Circulating vascular endothelial growth factor receptor 2 levels and their association with lipid abnormalities in patients on hemodialysis

LESZEK NIEPOLSKI¹, HANNA DRZEWIECKA² and WOJCIECH WARCHOŁ³

Departments of ¹Physiology, ²Biochemistry and Molecular Biology, and ³Ophthalmology and Optometry, Poznan University of Medical Sciences, Poznań 60-781, Poland

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Abstract. The aim of the present study was to examine the association between the levels of circulating vascular endothelial growth factor receptor (VEGFR)2 levels, serum lipid composition and plasma receptor for advanced glycation end-products (RAGE) expression in patients undergoing hemodialysis (HD). A total of 50 patients on HD (27 men and 23 women; median age, 66 years; age range 28-88 years; HD mean time, 29.0, 3.9‑157.0 months) were enrolled. Age‑matched healthy subjects (n=26) were used as the control group. Plasma VEGFR2 and RAGE levels were determined using ELISA. Dyslipidemia (D) in patients on HD was diagnosed according to the Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Managing Dyslipidemias in Chronic Kidney Disease. Circulating VEGFR2, RAGE and serum lipids were compared between dyslipidemic and non‑dyslipidemic patients on HD and controls. In patients on HD, the plasma VEGFR2 levels were lower compared with those in the healthy population. D was associated with high plasma VEGFR2 levels. The triglyceride/HDL‑cholesterol ratio was strongly associated with plasma VEGFR2 levels. The plasma VEGFR2 concentration was associated with circulating RAGE levels. Therefore, circulating VEGFR2 levels may be partly associated with lipid abnormalities and plasma RAGE levels in patients receiving HD.

Introduction

Patients with end‑stage renal disease (ESRD) on maintenance hemodialysis (HD) are susceptible to changes in their blood vessels. A number of studies have confirmed the high prevalence of atherosclerotic lesions in the arteries of patients on HD (1,2). It is well established that the function of the endothelium is intricately involved in atherogenesis (3). ESRD leads to altered properties and responses of the endothelium. However, the mechanisms through which uremia affects endothelial cells and causes atherosclerotic changes remain elusive. Therefore, it is worth exploring alternative metabolic pathways that may be involved in atherosclerotic formation in patients on HD. Vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) are crucial regulators of vasculogenesis and angiogenesis under physiological and pathophysiological conditions (3). The VEGF‑VEGFR axis regulates several biological functions in the endothelium. Human endothelial cells express three related membrane‑bound VEGFRs, VEGFR1, VEGFR2 and VEGFR3, which are encoded by different genes. All membrane‑bound receptors possess an extracellular, transmembrane and an intracellular domain that mediates critical signalling pathways in endothelial cells (4). In addition, circulating isoforms are present in human plasma. Due to the alternative splicing of VEGFR mRNA, and also due to proteolytic shedding of the extracellular domain, membranous receptors may exist as soluble truncated forms (sVEGFR1, 2 and 3). These soluble forms are secreted by endothelial cells or are proteolytically cleaved (5,6). The role of the splice variant sVEGFR‑1 is well established (7), less is known regarding sVEGFR2 and sVEGFR3. It has been reported that the membrane‑bound and spliced isoforms of VEGFR2 participate in several important pathophysiological processes, such as the production of vasoactive mediators involved in hypertension, thrombosis and inflammation (8‑12), non‑alcoholic fatty liver disease (NAFLD) (13,14), production of nitric oxide (1) and prostacyclin (2). VEGFR2 is...
activated through ligand-stimulated receptor dimerization and phosphorylation (15). In addition, the dimerization and phosphorylation of VEGFR2 may be modulated by modifying the lipid raft of endothelial cell membranes through high-density and low-density lipoproteins (16-19), advanced glycation end-products (AGEs) and their receptors (20,21). Therefore, metabolic abnormalities may lead to endothelial cell membrane VEGFR2 relocation and expression, conferring changes to the formation of circulating VEGFR2 isoforms. No clinical studies have assessed the association between plasma VEGFR2 concentrations, lipid abnormalities and receptor for AGE (RAGE) levels. Therefore, in the present pilot study, circulating (soluble truncated) VEGFR2 concentrations were examined in relation to lipid composition and plasma RAGE levels in patients on HD, and biochemical and anthropometric parameters were evaluated.

Materials and methods

Ethics statement. The protocol used in the present study was performed in accordance with the guidelines described in the Declaration of Helsinki (22) and was approved by the Institutional Review Board of Poznań University of Medical Sciences, Poland. Written informed consent was obtained from all subjects prior to participation.

Patients. A total of 50 Caucasian patients on HD (27 men and 23 women; median age, 66 years; age range, 28-88 years; HD mean time, 29.0, 3.9-157.0 months) who had been treated with maintenance HD for ≥6 months were recruited. All patients underwent HD 3 times for 4-4.5 h per week on low-flux polysulphone-based membranes with a surface area of 1.3-2.1 m², low-molecular-weight heparin was used as an anticoagulant. The dialysis efficiency was evaluated based on single-pool Kt/V urea nitrogen, according to the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (K/DOQI) Guidelines (23). Patients on HD who fell into one or more of the following categories were excluded: i) Suffered from a hepatic disease, neoplastic disease, active collagen disease, acute coronary syndrome and/or not well-controlled diabetes mellitus; ii) cerebral stroke in the 6 months preceding the commencement of the study; or iii) received statins or corticosteroids or immunosuppressant drugs at the time of the HD session, when a patient was more susceptible to developing symptoms of hypotension. DBW was determined by an experienced nephrologist. Anthropometric parameters measured included body mass index (BMI), waist circumference (WC), waist-to-height ratio (WHR) and waist-to-hip ratio (WHR). Weight was measured to the nearest 10 g. Height was measured to the nearest 5 mm, using a wall-mounted stadiometer. BMI (kg/m²) was calculated as DBW (kg)/height (m)². WC was measured midway between the lowest lateral border of the ribs and the uppermost lateral iliac crest. WHR was calculated as WC/height. WHR was measured as WC/hip circumference, fasting blood samples were collected from each patient at the start of a midweek HD session from the arterial site of the arterio-venous fistula, catheter, or antecubital vein of the participants. NAFLD was diagnosed by abdominal ultrasonography using a method established by Hamaguchi et al (25), which included hepatic parenchymal contrast, liver brightness, deep attenuation and vascular blurring.

Dyslipidaemia (D) in the control group was assessed according to The European Society of Cardiology and the European Atherosclerosis Society guidelines (26). D in patients on HD was diagnosed according to the K/DOQI Clinical Practice Guidelines for Managing Dyslipidemias in Chronic Kidney Disease (2003) (27). Patients diagnosed with D with a serum low-density cholesterol (LDL-chol) concentration ≥100 mg/dl were considered hyper-LDL cholesterolemic, whereas those with a non-high-density cholesterol (non-HDL-chol) levels of ≥130 mg/dl and triglycerides (TGs) levels of ≥200 mg/dl were considered hyper-TG/hyper-non-HDL cholesterolemic. Patients who met one of these criteria were included in the dyslipidemic group. The remaining patients were referred to as non-dyslipidemic, according to the K/DOQI criteria. To determine the atherogenic pattern of D, the TG/HDL-chol ratio was used as the atherogenic index (AI). A ratio value ≥3.8 was considered to indicate atherogenic D (27). HD subjects with a TG/HDL-chol ratio value <3.8 were described as patients without atherogenic D (28). Total chol (TC; cat. no. 03039773190), HDL-chol (cat. no. 04399803190) and TG (cat. no. 20767107322) were assessed using enzymatic and colorimetric methods, according to the manufacturer's protocol (Cobas Integra, Roche Diagnostics GmbH). The LDL-chol concentration was calculated using the Friedewald formula (29). In patients with serum TG concentrations of ≥400 mg/dl, LDL-chol was measured directly (BioSystems S.A.). Non-HDL-chol was estimated as the Tchol minus HDL-chol. The LDL-chol was calculated using the Friedewald formula.

Plasma VEGFR2, RAGE, insulin, glycated hemoglobin (HbA1c), glucose levels, lipid profiles, albumin levels and high-sensitivity C-reactive protein (hsCRP) concentration were measured. After a minimum of 8 h of overnight fasting, venous blood was drawn into an EDTA tube and promptly centrifuged at 2,000 x g for 10 min at 4°C. The obtained plasma was frozen at -80°C in aliquots until protein analysis was performed. The plasma VEGFR2 levels were measured.
using the Human VEGFR2 ELISA kit (cat. no. ab213476; Abcam), according to the manufacturer's protocols. No significant cross-reactivity or interference was observed. The limits of VEGFR2 concentration detection were 34.3–25,000 pg/ml. The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). Homeostasis model assessment of insulin resistance (HOMA-IR) was determined as fasting plasma insulin (µU/ml) x fasting plasma glucose (mmol/l)/22.5 (30). HbA1c was determined using the Human RAGE ELISA kit (cat. no. ab190807; Abcam). All measurements were performed in duplicate. Plasma insulin concentrations were determined using the electrochemiluminescence immunoassay method, according to the manufacturer's protocol (Cobas E411; cat. no. 12017547122; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%. The plasma RAGE levels were measured using a high-sensitivity latex turbidimetric inhibition immunoassay method (Cobas Integra; cat. no. 04628123 190; Roche Diagnostics GmbH). The concentration of hsCRP was determined using a high-sensitivity latex turbidimetry method, according to the manufacturer's protocol (Cobas Integra; cat. no. 04528123 190; Roche Diagnostics GmbH). The sensitivity of the assay was <70 pg/ml. The intra-assay coefficient of variation (CV) was 2.5%, and the CV for inter-assay precision was 5.8%

**Statistical analysis.** The normality of distribution of variables was assessed using the Shapiro-Wilk test for each group separately. Numeric variables are expressed as the mean ± standard deviation, or as a median and range, as appropriate; categorical variables are presented as percentages. A Student's t-test was used to compare normally distributed data, or otherwise a Mann-Whitney U test was used. As plasma VEGFR2 levels were not normally distributed, a Spearman's rank correlation was performed to determine the correlation between this variable and the other parameters. Univariate receiver operating characteristic (ROC) curves were evaluated using Medical kit version 4.0.67 (statssoft.pl). Statistical analysis was performed using STATISTICA version 13 (TIBCO Software Inc.). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Comparison between the entire HD group and control subjects.** The clinical and demographic characteristics of patients in the HD and control groups, with and without D, are summarized in Table I. Despite the prior declaration of good health, 57% of the subjects in the control group had D, with a similar percentage observed in the HD group (54%). The HD patients had a higher TG/HDL-chol ratio, as compared with age-matched controls (4.7 vs. 1.28; P<0.0005; Table II). The AI in the HD group was 56%, whereas there were no subjects with atherogenic D in the control group. A higher prevalence of MeS was recorded in patients on HD compared with the control subjects (60 vs. 15%, respectively; P=0.001). The HD group had a similar percentage of cigarette smokers, BMI and WHR to that of controls, but a higher WHR ratio (P=0.006). Compared with the control, the entire HD group exhibited a lower plasma VEGFR2 concentration (P=0.025; Fig. 1), Tchol, LDL-chol, HDL-chol and HDL/Tchol ratio. Additionally, patients on HD
exhibited a significantly elevated serum TCHOL, LDL, HDL, non-HDL, and TG levels, as well as the LDL/HDL and TG/HDL ratios, between patients on HD with or without D. The patients on HD with D had a significantly higher AI, compared with patients on HD without D (73% vs. 47%, respectively). In addition, the receiver operating characteristic (ROC) curve analysis revealed that a plasma VEGFR2 level of 1.33 was the ideal cut-off value for the prognosis of patients on HD with and without D [area under the ROC curve, 0.713; 95% confidence interval (CI): 0.569-0.858; Fig. 2].

Comparison of VEGFR2 levels between patients on HD with and without D. The laboratory parameters of patients on HD with and without D are presented in Table III. In patients on HD with D, the plasma VEGFR2 and serum TCHOL, TG, LDL, HDL, non-HDL, and TG/HDL ratios, as well as the LDL/HDL and TG/HDL ratios, were higher compared with those in patients on HD without D. The patients on HD with D had a significantly higher AI, compared with patients on HD without D (73% vs. 47%, respectively). In addition, the receiver operating characteristic (ROC) curve analysis revealed that a plasma VEGFR2 level of 1.33 was the ideal cut-off value for the prognosis of patients on HD with and without D [area under the ROC curve, 0.713; 95% confidence interval (CI): 0.569-0.858; Fig. 2].

Comparison of VEGFR2 levels in the control group with and without D. The laboratory parameters of controls with and without D are presented in Table IV. Control subjects with D did not differ significantly from those without D with respect to plasma VEGFR2, serum HDL, and TG concentrations. In the control subjects with D, the serum TCHOL, LDL, HDL, non-HDL, and TG/HDL ratios, as well as the LDL/HDL and TG/HDL ratios, were higher compared with those in control subjects without D.
Comparison of VEGFR2 levels between patients on HD with and without MeS. There was no difference in plasma VEGFR2 levels between patients with and those without MeS (1.38±0.915 vs. 1.10±0.785 ng/ml, respectively; P=0.338).

Correlation analysis of VEGFR2 levels and other parameters in patients on HD and controls. In the control group, plasma VEGFR2 levels were significantly positively correlated with TG/HDL-chol ratio and TG levels (Table V). In the entire HD group, there were positive correlations between plasma VEGFR2 levels and age, RAGE (Fig. 3), Tchol, LDL, TG, non-HDL, LDL/HDL-chol ratio and TG/HDL-chol ratio (Fig. 4), and a negative correlation between plasma VEGFR2 levels and HDL/Tchol ratio. Of note, there was no correlation between plasma VEGFR2 levels and residual diuresis, HOMA-IR, hsCRP or anthropometric parameters in any of the examined groups. In the HD group with D, there were positive correlations between the plasma levels of VEGFR2 with age and RAGE. Similarly, in the group without D, significant positive correlations were observed between plasma VEGFR2 and RAGE levels, whereas a significant negative correlation was observed between plasma VEGFR2 levels and platelet counts. There was no correlation between plasma VEGFR2 levels and the presence of NAFLD (P=0.818) based on the abdominal ultrasound examination of patients on HD. The significant correlations of plasma VEGFR2 levels in the examined groups are presented in Table V.

Discussion
To the best of our knowledge, there are no previous studies showing the association of plasma VEGFR2 levels with lipid abnormalities, MeS, plasma RAGE levels and other biochemical parameters in patients on HD. The following were the major findings of the present study in patients on HD: i) The plasma VEGFR2 levels were lower compared with those in the control subjects; ii) in patients on HD with D, the plasma VEGFR2 levels were higher compared with those in patients on HD without D; iii) there were positive correlations between plasma VEGFR2 levels and lipid abnormalities (Tchol, LDL, TG, Non-HDL, LDL/HDL-chol ratio, TG/HDL-chol ratio and plasma RAGE levels); iv) there was no difference in plasma VEGFR2 levels between patients with and without MeS.
In the present study, lower levels of VEGFR2 were observed in patients on HD compared with the control subjects, which was in line with the results reported by Sepe et al (32). In their study on 26 patients receiving regular HD, the plasma VEGFR2 levels were also significantly lower compared with those in the 9 age-matched healthy volunteers, and were inversely correlated with serum homocysteine levels. The results of the present study suggested that the atherogenic components of blood lipids may be partly involved in the regulation of VEGFR2 expression. A significant correlation was identified between atherogenic components of the lipid profile and plasma VEGFR2 levels in patients on HD and controls. In the examined groups, no marked difference was identified between HD and controls regarding the percentage of subjects with D (52 vs. 57%). Therefore, AI was assessed in these groups. Of note, ≤56% of patients on HD had atherogenic D, as calculated by AI. Conversely, there were no subjects with atherogenic D in the control group. In addition, a significant correlation was observed between the TG/HDL-chol ratio, which is a determinant of AI and plasma VEGFR2 levels in the HD group. This fact may suggest that AI may be more likely than D to affect plasma VEGFR2 levels in patients on HD.

There have been several reports of increased carbonyl stress (33), AGE production and RAGE expression (34) in patients with uraemia. In addition, Liu et al (20) demonstrated that AGEs and RAGE, whose activity in patients on HD was significantly elevated, may modulate VEGFR2 levels. It has been shown that the incubation of endothelial cells with methylglyoxal, a major source of AGEs and peroxynitrate (ONOO⁻) production, modulates VEGFR2 protein levels through RAGE-mediated, ONOO⁻-dependent and autophagy-induced VEGFR2 degradation on the cell membrane (20,33,35).

Furthermore, it has been shown that the formation of atherosclerotic lesions is accelerated in parallel with VEGFR2 and RAGE activity (21). The results of the present study were in line with those of these previous studies. In the present study,

### Table IV. Laboratory parameters in the control cohort with and without D.

| Parameter                | Controls with D | Controls without D | P-value |
|--------------------------|-----------------|--------------------|---------|
| VEGFR2, ng/ml            | 2.75±1.63       | 2.39±1.14          | 0.535   |
| Albumin, g/dl            | 4.31±0.18       | 4.34±0.24          | 0.724   |
| Total cholesterol, mg/dl | 225.0±38.0      | 174.0±30.0         | <0.001* |
| LDL-cholesterol, mg/dl   | 138.0±31.0      | 72.0±15.0          | <0.00001* |
| HDL-cholesterol, mg/dl   | 66.0±16.0       | 71.0±23.0          | 0.557   |
| TG, mg/dl                | 121.0±41.0      | 89.0±22.0          | 0.237   |
| Non-HDL, mg/dl           | 159.0±31.0      | 103.0±14.0         | <0.0001* |
| LDL/HDL-cholesterol ratio| 2.13±0.55       | 1.30±0.39          | 0.0002* |
| HDL/Total cholesterol, % | 29.5±5.6        | 40.0±7.9           | 0.0005* |
| TG/HDL-cholesterol ratio | 1.61±0.81       | 1.42±0.70          | 0.537   |
| hsCRP, mg/l              | 4.21±1.68       | 3.66±1.22          | 0.432   |
| HB, g/dl                 | 12.6±1.3        | 12.3±1.30          | 0.621   |

*P<0.001; *mean ± standard deviation D, dyslipidemia; HB, hemoglobin; HDL, high density lipoprotein; hsCRP, high sensitivity C reactive protein; LDL, low density lipoprotein; RAGE, receptor for advanced glycation end products; TG, triglyceride; VEGFR2, vascular endothelial growth factor receptor 2.

### Table V. Significant correlations between VEGFR2 and other clinicopathological characteristics in the examined groups treated with HD, with and without dyslipidemia.

#### A, Whole HD group, n=50

| Correlated parameter | R     | P-value |
|----------------------|-------|---------|
| VEGFR2 and age       | 0.334 | 0.017a  |
| VEGFR2 and RAGE      | 0.686 | <0.00001c |
| VEGFR2 and Tchol     | 0.297 | 0.036a  |
| VEGFR2 and LDL-chol  | 0.291 | 0.039a  |
| VEGFR2 and TG        | 0.362 | 0.009b  |
| VEGFR2 and non-HDL   | 0.361 | 0.009b  |
| VEGFR2 and LDL/HDL-chol ratio | 0.402 | 0.004b  |
| VEGFR2 and HDL/Tchol ratio | -0.403 | 0.004b  |
| VEGFR2 and TG/HDL-chol ratio | 0.362 | 0.009b  |

#### B, Dyslipidemic HD group, n=26

| Correlated parameter | R     | P-value |
|----------------------|-------|---------|
| VEGFR2 and age       | 0.397 | 0.04a  |
| VEGFR2 and RAGE      | 0.737 | 0.0002c |

#### C, Non-dyslipidemic HD group, n=24

| Correlated parameter | R     | P-value |
|----------------------|-------|---------|
| VEGFR2 and RAGE      | 0.677 | 0.00027c |
| VEGFR2 and PLT       | -0.457| 0.024a  |

*P<0.05; *P<0.01; *P<0.001. HDL-chol, high density lipoprotein cholesterol; LDL-chol, low density lipoprotein cholesterol; PLT, platelets; RAGE, receptor for advanced glycation end products; Tchol, total cholesterol; TG, triglyceride; VEGFR2, vascular endothelial growth factor receptor 2.
the plasma levels of VEGFR2 in patients on HD were posi-
tively correlated with plasma RAGE levels. Therefore, these
mechanisms may be at least partly be involved in the modula-
tion of plasma VEGFR2 levels in patients on HD.

MeS commonly occurs in patients on HD, and is closely
associated with endothelial cell dysfunction and VEGFR2
expression (36-38). The association between plasma VEGFR2
levels and MeS was evaluated in patients on HD. No differ-
ences were observed in the plasma VEGFR2 levels between
patients on HD with and without MeS. To the best of our
knowledge, the present study is one of few studies to report
an association between plasma VEGFR2 and MeS. The
conflicting data reported to date may be due to differences in
the measurements of sVEGFR2 (37,38). In previous studies,
compared with subjects with normal renal function, plasma
or serum sVEGFR2 levels were significantly decreased (37)
or increased (38) in subjects with MeS. In the present study,
circulating plasma VEGFR2 (secreted and proteolytic shedded
forms) were both measured, and no correlation was observed
between MeS and circulating plasma VEGFR2 levels. Further
studies are required to evaluate the association between the
components of MeS and plasma VEGFR2 levels.

NAFLD is known to be strongly associated with MeS, and
is considered as a novel risk factor of cardiovascular events
in patients undergoing HD (38-40). Wu et al (41) reported that
~24% of the examined patients on HD had NAFLD. In
the present study, 20% of patients on HD were found to have
NAFLD based on the ultrasonography examination. The
association between NAFLD and circulating VEGFR2 levels
was also examined, but no correlations were found, consistent
with previous studies (13,14).

D is the primary characteristic of MeS and a common
feature amongst patients on HD. In the present study, >50%
of the patients on HD were also diagnosed with D, based on the
diagnostic recommendations of the K/DOQI guidelines (27).
Hyper-LDL-cholesterolemia was also recorded in 91% of the
subjects. In addition, in dyslipidemic patients on HD, serum TG
was significantly higher compared with the non-dyslipidemic
HD group. A growing body of evidence supports the notion that
ESRD is associated with hypertriglyceridemia (27,8,39,40).
Recently, Vaziri et al (42) demonstrated that the novel endothe-
lium-derived molecule glycosylphosphatidylinositol-anchored
binding protein 1 (GPIHBP1) is involved in the development
of hypertriglyceridemia in patients with chronic kidney
disease. Other studies have found that the VEGF-VEGFR axis
modulates GPIHBP1 mRNA and protein expression levels in
endothelial cells (43). Consistent with these findings, the results
of the present study support the hypothesis that VEGFR2
may be partially involved in the development of hypertri-
glyceridemia through the VEGF-VEGFR axis. Additionally,
it was found that circulating VEGFR2 levels were positively
correlated with TG in patients on HD and control subjects.

The association between LDL-chol and plasma VEGFR2
levels was also examined. Data from previous studies indicated
that LDL-chol affects the structure and activity of VEGFR2,
but the mechanism underlying this phenomenon is not fully
understood. Jin et al (44) demonstrated that LDL-chol attenu-
ates endothelial VEGFR2 expression. It was revealed that the
loss of VEGFR2 resulted from its internalization and degrada-
tion in endosome-trans-Golgi network trafficking. Conversely,
a study by Rodrígues et al (45) on an animal model revealed
that native LDL-chol significantly upregulated VEGF and
VEGFR2 in endothelial cells, and this upregulation was
associated with an oxidative stress-mediated mechanism. In
addition, it has been established that oxidized phospholipids
may stimulate the expression of VEGFR2 (46). Since the
oxidized LDL-chol that accumulates during atheroscle-
rosis (47) contains large amounts of oxidized phospholipids,
a connection between LDL-chol metabolism and VEGFR2
may be considered. In the present study, a positive correlation
was identified between plasma circulating VEGFR2 levels and
LDL-chol, non-HDL and the LDL/HDL-chol ratio, in agree-
ment with previous studies (45,46).

Recently, the HDL/Tchol ratio was reported to be a more
reliable risk factor for atherosclerotic changes compared with
Tchol or HDL-chol alone (48). Since a low HDL/Tchol
ratio (47) and high endothelial expression of VEGFR2 (46)
are considered risk factors for atherosclerosis, the association
between HDL/Tchol ratio and plasma VEGFR2 levels was
evaluated in the present study. A negative correlation was
identified between plasma circulating VEGFR2 levels and the
HDL/Tchol ratio. These findings support the notion that not
only membranous VEGFR2, but also circulating VEGFR2
expression, may be partially modulated by lipid components.

The present study has several limitations. First, all patients
in this study were Caucasian, and any differences with other
ethnicities were not examined. Second, single-center trials are
associated with a potential bias. Third, the number of patients
and healthy subjects was relatively small.

In conclusion, the findings of the present study demons-
trated that circulating VEGFR2 levels were lower in patients
on HD compared with those in the healthy population.
D was associated with higher plasma VEGFR2 levels, the
tG/HDL-chol ratio (an index of atherogenic D) was strongly
associated with plasma VEGFR2 levels, and plasma VEGFR2
concentration was associated with circulating RAGE levels.
It may be inferred that, in patients on HD, circulating VEGFR2
levels may be partially associated with lipid abnormalities
and plasma RAGE levels. However, further studies are required
to define the pathophysiological role of circulating VEGFR2 and
its precise association with D.

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Availability of data and materials
The datasets used and/or analyzed during the present study are
available from the corresponding author on reasonable request.

Authors’ contributions
LN conceived and designed the study, and was involved
in data collection and analysis. HD performed the analyses
of VEGFR2 and RAGE levels and data analysis. WW was
involved in data analysis and performed the statistical analysis. All the authors have read and approved the final version of the manuscript. HD and WW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The protocol used in the present study was performed in accordance with the guidelines described in the Declaration of Helsinki and was approved by the Institutional Review Board of Poznań University of Medical Sciences, Poland. Written informed consent was obtained from all subjects before participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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