and LTA genes in HCV infection. Do they have protective role and predict sustained virological response? 45-th Annual Meeting of the EASL, April 14-18, 2010. Vienna

J Hepatol. 2010, 52. S457.
Papp M, Vitalis Z, Tornai I, Palatka K, Par G, Pár A, Szalay F, Lakatos PL: Mannose-binding lectin deficiency confers risk for infections in a large hungarian cohort of pastients with liver cirrhosis. 45-th Annual Meeting of the EASL, April 14-18, 2010. Vienna
J. Hepatol. 2010, 52. S79.

Pár G, Vincze Á, Pajor L, Miseta A, Hunyady B, Pár A.: Non-invasive fibrosis assessment in chronic hepatitis C: FibroScan is better than APRI score and hyaluronic acid for identifying liver fibrosis. 21-st Confrence of the Asian Pacific Association for the Study of the Liver 17-20 February 2011, Bangkok, **Hepatology International. 2011; 5: 310.**

Pár G, Berki T, Pálinkás L, Kisfali P, Melegh B, Szereday L, Hunyady B, Vincze Á, Pár A: Association between IL28B CC genotype and increased TNF-alfa and IL-2 production of peripheral bood mononuclear cells in patients with chronic hepatitis C virus (HCV) infection. Magyar Gasztroenterológiai Társaság 53. Nagygyűlése Tihany, 2011. jun.4-7.
Z. Gastroenterol. 2011; 49: 654.

Pár A, Kisfali P, Tornai I, Szalay F, Frater E, Papp M, Lengyel G, Fehér J, Varga M, Gervain J, Schuller J, Nemes Zs, Péterfi Z, Tusnádi A, Hunyady B, Harag A, Szinku Zs, Vincze Á, Pár G, Melegh B: Both IL28B CC and IL10R -1087 GG polymorphisms are protective in chronic HCV1 infection. A nationwide multicentric study in Hungary. 29-th United European Gastroenterology Week (UEGW) Stockholm Oct. 22-26. 2011. **Gut 2011; 60: suppl. 3 A58.**

Pár G, Berki T, Palinkas L, Szereday L, Hunyady B, Vincze Á, Pár A, Melegh B: Association between IL28B CC genotype and increased TNF-alfa and IL-2 production of peripheral blood mononuclear cells in patients with chronic hepatitis C virus (HCV) infection.
62-nd Annual Meeting of the American Association for the Study of the liver Diseases (AASLD). San Francisco 2011. November 4-8. **Hepatology 2011; 54: Suppl. 1329A.**

Par G, Berki T, Palinkas L, Szereday L, Kisfali P, Hunyady B, Vincze Á, Par A, Melegh B: IL28B CC genotype is associated with increased LSP induced Th1 type cytokine production by peripheral blood mononuclear cells in patients with chronic HCV-1 infection.
Gut. 2012; 61. (suppl.3): A228.

Halasz M, Szereday L, Berki T, Palinkás L, Vincze A, Pár A, Pár G:.
Impaired STAT4 phosphorylation is associated with non-response to PEG-IFN plus ribavirin treatment in chronic HCV hepatitis patients. Abstract.
Z. Gastroenterol. 2012; 50: 482.

Barakonyi A, Odpadlik C, Kőszegi B, Czéh A, Vincze Á, Pár A, Pár G: Increased cytotoxic potential of CD160 receptor positive natural immune cellls in chronic HCV infection.
Z. Gastroenterol. 2013; 51: 460.

Halász T, Horváth G, Pár G, Schaff Zs, Lendvai G: Association of MicroRNA expression and liver fibrosis detected by Fibroscan and histology.
Z.Gastroenterol. 2013; 51: 56.

Pár G, Szereday L, Berki T, Palinkas L, Miseta A, Vincze A, Par A: Increased baseline proinflammatory cytokine production in chronic hepatitis C patients with rapid virological response to peginterferon plus ribavirin. 21-st United European Gastroenterology Week Berlin 2013. Oct. 12-16. **UEG Journal. 2013; 1; suppl. 1: A114.**

Pár G, Trosits A, Pakodi F, Kenyeres P, Vincze A, Pár A: Liver stiffness measurement predicts the presence of large oesophageal varices in patients with chronic liver diseases.
21-st United European Gastroenterology Week Berlin 2013. Oct. 12-16.
UEG Journal. 2013; 1; suppl. 1: A296.

Pár G, Odpadlik C, Koszegi B, Czeh A, Par A, Barakonyi A: Increased cytotoxic potential of CD160 receptor positive natural immune cells in chronic HCV infection.
21-st United European Gastroenterology Week Berlin 2013. Oct. 12-16.
UEG Journal. 2013; 1; suppl. 1: A296.

Pár G, Szinku Zs, Haragh A, Nemes Zs, Vincze A, Hunyady B, Pár A: Sustained virologic response to antiviral therapy for chronic hepatitis C virus infection is associated with regression of liver fibrosis assessed by transient elastography.
Z. Gastroenterol. 2014; 52: A50.

Könyvfejezetek

Pár A. Hepatitis B virus (HBV) infekció és hepatocarcinogenesis. In: Fehér J, Lengyel G (szerk): Hepatocellularis carcinoma. Akadémiai Kiadó Budapest. 2010. 81-89. old.

Pár A. A Hepatitis B vírus (HBV) fertőzés terápiája. In: Takács M, Kárpáti J. (szerk): Klinikai és Járványügyi Virológia. Vox Medica Kiadói Kft, Budapest 2010. 695-699.

Pár A. A Hepatitis C vírus (HCV) fertőzés terápiája. In: Takács M, Kárpáti J. (szerk): Klinikai és Járványügyi Virológia. Vox Medica Kiadói Kft, Budapest 2010. 701-705.

Pár A. Autoimmun hepatitis. In: Lonovics J, Nemesánszky E, Simon L, Tulassay Zs, Wittman T (szerk): Varró Gasztroenterológia. Medicina. Budapest. 2011. 523-528

Pár A. Overlap szindromák. In: Lonovics J, Nemesánszky E, Simon L, Tulassay Zs, Wittman T (szerk): Varró Gasztroenterológia. Medicina. Budapest. 2011. 538-539.

Pár A. Májcirrhosis és szövődményei. In: Lonovics J, Nemesánszky E, Simon L, Tulassay Zs, Wittman T (szerk): Varró Gasztroenterológia. Medicina. Budapest. 2011. 564-582.

Pár A. A májrák és a hepatitis B-vírus fertőzés. In Tulassy Zs (szerk): A máj daganatai Medicina Könyvkiadó Zrt, Budapest 2013. 38-44.old.