Renal resistive index as an indicator of the presence and severity of anemia and its future development in patients with hypertension

Muneyoshi Tanimura1, Kaoru Dohi1*, Masumi Matsuda2, Yuichi Sato1, Emiyo Sugiura1, Naoto Kumagai1, Shiro Nakamori1, Tomomi Yamada3, Naoki Fujimoto4, Takashi Tanigawa1, Norikazu Yamada1, Mashio Nakamura1 and Masaaki Ito1

Abstract

Background: We examined whether renal resistive index (RI), a simple index of renal vascular resistance, is associated with the presence and severity of anemia, and can predict the future development of anemia in patients with hypertension.

Methods: We retrospectively examined 175 patients with hypertension (mean age 67 ± 11 years, 32-85 years, 134 males) who underwent renal ultrasonography. Anemia was defined as a reduction in the concentration of hemoglobin <13.0 g/dL for men and <12.0 g/dL for women. Renal RI was measured in the interlobar arteries.

Results: Anemia was present in 37% of men and 34% of women. The mean estimated glomerular filtration rate (eGFR) was 58 ± 23 ml/min/1.73 m² (median: 56 ml/min/1.73 m², range: 16-168 ml/min/1.73 m²) and the mean renal RI was 0.70 ± 0.09 (median: 0.70, range: 0.45-0.92). Proteinuria was present in 29% of patients. Both eGFR and renal RI correlated significantly with hemoglobin levels. In the stepwise multivariate linear regression analysis, renal RI was associated with hemoglobin levels independently of potential confounders including eGFR. During the follow-up period (median: 959 days, range: 7-3595 days), Kaplan–Meier curves demonstrated that patients with renal RI above the median value had a higher incidence of the future development of anemia than other patients. Cox regression analysis showed that renal RI (hazard ratio 1.18, 95% CI 1.02-1.37 per 0.05 rises in renal RI, p =0.03) and the presence of proteinuria were (hazard ratio 1.80, 95% CI 1.08-3.01, p =0.03) were independently associated with the future development of anemia after correcting for confounding factors.

Conclusions: Measurement of renal RI can be useful for elucidating the pathogenesis of anemia and for inferring its potential risk in patients with hypertension.

Keywords: Chronic kidney disease, Hypertension, Renal resistive index, Anemia

Background

Chronic kidney disease (CKD) is known to cause anemia mainly due to inappropriate erythropoietin (EPO) secretion [1,2], especially in patients with end-stage renal failure. However, anemia can occur early in the development of CKD, defined on the basis of the estimated glomerular filtration rate (eGFR). This indicates that the progression of renal anemia is not governed solely by glomerular function [3]. In recent studies, EPO-producing cells were identified in the renal tubulointerstitium surrounding the central vessels, not in the glomeruli [4,5]. Thus, unfavorable changes in the interstitial microenvironment, such as inflammation, oxidative stress and ischemia, can impair the function of EPO-producing cells. Recently, Souma et al. demonstrated that the phenotypic transition of EPO-producing cells to non-EPO-producing myofibroblasts is modulated by inflammatory molecules, and suggested the connection between anemia and renal fibrosis in CKD in a mouse model [6].

* Correspondence: dohik@clin.medic.mie-u.ac.jp
1Department of Cardiology and Nephrology, Mie University Graduate School of Medicine, Tsu, Japan
Full list of author information is available at the end of the article

© 2015 Tanimura et al; licensee BioMed Central. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Hypertension is one of the leading causes of CKD associated with arteriosclerosis, interstitial inflammation and fibrosis, as well as tubular insufficiency secondary to endothelial dysfunction and progressive infiltration of macrophages and T cells to the perivascular interstitium induced by chronic exposure to high blood pressure [7-10]. Therefore, the development of renal damage can contribute to the pathogenesis of anemia, even in earlier stages of CKD in patients with hypertension.

Renal resistive index (RI) in the intra-renal arteries at the level of the corticomedullary junction using pulsed Doppler ultrasonography is a simple index of renal vascular resistance [11,12], and high renal RI is known to be associated with severe interstitial fibrosis, arteriosclerosis and renal function decline [13-15]. Accordingly, we examined whether renal RI is associated with the presence and severity of anemia, and whether high renal RI predicts the future development of anemia in patients with hypertension.

**Methods**

**Study population**

We retrospectively reviewed patients with hypertension who underwent renal ultrasonography for the screening of renal artery stenosis and the evaluation of renal arteriosclerosis at their physician's discretion on the basis of their age, comorbidity and disease characteristics in Mie University Hospital between April 2004 and June 2012. Among them, we selected 175 subjects (mean age 67 ± 11 years, range 32-85 years, 134 males) after excluding patients with atrial fibrillation, moderate to severe aortic valvular heart diseases, congestive heart failure, primary glomerular and tubulointerstitial disease of the kidney, hydropneumothorax, neoplastic syndrome, renal artery stenosis, and diseases or conditions that could cause or improve anemia (such as advanced cancers, hematologic disorders, autoimmune diseases and active bleeding). Patients receiving hemodialysis or erythropoiesis-stimulating agent therapy were also excluded.

**Clinical evaluation**

Anemia was defined as a reduction in the concentration of hemoglobin in a sample of venous blood when compared with reference values (<12.0 g/dl for women and <13.0 g/dl for men) [16]. CKD was defined as the presence of eGFR <60 ml/min/1.73 m² for 3 months or more. The eGFR of each patient was calculated from their serum creatinine (SCr) value and their age using the following equation: eGFR (ml/min/1.73 m²) = 194 × Age⁻⁰.²⁰⁷ × SCr⁻¹.₀⁹⁴ (if female × 0.739) [17]. Urine proteins were determined under the following conditions: fasting glucose level >125 mg/dl, casual plasma glucose concentration >200 mg/dl in the presence of symptoms, 2-hour oral glucose tolerance test value of >200 mg/dl or if the patient was using antidiabetic agents, including insulin [18]. Hypertension was determined under the following conditions: systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or if the patient was receiving antihypertensive therapy [19]. Dyslipidemia was determined under the following conditions: low-density-lipoprotein cholesterol level ≥140 mg/dl, triglyceride level ≥150 mg/dl, high-density-lipoprotein cholesterol level <40 mg/dl or receiving antidyplipidemic medication [20]. Finally, chronic obstructive pulmonary disease (COPD) was diagnosed on the basis of a post-bronchodilator forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) value of <70 [21]. The protocol was approved by the Human Studies Subcommittee of Mie University Graduate School of Medicine.

**Renal doppler ultrasonography**

First, the flow velocities in the aorta and renal arteries were evaluated to rule out morphological abnormality or renal artery stenosis. Second, the renal RI was determined in the interlobar arteries of both kidneys and expressed as the mean of these values. The digital diagnostic ultrasound systems used were the Aplio XG SSA-790A with a PVT-375BT convex array transducer (Toshiba Medical Systems, Otawara, Tochigi, Japan) operating at a frequency of 3.5 MHz, and the LOGIQ P6 with a 4C convex array transducer (GE Medical Systems, Milwaukee, WI, US) operating in the frequency range of 4.0 MHz to 5.5 MHz. The ultrasound examinations were performed by two well-trained technicians. Renal RI was calculated as follows (Figure 1):

\[
\text{RenalRI} = \frac{\text{peak systolic velocity} - \text{end diastolic velocity}}{\text{peak systolic velocity}} = \frac{\text{peak systolic velocity} - \text{end diastolic velocity}}{\text{peak systolic velocity}}
\]

**Clinical outcome**

Medical records were retrospectively reviewed for each patient. The study end-point was 1) new anemia (<12.0 g/dl for women and <13.0 g/dl for men) for non-anemic patients, and 2) decreased hemoglobin levels greater than 1 g/dl and/or initiation of treatment including iron supplementation and EPO-stimulating agents for anemic patients. Anemic events within 3 months after minor surgery or cardiac catheterization and within 6 months after major surgery were excluded from the analysis.

**Statistical analysis**

All continuous variables were approximately normally distributed and presented as the mean ± standard deviation. In order to assess differences between the two groups, Pearson's chi-square test was used for nominal
scales and Student’s t test was used for all other scales. To make comparisons among three groups, we used Bonferroni’s multivariate comparison test. The linear correlations between the variables were parametrically evaluated using Pearson’s product moment correlation coefficient. We performed stepwise multivariate linear regression analysis to determine independent predictors of hemoglobin levels. Moreover, we examined the predictive value of potential clinical parameters for the study end-point, namely, the future development of anemia. Cumulative event rates were assessed using the Kaplan–Meier method and compared using the log-rank test. The impact of clinical predictors on the future development of anemia was assessed by univariate and multivariate Cox proportional hazards regression. Hazard ratios are given with 95% confidence intervals (CI). Reliability was assessed by calculating intraobserver and interobserver intraclass correlation coefficients. For all tests, a p value <0.05 was considered statistically significant. Data were analyzed using SPSS Statistics for Windows (Version 20.0, IBM Corp., Armonk, NY, US).

**Results**

**Patient characteristics**

Patient characteristics are presented in Table 1. Hemoglobin level ranged from 7.3 to 17.2 g/dL, and anemia was present in 37% of men and 34% of women. Among all 175 patients, eGFR ranged from 16 to 168 ml/min/1.73 m², and 102 patients (58%) had an eGFR of less than 60 ml/min/1.73 m². Only 19 patients (11%) had an eGFR of less than 30 ml/min/1.73 m². Fifty-one patients (29%) had a positive reaction in the urine protein test. The prevalence rates of coexisting diabetes mellitus, dyslipidemia and coronary artery diseases were 52%, 70% and 59%, respectively. When all subjects were divided into two groups according to the presence of anemia (Table 1), those with anemia were older, had greater body mass index (BMI), lower diastolic blood pressure, higher pulse pressure, higher renal RI, lower eGFR and higher prevalence of proteinuria than those without it. Patients with anemia were also more likely to receive renin-angiotensin-aldosterone system (RAAS) inhibitors and calcium channel blockers than those without it. There were statistically significant positive correlations between renal RI and pulse pressure, one of the surrogate measures of large artery stiffness (r = 0.45, p < 0.05), and age (r = 0.42, p < 0.05).

The relationships of eGFR and Renal RI to hemoglobin levels

All patients underwent successful renal Doppler ultrasonography, and the mean renal RI was 0.70 ± 0.09 (median: 0.70, range: 0.45–0.92). The measurements of renal RI were reproducible with intraobserver and interobserver intraclass correlation coefficients of 0.96 (p < 0.01) and 0.90 (p < 0.01), respectively. Figure 2 shows the relationships of renal RI and eGFR with hemoglobin levels. Both renal RI and eGFR had statistically significant correlations with hemoglobin levels. Further analysis revealed that there were significant correlations between renal RI and hemoglobin levels in the subgroups of 30 ≤ eGFR < 60 ml/min/1.73 m² (r = -0.42, p < 0.05) and eGFR ≥ 60 ml/min/1.73 m² (r = -0.24, p < 0.05) but not eGFR < 30 ml/min/1.73 m² (r = -0.11, p = ns). In addition, we assessed the relationship between renal RI and hemoglobin level in each stage of CKD with box and whisker plots (Figure 3). Bonferroni’s multivariate comparison test showed that there was a significant difference in the hemoglobin levels between patients with renal RI higher than the median value (0.70) and those with renal RI ≤ 0.7 only in the subgroup of 30 ≤ eGFR < 60 ml/min/1.73 m². Subsequently, univariate and stepwise multivariate linear regression analyses were applied to assess the relationships between hemoglobin levels and clinical variables (p < 0.1 for entry in Table 1: age, sex, BMI, diastolic blood pressure, pulse pressure, renal RI, eGFR, urine protein and administration of RAAS inhibitors and calcium channel blockers). As a
result, renal RI, eGFR, sex and BMI were independently associated with hemoglobin level (Table 2).

Predictive value of Renal RI for the future development of anemia

Among all 175 patients, we were able to perform follow-up evaluations on 174 (99%) of them. During the follow-up period (median 959 days, range: 7-3595 days), 84 (48%) patients developed anemia (new anemia in 48 patients, further development of anemia in 32 patients, initiation of iron supplementation in 2 patients and initiation of EPO in 2 patients). There was no relationship between the change in hemoglobin level and change in eGFR during the follow-up period. We performed Cox regression analysis in order to determine whether indices of renal damage including renal RI, eGFR and urine protein have potential to predict the future development of anemia. In terms of the results, renal RI per 0.05 rises and the presence of urine protein were independent predictors of the future development of anemia after correcting for sex, diabetes mellitus and baseline anemia (Table 3).

Kaplan–Meier curves demonstrated that patients with renal RI higher than the median value (0.70) in both the non-anemic and the anemic subgroups had a higher incidence of the future development of anemia than those with

### Table 1 Patient characteristics and comparison between patients with and without anemia

|                          | All | Anemia | No anemia | p value |
|--------------------------|-----|--------|-----------|---------|
| **Demographic data**     |     |        |           |         |
| Number                   | 175 | 64     | 111       |         |
| Age, years               | 67 ± 11 | 71 ± 10 | 65 ± 11 | <0.01   |
| Males, number (%)        | 134 (76.6) | 50 (78.1) | 84 (75.7) | 0.71    |
| Body mass index, kg/m²   | 242 ± 3.9 | 23.7 ± 4.5 | 24.6 ± 3.5 | 0.02    |
| Systolic blood pressure, mmHg | 135 ± 18 | 137 ± 20 | 135 ± 17 | 0.59    |
| Diastolic blood pressure, mmHg | 75 ± 13 | 72 ± 14 | 76 ± 13 | 0.01    |
| Pulse pressure, mmHg     | 61 ± 15 | 64 ± 17 | 59 ± 14 | 0.02    |
| Heart rate, bpm          | 67 ± 11 | 67 ± 10 | 67 ± 12 | 0.98    |
| Resistive index          | 0.70 ± 0.09 | 0.74 ± 0.09 | 0.67 ± 0.08 | <0.01  |
| Left ventricular ejection fraction | 0.63 ± 0.12 | 0.63 ± 0.14 | 0.64 ± 0.11 | 0.92   |
| **Laboratory data**      |     |        |           |         |
| Hemoglobin, g/dl         | 13.1 ± 1.7 | 11.4 ± 1.0 | 14.1 ± 1.2 | <0.01  |
| Estimated GFR, ml/min/1.73 m² | 57.7 ± 23.1 | 48.3 ± 24.8 | 63.1 ± 20.3 | <0.01  |
| Qualitative urine protein, number (%) | 51 (29.3) | 31 (49.2) | 20 (18.0) | <0.01  |
| Hemoglobin A1c, %        | 6.4 ± 1.7 | 6.6 ± 2.1 | 6.4 ± 1.4 | 0.98    |
| **Comorbidities, number (%)** |     |        |           |         |
| Diabetes mellitus        | 91 (52.0) | 35 (54.7) | 56 (50.5) | 0.60    |
| Dyslipidemia             | 122 (69.7) | 46 (71.9) | 76 (68.5) | 0.64    |
| Coronary artery disease  | 104 (59.4) | 41 (64.1) | 63 (56.8) | 0.34    |
| Peripheral artery disease| 34 (19.4) | 16 (25.0) | 18 (16.2) | 0.16    |
| History of congestive heart failure | 12 (6.8) | 6 (9.4) | 6 (5.4) | 0.35    |
| History of cerebral infarction | 26 (14.9) | 12 (18.8) | 14 (12.6) | 0.30    |
| Current smoking          | 53 (30.6) | 19 (30.2) | 34 (30.9) | 0.92    |
| COPD                     | 8 (4.6) | 4 (3.6) | 4 (3.6) | 0.41    |
| **Medications, number (%)** |     |        |           |         |
| RAAS inhibitors          | 125 (71.4) | 52 (81.3) | 73 (65.8) | 0.02    |
| Calcium channel blockers | 114 (65.1) | 47 (73.4) | 67 (60.4) | 0.07    |
| Beta blockers            | 57 (32.6) | 17 (26.6) | 40 (36.0) | 0.20    |
| Statins                  | 92 (52.6) | 37 (57.8) | 55 (49.5) | 0.29    |

Values are expressed as mean ± SD or numbers and percentages. Student’s t test was used to assess differences between the two groups except for sex, qualitative urine protein, comorbidities and medications, for which chi-square test was used.

GFR, glomerular filtration rate; COPD, chronic obstructive pulmonary disease; RAAS, renin-angiotensin-aldosterone system.
renal RI ≤0.70 (Figure 4, left top and left bottom). Patients with eGFR lower than the median value (56 ml/min/1.73 m²) only in the anemic subgroup had a higher incidence of the future development of anemia than those with eGFR ≥56 ml/min/1.73 m² (Figure 4, middle top and middle bottom). Patients with the presence of proteinuria had higher incidences of the future development of anemia than those without proteinuria only in the anemic subgroup (Figure 4, right top and right bottom). In addition, when all patients were divided into 4 risk groups according to median renal RI value and the presence or absence of proteinuria (group 1: renal RI ≤0.70 and no proteinuria, group 2: renal RI ≤0.70 and proteinuria, group 3: renal RI >0.70 and no proteinuria, and group 4: renal RI >0.70 and proteinuria), group 1 had a significantly lower risk for the future development of anemia than groups 3 and 4, and group 4 had a significantly higher risk than the other 3 groups (Figure 5).

**Discussion**

We demonstrated for the first time that renal RI was independently associated with hemoglobin level in patients with hypertension. Furthermore, high renal RI and the presence of proteinuria predicted the future development of anemia after correcting for confounding factors including age and diabetes mellitus in the present study.

According to Japan’s 2011 National Nutrition Survey, anemia was diagnosed in 8.9% of men and 8.5% of women aged 60-69 in the general population [24]. In the present study, however, the prevalence of anemia was as high as 37% in men and 34% in women in this study population. Renal damage secondary to hypertension is
characterized histologically by interstitial fibrosis, arteriosclerosis and glomerular sclerosis [10]. Chronic exposure to high blood pressure initially causes endothelial dysfunction and progressive infiltration of macrophages and T cells to the perivascular interstitium. Interactions of these cells and their cytokines with parenchymal cells cause interstitial fibrosis and tubular insufficiency [7-10]. In recent studies, EPO-producing cells were identified in the renal tubulointerstitium surrounding the central vessels [4,5]. Unfavorable changes in the interstitial microenvironment such as inflammation, oxidative stress and ischemia resulting from hypertension can impair the function of EPO-producing cells. Recently, Souma et al. demonstrated that the phenotypic transition of EPO-producing cells to non-EPO-producing myofibroblasts is modulated by inflammatory molecules, and suggested the connection between anemia and renal fibrosis in CKD in a mouse model [6]. Therefore, the development of CKD secondary to hypertension ought to affect hematopoiesis even in the earlier stages of CKD defined by eGFR. Indeed, there was a significant difference in the hemoglobin levels between patients with renal RI higher than the median value (0.70) and those with renal RI ≤0.7 only in the subgroup of 30 ≤ eGFR <60 ml/min/1.73 m².

We further demonstrated that renal RI and urine protein predicted the future development of anemia. Renal RI reflects the severity of vascular and tubulointerstitial lesions, and has been shown to correlate with an inflammatory state [13-15,25]. We also found that there was no relationship between the change in hemoglobin level

### Table 3 Cox proportional hazards regression for the development of anemia

|                     | Univariate Hazard ratio | p value | Multivariate Hazard ratio | p value |
|---------------------|-------------------------|---------|---------------------------|---------|
| Age                 | 1.04 (1.01-1.06)        | <0.01   | 1.03 (1.00-1.06)          | 0.03    |
| Male sex            | 1.02 (0.61-1.69)        | 0.95    | 1.07 (0.63-1.84)          | 0.80    |
| Diabetes mellitus   | 1.65 (1.06-2.55)        | 0.03    | 1.43 (0.89-2.30)          | 0.14    |
| Anemia at baseline  | 1.31 (0.86-2.00)        | 0.22    | 0.67 (0.40-1.11)          | 0.12    |
| Resistive index (per 0.05) | 1.30 (1.16-1.46) | <0.01   | 1.18 (1.02-1.37)          | 0.03    |
| Estimated GFR       | 0.99 (0.97-1.00)        | 0.11    | 1.00 (0.98-1.01)          | 0.57    |
| Qualitative urine protein | 2.21 (1.44-3.39) | <0.01   | 1.80 (1.08-3.01)          | 0.03    |

GFR, glomerular filtration rate.

Figure 4 Kaplan–Meier curves for new anemia in the non-anemic subgroup (top), and further development of anemia in the anemic group (bottom) stratified by median renal RI (>0.70 and ≤0.70, left), median eGFR (<55 and ≥55 ml/min/1.73 m², middle), and the presence or absence of proteinuria (right). RI, resistive index; eGFR, estimated glomerular filtration rate.
and change in eGFR during the follow-up period. These findings may suggest that baseline renal RI and urine protein, rather than worsening glomerular function defined by eGFR, had a greater impact on the development of anemia. Although it would be difficult to identify the underlying mechanisms responsible for the development of anemia precisely, damage of renal interstitial compartments and arteriosclerosis may contribute to the disease process independently of glomerular function, especially in the early stages of CKD defined by eGFR. The causal interrelationship between the progression of CKD and the development of anemia warrants further investigation.

We also demonstrated that the presence of proteinuria is a powerful independent predictor of the future development of anemia. Proteinuria is an important sign of CKD, which can result from hypertension, diabetes mellitus and diseases that cause inflammation in the kidneys. It is recognized that proteins abnormally filtered across the glomerular barrier have intrinsic renal toxicity linked to their over-reabsorption by proximal tubular cells and activation of tubular-dependent pathways of interstitial inflammation and fibrosis [26,27]. Protein overload causes a dose-dependent increase in nuclear factor kappa beta activation in proximal tubular cells that leads to myofibroblast transformation of EPO-producing cells [6,28,29]. We demonstrated that patients with high renal RA and proteinuria had the greatest risk for the future development of anemia. The relationship between proteinuria and renal RI as causal mechanisms underlying the future development of anemia in CKD warrants further investigation. It has been reported that the risk of anemia in patients with diabetes mellitus is approximately two to three times that of the general population with the same level of eGFR due to tubulointerstitial injury secondary to proteinuria and dysglycemia [30]. Diabetes mellitus is commonly found in patients with hypertension and vice versa. In the present study, 52% of patients had diabetes mellitus, and diabetic patients had a higher prevalence of proteinuria than non-diabetic ones. It is difficult to discriminate proteinuria secondary to diabetic nephropathy from that independent of diabetes mellitus per se. However, the presence of proteinuria was independently associated with the future development of anemia after correcting for diabetes mellitus in the present study.

The presence of anemia is known to result in a worse prognosis in terms of both morbidity and mortality. An earlier diagnosis and optimal treatment of anemia would reduce incidence rates of cardiovascular diseases, as well as slow the decline of renal function [31]. Measuring renal RI in addition to conventional markers of renal damage including eGFR and urine protein will help clinicians to decide whether or not the anemia is secondary to renal damage, especially in the early stages of CKD. In addition, high renal RI may aid in alerting them to monitor hemoglobin levels even if patients have no sign of anemia or advanced renal failure on the basis of eGFR.

The limitations of this study include the small sample size and the retrospective nature of the data collection. Renal ultrasonography was performed for the screening of renal artery stenosis and the evaluation of renal arteriosclerosis at the physician’s discretion on the basis of the patients’ age, comorbidity and disease characteristics. Accordingly, the prevalence of coexisting atherosclerotic diseases in patients who were enrolled in the present study can be higher than that of hypertensive patients among the general population. This selection bias may influence the relationship between renal RI and anemia, and the clinical impact of renal RI on the future development of anemia. Renal RI reflects systemic vascular stiffness as well as renal arteriolosclerosis. Indeed, we found a statistically significant positive correlation between renal RI and pulse pressure, one of the surrogate measures of large artery stiffness. Risk factors for systemic atherosclerosis such as inflammation and oxidative stress may affect renal RI and anemia via different pathways, which can confound precise understanding of the causal mechanism underlying the anemia in patients with hypertension and renal damage. However, stepwise multivariate linear regression analyses revealed that renal RI but not pulse pressure was independently associated with hemoglobin level. We were unable to obtain sufficient information to
examine precisely the etiology of anemia including iron metabolism, erythropoietin productivity and responsiveness, and inflammation. Iron deficiency is common in patients with CKD. Estrella et al. found that 42.6% of patients who had both anemia and CKD with eGFR between 15 and 59 ml/min/1.73 m² were classified as having functional or absolute iron-deficiency anemia (33.5% and 9.1%, respectively) [32]. Therefore, patients in the anemic group and even in the non-anemic group can have varying degrees of iron deficiency. The causal relationships between iron status and anemia in the hypertensive population, especially those with renal damage, warrant further investigation. Artunc and Risler reported that the correlation between hemoglobin level and EPO concentration was gradually attenuated with increasing stages of CKD and was mostly lost in CKD stages 4 and 5 in an observational study, indicating the presence of more prominent EPO deficiency in advanced CKD [2]. In other words, the pathogenesis of anemia in the early stages of CKD might be heterogeneous in terms of the severity of impairment of EPO productivity and responsiveness. Inflammation also influences EPO efficacy and production as well as renal atherosclerosis. Urine proteins were qualified by using a dipstick test in the present study. Therefore, the potential contribution of microalbuminuria to the pathogenesis of anemia, especially in patients with diabetes, was not investigated. The analysis of markers of tubulointerstitial damage such as beta-2 microglobulins or N-acetylglucosaminidase was also not carried out. Several factors including obesity-related obstructive sleep apnea, current smoking and COPD may contribute to persistent or intermittent hypoxia that lead to red cell production via EPO stimulation. Indeed, body mass index was positively related to hemoglobin level in the present study. However, the presence and severity of sleep apnea and their contribution to hemoglobin level were not assessed in the present study. Finally, follow-up data of renal RI, urine protein, and changes in nutritional status and medication were not included in our outcome evaluation.

Conclusion
In conclusion, renal RI correlated directly with hemoglobin levels and contributed to the development of anemia independently of glomerular function. The measurement of renal RI in patients with hypertension can be useful for elucidating the pathogenesis of anemia and for inferring its potential risk.

Abbreviations
CKD: Chronic kidney disease; EPO: Erythropoietin; RI: Resistive index; GFR: Glomerular filtration rate; eGFR: Estimated glomerular filtration rate; SCr: Serum creatinine; RAAS: Renin-angiotensin-aldosterone system.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MT and KD designed the study, performed the data analysis and wrote the manuscript. MT, YS, ES, NK, SN and NF collected the data. MM undertook ultrasonography examination and collected the data. NF, TT, NY and MN provided comments on the manuscript. TY supervised the statistical analysis. ML supervised and approved the manuscript. All authors read and approved the final manuscript.

Acknowledgements
The authors are grateful to GE Healthcare and Toshiba Medical Systems Corporation for their technical support, and to Yoko Sakurai, MT, CVT, and Harumi Fukuda, MT, CVT, for their technical assistance.

Author details
1Department of Cardiology and Nephrology, Mie University Graduate School of Medicine, Tsu, Japan. 2Central Clinical Laboratories, Mie University Hospital, Tsu, Japan. 3Department of Translational Medical Science, Mie University Graduate School of Medicine, Tsu, Japan. 4Department of Molecular and Laboratory Medicine, Mie University Graduate School of Medicine, Tsu, Japan.

Received: 3 February 2015 Accepted: 25 March 2015
Published online: 08 April 2015

References
1. Astor BC, Muntrner P, Levin A, Eustace JA, Coresh J. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med. 2002;162:1401–8.
2. Artunc F, Risler T. Serum erythropoietin concentrations and responses to anemia in patients with or without chronic kidney disease. Nephrol Dial Transplant. 2007;22:2900–8.
3. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int. 2011;80:17–28.
4. Suzuki N, Obara N, Yamamoto M. Use of gene-manipulated mice in the study of erythropoietin gene expression. Methods Enzymol. 2007;435:157–77.
5. Obara N, Suzuki N, Kim K, Nagasawa T, Imagawa S, Yamamoto M. Repression via the GATA box is essential for tissue-specific erythropoietin gene expression. Blood. 2008;111:5229–36.
6. Souma T, Yamaoaki S, Moriguchi T, Suzuki N, Hirano I, Pan X, et al. Plasticity of Renal Erythropoietin-Producing Cells Governs Fibrosis. J Am Soc Nephrol. 2013;24:1599–616.
7. Marín R, Gorostidi M, Fernández-Vega F, Alvarez-Navascués R. Systemic and glomerular hypertension and progression of chronic renal disease: the dilemma of nephroclerosis. Kidney Int Suppl. 2005;99:S2–5.
8. Reynolds K, Gu D, Muntner P, Kusek JW, Chen J, Wu X, et al. A population-based, prospective study of blood pressure and risk for end-stage renal disease in China. J Am Soc Nephrol. 2007;18:1928–35.
9. Yamagata K, Ishida K, Saienchi T, Takahashi H, Obha S, Shigai T, et al. Risk factors for chronic kidney disease in a community-based population: a 10-year follow-up study. Kidney Int. 2007;71:159–66.
10. Mai M, Geiger H, Hilger KF, Veelken R, Mann JF, Dämmrich J, et al. Early interstitial changes in hypertension-induced renal injury. Hypertension. 1993;22:754–65.
11. Kawai T, Kamide K, Oishi M, Yamamoto-Hanasaki H, Baba Y, Hongyo K, et al. Usefulness of the resistive index in renal Doppler ultrasonography as an indicator of vascular damage in patients with risks of atherosclerosis. Nephrol Dial Transplant. 2011;26:6256–62.
12. Portemoll R, Vazzi F, Martioli C, Ravira M, Nicolotta C, Berutti V, et al. Increased renal resistive index in patients with essential hypertension: a marker of target organ damage. Nephrol Dial Transplant. 1999;14:360–5.
13. Mostbeck GH, Kain R, Mallek R, Derfler K, Walter R, Havelec L, et al. Duplex Doppler sonography in renal parenchymal disease. Histopathologic correlation. J Ultrasound Med. 1991;10:189–94.
14. Ikei R, Kobayashi S, Hemmi N, Imakiire T, Kikuchi Y, Moriya H, et al. Correlation between the resistive index by Doppler ultrasound and kidney function and histology. Am J Kidney Dis. 2005;46(4):603–9.
15. Bigé N, Lévy PP, Callard P, Fantinchi JM, Chigot V, Jouesselin V, et al. Renal RI is associated with severe histological changes and poor renal outcome during chronic kidney disease. BMC Nephrol. 2012;13:139.
16. Nutritional anemias. Report of a WHO scientific group. World Health Organ Tech Rep Ser. 1968;405:5–37.
17. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Collaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
18. Inzucchi SE. Clinical practice. Diagnosis of diabetes. N Engl J Med. 2012;367:542–50.
19. Bertoia ML, Waring ME, Gupta PS, Roberts MB, Eaton CB. Implications of new hypertension guidelines in the United States. Hypertension. 2012;60:39–44.
20. Teramoto T, Sasaki J, Ueschima H, Egusa G, Kinoshita M, Shimamoto K, et al. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. J Atheroscler Thromb. 2007;14:45–50.
21. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD Executive Summary. Am J Respir Crit Care Med. 2007;176:532–55.
22. Radermacher J. Resistive index: an ideal test for renovascular disease or ischemic nephropathy? Nat Clin Pract Nephrol. 2006;2:232–3.
23. Tublin ME, Bude RO, Platt JF. The resistive index in renal Doppler sonography: where do we stand? AJR Am J Roentgenol. 2003;180:885–92.
24. National Nutrition Survey 2010 in Japan. [http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h23-houkoku-05.pdf].
25. Berni A, Ciani E, Bernetti M, Cecioni I, Berardino S, Poggesi L, et al. Renal resistive index and low-grade inflammation in patients with essential hypertension. J Human Hypertens. 2012;26:723–30.
26. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. N Engl J Med. 1998;339:1448–56.
27. Zoja C, Morigi M, Remuzzi G. Proteinuria and phenotypic change of proximal tubular cells. J Am Soc Nephrol. 2003;14 Suppl 1:356–41.
28. Zoja C, Donadelli R, Colleoni S, Figliuzzi M, Bonazolala S, Morigi M, et al. Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kB activation. Kidney Int. 1998;53:1608–15.
29. Morigi M, Macconi D, Zoja C, Donadelli R, Buelli S, Amand S, et al. Protein overload-induced NF-kB activation in proximal tubular cells requires H2O2 through a PKC dependent pathway. J Am Soc Nephrol. 2002;13:1179–89.
30. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G. Unrecognized anemia in patients with diabetes: a cross-sectional survey. Diabetes Care. 2003;26:1164–9.
31. Gouva C, Nikolopoulos P, Ioannidis JP, Siamopoulos KC. Treating anemia early in renal failure patients slows the decline of renal function: a randomized controlled trial. Kidney Int. 2004;66:753–60.
32. Estrella MM, Astor RC, Köttgen A, Selvin E, Coresh J, Parekh RS. Prevalence of kidney disease in anemia differs by GFR-estimating method: the Third National Health and Nutrition Examination Survey (1988-94). Nephrol Dial Transplant. 2010;25:2542–8.