Markers of the Hepatic Component of the Metabolic Syndrome as Predictors of Mortality in Renal Transplant Recipients


*Department of Nephrology, bDepartment of Epidemiology, cDepartment of Laboratory Medicine and dDepartment of Internal Medicine, University Medical Center Groningen, The Netherlands
eRenal Transplant Program, University of Groningen and University Medical Center Groningen, The Netherlands
*Corresponding author: Stephan J.L. Bakker, s.j.l.bakker@int.umcg.nl

Cardiovascular disease (CVD) is a leading cause of mortality in renal transplant recipients (RTRs). Metabolic syndrome (MS) is highly prevalent in RTRs. Nonalcoholic fatty liver disease (NAFLD) is considered the hepatic component of MS. We investigated associations of NAFLD markers with MS and mortality. RTRs were investigated between 2001 and 2003. NAFLD markers, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (AP) were measured. Bone and nonbone fractions of AP were also determined. Death was recorded until August 2007. Six hundred and two RTRs were studied (age 52 ± 12 years, 55% men). At baseline 388 RTRs had MS. Prevalence of MS was positively associated with liver enzymes. During follow-up for 5.3[4.5–5.7] years, 95 recipients died (49 cardiovascular). In univariate Cox regression analyses, GGT (HR = 1.43[1.21–1.69], p < 0.001) and AP (HR = 1.34[1.11–1.63], p = 0.003) were associated with mortality, whereas ALT was not. Similar associations were found for cardiovascular mortality. Adjustment for potential confounders, including MS, diabetes and traditional risk factors did not materially change these associations. Results for nonbone AP mirrored that for total AP. ALT, GGT and AP are associated with MS. Of these three enzymes, GGT and AP are associated with mortality, independent of MS. These findings suggest that GGT and AP are independently related to mortality in RTRs.

Key words: Cardiovascular risk factors, fatty liver disease, mortality, renal transplant patients

Introduction

Despite major improvements in short-term survival after renal transplantation, long-term prognoses are still poor. Many renal transplant recipients (RTRs) die prematurely from cardiovascular disease (CVD) (1). This excess in mortality can only partly be explained by the classical risk factors such as dyslipidemia, hypertension and smoking (1–3). The metabolic syndrome (MS), characterized by a clustering of risk factors, is a herald of CVD in the general population. Prevalence of MS and its accompanying cardiovascular risks is increased in the renal transplant population (3).

All components of MS, including waist circumference, glucose, triglycerides, high-density lipoprotein (HDL)-cholesterol levels and blood pressure have been shown to correlate with liver fat in the general population (4). In this population, nonalcoholic fatty liver disease (NAFLD) is a common hepatic disorder characterized by liver fat accumulation and an increased risk for cardiovascular death (5). NAFLD is considered to be the hepatic component of MS (6). Prevalence of NAFLD is varying between studies, but it is estimated that approximately one fourth of healthy adults are affected (4).

Prevalence of NAFLD is much higher in patients with type 2 diabetes mellitus and in morbidly obese patients (7).

In most cases of NAFLD, enzyme activity of alanine aminotransferase (ALT) in the circulation is elevated and consequently it is used as a marker of NAFLD (8–12). Serum gamma-glutamyl transferase (GGT) is well known as a marker of hepatobiliary disease and excessive alcohol consumption (13), but recent studies in the general population indicate that GGT is also associated with NAFLD and increased risk for CVD (9,13–18). In the same population, both ALT and GGT are strongly correlated with MS (19–22). Alkaline phosphatase (AP) is most commonly used to monitor metabolic bone disease, but is also associated with MS in the general population and with increased risk for death in hemodialysis patients (23,24).

We aimed to cross-sectionally investigate whether the liver enzymes ALT, GGT and AP are associated with MS in RTRs. We furthermore aimed to prospectively investigate
Materials and Methods

Research design and subjects
In this prospective cohort study, we invited all RTRs, with a functioning graft for more than 1 year, who visited our outpatient clinic between 2001 and 2003. The group that did not sign informed consent was comparable with the group that did sign informed consent with respect to age, sex, body mass index, serum creatinine, creatinine clearance and proteinuria. In patients with fever or other signs of infection (e.g. complaints of upper respiratory tract infection or urinary tract infection), baseline visits were postponed until symptoms had resolved. Patients with overt congestive heart failure and patients diagnosed with cancer other than cured skin cancer were not considered eligible for the study. A total of 606 out of 847 eligible RTRs signed written informed consent. Liver enzymes were available in 602 recipients. Full details on the study design have been previously reported (28). The Institutional Review Board approved the study protocol (METc 2001/039).

Endpoints of the study
The primary end point of this study was RTR mortality. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status and cause of death. We contacted general practitioners or referring nephrologists in case the status of a patient was unknown. Mortality was recorded until August 2007. Cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Central Bureau of Statistics. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9). Cardiovascular death was defined as deaths in which the principal cause of death was cardiovascular in nature, using ICD-9 codes 410–447. There was no loss due to follow up.

Renal transplant characteristics
Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant characteristics such as age, gender and date of transplantation were extracted from this database. Current medication was taken from the medical record. Smoking status and CVD history were obtained using a self-report questionnaire. CVD history was considered positive if participants had a myocardial infarction, transient ischemic attack or cerebrovascular accident.

Measurements and definitions
Body mass index (BMI) was calculated as weight in kilograms, divided by height in meters squared. Hip circumference was measured at the widest point at the level of the trochanter major and waist circumference was measured at the point halfway between the spina iliaca and the lower rib using a plastic tape measure. Blood pressure was measured as the average of three automated (Omron M4; Omron Europe B.V., The Netherlands) measurements with 1-min intervals after a 6-min rest in supine position. Blood was drawn after an overnight fasting period. Serum creatinine concentrations were determined using the Jaffé method, and serum triglycerides were determined with the GPO-PAP method. Total cholesterol was determined using the CHOD PAP method on a Technikon RA-1000 (Bayer Diagnostics b.v., Mijdrecht, The Netherlands) and low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula (27). Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH). HbA1c was determined by high performance liquid chromatography (VARIANT™ HbA1c Program with Bio-Rad CARITANt Bt Imaging System, Bio-Rad, Hercules, CA). Serum high-sensitive C-reactive protein (hsCRP) was assessed with a high-sensitivity CRP ELISA assay as described earlier (28).

Creatinine clearance was calculated from 24-h urinary creatinine excretion and serum creatinine. Total urinary protein concentration was analyzed using the Biuret reaction (MEGA AU 510; Merck Diagnostica) and proteinuria was defined as urinary protein excretion > 0.5 g per 24 h.

In this study, MS was defined by the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATP) (31). MS is defined by the NCEP-ATP when three or more of the following components are present: (1) a waist circumference > 102 cm in men and > 88 cm in women; (2) serum triglycerides ≥ 1.70 mmol/L; (3) serum HDLc < 1.03 mmol/L in men and <1.29 mmol/L in women; (4) blood pressure > 130/85 mmHg or use of antihypertensive medication; and (5) fasting plasma glucose ≥ 6.1 mmol/L or use of antidiabetic medication. Recently, the American Diabetes Association (ADA) lowered the cut-off point for impaired fasting glucose to ≥5.6 mmol/L (26). For our analysis of the prevalence of the MS we used this ADA cut-off point. In our laboratory, the normal ranges for ALT, GGT and AP are < 45 U/L for ALT, < 40 U/L in females and < 55 U/L in males for GGT and < 120 U/L for AP.

Statistical analysis
Data were analyzed with SPSS version 16.0 (SPSS Inc., Chicago, IL) and GraphPad Prism version 4.03 (GraphPad Software Inc., San Diego, CA). Continuous variables were summarized using means (standard deviations) and medians (interquartile range); percentages were used to summarize categorical variables. Log-transformation was used for variables with a skewed distribution. Hazard ratios (HRs) are reported with 95% confidence interval [95% CI].

Recipient- and transplantation-related characteristics were analyzed separately for quartiles of liver enzymes. Student’s t-test or Kruskal–Wallis test was used to compare means for continuous variables and with Chi-square for categorical variables. The association between quartiles of ALT, GGT and AP and prevalence of MS was tested with Chi-square test. Subsequently, the relationship between single components of MS and liver enzymes were analyzed and tested with Chi-square test.

To analyze whether ALT, GGT and AP are associated with mortality, we first performed Kaplan–Meier analyses with log-rank test. For these analyses, levels of ALT, GGT and AP were divided into quartiles. Multivariate Cox regression analyses were performed to investigate whether ALT, GGT and AP were independently associated with all-cause and cardiovascular mortality. For these analyses, ALT, GGT and AP were first log-transformed to achieve a normal distribution and then transformed into z-scores, which results in expression of HRs per standard deviation change in the log-transformed variables. In this way, strengths of associations can be compared between
variables. In the Cox regression analyses, we adjusted for recipient age, sex, creatinine clearance, urinary protein excretion (Model 2), additionally adjusted for presence of diabetes, HbA1c, fasting glucose, fasting insulin, use of antidiabetic drugs and duration of diabetes (Model 3), all individual components of the MS (Model 4) and other cardiovascular risk factors (Model 5). As secondary analyses, we also performed similar Cox regression analyses with all-cause mortality as end point, after exclusion of all subjects with diabetes. To allow for comparison of strength of associations of GGT and AP with mortality with associations of more classical risk factors for mortality, we performed age- and sex-adjusted analyses for several risk factors, including HbA1c and HDL cholesterol (Table 4). To allow for mutual comparison of strengths of associations, variables were transformed to z-scores prior to entrance in the Cox regression analyses, which results in expression of HRs per standard deviation change in the respective variables.

Results

Cross-sectional association of ALT, GGT and AP with features of the MS

A total of 602 RTRs were studied (mean age 52 ± 12 years, 55% men). Baseline characteristics according to quartiles of serum activity levels of ALT, GGT and AP are shown in Table 1. Median [interquartile range] activity of ALT, GGT and AP were 18.0 [14–25] U/L, 24.0 [17.8–39.0] U/L, and 72.0 [57.0–93.3] U/L respectively, with bone and nonbone AP respectively accounting for 42.9 ± 11% and 57.1 ± 12% of total AP activity. Enzyme activities exceeding the normal ranges of ALT, GGT and AP, respectively, were present in 29 (4.8%), 118 (19.7%) and 59 (9.8%) of RTRs. Levels of ALT are positively associated with male gender, MS, BMI, waist, fasting triglycerides, use of statins, fasting insulin concentrations, prevalence of posttransplant diabetes, use of antidiabetic drugs and creatinine clearance, whereas there are inverse associations with smoking status and prevalence of polycystic renal disease as primary renal disease. Similar associations were found for GGT, except for absence of associations with smoking and creatinine clearance and a positive rather than an inverse association with polycystic renal disease. GGT appeared also positively associated with age, fasting glucose and HbA1c. For AP, also similar associations were present as for ALT, except for absence of associations with male gender, smoking status, use of statins and polycystic renal disease, presence of inverse associations with alcohol intake and HDL cholesterol and positive associations with systolic blood pressure, fasting glucose, HbA1c, pretransplant diabetes, use of insulin and hsCRP.

A total of 388 out of 602 recipients suffered from MS. The association between liver enzymes and MS is shown in Figure 1, with significantly increasing prevalence of MS over respective quartiles of liver enzymes. Prevalence of MS in the first versus fourth quartile of liver enzymes was 57.9% versus 72.7% for ALT, 52.3% versus 73.7% for GGT and 50.3% versus 82.2% for AP. To investigate which component of the MS contributed most to the relationship with liver enzymes, components were analyzed separately. Percentages of patients meeting the component criteria of MS according to quartiles of liver enzymes are shown in Table 2. Levels of ALT, GGT and AP were significantly associated with abdominal obesity, hypertriglyceridemia and impaired fasting glucose (Table 2). Higher levels of AP were also associated with low HDL cholesterol concentrations. If the in-total 107 RTRs with either pre- or posttransplant diabetes were excluded, existing associations of liver enzymes with abdominal obesity, hypertriglyceridemia and HDL cholesterol remained essentially unchanged, whereas associations with the impaired fasting glucose criterion of the MS became insignificant. If after exclusion, analyses were not restricted to individual components but to prevalence of MS overall, the association with ALT lost significance (prevalence of MS 53.5%, 53.7%, 57.6% and 63.3% according to increasing quartiles, p = 0.36), whereas associations with GGT (45.1%, 59.9%, 57.7% and 64.8% respectively, p = 0.02) and AP (43.5%, 53.7%, 57.1% and 74.4% respectively, p < 0.001) remained.

Prospective association of ALT, GGT and AP with mortality

During (median) follow-up for 5.3 [4.5–5.7] years, 95 recipients died, with 49 deaths cardiovascular in origin. We first performed Kaplan–Meier analyses for quartiles of liver enzymes. The incidence of death was almost equally distributed over the different quartiles of ALT, with respective numbers of 21 (14.9%), 25 (14.8%), 29 (20.3%) and 20 (13.2%) according to respective quartiles (log-rank test: p = 0.46). It, however, significantly increased according to increasing quartiles of GGT, with respective numbers of 14 (9.3%), 17 (11.7%), 29 (18.7%) and 35 (23.0%) (log-rank test: p = 0.001; Figure 2A). A similar association with death was present for total AP, with incidences of death of 18 (11.8%), 24 (16.0%), 21 (14.3%) and 32 (21.1%) according to increasing quartiles respectively (log-rank test: p = 0.02; Figure 2B). This association was not present for bone-specific AP, with incidences of death of 20 (12.9%), 27 (18.4%), 25 (16.6%) and 23 (15%) according to increasing quartiles respectively (log-rank test: p = 0.30). For nonbone AP, however, a similar—albeit somewhat weaker—association was present as for total AP, with incidences of death of 15 (10.0%), 21 (14.0%), 28 (18.4%) and 31 (20.8%) according to increasing quartiles respectively (log-rank test: p = 0.04). Of note, increases in GGT and total AP within the normal range are already associated with higher mortality, because the second and the third quartiles in which levels are still within the normal range (the second and third quartiles were analyzed together because lines were comparable) are at increased risk compared to the first quartile.

Results of univariate and multivariate Cox regression analyses are shown in Table 3. In univariate analyses, GGT strongly predicted all-cause and cardiovascular mortality in RTRs (Model 1). These associations weakened after adjustment for age, sex, creatinine clearance and urinary protein...
<table>
<thead>
<tr>
<th></th>
<th>First</th>
<th>Second + Third</th>
<th>Fourth</th>
<th>First</th>
<th>Second + Third</th>
<th>Fourth</th>
<th>First</th>
<th>Second + Third</th>
<th>Fourth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recipient demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.5 ± 13</td>
<td>51.8 ± 12</td>
<td>51.8 ± 11</td>
<td>47.6 ± 13</td>
<td>52.4 ± 11(^1)</td>
<td>53.6 ± 11(^1)</td>
<td>50.6 ± 12</td>
<td>51.2 ± 12</td>
<td>53.0 ± 11</td>
</tr>
<tr>
<td>Male (%)</td>
<td>42.9</td>
<td>55.5(^1)</td>
<td>63.6(^1)</td>
<td>47.3</td>
<td>58.0(^1)</td>
<td>54.6(^1)</td>
<td>51.3</td>
<td>54.5</td>
<td>58.6</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>31.4</td>
<td>21.4(^1)</td>
<td>14.9(^1)</td>
<td>24.0</td>
<td>21.0</td>
<td>22.4</td>
<td>22.2</td>
<td>20.2</td>
<td>25.7</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>15.1</td>
<td>13.4</td>
<td>15.1</td>
<td>14.8</td>
<td>15.4</td>
<td>11.4</td>
<td>21.6</td>
<td>11.4(^1)</td>
<td>12.3(^1)</td>
</tr>
<tr>
<td>MS (%)</td>
<td>57.9</td>
<td>63.3</td>
<td>72.7(^1,2)</td>
<td>53.3</td>
<td>65.3(^1)</td>
<td>73.7(^1,2)</td>
<td>50.3</td>
<td>62.6(^1)</td>
<td>82.2(^1,2)</td>
</tr>
<tr>
<td>History of CVD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI (%)</td>
<td>7.1</td>
<td>7.2</td>
<td>10.5</td>
<td>6.8</td>
<td>8.0</td>
<td>9.2</td>
<td>11.8</td>
<td>5.8</td>
<td>8.6</td>
</tr>
<tr>
<td>TIA/CVA (%)</td>
<td>6.4</td>
<td>5.2</td>
<td>5.2</td>
<td>4.7</td>
<td>5.4</td>
<td>6.6</td>
<td>6.6</td>
<td>4.1</td>
<td>7.2</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.8 ± 4</td>
<td>26.3 ± 4(^1)</td>
<td>26.9 ± 5(^1)</td>
<td>24.9 ± 4</td>
<td>26.2 ± 4(^1)</td>
<td>27. ± 5(^1,2)</td>
<td>24.9 ± 4</td>
<td>25.2 ± 4(^1)</td>
<td>27.1 ± 5(^1,2)</td>
</tr>
<tr>
<td>Waist (cm) women</td>
<td>90 ± 14</td>
<td>96 ± 15(^1)</td>
<td>96 ± 15(^1)</td>
<td>88 ± 12</td>
<td>96 ± 14(^1)</td>
<td>99 ± 16(^1)</td>
<td>89 ± 14</td>
<td>95 ± 14(^1)</td>
<td>99 ± 15(^1,2)</td>
</tr>
<tr>
<td>Waist (cm) men</td>
<td>94 ± 12</td>
<td>100 ± 12(^1)</td>
<td>103 ± 13(^1)</td>
<td>95 ± 12</td>
<td>99 ± 12(^1)</td>
<td>105 ± 12(^1,2)</td>
<td>97 ± 13</td>
<td>99 ± 12</td>
<td>104 ± 13(^1,2)</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>153 ± 25</td>
<td>153 ± 22</td>
<td>153 ± 21</td>
<td>151 ± 22</td>
<td>154 ± 23</td>
<td>153 ± 22</td>
<td>149 ± 20</td>
<td>153 ± 23</td>
<td>157 ± 23(^1,2)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>90 ± 10</td>
<td>90 ± 10</td>
<td>90 ± 9</td>
<td>90 ± 10</td>
<td>90 ± 10</td>
<td>89 ± 10</td>
<td>89 ± 10</td>
<td>90 ± 10</td>
<td>91 ± 10</td>
</tr>
<tr>
<td>Use of ACE-i (%)</td>
<td>27.1</td>
<td>40.9</td>
<td>26.6</td>
<td>37.3</td>
<td>34.0</td>
<td>30.9</td>
<td>39.2</td>
<td>33.0</td>
<td>30.9</td>
</tr>
<tr>
<td>Use of β-blocker (%)</td>
<td>57.9</td>
<td>62.7</td>
<td>63.6</td>
<td>59.3</td>
<td>61.0</td>
<td>65.8</td>
<td>62.1</td>
<td>60.9</td>
<td>63.2</td>
</tr>
<tr>
<td>Number of AHD</td>
<td>1.9 ± 1</td>
<td>2.0 ± 1</td>
<td>1.8 ± 1</td>
<td>1.8 ± 1</td>
<td>1.9 ± 1</td>
<td>2.0 ± 1</td>
<td>1.9 ± 1</td>
<td>1.9 ± 1</td>
<td>2.0 ± 1</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.6 ± 1</td>
<td>5.6 ± 1</td>
<td>5.6 ± 1</td>
<td>5.5 ± 1</td>
<td>5.6 ± 1</td>
<td>5.7 ± 1</td>
<td>5.5 ± 1</td>
<td>5.7 ± 1</td>
<td>5.6 ± 1</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.6 ± 1</td>
<td>3.5 ± 1</td>
<td>3.5 ± 1</td>
<td>3.6 ± 1</td>
<td>3.6 ± 1</td>
<td>3.6 ± 1</td>
<td>3.5 ± 1</td>
<td>3.7 ± 1</td>
<td>3.4 ± 1</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3(^1,2)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.8 [1–2]</td>
<td>1.9 [1–3]</td>
<td>2.0 [2–3](^1,2)</td>
<td>1.7 [1–2]</td>
<td>1.9 [1–3]</td>
<td>2.1 [2–3](^1,2)</td>
<td>1.7 [1–2]</td>
<td>1.9 [1–3]</td>
<td>2.2 [2–3](^1,2)</td>
</tr>
<tr>
<td><strong>Glucose homeostasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.7 ± 1</td>
<td>4.8 ± 1</td>
<td>5.0 ± 2</td>
<td>4.6 ± 1</td>
<td>4.8 ± 1</td>
<td>5.1 ± 2(^1)</td>
<td>4.5 ± 1</td>
<td>4.8 ± 1</td>
<td>5.3 ± 2(^1,2)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.4 ± 1</td>
<td>6.5 ± 1</td>
<td>6.6 ± 1</td>
<td>6.2 ± 1</td>
<td>6.5 ± 1 (^1)</td>
<td>6.8 ± 1(^1,2)</td>
<td>6.3 ± 1</td>
<td>6.5 ± 1 (^1)</td>
<td>6.9 ± 1(^1,2)</td>
</tr>
<tr>
<td>Post-Tx DM (%)</td>
<td>5.7</td>
<td>15.6(^1)</td>
<td>22.1(^1,2)</td>
<td>8</td>
<td>12.7</td>
<td>26.3(^1,2)</td>
<td>10.5</td>
<td>12.1</td>
<td>25.0(^1,2)</td>
</tr>
<tr>
<td>Pre-Tx DM (%)</td>
<td>3.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.0</td>
<td>3.3</td>
<td>2.6</td>
<td>0.0</td>
<td>3.0(^1)</td>
<td>5.3(^1)</td>
</tr>
<tr>
<td>Duration of diabetes(^3)</td>
<td>2.9 [0–26]</td>
<td>4.1 [0–8](^1)</td>
<td>1.4 [0–4]</td>
<td>4.1 [0–12]</td>
<td>2.3 [0–10]</td>
<td>1.5 [0–6]</td>
<td>0.0 [0–4.3]</td>
<td>2.7 [1–12]</td>
<td>1.8 [0–7]</td>
</tr>
<tr>
<td>Use of ADD (%)</td>
<td>6.4</td>
<td>14.0(^1)</td>
<td>18.2(^1)</td>
<td>7.3</td>
<td>12.0</td>
<td>21.7(^1,2)</td>
<td>5.2</td>
<td>12.8(^1)</td>
<td>22.4(^1,2)</td>
</tr>
<tr>
<td>Use of insulin (%)</td>
<td>4.3</td>
<td>7.1</td>
<td>7.1</td>
<td>6.7</td>
<td>6.0</td>
<td>7.2</td>
<td>2.6</td>
<td>6.4</td>
<td>10.5(^1)</td>
</tr>
<tr>
<td><strong>Renal transplant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>156 ± 74</td>
<td>146 ± 58</td>
<td>143 ± 44</td>
<td>154 ± 70</td>
<td>144 ± 53</td>
<td>148 ± 59</td>
<td>151 ± 67</td>
<td>147 ± 58</td>
<td>145 ± 54</td>
</tr>
<tr>
<td>Creat Clear (mL/min)</td>
<td>56 ± 22</td>
<td>64 ± 22(^1)</td>
<td>66 ± 23(^1)</td>
<td>60 ± 22</td>
<td>64 ± 23</td>
<td>61 ± 22</td>
<td>61 ± 22</td>
<td>62 ± 22</td>
<td>63 ± 23</td>
</tr>
</tbody>
</table>

Continued...
Table 1: Continued

<table>
<thead>
<tr>
<th>ALT</th>
<th>GGT</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second + Third</td>
</tr>
<tr>
<td></td>
<td>N = 140</td>
<td>N = 308</td>
</tr>
<tr>
<td>UPE (g/24 h)</td>
<td>0.3 [0–1]</td>
<td>0.2 [0–1]</td>
</tr>
<tr>
<td>Proteinuria (%)</td>
<td>27.1</td>
<td>29.4</td>
</tr>
<tr>
<td>Prior transplants (n)</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Primary renal disease Polycystic vs. other (%)</td>
<td>19.3</td>
<td>19.8</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SD, or median [95% CI]. Differences were tested by t-test or Kruskal–Wallis test for continuous variables and with Chi-square for categorical variables.

ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; AP = alkaline phosphatase; UPE = urinary protein excretion.

1Quartile significantly different from first quartile, p < 0.05.

3Duration of diabetes is expressed in years and only applicable to patients with diabetes.

Figure 1: Prevalence of the metabolic syndrome according to quartiles of ALT, GGT and AP

Differences were tested with Chi-square: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
In univariate analyses, AP was also strongly associated with all-cause and cardiovascular mortality (Model 1). Adjustment for age, sex, creatinine clearance and urinary protein excretion strengthened the association (Model 2), whereas it was weakened, but remaining significant after adjustment for diabetes and factors related to diabetes (Model 3), components of MS (Model 4) and cardiovascular risk factors, number of previous transplantations and total time on renal replacement therapy (Model 5). In univariate analysis, bone-specific AP was associated with neither all-cause mortality (HR = 1.10[0.90–1.35], p = 0.36) nor cardiovascular mortality (HR = 1.09[0.82–1.44], p = 0.56). Contrastingly, nonbone AP was strongly associated with all-cause and cardiovascular mortality, with HRs of 1.45[1.20–1.76], p < 0.001 and 1.63[1.27–2.08], p < 0.001 respectively for univariate analyses. Results for multivariate Cox regression analyses with nonbone AP were essentially similar to those for total AP.

In Table 4, results of age- and sex-adjusted Cox regression analyses for z-scores of classic cardiovascular risk factors, including HbA1c, HDL-cholesterol, systolic blood pressure, C-reactive protein and glucose are compared with those for GGT and AP. By using z-scores, strengths of associations are comparable because derived HRs are expressed per standard deviation change in variables. HRs of GGT and AP appeared of similar strength as those of HbA1c and hsCRP.

**Discussion**

This study shows that liver enzymes ALT, GGT and AP are positively associated with prevalence of MS in RTRs. Not all components of the MS contributed equally to this relationship. The strongest contributors for associations of MS with liver enzymes were abdominal obesity, hypertriglyceridemia and impaired fasting glucose. Only for AP low HDL-cholesterol concentrations had significant impact. If RTRs with diabetes were excluded, the association of ALT with prevalence of MS lost significance, but associations of GGT and AP remained. This study furthermore shows that liver enzymes GGT and AP strongly predicted mortality in RTRs. Increasing levels of GGT and AP—even within the normal range—are associated with increasing all-cause and cardiovascular mortality. Separate analyses for bone and nonbone AP—mainly representing liver isoenzyme activity—showed that associations present for total AP also exist for nonbone AP, whereas bone AP is unrelated. Associations of liver enzymes with all-cause and cardiovascular mortality were not materially affected by adjustments for age, sex, creatinine clearance, urinary protein excretion, diabetes and factors related to diabetes, MS and other cardiovascular risk factors. Analyses performed after exclusion of all patients with diabetes showed that the associations of GGT and AP with mortality are independent of diabetes. When GGT and AP are compared with classical cardiovascular risk factors, including HbA1c, HDL-cholesterol, systolic blood pressure and C-reactive protein, GGT and AP appeared to have associations of similar strength as the strongest of established cardiovascular risk factors.

CVD is the main cause of death in renal transplant patients (1). Traditional risk factors such as diabetes mellitus, dyslipidemia and hypertension are often seen in transplantation patients but could not fully explain the high prevalence of cardiovascular death among this patient group (3). The clustering of risk factors in the MS is associated with an increased risk of CVD and type 2 diabetes mellitus (32). It is also associated with a broad spectrum of other cardiovascular risk factors such as coagulation abnormalities, chronic inflammation, increased oxidative stress and endothelial dysfunction (33). There is a body of evidence that supports that NAFLD is the new hepatic component of the MS (6,20,22). Our findings are consistent with earlier findings where higher GGT levels predicted CVD, mortality and development of the MS in the general population (15,18–20). Other prior community-based studies found that
increased levels of GGT were associated with insulin resistance and an increased risk of coronary heart disease and stroke (17,21). Our observations relating AP to mortality in RTRs complement a prior study by Regidor et al. (23), where a rise in AP was associated with an increased risk for death in hemodialysis patients. In this study, no data on GGT were available, and it could not be investigated whether there was an association between AP and GGT, neither was it investigated whether AP was associated with MS and its components.

We hypothesized that the hepatic manifestation of the MS could be a new risk factor for cardiovascular-related death. As our findings point toward an association between GGT, AP and mortality independent of the components of MS, it can be suggested that other underlying mechanisms play a role. Our observations relating GGT and AP to mortality could be elucidated by the mechanism of oxidative stress. Serum GGT activity levels within its normal range might be related to oxidative stress (34). Recent studies indicate that GGT may have a direct involvement in atherosclerotic plaque formation and thereby could be a key factor in the pathogenesis of CVD (35,36). This is in line with evidence for an association of cytosolic triglycerides with oxidative stress (37). Increased storage of cytosolic triglycerides in nonadipose tissues such as the liver leads to elevated concentrations of cytosolic long-chain acetyl-CoA esters. An increase in these esters would lead to inhibition of mitochondrial adenine nucleotide translocators, causing an ADP deficiency. It was speculated that this deficiency stimulates production of free oxygen radicals, which leads to atherosclerosis (37).

### Table 3: Hazard ratios for mortality according to z-scores of ALT, AP and GGT

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>All-cause mortality</th>
<th>Cardiovascular mortality</th>
<th>All-cause mortality^1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95% CI]</td>
<td>p</td>
<td>HR [95% CI]</td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.03 [0.8–1.3]</td>
<td>0.8</td>
<td>1.06 [0.8–1.4]</td>
</tr>
<tr>
<td>2</td>
<td>1.13 [0.9–1.4]</td>
<td>0.2</td>
<td>1.15 [0.9–1.5]</td>
</tr>
<tr>
<td>3</td>
<td>1.10 [0.9–1.3]</td>
<td>0.4</td>
<td>1.10 [0.8–1.4]</td>
</tr>
<tr>
<td>4</td>
<td>1.07 [0.9–1.3]</td>
<td>0.5</td>
<td>1.05 [0.8–1.4]</td>
</tr>
<tr>
<td>5</td>
<td>1.07 [0.9–1.3]</td>
<td>0.5</td>
<td>1.04 [0.8–1.3]</td>
</tr>
<tr>
<td>GGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.43 [1.2–1.7]</td>
<td>&lt;0.001</td>
<td>1.55 [1.3–1.9]</td>
</tr>
<tr>
<td>2</td>
<td>1.32 [1.1–1.6]</td>
<td>0.001</td>
<td>1.40 [1.1–1.7]</td>
</tr>
<tr>
<td>3</td>
<td>1.30 [1.1–1.5]</td>
<td>0.003</td>
<td>1.35 [1.1–1.7]</td>
</tr>
<tr>
<td>4</td>
<td>1.21 [1.0–1.4]</td>
<td>0.03</td>
<td>1.27 [1.0–1.6]</td>
</tr>
<tr>
<td>5</td>
<td>1.21 [1.0–1.4]</td>
<td>0.03</td>
<td>1.25 [1.0–1.4]</td>
</tr>
<tr>
<td>AP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.34 [1.1–1.6]</td>
<td>0.003</td>
<td>1.50 [1.2–1.9]</td>
</tr>
<tr>
<td>2</td>
<td>1.39 [1.2–1.7]</td>
<td>&lt;0.001</td>
<td>1.51 [1.2–1.9]</td>
</tr>
<tr>
<td>3</td>
<td>1.32 [1.1–1.6]</td>
<td>0.005</td>
<td>1.37 [1.1–1.8]</td>
</tr>
<tr>
<td>4</td>
<td>1.24 [1.0–1.5]</td>
<td>0.03</td>
<td>1.33 [1.0–1.7]</td>
</tr>
<tr>
<td>5</td>
<td>1.29 [1.1–1.6]</td>
<td>0.01</td>
<td>1.36 [1.1–1.8]</td>
</tr>
</tbody>
</table>

Model 1: Univariate analyses.
Model 2: Model 1 + adjustment for recipient age, sex, creatinine clearance and urinary protein excretion.
Model 3: Model 2 + adjustment for presence of diabetes, HbA1c, glucose, use of insulin, use of anti diabetic drugs and years of diabetes.
Model 4: Model 2 + adjustment for all components of the MS: waist circumference, triglycerides, HDL cholesterol, blood pressure and impaired fasting plasma glucose or diabetes.
Model 5: Model 4 + adjustment for HbA1c, LDL cholesterol, hsCRP, smoking, previous transplantations, total time on renal replacement therapy.

^1Patients with pre- and posttransplant diabetes were excluded from these analyses.
^2Only additional adjustment for HbA1c and glucose, because all subjects with diabetes were excluded in this column.
^3No additional adjustment for diabetes, because all subjects with diabetes were excluded in this column.

### Table 4: Age- and sex-adjusted hazard ratios for prediction of all-cause mortality expressed per standard deviation change in risk factor

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>All-cause mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>1.30 [1.10–1.54]</td>
</tr>
<tr>
<td>SBP</td>
<td>1.28 [1.05–1.55]</td>
</tr>
<tr>
<td>hsCRP</td>
<td>1.26 [1.12–1.42]</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.16 [1.00–1.34]</td>
</tr>
<tr>
<td>HDLC</td>
<td>0.80 [0.64–0.99]</td>
</tr>
<tr>
<td>GGT</td>
<td>1.32 [1.12–1.56]</td>
</tr>
<tr>
<td>AP</td>
<td>1.31 [1.08–1.59]</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure; hsCRP = high-sensitive C-reactive protein; HDLC = high- density lipoprotein cholesterol; GGT = gamma-transferase; AP = alkaline phosphatase.
RTRs are prone to develop high bone turnover, which can lead to elevations in AP activity. In separate analyses for the bone and nonbone part of AP activity, we found that the association with all-cause and cardiovascular mortality was only present for nonbone AP. Nonbone AP is usually considered equivalent to liver AP because intestinal and kidney iso-enzymes contribute little to total nonbone AP activity (29,30). Alcohol abuse can also alter the level of GGT but in this study alcohol consumption was not higher in the group with elevated levels of GGT. We also explored the possibility that polycystic renal disease as underlying disorder could have influenced the relationship between GGT and mortality; nevertheless it did not remain significant in our regression analyses.

The strength of our study is its prospective design. RTRs in this study were closely monitored by regular checkup in our clinic, this gives complete information on patient status. A limitation of our study is that we only measured liver enzymes in baseline samples. Most epidemiological studies use a single baseline measurement to predict outcome, which adversely affects predictive properties of variables associated with outcome. If intra-individual variability of predictive biomarkers is taken into account, this results in strengthening of predictive properties that—despite sometimes considerable intra-individual variation day-to-day variation—also existed for single measurements of these biomarkers. The higher the intra-individual day-to-day variation would be, the greater one would expect the benefit of repeated measurement for prediction of outcomes. Further, this study is a single center study and the predictive value of GGT and AP need to be confirmed in other centers and/or multicenter studies. Our study includes RTRs that were transplanted in multiple immunosuppressive eras. However, immunosuppressive therapy was not significantly associated with liver enzymes. Furthermore, our study only includes stable RTR, relatively long-term after transplantation. Future studies could investigate whether liver enzymes, measured in the early posttransplant period also predict mortality.

In conclusion, liver enzymes ALT, GGT and AP are strongly related to prevalence of the MS. Waist circumference, triglycerides and glucose levels were the strongest contributors to this relationship. Increasing levels of GGT and AP within the normal ranges are associated with a higher risk for mortality independently of age, sex, creatinine clearance, protein excretion, diabetes and factors related to diabetes, MS and classic cardiovascular risk factors. These findings suggest that a raise in serum GGT and AP levels are independent risk factors for early all-cause and cardiovascular mortality in RTRs.

**Conflict of Interest Statement**

The authors have no conflicting interests.

**Acknowledgments**

This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl), project PREDICT (grant 01C-104), and supported by the Netherlands Heart Foundation, Dutch Diabetes Research Foundation and Dutch Kidney Foundation.

**References**


