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Relationship Between Arterial Stiffness and Immature Granulocyte, Delta Neutrophil Index, and Systemic Immune-Inflammation Index in Hemodialysis Patients

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Abstract

Introduction: Several inflammatory markers are thought to be potential predictors of disease progression and mortality in end-stage renal disease. In the present study, we aimed to assess the potential association between newly described inflammatory markers immature granulocyte (IG), delta neutrophil index (DNI), and systemic immune-inflammation index (SIII) and arterial stiffness (AS) in hemodialysis (HD) patients.

Materials and Methods: Medical records of HD patients undergoing outpatient clinic were retrospectively reviewed. A single-cuff arteriograph device was used to measure AS. IG and DNI levels were determined using a specific automated hematology analyzer. SIII was calculated using the formula: (neutrophil count × platelet count) / lymphocyte count.

Results: A total of 117 patients were enrolled, with a mean age of 53.6±14.5 years; 55.6% were male and 44.4% were female. Correlation analysis showed that pulse wave velocity (PWV) was strongly correlated with age, body mass index, and systolic blood pressure; positively correlated with IG and DNI; and negatively correlated with serum albumin levels. When the effect of age was eliminated in the partial correlation analysis, the relationship between PWV and inflammation markers disappeared. Regression analysis revealed that the most important determinants of PWV were age and systolic blood pressure (SBP).

Conclusion: In HD patients, the relationship between PWV and IG & DNI is mediated by age, but there is no relationship between PWV and SIII. While these routinely obtainable hematological parameters cannot be directly used for cardiovascular risk assessment in this population, they can provide insights into patients at risk.

Key words: Arterial stiffness; delta neutrophil index; hemodialysis; immature granulocyte; systemic immune-inflammation index.

Introduction

Cardiovascular disease represents the primary cause of mortality among patients undergoing hemodialysis (HD) (1). Arterial stiffness (AS) is widely used as a predictor of cardiovascular risk assessment (2). Because AS cannot be routinely measured in daily clinical practice, there is ongoing interest in noninvasive and easily obtainable laboratory markers. In end-stage renal disease (ESRD), chronic inflammation, oxidative stress, and the accumulation of uremic toxins can lead to elevations in inflammatory biomarkers (3). All of these conditions are also considered to be among the causes of atherosclerosis and AS (2). Several inflammatory markers have been reported as predictors of disease progression and mortality in ESRD patients (4-6). Inflammation and endothelial dysfunction play essential roles in the pathogenesis of vascular calcification (7). Therefore, inflammatory markers may be clinically useful in assessing cardiovascular risk. These biomarkers may also faciliate the identification high-risk patients, guide prophylactic treatments, determine follow-up frequency, and contribute to the development of new therapeutic strategies (8). Although previous studies have evaluated markers such as white blood cell count (WBC), neutrophil count (PMN), neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNF-α), and other specific molecules, no consensus has been established regarding an ideal marker. Immature granulocytes (IGs) are post-myeloblast myeloid cells including promyelocytes, myelocytes, and

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metamyelocytes that have not yet matured into fully developed neutrophils, basophils, or eosinophils (9). An increase in circulating IGs reflects bone marrow activation and is not observed under physiological conditions (10). In the literature, IGs have been suggested as inflammatory markers in pre-dialysis children with ESRD (11). IG, delta neutrophil index (DNI), and systemic immune-inflammation index (SIII) are newly described inflammatory markers. Their associations with several clinical conditions have been explored, and their potential roles as universal indicators of systemic inflammation have gained interest. This study aimed, for the first time in the literature, to evaluate the potential relationship between these inflammatory markers and AS in patients undergoing HD.

Materials and Methods

Study design and population: This single-center retrospective observational study was conducted in the HD Unit of Kahramanmaras Sutcu Imam University Medical Faculty Hospital. Patients aged 18-90 years who had been undergoing HD treatment for at least 3 months, had no active infection, and provided informed consent were included. Exclusion criteria were: age <18 or >90 years, HD duration <3 months, temporary HD initiated for acute kidney injury, severe electrolyte imbalances, or urgent uremic complications, peritoneal dialysis treatment, active infection, malignancy, corticosteroid or immunosuppressive therapy, or refusal to participate.

Data collection: AS was measured using a single-cuff arteriograph device (Mobil-O-Graph PWA, I.E.M. GmbH). This device measures AS non-invasively and oscillometrically. Similar in structure to a blood pressure monitor, it uses a cuff to measure not only blood pressure but also pulse wave forms. Measurements were performed on HD days, before the dialysis session, following at least 30 minutes of rest. The cuff was positioned on the arm without the arteriovenous fistula, and three consecutive measurements were recorded at 30 second intervals; the mean values were used for analysis. Measurements obtained included heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse wave velocity (PWV), and augmentation index (AI).

Laboratory measurements were obtained from routine pre-dialysis blood samples. IG and DNI values were measured with an automated hematology analyzer (XN-1000; Sysmex, Kobe, Japan). This device calculates these parameters using two independent leukocyte measurement methods: cytochemical analysis and fluorescent flow cytometry. IG values were expressed as absolute counts per milliliter (IG × 10^6/mL), and DNI values as the percentage of IGs within the total leukocyte count. SIII was calculated using the formula: (neutrophil count × platelet count) / lymphocyte count.

Ethical Consent: Ethical approval was obtained from the Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Research Ethics Committee (Date: March 4, 2024, Session No: 5, Decision No: 2). This study adhered to the principles outlined in the Declaration of Helsinki, with written informed consent obtained from all participants before inclusion.

Statistical Analysis: Continuous variables are expressed as mean±standard deviation (SD), whereas categorical variables are reported as frequencies or percentages. Assessment of data distribution normality was used the Kolmogorov Smirnov and/or Shapiro Wilk tests. Comparisons of continuous variables were performed using the Student's t-test or the Mann–Whitney U test, based on the underlying data distribution. Correlation analyses were performed using Pearson or Spearman correlation tests as appropriate. Multiple linear regression analysis was used to identify factors independently associated with PWV. Beta coefficients and confidence intervals were calculated across two distinct analytical models. Statistical significance was defined as a p-value <0.05.

Results

Data from 137 HD patients who met the inclusion criteria and were selected through consecutive sampling were retrospectively analyzed. Twenty individuals were excluded due to missing IG and DNI data, resulting in a final cohort of 117 patients for analysis. The study population had a mean age of 53.6±14.5 years; 55.6% were male and 44.4% were female. Regarding comorbidities, 79.5% had hypertension (HT), 61.5% had diabetes mellitus (DM), and 29.1% had coronary artery disease (CAD). Demographic characteristics and laboratory findings are presented in detail in Table 1. Correlation analysis showed that PWV was strongly correlated with age, body mass index (BMI), and systolic blood pressure (SBP) (p=0.001, r=0.893; p=0.001, r=0.402; p=0.001, r=0.338, respectively). PWV also demonstrated a significant positive correlation with both IG and DNI (p=0.011, r=0.233; p=0.013, r=0.230, respectively), while showing a negative correlation with serum albumin levels (p=0.003, r = –0.273). IG was correlated with age, PWV, and AI (p=0.007, r=0.248; p=0.011, r=0.233; p = 0.026,

Table 1: Demographic Data and Laboratory of Patients

Parameters	n=117 Mean±SD	min-max
Age (year)	53.6±14.5	20-82
Gender (m) (%)	55.6	
HD duration (m)	61.2±50.7	4-204
BMI (kg/m²)	25.9±5.2	15.7-39.9
SBP (mmHg)	140±24	80-200
DBP (mmHg)	87±18	48-138
DM (%)	61.5	
HT (%)	79.5	
CAD (%)	29.1	
BUN (mg/dL)	69±35	12-262
Creatinine (mg/dL)	8.0±2.3	3.3-13.2
Sodium (mmol/L)	136±4	121-145
Potassium (mmol/L)	5.0±0.7	3.3-7.9
Calcium (mg/dL)	8.5±0.8	6.2-11.6
Phosphorus (mg/dL)	5.0±1.6	1.1-9.8
iPTH (pg/mL)	431±445	3-2924
Glucose (mg/dL)	144±90	65-597
LDL cholesterol (mg/dL)	79±24	13-157
Triglyceride (mg/dL)	162±110	34-772
CRP (mg/L)	21±26	2-45
Albumin (mg/dL)	38.6±5.6	18-55
Uric acid (mg/dL)	6.0±1.5	1.4-11.9
PMNL (10^9/L)	5.1±2.4	1.5-13.6
Lymphocyte (10^9/L)	1.5±0.6	0.3-3.3
Hemoglobin (g/dL)	11.8±7.1	7.1-16.4
Platelet (10^9/L)	215±89	75-558
IG (10^9/L)	0.046±0.048	0.01-0.25
DNI (%)	0.57±0.50	0.04-3.60
SIII	947±981	156-6168
Kt/V	1.58±0.19	1.07-2.36
URR (%)	73±6	59-97
Mean PWV (m/s)	8.3±1.7	4.5-12.3
Mean AI (%)	26.6±10.5	3.7-58.3

HD: hemodialysis, BMI: body mass index, SBP: systolic blood pressure, DKB: diastolic blood pressure, DM: diabetes mellitus, HT: hypertension, CAD: coroner artery disease, BUN: blood urea nitrogen, iPTH: intact parathyroid hormone, LDL: low density lipoprotein, CRP: C-reactive protein, PMNL: polymorphonuclear leukocyte, IG: immature granulocytes, DNI: delta neutrophil index, SIII: systemic immune-inflammation index, URR: urea reduction rate, PWV: pulse wave velocity, AI: augmentation index.

r=0.206, respectively). DNI was positively correlated with age and PWV (p=0.006, r=0.254; p=0.013, r=0.230, respectively) and negatively correlated with DBP (p=0.013, r=-0.229). SIII was inversely correlated with serum albumin (p=0.008, r=-0.242). Correlation analyses are summarized in Table 2. In partial correlation analysis, the correlations between PWV and IG/DNI disappeared. In the unadjusted regression analysis, age (β=0.10; 95% CI: 0.09–0.11; p<0.001), IG (β=10.62; 95% CI: 4.08–17.16; p=0.002), albumin (β=−0.06; 95% CI: −0.11 to −0.003; p=0.03), and SBP (β=0.02; 95% CI: 0.012–0.038; p<0.001) were significantly associated with PWV. In the adjusted regression analysis, age (β=0.11; 95% CI: 0.10–0.11; p<0.001), SBP (β=0.03; 95% CI: 0.02–0.03; p<0.001), and CRP (β=−0.005; 95% CI: −0.008 to −0.001; p=0.006) were identified as independent determinants of PWV. IG and albumin levels, which were significant in the unadjusted analysis, lost their statistical significance after adjustment

Table 2: Correlation Analysis for PWV, AI, IG, DNI and SIII

Parameters	PWV		AI		IG		DNI		SIII	
	p	r	p	r	p	r	p	r	p	R
Age (year)	0.001	0.893	0.839	0.019	0.007	0.248	0.006	0.254	0.182	0.124
HD period (m)	0.401	0.078	0.609	0.048	0.635	-0.044	0.806	-0.023	0.684	0.038
BMI (kg/m^2)	0.001	0.402	0.501	0.63	0.157	0.132	0.611	0.047	0.460	0.069
SBP (mmHg)	0.001	0.338	0.001	0.376	0.696	-0.037	0.362	-0.085	0.915	0.010
DBP (mmHg)	0.508	-0.062	0.048	0.184	0.052	-0.180	0.013	-0.229	0.086	-0.159
LDL cholesterol (mg/dL)	0.176	0.128	0.889	-0.013	0.256	0.108	0.993	-0.001	0.129	0.144
CRP (mg/L)	0.735	0.032	0.178	-0.126	0.914	0.010	0.956	-0.005	0.123	0.145
Albumin (mg/dL)	0.003	-0.273	0.173	-0.127	0.101	-0.152	0.175	-0.126	0.008	-0.242
Uric acid (mg/dL)	0.964	-0.004	0.895	-0.012	0.869	0.015	0.961	0.005	0.589	-0.050
IG (10^9/L)	0.011	0.233	0.026	0.206	-	-	0.001	0.883	0.001	0.391
DNI (%)	0.013	0.230	0.089	0.158	0.001	0.883	-	-	0.014	0.227
SIII	0.116	0.146	0.754	0.029	0.001	0.391	0.014	0.227	-	-
Mean PWV (m/s)	-	-	0.052	0.180	0.011	0.233	0.013	0.230	0.116	0.146
Mean AI (%)	0.052	0.180	-	-	0.026	0.206	0.089	0.158	0.754	0.029

PWV: pulse wave velocity, AI: augmentation index, IG: immature granulocytes, DNI: delta neutrophil index, SIII: systemic immune-inflammation index, HD: hemodialysis, BMI: body mass index, SBP: systolic blood pressure, DKB: diastolic blood pressure, LDL: low density lipoprotein, CRP: C-reactive protein

Table 3: Multiple Linear Regression Analysis of Factors Affecting PWV

Parameters	Unadjusted			Udjusted		
	b	P	%95 CI	b	p	%95 CI
Age (year)	0.10	0.001	0.09 to 0.11	0.11	0.001	0.10 to 0.11
HD period (m)	0.001	0.71	-0.005 to 0.008	-0.001	0.16	-0.003 to 0.000
IG (10^9/L)	10.62	0.002	4.08 to 17.16	0.75	0.44	-1.2 to 2.7
Calcium (mg/dL)	0.14	0.45	-0.24 to 0.54	0.18	0.72	-0.08 to 0.11
Phosphorus (mg/dL)	-0.09	0.34	-0.29 to 0.10	0.05	0.07	-0.006 to 0.10
iPTH (pg/mL)	-0.001	0.10	-0.001 to 0.000	0.001	0.88	0.000 to 0.000
LDL cholesterol (mg/dL)	0.009	0.17	-0.004 to 0.022	-0.002	0.35	-0.005 to 0.002
CRP (mg/L)	-0.001	0.83	-0.14 to 0.011	-0.005	0.006	-0.008 to -0.001
Albumin (mg/dL)	-0.06	0.03	-0.11 to -0.003	0.007	0.44	-0.01 to 0.02
Uric acid (mg/dL)	-0.04	0.67	-0.26 to 0.17	-0.04	0.11	-0.10 to 0.01
SBP (mmHg)	0.02	0.001	0.012 to 0.038	0.03	0.001	0.02 to 0.03

HD: hemodialysis, IG: immature granulocytes, iPTH: intact parathyroid hormone, LDL: low density lipoprotein, CRP: C-reactive protein, SBP: systolic blood pressure

for potential confounding variables. Although phosphorus levels showed a borderline association in the multivariable analysis, this association did not reach statistical significance (p=0.07). Detailed results of the multiple linear regression analysis identifying predictors of PWV are presented in Table 3.

Discussion

To our knowledge, no prior research have evaluated the relationship between AS and the inflammatory markers IG, DNI, and SIII in HD patients, making this the first study to investigate this topic. One study demonstrated that IG and DNI levels were higher in pre-dialysis ESRD patients compared to the normal population (11). Another study indicated that DNI was elevated in patients with Henoch–Schönlein purpura with renal involvement (12). Similarly, SIII has been suggested as a predictor of dialysis requirement and survival in pre-dialysis ESRD patients (13). These findings have been linked to endothelial dysfunction as a key mechanism and the potential of IG, DNI, and SIII to reflect this pathological process. In our cross-sectional analysis, we showed that PWV, an indicator of AS, was age-dependently associated with IG and DNI in HD patients. The relationship between leukocyte counts or leukocyte subtypes and endothelial dysfunction, AS, and cardiovascular events has been extensively studied both in renal and non-renal populations. For example, an association between AS and total leukocyte count, neutrophil count, monocyte count, and lymphocyte count has been demonstrated in patients with hematologic or solid organ malignancies (14,15). This relationship has been explained by enhanced leukocyte endothelial adhesion and subsequent cytokine and enzyme-mediated vascular injury (14). In patients with acute coronary syndromes, AS has been associated with the neutrophil-to-lymphocyte ratio, and decreases in this ratio have been accompanied by improvements in AS in non-ST elevation myocardial infarction (NSTEMI) patients (16,17). Similar relationships have been described in patients with type 1 and type 2 DM, familial Mediterranean fever (FMF) and autosomal dominant polycystic kidney disease (ADPKD) (18-21). In ESRD and HD patients, ratios such as NLR, platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) have been reported as predictors of mortality (4,22). As can be seen, the relationship between hemogram parameters, their resulting ratios, or other inflammatory markers, and AS and mortality has been a frequently researched topic in the literature for many diseases. Because these easily obtained and routinely used tests allow clinicians to predict disease progression, they provide useful information to guide treatment approaches. However, despite extensive research involving leukocyte subtypes, studies on newer inflammatory markers remain limited. IG and DNI have been shown to be elevated in pre-dialysis children with ESRD (11). Similarly, elevated DNI was reported in children with Henoch–Schönlein purpura, particularly those with renal or visceral involvement (12). Tanindi et al. (16) proposed that granulocyte function and count may be influenced by sympathetic activation via adrenergic receptors, potentially linking them to endothelial dysfunction. Furthermore, IG has been suggested as a marker for disease activity, treatment response, and follow-up in patients with ankylosing spondylitis (23). In intensive care settings, IG was reported to be more sensitive and specific than CRP, IL-6, and lipopolysaccharide-binding protein (LBP) in diagnosing sepsis and systemic inflammatory response syndrome (SIRS), though not predictive of mortality (24). Conversely, Agarwal et al. (25) found that ESRD patients demonstrated increased granulocyte counts compared to healthy individuals, and this increase was associated with disease progression and mortality. Additionally, postoperative increases in IG levels have been associated with organ dysfunction in patients undergoing open heart surgery, potentially aiding early detection of patients at risk for infection (26). No previous studies have examined the association between AS and IG or DNI. Our study, for the first time, revealed a significant association between AS and both markers; however, regression analysis indicated that this relationship was age-dependent. The aging process is characterized by a chronic, low-grade pro-inflammatory state called "inflammaging" (27). In HD patients, advanced age often brings with it a higher inflammatory burden, increased risk of infection, and more comorbidities. Therefore, it is possible to find higher IG and DNI levels in elderly HD patients.

SIII has also been studied extensively in renal disease and has been shown to serve as a marker in various clinical scenarios. For example, SIII has been shown to predict the need for dialysis in ESRD children (13). In HD patients, elevated SIII has been associated with reduced survival and recognized as an independent predictor for protein-energy wasting (28). It has also been proposed as a predictor of peritoneal dialysis associated peritonitis, delayed graft function, and acute rejection following kidney transplantation (29-30). In our study, the association between SIII and AS was evaluated for the first time in the literature, and no significant relationship was detected. However, the positive correlation of SIII with IG and DNI and its negative correlation with serum albumin support its role as an inflammatory indicator. Our study remains significant as it is the first to evaluate the relationship between IG, DNI, SIII, and AS in HD patients.

Study Limitations: Only baseline IG, DNI, and SIII values were available, and a single time-point measurement may yield misleading results; repeated measurements over time could provide more accurate assessments. Additionally, simultaneous evaluation of specific inflammatory markers such as IL-6 and TNF-α could have strengthened the findings. Moreover, the use of the Sysmex XN-1000 hematology analyzer is not yet widespread, and broader availability may require time. Furthermore, since only one measurement was taken in our study, interdialytic weight gain, which varies between sessions, was not examined.

Conclusion

IG and DNI are age-dependent parameters associated with AS in HD patients. These parameters, obtained from standard complete blood count tests that require no additional cost, can offer valuable information about at-risk patients, even if they are not directly applicable to cardiovascular risk assessment in HD patients. Larger, multi-center prospective studies are needed to support and expand upon these findings.

Ethical approval: Ethical approval was obtained from the Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Research Ethics Committee (Date: March 4, 2024, Session No: 5, Decision No: 2).

Conflict of interest statement: The authors declare no conflict of interest.

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