Monocyte chemoattractant protein-1 gene (MCP-1-2518 A/G) polymorphism and serological markers of hepatitis B virus infection in hemodialysis patients

The role of MCP1-2518 A/G in hepatitis B virus (HBV) infection is controversial. Our aim was to evaluate the frequency distribution of MCP1-2518 A/G (rs1024611) polymorphic variants in hemodialysis (HD) patients without or with type 2 diabetes in relation to serological markers of HBV infection.

Material/Methods: HD patients (n=170, 48 with diagnosis of type 2 diabetes), who tested positive for total antibodies to HBV core antigen (anti-HBc), underwent MCP1 genotyping using polymerase chain reaction-restriction fragment length polymorphism assay. Anti-HBc was accompanied by antibodies to HBV surface antigen (anti-HBs) in 127 individuals. In anti-HBc-positive/anti-HBs-negative patients, HBV surface antigen (HBsAg) was shown in 15 patients and isolated anti-HBc were present in 28 patients. The distribution of MCP1 genotypes in anti-HBc-positive patients was compared to that in healthy subjects (n=437) and anti-HBc-negative HD patients (n=754).

Results: There were no significant differences (P_trend >0.05) in distribution of MCP1 genotypes between anti-HBc-positive patients, anti-HBc-negative subjects, and controls, regardless of anti-HBs or diabetic status. The MCP1-2518G allele prevalence was higher in HBsAg-positive/anti-HBs-negative patients defined as HBV carriers compared to MCP1-2518G allele frequency shown in groups composed of HBsAg-negative HD individuals and controls (50% vs. 28%, P_trend 0.022).

Conclusions: A frequency distribution of MCP1 polymorphic variants is not associated with anti-HBs development in response to HBV infection in HD patients, independent of diabetic status, but the MCP1-2518G allele may predispose to HBsAg persistence (HBV carrier status).

MeSH Keywords: Chemokine CCL2 • Diabetes Complications • Dialysis • Hepatitis B Antibodies • Polymorphism, Genetic

Abbreviations: ALT – alanine aminotransferase; anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; AST – aspartate aminotransferase; cccDNA – covalently closed circular DNA; CI – confidence interval; DM – diabetes mellitus; DNA – deoxyribonucleic acid; GGT – gamma-glutamyltranspeptidase; HBsAg – surface antigen of hepatitis B virus; HBV – hepatitis B virus; HD – hemodialysis; HWE – Hardy-Weinberg equilibrium; IL – interleukin; MAF – minor allele frequency; MCP-1 – monocyte chemoattractant protein-1; MCP1 – monocyte chemoattractant protein-1 gene; OR – odds ratio; RRT – renal replacement therapy; TNF-α – tumor necrosis factor interleukin

Full-text PDF: http://www.medscimonit.com/download/index/idArt/891009
Background

Patients undergoing chronic hemodialysis (HD) treatment due to end-stage renal disease (ESRD) are at risk of infection with blood-borne viruses, including hepatitis B virus (HBV). Total antibodies to HBV core antigen (anti-HBc) are an established marker of current (IgM) or previous (IgG) infection with HBV if they are positive in the confirmatory tests and reactive in determinations repeated over time [1,2]. Anti-HBC appear as a result of HBV transmission to non-vaccinated or non-successfully hepatitis B vaccinated individuals, but they may also elicit in vaccinated HD patients with maintained protective levels (>10 U/l) of antibodies to HBV surface antigen (anti-HBs) [3]. Immune tolerance to viral antigens, like HBV surface antigen (HBsAg), results in a lack of development of anti-HBs and persistence of HBsAg in the bloodstream. Patients who are HBsAg-positive and simultaneously anti-HBs-negative are commonly defined as HBV carriers. The mechanisms responsible for promotion or inhibition of HBsAg generation and HBsAg clearance are not fully understood. Monocyte chemoattractant protein-1 (MCP-1), referred also as chemokine (C-C motif) ligand 2 (CCL2), has been suggested to be a link in the chain involved in the hepatitis B outcome [4,5].

Individuals with occult hepatitis B – defined as the presence of HBV DNA in liver-serum with undetectable HBsAg – had significantly increased levels of MCP-1 compared to the healthy controls and patients that had resolved HBV infection (HBsAg-negative, anti-HBs-positive) [5]. MCP-1 expression level in the liver was higher in chronic hepatitis B complicated with non-alcoholic fatty liver diseases than that shown in hepatitis B without such concomitant diseases [6]. MCP-1 was significantly up-regulated in patients with hepatocellular carcinoma, showing HBV infection in over 50% of cases [7]. These data indicate that higher MCP-1 level is generally associated with worse clinical condition in HBV infection. Serum levels of MCP-1 increase with deterioration of renal function and are higher in HD patients than in healthy individuals [8–12]. The promoter region of the MCP-1 gene (MCP1) was shown to influence MCP-1 expression [13,14]. The MCP1-2518G allele was associated with up-regulation of both MCP-1 transcript and protein levels in many studies [11,13–16], but not all [17]. HD subjects with AG+GG genotypes of the MCP-1 gene (MCP1 rs1024611) had higher MCP-1 levels than those with the AA variant [11]; this may predispose HD patients to HBV infection. In the study by Park et al. [18] on Korean subjects, promoter polymorphism of MCP1 (MCP1-2518G>A) was involved in HBV clearance, but Cheong et al. [19] did not demonstrate an association of MCP1-2518G>A with the outcome of HBV infection in Korean patients.

The aim of our study was to evaluate the frequency distribution of MCP1-2518 A/G (rs1024611) polymorphic variants in patients who are non-hepatitis B vaccinated and HBV-infected HD patients in respect to commonly used HBV serological markers present in response to HBV infection. In particular, we would like to determine whether MCP1-2518 A/G polymorphism is associated with the development of anti-HBs that usually follows HBsAg disappearance from the bloodstream, and spontaneous recovery from HBV infection indicated by negative HBsAg and positive anti-HBs.

Material and methods

Patients and controls

One hundred seventy HD patients showing positive total anti-HBc were enrolled into the study (99 men, age 61.0±14.7 years, renal replacement therapy vintage 3.1, 0.05–26.3 years). Subjects with isolated anti-HBc positivity (HBsAg-negative, anti-HBc-positive, anti-HBs-negative) were also included. Only patients who had confirmatory assays and consistently maintained positive anti-HBc status were enrolled.

Anti-HBc-positive patients were never hepatitis B vaccinated and accounted for 18.4% of HD subjects (n=924) tested for serologic markers of HBV infection. Thirteen patients had a history of acute hepatitis B. Anti-HBc was accompanied by anti-HBs in 127 individuals: 126 patients showed classical serologic pattern of HBV resolution (HBsAg-negative, anti-HBs-positive), indicating spontaneous recovery from HBV infection; 1 patient in this group was both HBsAg- and HBV DNA-positive. In anti-HBc-positive/anti-HBs-negative patients (n=43), HBsAg positivity was shown in 15 patients (classical serologic pattern of HBV carrier status), and isolated anti-Hbc seropositivity (HBsAg-negative, anti-HBc-positive, anti-HBs-negative) was present in 28 patients. HBV DNA testing (detection limit 250 copies ml-1) was positive in 11 HBV carriers (1 patient had a negative test result for HBV DNA, and 3 patients were not investigated). All HBsAg-positive patients (n=16) accounted for 1.7% of all tested subjects.

In the anti-HBc-positive HD group there were 48 patients with type 2 diabetes mellitus (DM), and no patients with type 1 DM. Type 2 DM was a cause of diabetic nephropathy leading to ESRD and HD treatment in all 48 patients. Selected demographic and clinical data of main groups of anti-HBc-positive HD patients are shown in Table 1.

Unrelated blood donors and healthy volunteers served as controls for distribution of MCP1-2518 A/G (rs1024611) polymorphic variants (n=437). This control group was also used in our earlier studies [20,21]. Additionally, results of MCP1 genotype distribution in anti-HBc-positive HD patients were compared to those of anti-HBc-negative HD patients (n=754) described...
Table 1. Selected demographic and clinical data of main groups of anti-HBc positive HD patients.

| Parameter                      | All anti-HBc positive HD patients (n=170) | Anti-HBs positive (n=127) | Anti-HBs negative (n=43) | P value |
|-------------------------------|------------------------------------------|----------------------------|--------------------------|---------|
| Men, n (% of all)             |                                          | 71 (55.9)                  | 28 (65.1)                | 0.371a  |
| Age, years                    |                                          | 61.6±14.4                  | 59.3±15.5                | 0.391b  |
| RRT duration, years           |                                          | 2.8 (0.05–26.3)            | 3.6 (0.05–25.1)          | 0.139a  |

Causes of end-stage renal disease, n (% of all)

| Cause                                | All anti-HBc positive HD patients (n=170) | Anti-HBs positive (n=127) | Anti-HBs negative (n=43) | P value |
|--------------------------------------|------------------------------------------|----------------------------|--------------------------|---------|
| Diabetic nephropathy                 |                                          | 37 (29.1)                  | 11 (25.6)                | 0.700a  |
| Hypertensive nephropathy             |                                          | 20 (15.7)                  | 6 (14.0)                 | 0.778d  |
| Chronic glomerulonephritis           |                                          | 18 (14.2)                  | 14 (32.6)                | 0.008d  |
| Chronic tubulointerstitial nephritis |                                          | 10 (7.9)                   | 3 (7.0)                  | 0.888e  |
| Polycystic kidney disease            |                                          | 7 (5.5)                    | 1 (2.3)                  | 0.663d  |
| 346a, 1752a, 3691a, 1884a or other   |                                          | 35 (27.6)                  | 8 (18.6)                 | 0.311a  |

| Parameter                      | Diabetics (n=37) | Non-diabetics (n=90) | P value |
|-------------------------------|-----------------|---------------------|---------|
| Men, n (% of all)             | 18 (48.6)       | 53 (58.9)           | 0.329a  |
| Age, years                    | 63.3±13.1       | 60.8±14.9           | 0.381b  |
| RRT duration, years           | 2.0 (0.05–15.7) | 3.4 (0.14–26.3)     | 0.008c  |

Causes of end-stage renal disease, n (% of all)

| Cause                                | Diabetics (n=37) | Non-diabetics (n=90) | P value |
|--------------------------------------|-----------------|---------------------|---------|
| Diabetic nephropathy                 | 37 (100)        | 0 (0)               |         |
| Chronic glomerulonephritis           | --              | 18 (20.0)           |         |
| Hypertensive nephropathy             | --              | 20 (22.2)           |         |
| Chronic tubulointerstitial nephritis | --              | 10 (11.1)           |         |
| Polycystic kidney disease            | --              | 7 (7.8)             |         |
| 346a, 1752a, 3691a, 1884a or other   | --              | 35 (38.9)           |         |
| ALT (U/l)                            | 16 (2–195)      | 18 (4–53)           | 0.446f  |
| AST (U/l)                            | 18 (6–152)      | 18 (6–81)           | 0.651f  |
| GGT (U/l)                            | 26 (4–498)      | 25 (7–284)          | 0.936f  |

Anti-HBs negative (n=43)

| Parameter                      | Diabetics (n=11) | Non-diabetics (n=32) | P value |
|-------------------------------|-----------------|---------------------|---------|
| Men, n (% of all)             | 6 (54.5)        | 22 (68.8)           | 0.627a  |
| Age, years                    | 64.7±12.4       | 57.5±16.2           | 0.189o  |
| RRT duration, years           | 3.6 (0.24–24.2) | 4.7 (0.05–25.1)     | 0.770o  |
in our recent study [21]. The latter group consisted of 601 anti-HBs-positive patients due hepatitis B vaccination and 153 non-responders to hepatitis B vaccination (anti-HBs-negative).

All examined subjects were of white race.

**Genotyping**

*MCP1* rs1024611 genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism, as previously described [20].

**Laboratory methods**

Sero logic markers of HBV infection and serum activities of liver enzymes were determined by the methods previously described [22].

**Statistical methods**

Results are presented as percentage for categorical variables, as mean with 1 standard deviation for normally distributed continuous variables, or as median with range for not normally distributed continuous variables. Statistical tests used for comparison of data obtained in selected groups are indicated at each P value.

Hardy-Weinberg equilibrium (HWE) was tested to compare the observed genotype frequencies to the expected ones using the chi-square test. The Fisher exact probability test or chi-square test were used to evaluate differences in genotype and allele prevalence between the examined groups. The odds ratio (OR) with p value and 95% confidence intervals (95% CI) value were calculated. Polymorphisms were tested for association using the chi-square test for trend \( \left( \chi^2_{\text{trend}} \right) \). The Fisher exact test was used for power analysis.

Values of \( P<0.05 \) were judged to be significant. All probabilities were 2-tailed.

Statistical calculations were performed using GraphPad InStat 3.10, 32 bit for Windows, created July 9, 2009 (GraphPad Software, Inc., La Jolla, USA), CytelStudio version 10.0, created January 16, 2013 (CytelStudio Software Corporation, Cambridge, USA), and Statistica version 10, 2011 (Stat Soft, Inc., Tulsa, USA).

**Ethical approval**

The research design was approved by the Institutional Review Board of Poznań University of Medical Sciences, Poland. Informed consent was obtained from all study participants.

**Results**

There was no significant deviation from the HWE in the genotype frequencies in all anti-HBc-positive HD patients, non-DM and DM groups, as well as anti-HBs-positive and anti-HBs-negative groups (Supplementary Table 1).
Statistical analyses did not show significant differences in MCP1 genotype frequencies between anti-HBc-positive HD patients and controls, independent of occurrence of type 2 DM or anti-HBs status (Tables 2 and 3). There were also no significant differences in MCP1 genotype frequencies when anti-HBc-positive patients were categorized as anti-HBs-positive or -negative (Table 4). Similar comparisons between anti-HBc-positive and anti-HBc-negative HD groups did not reveal a significant difference (P<0.05, Supplementary Tables 2–4).

The significant differences in MCP1 genotype frequencies were shown between the anti-HBc-positive HD group that represented HBV carriers and HD individuals with HBV resolution (Table 5), as well as between HBV carriers, healthy controls, and HD patients with isolated anti-HBc positivity and HD patients with HBV resolution (in our study both these groups differed only in anti-HBs status) were non-significant (Supplementary Table 5).

Table 2. Comparison of the distribution of MCP1 rs1024611 polymorphic variants in anti-HBc positive/anti-HBs positive hemodialysis (HD) patients and controls

| Genotype | Anti-HBc positive/anti-HBs positive patients (frequency) | Controls (frequency) | Odds ratio (95%CI) | Two-tailed P | \(P_{\text{trend}}\) | \(P_{\text{genotyping}}\) | Power (%) |
|----------|-------------------------------------------------|-------------------------|-----------------|-------------|--------|-----------------|-----------|
| AA       | n=127                                           | n=437                   | Referent        | –           | 0.979  | 0.796           |           |
|          | 67 (0.53)                                       | 225 (0.51)              |                 |             |        |                 |           |
| AG       | 48 (0.38)                                       | 177 (0.41)              | 0.911 (0.584–1.414) | 0.743       | 0.667  | 0.681           | 10.7      |
| GG       | 12 (0.09)                                       | 35 (0.08)               | 1.151 (0.514–2.428) | 0.821       | 0.446  | 0.743           | 7.2       |
| AG+GG    | 60 (0.47)                                       | 212 (0.49)              | 0.950 (0.627–1.439) | 0.881       | 0.446  | 0.743           | 8.2       |
| MAF      | 72 (0.28)                                       | 247 (0.28)              | 1.004 (0.725–1.382) | 1.000       | 4.6    |

| Genotype | HD cases without DM vs. controls n=90 n=437 |
|----------|---------------------------------------------|
| AA       | 50 (0.56)                                   | 225 (0.51) |
| AG       | 32 (0.36)                                   | 177 (0.41) |
| GG       | 8 (0.09)                                    | 35 (0.08) |
| AG+GG    | 40 (0.44)                                   | 212 (0.49) |
| MAF      | 48 (0.27)                                   | 247 (0.28) |

| Genotype | HD cases with DM vs. controls n=37 n=437 |
|----------|-----------------------------------------|
| AA       | 17 (0.46)                                | 225 (0.51) |
| AG       | 16 (0.43)                                | 177 (0.41) |
| GG       | 4 (0.11)                                 | 35 (0.08) |
| AG+GG    | 20 (0.54)                                | 212 (0.49) |
| MAF      | 24 (0.32)                                | 247 (0.28) |

anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus; MAF – minor allele frequency.

Table 3. Comparison of the distribution of MCP1 rs1024611 genotype frequencies between HD patients with isolated anti-HBc positivity and HD patients with HBV resolution

| Genotype | Anti-HBc positive/anti-HBs positive patients (frequency) | Controls (frequency) | Odds ratio (95%CI) | Two-tailed P | \(P_{\text{trend}}\) | \(P_{\text{genotyping}}\) |
|----------|-------------------------------------------------|-------------------------|-----------------|-------------|--------|-----------------|
| AA       | n=127                                           | n=437                   | Referent        | –           | 0.979  | 0.796           |
| AG       | 48 (0.38)                                       | 177 (0.41)              | 0.911 (0.584–1.414) | 0.743       | 0.667  | 0.681           |
| GG       | 12 (0.09)                                       | 35 (0.08)               | 1.151 (0.514–2.428) | 0.821       | 0.446  | 0.743           |
| AG+GG    | 60 (0.47)                                       | 212 (0.49)              | 0.950 (0.627–1.439) | 0.881       | 0.446  | 0.743           |
| MAF      | 72 (0.28)                                       | 247 (0.28)              | 1.004 (0.725–1.382) | 1.000       | 4.6    |

| Genotype | HD cases without DM vs. controls n=90 n=437 |
|----------|---------------------------------------------|
| AA       | 50 (0.56)                                   | 225 (0.51) |
| AG       | 32 (0.36)                                   | 177 (0.41) |
| GG       | 8 (0.09)                                    | 35 (0.08) |
| AG+GG    | 40 (0.44)                                   | 212 (0.49) |
| MAF      | 48 (0.27)                                   | 247 (0.28) |

| Genotype | HD cases with DM vs. controls n=37 n=437 |
|----------|-----------------------------------------|
| AA       | 17 (0.46)                                | 225 (0.51) |
| AG       | 16 (0.43)                                | 177 (0.41) |
| GG       | 4 (0.11)                                 | 35 (0.08) |
| AG+GG    | 20 (0.54)                                | 212 (0.49) |
| MAF      | 24 (0.32)                                | 247 (0.28) |

The significant differences in MCP1 genotype frequencies were shown between the anti-HBc-positive HD group that represented HBV carriers and HD individuals with HBV resolution (Table 5), as well as between HBV carriers, healthy controls, and HD patients with isolated anti-HBc positivity and HD patients with HBV resolution.

Grzegorzewska A.E. et al.: MCP-1-2518 A/G and HBV infection
© Med Sci Monit, 2014; 20: 1101-1116

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System]
[ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica]
[Chemical Abstracts/CAS] [Index Copernicus]
anti-HBc negative HD patients, and HD patients with isolated anti-HBc positivity (Table 6). There was a higher prevalence of the MCP1-2518G allele in HBV carriers compared to the MCP1-2518G allele frequency in patients of all aforementioned groups. Among anti-HBc-positive HD patients, the highest prevalence of HBsAg-positive/anti-HBs-negative subjects (HBV carriers) was in the group bearing the GG genotype (Supplementary Table 6).

Table 3. Comparison of the distribution of MCP1 rs1024611 polymorphic variants in anti-HBc positive/anti-HBs negative hemodialysis (HD) and controls

| Genotype | Anti-HBc positive/anti-HBs negative HD patients (frequency) | Controls (frequency) | Odds ratio (95%CI) | Two-tailed P | P<sub>trend</sub> | P<sub>genotyping</sub> | Power (%) |
|----------|----------------------------------------------------------|----------------------|--------------------|--------------|-----------------|-------------------|-----------|
|          | All HD cases vs. controls                                |                      |                    |              |                 |                   |           |
| n=43     | n=437                                                    |                      |                    |              |                 |                   |           |
| AA       | 20 (0.47)                                                | 225 (0.51)           | Referent           | –            | 0.402           | 0.663             |           |
| AG       | 18 (0.42)                                                | 177 (0.41)           | 1.144 (0.552–2.354)| 0.818        |                 |                   |           |
| GG       | 5 (0.11)                                                 | 35 (0.08)            | 1.607 (0.442–4.796)| 0.525        |                 |                   |           |
| AG+GG    | 23 (0.53)                                                | 212 (0.49)           | 1.221 (0.621–2.417)| 0.643        |                 |                   |           |
| MAF      | 28 (0.32)                                                | 247 (0.28)           | 1.225 (0.733–2.008)| 0.470        |                 |                   |           |
|          | HD cases without DM vs. controls                         |                      |                    |              |                 |                   |           |
| n=32     | n=437                                                    |                      |                    |              |                 |                   |           |
| AA       | 16 (0.50)                                                | 225 (0.51)           | Referent           | –            | 0.611           | 0.669             |           |
| AG       | 12 (0.38)                                                | 177 (0.41)           | 0.953 (0.401–2.211)| 1.000        |                 |                   |           |
| GG       | 4 (0.13)                                                 | 35 (0.08)            | 1.607 (0.369–5.374)| 0.595        |                 |                   |           |
| AG+GG    | 16 (0.50)                                                | 212 (0.49)           | 1.061 (0.483–2.330)| 1.000        |                 |                   |           |
| MAF      | 20 (0.31)                                                | 247 (0.28)           | 1.154 (0.631–2.046)| 0.702        |                 |                   |           |
|          | HD cases with DM vs. controls                            |                      |                    |              |                 |                   |           |
| n=37     | n=437                                                    |                      |                    |              |                 |                   |           |
| AA       | 4 (0.36)                                                 | 225 (0.51)           | Referent           | –            | 0.405           | 0.602             |           |
| AG       | 6 (0.55)                                                 | 177 (0.41)           | 1.907 (0.444–9.317)| 0.494        |                 |                   |           |
| GG       | 1 (0.09)                                                 | 35 (0.08)            | 1.607 (0.032–16.83)| 1.000        |                 |                   |           |
| AG+GG    | 7 (0.64)                                                 | 212 (0.49)           | 1.857 (0.464–8.767)| 0.494        |                 |                   |           |
| MAF      | 8 (0.36)                                                 | 247 (0.28)           | 1.451 (0.520–3.758)| 0.540        |                 |                   |           |

anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus; MAF – minor allele frequency.

Discussion

The past decades have brought important changes in recognition of outcome of HBV infection. A discovery of HBV covalently closed circular DNA (cccDNA) organized into minichromosomes within the nucleus of HBV-infected cells have presented new challenges for researchers and clinicians who focus on complete cccDNA eradication as a target for antiviral
Table 4. Comparison of the distribution of MCP1 rs1024611 polymorphic variants in anti-HBc positive/anti-HBs negative patients undergoing hemodialysis (HD) and anti-HBc positive/anti-HBs positive HD patients.

| Genotype | Anti-HBc positive/anti-HBs negative (frequency) | Anti-HBc positive/anti-HBs positive (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | P_genotyping | Power (%) |
|----------|-----------------------------------------------|-----------------------------------------------|--------------------|--------------|---------|----------|-----------|
| AA       | 20 (0.47)                                     | 67 (0.53)                                     | Referent           | 0.766        | 0.473   |          |           |
| AG       | 19 (0.42)                                     | 48 (0.38)                                     | 1.256 (0.560–2.798) | 0.673        | 7.7     |          |           |
| GG       | 5 (0.11)                                      | 12 (0.09)                                     | 1.396 (0.342–4.911) | 0.772        | 8.0     |          |           |
| AG+GG    | 23 (0.53)                                     | 60 (0.47)                                     | 1.284 (0.606–2.732) | 0.595        | 8.5     |          |           |
| MAF      | 28 (0.33)                                     | 72 (0.28)                                     | 1.220 (0.690–2.125) | 0.542        | 10.4    |          |           |
| **All HD cases** | **n=43**                                    | **n=127**                                    |                    |              |         |          |           |
| AA       | 16 (0.50)                                     | 50 (0.56)                                     | Referent           | 0.790        | 0.504   |          |           |
| AG       | 12 (0.38)                                     | 32 (0.36)                                     | 1.172 (0.442–3.039) | 0.888        | 5.9     |          |           |
| GG       | 4 (0.13)                                      | 8 (0.09)                                      | 1.563 (0.302–6.791) | 0.734        | 8.7     |          |           |
| AG+GG    | 16 (0.50)                                     | 40 (0.44)                                     | 1.250 (0.514–3.036) | 0.736        | 6.8     |          |           |
| MAF      | 20 (0.31)                                     | 48 (0.27)                                     | 1.250 (0.631–2.421) | 0.534        | 9.8     |          |           |
| **HD cases without DM** | **n=32**                                    | **n=90**                                     |                    |              |         |          |           |
| AA       | 4 (0.36)                                      | 17 (0.46)                                     | Referent           | 0.803        | 0.727   |          |           |
| AG       | 6 (0.55)                                      | 16 (0.43)                                     | 1.594 (0.306–9.097) | 0.784        | 6.1     |          |           |
| GG       | 1 (0.09)                                      | 4 (0.11)                                      | 1.063 (0.017–15.84) | 1.000        | 1.9     |          |           |
| AG+GG    | 7 (0.64)                                      | 20 (0.54)                                     | 1.488 (0.310–8.103) | 0.836        | 6.4     |          |           |
| MAF      | 8 (0.36)                                      | 24 (0.32)                                     | 1.190 (0.378–3.544) | 0.919        | 5.1     |          |           |

**HD cases with DM**

| Genotype | Anti-HBc positive/anti-HBs negative (frequency) | Anti-HBc positive/anti-HBs positive (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | P_genotyping | Power (%) |
|----------|-----------------------------------------------|-----------------------------------------------|--------------------|--------------|---------|----------|-----------|
| AA       | 4 (0.36)                                      | 17 (0.46)                                     | Referent           | 0.803        | 0.727   |          |           |
| AG       | 6 (0.55)                                      | 16 (0.43)                                     | 1.594 (0.306–9.097) | 0.784        | 6.1     |          |           |
| GG       | 1 (0.09)                                      | 4 (0.11)                                      | 1.063 (0.017–15.84) | 1.000        | 1.9     |          |           |
| AG+GG    | 7 (0.64)                                      | 20 (0.54)                                     | 1.488 (0.310–8.103) | 0.836        | 6.4     |          |           |
| MAF      | 8 (0.36)                                      | 24 (0.32)                                     | 1.190 (0.378–3.544) | 0.919        | 5.1     |          |           |

anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus; MAF – minor allele frequency.

therapy [23,24]. Therefore, disappearance of cccDNA from infected cells (hepatocytes) could be an indicator of resolution of HBV infection. Commonly used serologic markers of HBV infection help to stratify the HBV-infected individuals according to their infectivity rather than in respect to HBV eradication and total dissolution of hepatitis B infection. They change over time and may disappear throughout the lifespan. Such a possibility needs to be taken into account in stratification of infected patients for those with a high probability of HBV replication (HBV DNA usually detectable using standard determinations) or those who currently do not replicate HBV or replicate at low levels, routinely undetectable. HD subjects are in good position in diagnosis of HBV infection because they undergo periodic examinations of basic serologic HBV markers on a mandatory basis. However, it is also possible that HBV-infected patients with occult hepatitis B may be negative...
for all serological markers of HBV infection except HBV DNA [25]; this indicates a tremendous variability in chronic immunological reactions to HBV transmission. Our main purpose was to examine the possible association of MCP1-2518 A/G (rs1024611) polymorphism with anti-HBs development. Patients stratified by anti-HBs status represented different serological constellations, especially anti-HBc-positive/anti-HBs-negative subjects. Therefore, the anti-HBs-sorted groups were also analyzed by HBsAg status.

Comparison of MCP1-2518 A/G (rs1024611) polymorphic variant frequency between anti-HBc-positive/anti-HBs-negative HD patients and anti-HBc-positive/anti-HBs-negative HD patients without or with DM.

**Table 5.** Comparison of the distribution of MCP1 rs1024611 polymorphic variants in HBsAg positive/anti-HBs negative HD patients and anti-HBc positive/HBsAg negative/anti-HBs positive HD without or with DM.

| Genotype | HD patients anti-HBc positive/ HBsAg negative (frequency) | HD patients anti-HBc negative/ HBsAg positive (frequency) | Odds ratio (95%CI) | Two-tailed P | P trend | P genotyping | Power (%) |
|----------|--------------------------------------------------------|---------------------------------------------------------|-------------------|--------------|----------|--------------|-----------|
|          | n=15                                                  | n=126                                                  |                   |              |          |              |           |
| AA       | 4 (0.27)                                              | 66 (0.52)                                              | Referent                  | 0.021        | 0.064    |              |           |
| AG       | 7 (0.47)                                              | 48 (0.38)                                              | 0.416 (0.085–1.756)     | 0.292        | 20.2     |              |           |
| GG       | 4 (0.27)                                              | 12 (0.10)                                              | 0.182 (0.030–1.147)     | 0.073        | 56.3     |              |           |
| AG+GG    | 11 (0.73)                                             | 60 (0.48)                                              | 0.331 (0.073–1.201)     | 0.105        | 38.4     |              |           |
| MAF      | 15 (0.50)                                             | 72 (0.29)                                              | 0.400 (0.173–0.932)     | 0.033        | 61.4     |              |           |
|          | n=10                                                  | n=89                                                   |                   |              |          |              |           |
| AA       | 3 (0.3)                                               | 49 (0.55)                                              | Referent                  | 0.043        | 0.096    |              |           |
| AG       | 4 (0.4)                                               | 32 (0.36)                                              | 0.490 (0.068–3.130)     | 0.602        | 11.9     |              |           |
| GG       | 3 (0.3)                                               | 8 (0.09)                                               | 0.163 (0.019–1.494)     | 0.121        | 47.9     |              |           |
| AG+GG    | 7 (0.7)                                               | 40 (0.45)                                              | 0.350 (0.055–1.672)     | 0.242        | 28.9     |              |           |
| MAF      | 10 (0.5)                                              | 48 (0.27)                                              | 0.369 (0.130–1.062)     | 0.066        | 51.2     |              |           |
|          | n=5                                                   | n=37                                                   |                   |              |          |              |           |
| AA       | 1 (0.2)                                               | 17 (0.46)                                              | Referent                  | 0.273        | 0.528    |              |           |
| AG       | 3 (0.6)                                               | 16 (0.43)                                              | 0.314 (0.006–4.509)     | 0.646        | 10.1     |              |           |
| GG       | 1 (0.2)                                               | 4 (0.11)                                               | 0.235 (0.003–23.03)     | 0.791        | 10.4     |              |           |
| AG+GG    | 4 (0.8)                                               | 20 (0.54)                                              | 0.294 (0.006–3.444)     | 0.550        | 6.9      |              |           |
| MAF      | 5 (0.5)                                               | 24 (0.32)                                              | 0.480 (0.101–2.323)     | 0.451        | 15.9     |              |           |

anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus, hemodialysis; HBsAg – surface antigen of hepatitis B virus; HD – hemodialysis; MAF – minor allele frequency.

Significant differences are indicated using bold font.
healthy controls indicate no association between MCP1 genotypes and susceptibility to HBV infection, or anti-HBs development in HD patients already infected. Comparisons performed inside the entire anti-HBc-positive HD group also did not reveal any associations between MCP1 genotypes and anti-HBs development in response to HBV infection. This lack of association was also evident in analyses in which DM and non-DM patients were analyzed separately. Associations of MCP1 polymorphism with type 2 DM have been demonstrated [26,27], but in our studies there were no differences in MCP1 genotype frequencies between type 2 DM subjects with diabetic nephropathy as a cause of ESRD and HD treatment, healthy controls, anti-HBc-negative HD patients [21], or anti-HBc-positive HD subjects (Supplementary Table 7). On the other hand, DM is a well-known predictor of hypo- or non-responsiveness to hepatitis B vaccination in patients with chronic renal diseases [28]. Therefore, DM could also influence anti-HBs production in response to HBV infection. However, the distribution of MCP1 polymorphic variants was not associated with development of protective anti-HBs in response to hepatitis B vaccination, in DM as well as non-DM HD subjects not infected with HBV [21]. In the present study, the lack of MCP1-2518 A/G association with anti-HBs development was extended to HBV-infected HD patients with or without type 2 DM.

Stimulations with HBsAg and different fusion proteins eliciting moderate or high MCP-1 levels [with concomitant differences in tumor necrosis factor α (TNF-α), interleukin (IL)-12, IL-10, interferon-γ, and IL-6]) did not result in a significant difference in anti-HBs levels in transgenic mice [4], and reductions in serum and liver HBsAg levels were dependent on stimulation. High level productions of TNF-α and MCP-1 caused a more severe cytotoxicity in hepatocytes and were less effective in reducing serum HBsAg level. Studies by Meng et al. [4], although not exclusively related to MCP-1, clearly demonstrate that differences in MCP-1 concentrations do not correlate with anti-HBs levels but may be important for HBsAg clearance. It has been suggested that the anti-HBs response alone cannot account for the reduction of HBsAg level. Studies by Meng et al. [4], although not exclusively related to MCP-1, clearly demonstrate that differences in MCP-1 concentrations do not correlate with anti-HBs levels but may be important for HBsAg clearance. It has been suggested that the anti-HBs response alone cannot account for the reduction of HBsAg level.

Table 6. Comparison of MCP1 rs1024611 genotype frequencies between hepatitis B virus carriers (AA 4 (0.27), AG 7 (0.47), GG 4 (0.27), MAF (0.50)) and other selected groups.

| Description of HD group | rs1024611 | Odds ratio (95%CI) | Two-tailed P | P trend | P genotyping | Power (%) |
|-------------------------|-----------|--------------------|--------------|---------|--------------|-----------|
| Controls (n=437)        | AA        | 225 (0.51)         | 2.919 (0.846–12.73) | 0.101   | 0.010        | 0.021     | 42.0 |
|                         | AG        | 177 (0.41)         | 0.778 (0.242–2.571) | 0.822   | 5.3          |           |      |
|                         | GG        | 35 (0.08)          | 0.239 (0.067–1.091) | 0.065   | 56.8         |           |      |
|                         | AG+GG     | 212 (0.49)         | 0.343 (0.079–1.811) | 0.101   | 41.9         |           |      |
|                         | MAF       | 247 (0.28)         | 0.394 (0.177–0.880) | 0.022   | 67.7         |           |      |
| HD anti-HBc negative patients (n=754) | AA | 349 (0.46) | 2.370 (0.694–10.29) | 0.209  | 0.015 | 0.012 | 26.9 |
|                         | AG        | 352 (0.47)         | 1.001 (0.314–3.277) | 1.000   | 3.6          |           |      |
|                         | GG        | 53 (0.07)          | 0.208 (0.059–0.929) | 0.040   | 61.4         |           |      |
|                         | AG+GG     | 405 (0.54)         | 0.422 (0.097–1.442) | 0.209   | 26.9         |           |      |
|                         | MAF       | 458 (0.30)         | 0.436 (0.197–0.967) | 0.041   | 59.5         |           |      |
| HD patients with isolated anti-HBc positivity (n=28) | AA | 16 (0.57) | 3.667 (0.797–19.27) | 0.110  | 0.014 | 0.038 | 39.0 |
|                         | AG        | 11 (0.39)          | 0.740 (0.175–3.184) | 0.882   | 5.0          |           |      |
|                         | GG        | 1 (0.04)           | 0.102 (0.002–1.248) | 0.086   | 60.3         |           |      |
|                         | AG+GG     | 12 (0.43)          | 0.273 (0.052–1.254) | 0.110   | 38.9         |           |      |
|                         | MAF       | 13 (0.23)          | 0.302 (0.106–0.866) | 0.023   | 66.8         |           |      |

anti-HBc – antibodies to core antigen of hepatitis B virus; HD – hemodialysis; MAF – minor allele frequency. Significant differences are indicated using bold font.
Differences in MCP1-2518 A/G genotype frequencies are reflected in variations of MCP-1 blood concentrations [11,13–16]. In accordance with the available data, the involvement of MCP1-2518 A/G polymorphism in the outcome of HBV infection is, however, controversial [18,19]. To approach this problem, our further analyses on anti-HBc-positive HD patients were focused not only on anti-HBs status, but also on coexistence of HBsAg positivity and anti-HBs negativity, as well as the HBsAg negativity and anti-HBs positivity that represent serological profiles of HBV carrier status and recovery from HBV infection, respectively. For such analyses, patients with isolated anti-HBc positivity were excluded from the group of anti-HBc-positive/anti-HBs-negative subjects, as well as a unique HBsAg-positive/anti-HBs-positive patient was excluded from the group of anti-HBc-positive/anti-HBs-positive subjects. As a result, it became possible to show that the MCP1-2518G allele predisposes to maintenance of HBV infection (HBV carrier status). Therefore, our results support Korean findings indicating associations of MCP1-2518 A/G polymorphism with resolution/persistence of HBV infection (renal function in the examined subjects was not shown).

A weak point of this study is the small number of HBsAg-positive patients (HBV carriers). In the Greater Poland region of our country, the prevalence of HD patients infected with blood-borne viruses decreases every year due to rigorous sanitary regimen in dialysis facilities, and full implementation of hepatitis B vaccination in dialysis patients and medical staff. We consider this part of our study as preliminary field research, although it appears to be the first study on the association of MCP1-2518 A/G polymorphism with serological markers of HBV infection in HD patients.

### Supplementary Table 1. The distribution of MCP1 rs1024611 genotypes in anti-HBc positive HD patients in respect to HWE.

| MCP1 rs1024611 genotype frequencies | All HD cases | HD cases without DM | HD cases with DM |
|-------------------------------------|-------------|---------------------|-----------------|
|                                     | Observed    | Expected            | Observed        | expected        | Observed        | expected        |
| All anti-HBc positive HD patients (n=170) |             |                     |                 |                 |                 |
| AA                                  | 87 (0.51)   | 85 (0.50)           | 66 (0.54)       | 63 (0.52)       | 21 (0.44)       | 21 (0.44)       |
| AG                                  | 66 (0.39)   | 70 (0.41)           | 44 (0.36)       | 49 (0.40)       | 22 (0.46)       | 21 (0.44)       |
| GG                                  | 17 (0.10)   | 15 (0.09)           | 12 (0.10)       | 10 (0.08)       | 5 (0.10)        | 6 (0.12)        |
| P value for deviation from HWE      | 0.397       | 0.256               | 0.829           |                 |                 |
| Anti-HBs positive patients (n=127)  |             |                     |                 |                 |                 |
| AA                                  | 67 (0.53)   | 65 (0.51)           | 50 (0.56)       | 48 (0.53)       | 17 (0.46)       | 17 (0.46)       |
| AG                                  | 48 (0.38)   | 52 (0.41)           | 32 (0.35)       | 35 (0.39)       | 16 (0.43)       | 16 (0.43)       |
| GG                                  | 12 (0.09)   | 10 (0.08)           | 8 (0.09)        | 7 (0.08)        | 4 (0.11)        | 4 (0.11)        |
| P value for deviation from HWE      | 0.433       | 0.388               | 0.935           |                 |                 |
| Anti-HBs negative patients (n=43)   |             |                     |                 |                 |                 |
| AA                                  | 20 (0.46)   | 19 (0.44)           | 16 (0.50)       | 15 (0.47)       | 4 (0.35)        | 5 (0.45)        |
| AG                                  | 18 (0.42)   | 19 (0.44)           | 12 (0.38)       | 14 (0.44)       | 6 (0.55)        | 5 (0.45)        |
| GG                                  | 5 (0.12)    | 5 (0.12)            | 4 (0.12)        | 3 (0.09)        | 1 (0.10)        | 1 (0.10)        |
| P value for deviation from HWE      | 0.759       | 0.472               | 0.554           |                 |                 |

anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus; HD – hemodialysis; HWE – Hardy-Weinberg equilibrium.
Conclusions

In this study we have demonstrated that MCP1-2518 A/G (rs1024611) polymorphism is not associated with anti-HBs development in response to hepatitis B infection in HD patients, independent of whether they are type 2 diabetics. In our previous study on HD patients [21], we documented that this polymorphism is also not associated with response to hepatitis B vaccination characterized by seroconversion to anti-HBs >10 U/L. However, the role of MCP1-2518 A/G polymorphism in the HBsAg clearance may be seen from our current studies, and seems to be worth further investigation, especially in immunocompromised patients.

| Genotype | HD patients anti-HBc negative (frequency) | HD patients anti-HBc positive (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | P_genotyping | Power (%) |
|----------|------------------------------------------|------------------------------------------|--------------------|--------------|---------|--------------|-----------|
|          | n=754                                    | n=170                                    |                    |              |         |              |           |
| AA       | 349 (0.46)                               | 87 (0.51)                                | Referent           | –            | 0.718   | 0.122        |           |
| AG       | 352 (0.47)                               | 66 (0.39)                                | 0.752 (0.520–1.086)| 0.134        |         |              |           |
| GG       | 53 (0.07)                                | 17 (0.10)                                | 1.287 (0.664–2.392)| 0.493        |         |              |           |
| AG+GG    | 405 (0.54)                               | 83 (0.49)                                | 0.822 (0.581–1.163)| 0.285        |         |              |           |
| MAF      | 458 (0.30)                               | 100 (0.29)                               | 0.955 (0.730–1.244)| 0.781        |         |              |           |
|          | n=532                                    | n=122                                    |                    |              |         |              |           |
| AA       | 245 (0.46)                               | 66 (0.54)                                | Referent           | –            | 0.493   | 0.038        |           |
| AG       | 255 (0.48)                               | 44 (0.36)                                | 0.641 (0.410–0.994)| 0.047        |         |              |           |
| GG       | 32 (0.06)                                | 12 (0.10)                                | 1.392 (0.617–2.961)| 0.468        |         |              |           |
| AG+GG    | 287 (0.54)                               | 56 (0.46)                                | 0.724 (0.478–1.096)| 0.133        |         |              |           |
| MAF      | 319 (0.30)                               | 68 (0.28)                                | 0.902 (0.652–1.240)| 0.569        |         |              |           |
|          | n=222                                    | n=48                                     |                    |              |         |              |           |
| AA       | 104 (0.47)                               | 21 (0.44)                                | Referent           | –            | 0.696   | 0.923        |           |
| AG       | 97 (0.44)                                | 22 (0.46)                                | 1.123 (0.550–2.296)| 0.858        |         |              |           |
| GG       | 21 (0.09)                                | 5 (0.10)                                 | 1.179 (0.312–3.721)| 0.956        |         |              |           |
| AG+GG    | 118 (0.53)                               | 27 (0.56)                                | 1.133 (0.603–2.245)| 0.820        |         |              |           |
| MAF      | 139 (0.31)                               | 32 (0.33)                                | 1.097 (0.662–1.791)| 0.783        |         |              |           |

anti-HBc – antibodies to core antigen of hepatitis B virus; DM – diabetes mellitus, hemodialysis; HD – hemodialysis; MAF – minor allele frequency. Significant differences are indicated using bold font.

Supplementary Table 2. Comparison of the distribution of MCP1 rs1024611 polymorphic variants in anti-HBc negative and anti-HBc positive HD without or with DM.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License
**Supplementary Table 3.** Comparison of the distribution of *MCP1* rs1024611 polymorphic variants in anti-HBs-positive HD due to vaccination or infection

| Genotype | Anti-HBs positive HD patients due to vaccination (frequency) | Anti-HBs positive HD patients due to infection (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | P_genotyping | Power (%) |
|----------|----------------------------------------------------------|--------------------------------------------------------|--------------------|-------------|----------|-------------|-----------|
|          | n=601                                                   | n=127                                                  |                    |              |          |              |           |
| AA       | 284 (0.47)                                              | 67 (0.53)                                              | Referent           | –           | 0.693    | 0.143       |           |
| AG       | 279 (0.46)                                              | 48 (0.38)                                              | 0.729 (0.475–1.115) | 0.153       |          | 30.1        |           |
| GG       | 38 (0.07)                                               | 12 (0.09)                                              | 1.339 (0.603–2.790) | 0.520       |          | 12.0        |           |
| AG+GG    | 317 (0.53)                                              | 60 (0.47)                                              | 0.802 (0.536–1.199) | 0.303       |          | 18.1        |           |
| MAF      | 355 (0.29)                                              | 72 (0.28)                                              | 0.944 (0.689–1.284) | 0.768       |          | 6.0         |           |
|          | n=426                                                   | n=90                                                   |                    |              |          |              |           |
| AA       | 201 (0.47)                                              | 50 (0.56)                                              | Referent           | –           | 0.505    | 0.073       | 41.6      |
| AG       | 203 (0.48)                                              | 32 (0.36)                                              | 0.634 (0.377–1.055) | 0.082       |          | 13.1        |           |
| GG       | 22 (0.05)                                               | 8 (0.09)                                               | 1.462 (0.530–3.659) | 0.518       |          | 29.8        |           |
| AG+GG    | 225 (0.53)                                              | 40 (0.44)                                              | 0.715 (0.440–1.157) | 0.184       |          | 8.6         |           |
| MAF      | 247 (0.29)                                              | 48 (0.27)                                              | 0.891 (0.606–1.293) | 0.596       |          |             |           |
|          | n=175                                                   | n=37                                                   |                    |              |          |              |           |
| AA       | 83 (0.47)                                               | 17 (0.46)                                              | Referent           | –           | 0.789    | 0.949       | 3.5       |
| AG       | 76 (0.43)                                               | 16 (0.43)                                              | 1.028 (0.451–2.334) | 1.000       |          | 5.2         |           |
| GG       | 16 (0.09)                                               | 4 (0.11)                                               | 1.221 (0.264–4.460) | 0.961       |          | 4.8         |           |
| AG+GG    | 92 (0.53)                                               | 20 (0.54)                                              | 1.061 (0.491–2.316) | 1.000       |          | 5.1         |           |
| MAF      | 108 (0.31)                                              | 24 (0.32)                                              | 1.076 (0.600–1.889) | 0.890       |          |             |           |

anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus; MAF – minor allele frequency.
Supplementary Table 4. Comparison of the distribution of MCP1 rs1024611 polymorphic variants in anti-HBs-negative hemodialysis (HD) patients despite vaccination or infection.

| Genotype | Anti-HBs negative HD patients despite vaccination (frequency) | Anti-HBs negative HD patients despite infection (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | Power (%) |
|----------|---------------------------------------------------------------|------------------------------------------------------------|--------------------|--------------|----------|-----------|
|          | All HD patients n=153 n=43                                   |                                                            |                    |              |          |           |
|          | AA 65 (0.42) 20 (0.47) Referent – 0.845 0.786               |                                                            |                    |              |          |           |
|          | AG 73 (0.48) 18 (0.42) 0.801 (0.365–1.752) 0.674           |                                                            |                    |              |          |           |
|          | GG 15 (0.10) 5 (0.11) 1.083 (0.273–3.662) 1.000           |                                                            |                    |              |          |           |
|          | AG+GG 88 (0.57) 23 (0.53) 0.849 (0.408–1.782) 0.764        |                                                            |                    |              |          |           |
|          | MAF 103 (0.34) 28 (0.33) 0.952 (0.549–1.624) 0.957         |                                                            |                    |              |          |           |
|          | HD cases without DM n=106 n=32                              |                                                            |                    |              |          |           |
|          | AA 44 (0.42) 16 (0.50) Referent – 0.680 0.511              |                                                            |                    |              |          |           |
|          | AG 52 (0.49) 12 (0.38) 0.635 (0.246–1.160) 0.402          |                                                            |                    |              |          |           |
|          | GG 10 (0.09) 4 (0.13) 1.100 (0.220–4.536) 1.000           |                                                            |                    |              |          |           |
|          | AG+GG 62 (0.58) 16 (0.50) 0.710 (0.297–1.699) 0.517        |                                                            |                    |              |          |           |
|          | MAF 72 (0.34) 20 (0.31) 0.884 (0.478–1.666) 0.808          |                                                            |                    |              |          |           |
|          | HD cases with DM n=47 n=11                                  |                                                            |                    |              |          |           |
|          | AA 21 (0.45) 4 (0.36) Referent – 0.757 0.839              |                                                            |                    |              |          |           |
|          | AG 21 (0.45) 6 (0.55) 1.500 (0.301–8.269) 0.832          |                                                            |                    |              |          |           |
|          | GG 5 (0.11) 1 (0.09) 1.050 (0.018–14.37) 1.000           |                                                            |                    |              |          |           |
|          | AG+GG 26 (0.55) 7 (0.64) 1.413 (0.307–7.465) 0.879        |                                                            |                    |              |          |           |
|          | MAF 31 (0.33) 8 (0.36) 1.161 (0.379–3.345) 0.945          |                                                            |                    |              |          |           |

anti-HBs – antibodies to surface antigen of hepatitis B virus; DM – diabetes mellitus; MAF – minor allele frequency.
**Supplementary Table 5.** Comparison of the distribution of MCP1 rs1024611 polymorphic variants between HD patients with isolated anti-HBc positivity and HD patients with HBV resolution.

| Genotype | HD patients with isolated anti-HBc positivity (frequency) | HD patients anti-HBc positive/HBsAg negative/anti-HBs positive HD (frequency) | Odds ratio (95%CI) | Two-tailed P | P_trend | P_genotyping | Power (%) |
|----------|-----------------------------------------------------------|------------------------------------------------------------------------|------------------|--------------|---------|-------------|-----------|
| AA       | n=28                                                      | n=126                                                                  |                  |              |         |             |           |
|          | 16 (0.57)                                                 | 66 (0.52)                                                              | Referent         | 0.427        | 0.586   |             | 3.8       |
| AG       | 11 (0.39)                                                 | 48 (0.38)                                                              | 1.058 (0.417–2.765) | 1.000 |         |             |           |
| GG       | 1 (0.04)                                                  | 12 (0.10)                                                              | 2.909 (0.373–132.0) | 0.550 |         |             | 2.6       |
| AG+GG    | 12 (0.43)                                                 | 60 (0.48)                                                              | 1.212 (0.492–3.052) | 0.807 |         |             | 5.4       |
| MAF      | 13 (0.23)                                                 | 72 (0.29)                                                              | 1.323 (0.650–2.844) | 0.525 |         |             | 10.1      |

anti-HBc – antibodies to core antigen of hepatitis B virus; HBV – hepatitis B virus; HD – hemodialysis.

**Supplementary Table 6.** Distribution of main demographic and clinical data in the entire group of anti-HBc positive hemodialysis patients selected according to genotypes of MCP1 rs1024611.

| Parameter                                             | AA n=87 | AG n=66 | GG n=17 | P value between all groups |
|-------------------------------------------------------|---------|---------|---------|---------------------------|
| Male gender (n,%)                                      | 53 (60.9) | 34 (51.5) | 12 (70.6) | 0.290*                    |
| Age (years)                                           | 61.1±14.7 | 62.7±14.3 | 61.4±16.8 | 0.979*                    |
| Diabetic nephropathy (n,%)                            | 21 (24.1) | 22 (33.3) | 5 (29.4) | 0.462*                    |
| Chronic glomerulonephritis (n,%)                      | 16 (18.4) | 12 (18.2) | 4 (23.5) | 0.917*                    |
| Hypertensive nephropathy (n,%)                        | 18 (20.7) | 8 (12.1)  | 0 (0.0)  | 0.066*                    |
| Chronic tubulointerstitial nephritis (n,%)            | 5 (5.7)  | 5 (7.6)  | 3 (17.6) | 0.237*                    |
| Polycystic kidney disease (n,%)                       | 4 (4.6)  | 4 (6.1)  | 0 (0.0)  | 0.766*                    |
| RRT vintage (years)                                   | 2.5 (0.05–25.1) | 3.6 (0.05–26.3) | 2.3 (0.14–24.8) | 0.512*                    |
| HBsAg positive/anti-HBs negative (n,%)                | 4 (4.6)  | 7 (10.6) | 4 (23.5) | AA vs. AG p=0.268*        |
| HBsAg negative/anti-HBs positive (n,%)                | 66 (75.9) | 48 (72.7) | 12 (70.6) | 0.844*                    |
| Isolated anti-HBc positivity (n,%)                    | 16 (18.4) | 11 (16.7) | 1 (5.9)  | 0.472*                    |
| HBsAg positive/anti-HBs positive (n,%)                | 1 (1.1)  | 0 (0.0)  | 0 (0.0)  | 1.000*                    |
| ALT (U/L)                                             | 17 (3–50) | 15 (2–195) | 19 (9–95) | 0.266*                    |
| AST (U/L)                                             | 16 (6–72) | 19 (9–152) | 18 (9–64) | 0.571*                    |
| GGT (U/L)                                             | 25 (4–498) | 27 (5–211) | 35 (10–147) | 0.757*                    |

ALT – alanine aminotransferase; anti-HBc – antibodies to core antigen of hepatitis B virus; anti-HBs – antibodies to surface antigen of hepatitis B virus; AST – aspartate aminotransferase; GGT – gamma-glutamyltranspeptidase; HBsAg – surface antigen of hepatitis B virus; RRT – renal replacement therapy. Statistical tests: a – Chi square; b – ANOVA; c – Fisher Freeman Halton; d – Kruskal-Wallis; e – Yates corrected Chi-square. Significant differences are indicated using bold font.
Supplementary Table 7. Comparison of the distribution of MCP1 rs1024611 polymorphic variants in all anti-HBc positive HD patients as well as in non-DM and DM patients to respective genotype frequencies in controls.

| Genotype | HD patients (frequency) | Controls (frequency) | Odds ratio (95%CI) | Two-tailed P | P trend | Power (%) |
|----------|------------------------|---------------------|-------------------|--------------|----------|-----------|
|          | n=170                  | n=437               |                   |              |          |           |
| AA       | 87 (0.51)              | 225 (0.51)          | Referent          | –            | 0.693    | 0.721     |
| AG       | 66 (0.39)              | 177 (0.41)          | 0.964 (0.650–1.428) | 0.927        |          |           |
| GG       | 17 (0.10)              | 35 (0.08)           | 1.256 (0.625–2.443) | 0.578        |          |           |
| AG+GG    | 83 (0.49)              | 212 (0.49)          | 1.013 (0.699–1.466) | 1.000        |          |           |
| MAF      | 100 (0.29)             | 247 (0.28)          | 1.058 (0.793–1.405) | 0.740        |          |           |
|          | n=122                  | n=437               |                   |              |          |           |
| AA       | 66 (0.54)              | 225 (0.51)          | Referent          | –            | 0.905    | 0.613     |
| AG       | 44 (0.36)              | 177 (0.41)          | 0.848 (0.531–1.329) | 0.519        |          | 10.2      |
| GG       | 12 (0.10)              | 35 (0.08)           | 1.169 (0.522–2.466) | 0.790        |          | 6.6       |
| AG+GG    | 56 (0.46)              | 212 (0.49)          | 0.901 (0.589–1.373) | 0.684        |          | 6.7       |
| MAF      | 68 (0.28)              | 247 (0.28)          | 0.981 (0.703–1.358) | 0.973        |          | 4.6       |
|          | n=48                   | n=437               |                   |              |          |           |
| AA       | 21 (0.44)              | 225 (0.51)          | Referent          | –            | 0.297    | 0.573     |
| AG       | 22 (0.46)              | 177 (0.41)          | 1.332 (0.674–2.634) | 0.463        |          | 12.0      |
| GG       | 5 (0.10)               | 35 (0.08)           | 1.531 (0.423–4.538) | 0.579        |          | 11.7      |
| AG+GG    | 27 (0.56)              | 212 (0.49)          | 1.365 (0.718–2.621) | 0.387        |          | 17.0      |
| MAF      | 32 (0.33)              | 247 (0.28)          | 1.269 (0.782–2.025) | 0.355        |          | 17.1      |

anti-HBc – antibodies to core antigen of hepatitis B virus; DM – diabetes mellitus, hemodialysis; HD – hemodialysis; MAF – minor allele frequency.

Acknowledgements

We would like to express our gratitude to physicians of the dialysis centers for their consent in collecting the participants’ data during the study period.

Conflict of interest

None declared.

References:

1. Grob P, Jilg W, Bornhak H et al: Serological pattern ‘anti-HBc alone’: report on a workshop. J Med Virol, 2000; 62: 450–55
2. Juhl D, Luhm J, Görg S et al: Evaluation of algorithms for the diagnostic assessment and the reentry of blood donors who tested reactive for antibodies against hepatitis B core antigen. Transfusion, 2011; 51: 1477–85
3. Grzegorzewska AE, Kaczmarek-Leki V, Motł-Michalska M, Niepolski L: Seroconversion rate to positivity for antibodies against core antigen of hepatitis B virus and duration of renal replacement therapy. Nephrol Dial Transplant, 2011; 26: 970–76
4. Meng ZF, Wang HJ, Yao X et al: Immunization with HBsAg-Fc fusion protein induces a predominant production of Th1 cytokines and reduces HBsAg level in transgenic mice. Chin Med J (Engl), 2012; 125: 3266–72
5. Fierro NA, Roman S, Realpe M et al: Multiple cytokine expression profiles reveal immune-based differences in occult hepatitis B genotype B infected Mexican Nahua patients. Mem Inst Oswaldo Cruz, 2011; 106: 1007–13
6. Shen HY, Deng YC, Wang QM et al: Expression of MCP-1 in the patients of chronic hepatitis B complicated with nonalcoholic fatty liver disease [Article in Chinese]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi, 2012; 28: 975–78
7. Wang WW, Ang SF, Kumar R et al: Identification of serum monocyte chemoattractant protein-1 and prolactin as potential tumor markers in hepatocellular carcinoma. PLoS One, 2013; 8: e68904
8. Uchida E, Anan F, Masaki T et al: Monocyte chemoattractant protein-1 is associated with silent cerebral infarction in patients on haemodialysis. Intern Med, 2012; 42: 29–34
9. Morena M, Jaussent I, Chalabi L et al: Biocompatibility of heparin-grafted membranes: impact on monocyte chemoattractant protein-1 circulating level and oxidative status. Hemodial Int, 2010; 14: 403–10
10. Olsson J, Paulsson J, Dadfar E et al: Monocyte and neutrophil chemotactic activity at the site of interstitial inflammation in patients on high-flux haemodialysis or hemodiafiltration. Blood Purif, 2009; 28: 47–52
11. Buraczyńska M, Bednarek-Skublewska A, Buraczyńska K, Książek A: Monocyte chemoattractant protein-1 (MCP-1) gene polymorphism as a potential risk factor for cardiovascular disease in hemodialyzed patients. Cytokine, 2008; 44: 361–65
12. Papayianni A, Alexopoulos E, Giamalis P et al: Circulating levels of ICAM-1, VCAM-1, and MCP-1 are increased in haemodialysis patients: association with inflammation, dyslipidaemia, and vascular events. Nephrol Dial Transplant, 2002; 17: 435–41
13. Rovin BH, Lu L, Saxena R: A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem Biophys Res Commun, 1999; 259: 344–48
14. Fenoglio C, Galimberti D, Lovati C et al: MCP-1 in Alzheimer’s disease patients: A -2518G polymorphism and serum levels. Neurobiol Aging, 2004; 25: 1169–73
15. Xu ZE, Xie YY, Chen JH et al: Monocyte chemoattractant protein-1 gene polymorphism and monocyte chemotactic protein-1 expression in Chongqing Han children with tuberculosis. Zhonghua Er Ke Za Zhi, 2009; 47: 200–3 [in Chinese]
16. Flores-Villanueva PO, Ruiz-Morales JA, Song CH et al: A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. J Exp Med, 2005; 202: 1649–58
17. El-Gezawy EM, Eldin EN, Mohamed WS et al: Tumor necrosis factor-alfa and monocyte chemoattractant protein-1 gene polymorphisms in kidney transplant recipients. Saudi J Kidney Dis Transpl, 2013; 24: 688–95
18. Park BL, Kim YJ, Cheong HS et al: Association of common promoter polymorphisms of MCP1 with hepatitis B virus clearance. Exp Mol Med, 2006; 38: 694–702
19. Cheong JY, Cho SW, Choi JY et al: RANTES, MCP-1, CCR2, CCR5, CXCR1 and CXCR4 gene polymorphisms are not associated with the outcome of hepatitis B virus infection: results from a large scale single ethnic population. J Korean Med Sci, 2007; 22: 529–35
20. Mostowska M, Lianeri M, Oko A et al: No association of monocyte chemoattractant protein-1 22518 A/G polymorphism with the risk of primary glomerulonephritis in the Polish population. Mol Biol Rep, 2012; 39: 5933–41
21. Grzegorzewska AE, Pajdzerski D, Sowińska A, Jagodziński PP: Distribution of Monocyte Chemoattractant Protein-1 -2518 A/G Polymorphism and Responsiveness to Hepatitis B Vaccination in Hemodialysis Patients. Pol Arch Med Wewn, 2014; 124: 10–18
22. Grzegorzewska AE, Pajdzerski D, Sowińska A et al: IL4R and IL13 polymorphic variants and development of antibodies to surface antigen of hepatitis B virus in hemodialysis patients in response to HBV vaccination or infection. Vaccine, 2013; 31: 1766–70
23. Schiffer JT, Swan DA, Stone D, Jerome KR: Predictors of hepatitis B cure using gene therapy to deliver DNA cleavage enzymes: a mathematical modeling approach. PLoS Comput Biol, 2013; 9: e1003131
24. Cheng PN, Liu WC, Tsai HW et al: Association of intrahepatic cccDNA reduction with the improvement of liver histology in chronic hepatitis B patients receiving oral antiviral agents. J Med Virol, 2011; 83: 602–7
25. Hällinger FB, Sood G: Occult hepatitis B virus infection: a covert operation. J Viral Hepat, 2010; 17: 1–15
26. Karadeniz M, Erdogan M, Cetinkalp S et al: Monocyte chemoattractant protein-1 (MCP-1) 2518G/A gene polymorphism in Turkish type 2 diabetes patients with nephropathy. Endocrine, 2010; 37: 513–17
27. Simeoni E, Hoffmann MM, Winkelmann BR et al: Association between the A-2518G polymorphism in the monocyte chemoattractant protein-1 gene and insulin resistance and Type 2 diabetes mellitus. Diabetologia, 2004; 47: 1574–80
28. Alavian SM, Tabatabaie SV: The effect of diabetes mellitus on immunological response to hepatitis B virus vaccine in individuals with chronic kidney disease: A meta-analysis of current literature. Vaccine, 2010; 28: 3773–77
29. Venkat-Raman G, Tomson CR, Gao Y et al: New primary renal diagnosis codes for the ERA-EDTA. Nephrol Dial Transplant, 2012; 27: 4414–19