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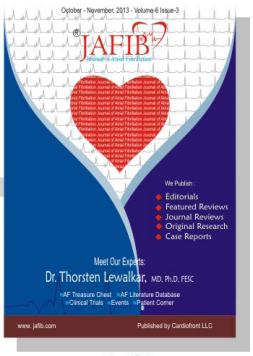

Impaired Erythrocyte Deformability in Patients with Coronary Risk Factors: Significance of Nonvalvular Atrial Fibrillation

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Abstract

Although coronary risk factors promote the formation of atherosclerotic plaque containing activated platelets and inflammatory leukocytes, and play a pivotal role in the development of coronary artery diseases (CAD), the hemorheological effects of these risk factors on circulating intact erythrocytes, a major component of whole blood cells, are poorly understood. Therefore, this study aimed to quantify erythrocyte deformability in patients with coronary risk factors, and enrolled 320 consecutive cardiac outpatients including 33 patients with nonvalvular atrial fibrillation (AF). Patients with acute coronary syndrome or valvular AF were excluded. Demographic variables obtained by medical records were correlated with erythrocyte deformability investigated by our highly sensitive and reproducible filtration technique. Among demographic variables, triglyceride ($p = 0.004$), HbA1c ($p = 0.014$) and body weight ($p = 0.020$) showed significant inverse correlation to the erythrocyte deformability. This deformability was not associated with types of CAD (old myocardial infarction vs. stable angina) or modality of treatment (percutaneous intervention vs. coronary artery bypass grafting). Unexpectedly, stepwise multiple regression analysis demonstrated that nonvalvular AF was the most significant contributor to the impaired erythrocyte deformability ($p = 0.002$). Hypertension and dyslipidemia are more prevalent in the AF patients ($p < 0.001$), and the erythrocyte deformability was found to be impaired synergistically and significantly ($p < 0.001$) during the stepwise accumulation of the coronary risk factors in addition to AF. In conclusion coronary risk factors synergistically impair the erythrocyte deformability, which may play an important role in critically stenotic coronary arteries. Since the impairment of intact erythrocyte deformability is mostly associated with nonvalvular AF, this common arrhythmia may reflect the coronary risk accumulation.

Introduction

Hemorheology in association with coronary atherosclerotic plaque formation has drawing increasing attention. Much effort has been devoted to elucidate the role of activated platelets and leukocyte (scavenger macrophages, activated lymphocytes and inflammatory polymorphonuclear neutrophils) in atherosclerotic progression and prothrombotic tendencies. In contrast, the effects of coronary risk factors on the behaviors of circulating intact erythrocytes, major component of whole blood cells, have been poorly understood in actual cardiac patients. The deformability of erythrocytes that pass through the microvascular network is an essential factor to maintain the physiological fluent microcirculation. Physiological and hematological values of erythrocyte deformability are equivalent to those of platelet aggregating and leukocyte migratory functions. However, the concept of erythrocyte deformability has not been strictly defined as a physical quantity, and the evaluation of deformability depends on the measurement technique and its relative sensitivity.[1] Erythrocytes deformability is supposed to be impaired in patients with coronary artery disease (CAD) by mechanical stress depending on plaque morphology such as irregular surface, calcified plaque edge and sharp plaque shoulder. This deformability is also impaired by shear stress of high blood flow velocity observed in stenotic coronary arteries. Nevertheless, erythrocytes deformability is a fundamental prerequisite of coronary microcirculation maintaining patency of critically stenotic coronary arteries or collateral circulation.[2] Therefore, the aim of this study is to investigate the homerheologic effects of coronary risk factors on the intact erythrocyte deformability, using our highly sensitive and reproducible filtration technique. In this setting, erythrocyte

Key Words:
Atrial fibrillation, Coronary risk factors, Deformability, Erythrocytes

Disclosures:
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deformability is considered as filterability of erythrocyte suspension. As mMicropipette technique, a representative hemorheologic methods other than filtration technique, estimates single erythrocyte membrane rigidity, one of the components regulating whole cell deformability.[3] Therefore, erythrocyte suspension filterability is considered as whole cell deformabilty.

Methods

Study Population

This study was approved by the internal ethics committee of the Institute of Rheological Function of Foods Co. Ltd. (Fukuoka, Japan) and was performed in accordance with the Declaration of Helsinki, i.e., signed informed consent was obtained from each subject prior to enrollment into the study. The study population consisted of 320 Japanese patients (209 males and 111 females) with common cardiac and/or systemic diseases. All patients were managed monthly at least over 1.5 years and treated at the discretion of attending physicians in the outpatients section of the Kyushu University Hospital (Fukuoka, Japan) or in several teaching hospitals and community clinics affiliated to the University Hospital. Hypertension was defined as casual blood pressure > 140/90 mmHg or treatment with antihypertensive drugs. Type 2 diabetes mellitus was defined as fasting serum glucose > 126 mg/dl, casual serum glucose > 200 mg/dl, HbA1c(NGSP) > 6.5% and/or current antidiabetic medication.4 Patients with type 1 diabetes mellitus were not enrolled. Dyslipidemia was defined as serum LDL cholesterol > 140 mg/dl, serum HDL cholesterol < 40 mg/dl or prescription of lipid-lowering agents.[4] Coronary artery disease (CAD) included old myocardial infarction and stable angina pectoris irrespective of conservative, endovascular or surgical treatment. Activity of CAD was stable in these patients and those with acute coronary syndrome (ACS) were excluded. Chronic kidney disease (CKD) covers all the stages of impaired renal function, indicating estimated glomerular filtration rate (eGFR) ≤ 60 ml/min/1.73m2.[5] Patients with atrial fibrillation (AF) due to evident rheumatic mitral valve diseases, episode of mitral valve replacement or valvuloplasty were excluded. Therefore, AF was nonvalvular. Paroxysmal AF was defined according to the HRS/EHRA/ECAS 2012 Consensus Statement on Catheter and Surgical Ablation of AF.[6] In this Consensus Statement, the concept of permanent AF was considered inappropriate in that patient and physician ceased further attempts to restore and maintain sinus rhythm, whenever this joint decision is made. However, the term of permanent AF, defined as longstanding AF impossible to be terminated by any means, was used in this study in contrast to paroxysmal AF. AF outpatients (n = 33) were followed every month for anticoagulation, i.e., 20 patients showed permanent AF whereas 13 patients had paroxysmal AF. In AF patients treated with warfarin, time in therapeutic range (TTR) of the international normalized ratio of prothrombin time (PT-INR) ranged from 51 to 72% (65.6 ± 0.9%). Dabigatran (n = 7) and rivaroxaban (n = 5) were also prescribed newly or converted from warfarin. Transthoracic echocardiography and Holter monitoring were conducted in all AF patients. Treatment of the aforementioned coronary risk factors was under the discretion of the treating physicians in outpatient clinics. Medication and lifestyle including smoking were not altered in any patient during the study period.

Erythrocyte Filterability

Erythrocyte suspensions were prepared as described elsewhere.[7] In brief, venous blood (approximately 10 ml) was sampled in the morning after an overnight fast. Blood cell counting and hematocrit measurement were performed using a hemocytometer (Ace Counter, FLC-240A, Fukuda Denshi Co. Ltd., Tokyo, Japan). After centrifugation at 1300 x g for 10 min, supernatant was aspirated to replace buffy coat and plasma with HEPES-buffered saline. Intact erythrocytes were then washed three times (800 x g, 600 x g and 500 x g for 10 min each) by re-suspension with HEPES-buffered saline and the final hematocrit of erythrocyte suspension was adjusted to 3.0% to prevent erythrocytes sticking within the filter pores and to reuse the specific filter. These procedures were performed within 2 hours after blood sampling for subsequent filtration experiments.[7]

Erythrocyte filterability (whole cell deformability) was investigated blindly by filtration technique (Model NOBU-II, Tsukasa Sokken Co. Ltd., Tokyo, Japan) as reported elsewhere.[8,9] Nickel mesh filters were produced by a photo-fabrication technique (Dainippon Printing Co. Ltd., Tokyo, Japan) and characterized by regularly distributed pores of identical size and shape (Fig. 1A), which guarantees the sensitivity of filtration experiments even in the erythrocyte suspension with low (3.0%) hematocrit value. A specific filter with appropriate pore size (4.94 μm in diameter) was used repetitively by sonication during a series of experiments to guarantee the reproducibility. The relationship between hydrostatic pressure (P; mmH2O) and time (t; sec) was obtained during continuous filtration under gravity using a pressure transducer. P was transformed to the height of the meniscus in the vertical tube (h; mm) and flow rate (Q; ml/min) was calculated by the rate of fall of the meniscus (Δh/Δt) and internal cross-sectional area of the vertical tube. P-Q relationship was obtained automatically by software installed on a personal computer (DELL Latitude CS, Dell Inc., Round Rock, TX, USA) and stored simultaneously on Microsoft Office Excel 2003 on Windows XP (Microsoft, Tokyo, Japan). The percentage of Q of erythrocyte suspension relative to Q of saline at 100 mmH2O was used as an index of erythrocyte filterability. Temperature of the specimens was kept at 25°C by circulating isothermal water within a water jacket around the

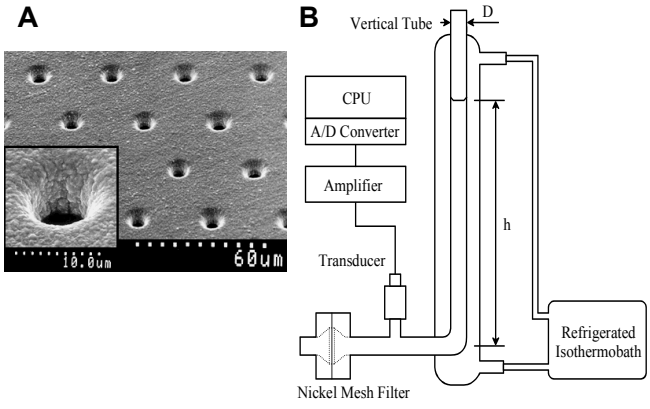

Figure 1: **A: Scanning electron microscopic photograph of a nickel mesh filter. Inset shows magnification of a single pore in this filter. B: Schematic illustration of nickel mesh filtration system. Filtration pressure (P; mmH2O) is converted to the height of the meniscus within the vertical tube (h; mm) using the equation h = P/ρg. Flow rate (Q; ml/min) is calculated using the equation Q = π·(D/2)2·(Δh/Δt). D, internal diameter of vertical tube; Δh/Δt, first time derivatives of h; g, acceleration of gravity; ρ, specific gravity of specimens.**

vertical tube (Fig. 1B). These examinations were performed at room temperature (22 ± 2°C).

An aliquot of the erythrocyte suspension was fixed with isotonic 1.0% glutaraldehyde solution containing 24.5 mM NaCl and 50 mM phosphate buffer (pH 7.4). Thereafter, the shape of erythrocytes was observed blindly using a differential interference contrast microscope (Diaphoto 300, Nikon Co. Ltd., Tokyo, Japan) at 400 x magnification.

Data Analyses

All data are expressed as means ± SEM. For statistical analyses, human sample size was chosen to provide 90% power with an α error of 0.05. A total of ≥ 310 cases was required provided that the significant intergroup difference of human erythrocyte filterability investigated by this technique is 1.0%.[9] Continuous variables were compared with unpaired Student's t test or Mann-Whitney U test. Erythrocyte filterability (%) was compared by the latter, because Kolmogorov-Smirnov test confirmed that this was not normally distributed ($p < 0.001$). Discrete variables were analyzed by Fisher's exact test or Pearson's $\chi 2$ test. Linear regression was fitted by the standard least square method. Significant contributors to erythrocyte filterability impairment were determined by stepwise multiple regression analysis. None of the variables with missing data qualified. The criteria for entering into the regression model were significant partial correlation coefficient (r) to the filterability, greatest r among the same category even if it was not significant, or otherwise clinically meaningful variables. These analyses were performed using Predictive Analytics Software (PASW) 18.0 version for Windows (SPSS, Inc., IBM, Chicago, IL, USA). Differences with two-sided $p < 0.05$ were considered significant.

Results

Patients' Profile

Baseline characteristics of enrolled subjects are detailed in Table 1. Averages of some listed variables had already reached the upper limit of normal range (systolic blood pressure) or even exceeded the ranges (HbA1c, fasting serum glucose, triglyceride). None of these patients had experienced an episode of evident heart failure (NYHA class ≥III). In patients with CAD, 25 patients had a history of old myocardial infarction and the remaining 23 patients showed stable angina pectoris. Percutaneous coronary intervention (PCI) was performed in 38 patients, and coronary artery bypass grafting surgery (CABG) was conducted in 21 patients. Medication included Ca antagonists (n = 46, 14%), statins (n = 34, 11%), angiotensin receptor blockers (n = 28, 9%), antiplatelet agents (n = 27, 8%), β-blockers (n = 18, 6%), angiotensin converting enzyme inhibitors (n = 16, 5%) and do on.

Table 1: Baseline Characteristics of Subjects (N = 320)

Variables	Means ± SEM	Variables	Means ± SEM
Age (years)	61.5 ± 0.7	LDL cholesterol (mg/dl)	120 ± 2
Body weight (kg)	62.6 ± 0.7	Triglyceride (mg/dl)	160 ± 8
Fat (%)	27.2 ± 0.5	WBC (x 102/μl)	60.2 ± 1.1
BMI (kg/m2)	24.2 ± 0.2	RBC (x 104/μl)	442 ± 3
SBP (mmHg)	140 ± 1	Hb (g/dl)	14.1 ± 0.1
DBP (mmHg)	80 ± 1	Ht (%)	40.9 ± 0.3
HbA1c (%)	7.0 ± 0.1	MCH (pg)	31.9 ± 0.1
FSG (mg/dl)	149 ± 3	MCV (fl)	92.7 ± 0.3
Total Cholesterol (mg/dl)	208 ± 2	MCHC (g/dl)	34.4 ± 0.1
HDL cholesterol (mg/dl)	56 ± 1	Erythrocyte filterability (%)	87.6 ± 0.2

BMI, body mass index; DBP, diastolic blood pressure; FSG, fasting serum glucose; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; SBP, systolic blood pressure.

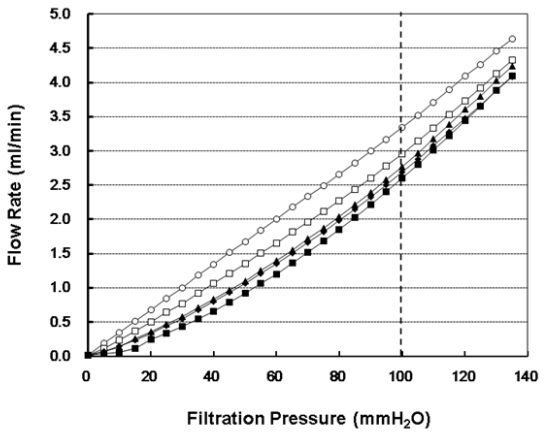

Figure 2A: A: Representative P-Q relationships during continuous filtration experiment using erythrocyte suspensions and HEPES-buffered saline. The P-Q relationships with open symbols correspond to filtrations of saline (open circles) or erythrocyte suspension obtained from a healthy non-smoker without coronary risk factors (open squares). Closed symbols indicate representative patients with diabetes (closed triangles), dyslipidemia (closed diamonds) and permanent atrial fibrillation (closed squares). Q in patients with these diseases (closed symbols) is less than Q in a subject without them (open squares) at any given P. B: Magnification of the P-Q curves at filtration pressure around 100 mmH2O.

Representative Filtration Data

Hematologically, there were no remarkable erythrocytes shape changes observed in the enrolled subjects. Figure 2 shows representative results of the nickel mesh filtration experiments. The continuous filtration process yielded P-Q relationships for control

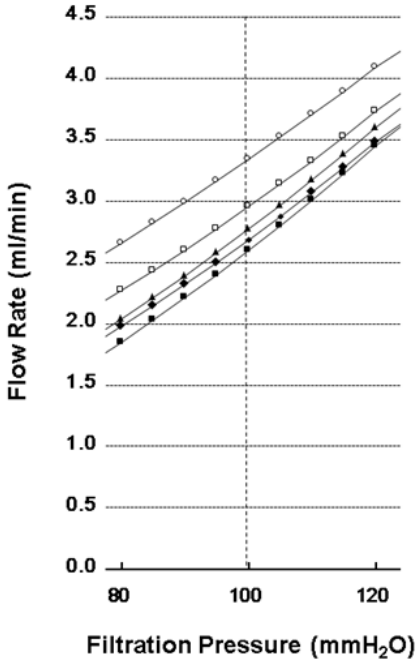

Figure 2B: B: Magnification of the P-Q curves at filtration pressure around 100 mmH2O.

Table 2: Correlation of Categorical Factors and Erythrocyte Filterability

Factors		n	Erythrocyte filterability (%)	p
Gender	male	209	87.4 ± 0.2	
	female	111	88.0 ± 0.3	0.072
Current Smoking	(−)	201	87.9 ± 0.2	
	(+)	119	87.2 ± 0.3	0.059
Hypertension	(−)	213	87.6 ± 0.2	
	(+)	107	87.8 ± 0.3	0.867
Dyslipidemia	(−)	217	87.6 ± 0.2	
	(+)	103	87.7 ± 0.3	0.841
Atrial fibrillation	(−)	287	87.8 ± 0.2	
	(+)	33	86.3 ± 0.9	0.308
Diabetes mellitus	(−)	100	88.1 ± 0.2	
	(+)	220	87.4 ± 0.2	0.200
CKD	(−)	184	87.9 ± 0.2	
	(+)	136	87.2 ± 0.3	0.041
CAD	(−)	272	88.2 ± 0.2	
	(+)	48	87.5 ± 0.3	0.407

Erythrocyte filterability is expressed as mean ± SEM. CAD, coronary artery disease; CKD, chronic kidney disease;
n, number of subjects in a specific group; p, probability.

saline and erythrocyte suspensions. Reproducibility of the filtration experiment was confirmed in that P-Q curves obtained by filtration of the same specimen were superimposable as in our recent study.[8, 9] Saline demonstrated a linear P-Q relationship passing through the origin, which is compatible with Newtonian fluid. P-Q relationships for the erythrocyte suspensions displayed smooth and upward concave curves over the low-pressure region. These findings indicate that erythrocyte suspension shows non-Newtonian behavior, which is evident under a low-shear-rate condition. Open squares correspond to erythrocyte suspensions obtained from subjects without any coronary risk factors. Closed symbols indicate those obtained from patients with at least one risk factor. Q of erythrocyte suspensions obtained from patients with these risk factors were always less than Q in patients without them at any given pressure. These findings indicate that these coronary risk factors impair the human erythrocyte filterability.

Table 3: Correlation of Continuous Variables and Erythrocyte Filterability

	(N = 320)	
Variables	r	p
Age (years)	0.041	0.460
Body weight (kg)	−0.130	0.020
BMI (kg/m2)	−0.094	0.095
Fat (%)	−0.074	0.293
SBP (mmHg)	−0.033	0.557
DBP (mmHg)	−0.027	0.639
HbA1c (%)	−0.137	0.014
Total cholesterol (mg/dl)	−0.062	0.271
HDL cholesterol (mg/dl)	0.100	0.076
LDL cholesterol (mg/dl)	−0.101	0.073
Triglyceride (mg/dl)	−0.159	0.004
MCH (pg)	−0.014	0.798
MCV (fl)	−0.018	0.742
MCHC (g/dl)	0.003	0.954

,p, probability; r, partial correlation coefficient. Other abbreviations are the same as in Table 1.

Erythrocyte Filterability and Coronary Risk Factors

Relationships between categorical factors and erythrocyte filterability are shown in Table 2. CKD alone showed significant impairment of erythrocyte filterability (p = 0.041). The filterability in AF patients did not differ from that in the remaining subjects, and the filterability in permanent AF was equivalent to that in paroxysmal AF. Correlations of continuous variables and erythrocyte filterability are demonstrated in Table 3. Body weight, HbA1c and triglyceride showed significant inverse correlation, whereas hematological parameters relating to erythrocyte size (mean corpuscular volume; MCV) and internal density (mean corpuscular hemoglobin concentration; MCHC) did not show any correlation with filterability.

Prior to the multiple regression analysis, demographic variables suitable for inclusion into the regression model were selected. For categorical variables, CKD was selected for significance (p = 0.041), current smoking was included as a potent risk of hemorheology,10 and AF was selected as an outcome of overall risk accumulation.6 For continuous variables, body mass index (BMI, p = 0.095) instead of body weight (p = 0.020) was selected as a surrogate of obesity. HbA1c (p = 0.014) and triglyceride (p = 0.004) were selected for respective significance. Although hematological indices were not significant, MCV was selected for its greatest r.

Multiple regression analysis was performed to identify significant covariates contributing to the impairment of erythrocyte filterability with concurrent avoidance of multi-colinearity by monitoring the variance inflation factors. Table 4 shows five regression models including selected continuous or categorical variables according to stepwise reduction. Multiple correlation coefficients were highly significant in this series of regression models. AF was the greatest contributor to the impaired erythrocyte filterability in all the listed models with high significance (p = 0.002 - 0.023).

Erythrocyte filterability of AF patients did not differ from that of the remaining patients (Table 2). However, AF remained alone as the greatest contributor to the impaired erythrocyte filterability (Table 4). Table 5 shows the distribution of AF in patients with coronary risk factors. AF was prevalent significantly (p < 0.001) in hypertensive and dyslipidemic patients. Table 6 further indicates the effects of coronary risk accumulation on the erythrocyte filterability. Multiple comparison of the filterability under the stepwise accumulation of coronary risk factors showed highly significance (p < 0.001), i.e., the erythrocyte filterability was impaired synergistically during the accumulation of coronary risk factors in addition to AF. However, the filterability was not different with respect to the types of CAD (old myocardial infarction vs. stable angina), modality of treatment (PCI vs. CABG), and choice of medication (not shown).

Discussion

Main Findings

The erythrocyte deformability has significant impact on the apparent blood viscosity, which has profound influence on the coronary blood flow,2 development of myocardial infarction and the resultant infarct size.[11] However, the involvement of abnormal erythrocyte behaviors

Table 4: Stepwise Multiple Regression Analysis Predicting Contributors to Erythrocyte Filterability (N = 320)

model	F	R	P	Covariates (p)
1	2.650	0.259	0.008	Atrial fibrillation (0.023), Triglyceride (0.025), HbA1c (0.040), BMI (0.295) Current smoking (0.346), MCV (0.367), SBP (0.538), CKD (0.848)
2	3.034	0.259	0.004	Atrial fibrillation (0.023), Triglyceride (0.024), HbA1c (0.036), BMI (0.298) Current smoking (0.346), MCV (0.366), SBP (0.557)
3	4.077	0.273	0.001	Atrial fibrillation (0.004), Triglyceride (0.022), HbA1c (0.061), BMI (0.258) Current smoking (0.224), MCV (0.389)
4	4.748	0.269	< 0.001	Atrial fibrillation (0.003), Triglyceride (0.019), HbA1c (0.088), BMI (0.326) Current smoking (0.177)
5	5.835	0.265	< 0.001	Atrial fibrillation (0.002), Triglyceride (0.010), HbA1c (0.070), Current smoking (0.196)

F, F-value for fitness of multiple linear regression; P, probability for trend toward fitting the regression; p, probability of significant covariate contribution; R, multiple correlation coefficient. Other abbreviations are the same as in Table 1.

on the progression of CAD is not fully elucidated. In the present study, a highly sensitive and reproducible filtration technique was applied to the actual cardiac outpatients to assess the circulating intact erythrocyte filterability (whole cell deformability) in relation to coronary risk factors. Univariate analyses found significant impact of triglyceride (p = 0.004), HbA1c (p = 0.014), body weight (p = 0.020), and CKD (p = 0.041) on the impairment of the erythrocyte deformability, but multiple regression analysis demonstrated that nonvalvular AF was the greatest contributor to the impaired deformability, indicating that coronary risk factors including AF impair the erythrocyte filterability synergistically.

Erythrocyte Deformability and Coronary Risk Factors

It is generally accepted that erythrocyte deformability is determined by 1) erythrocyte membrane material properties; 2) internal density as reflected by MCHC; and 3) cellular geometric factors as reflected by MCV, surface-to-volume ratio and erythrocyte shape.[1] Therefore, abnormal erythrocyte membrane properties, increased MCHC or MCV, and several kinds of shape changes impair the deformability individually or in concert. There were no discernible erythrocyte shape changes in this study population. There were contributions of AF, triglyceride and HbA1c, but not of MCV or MCHC, to the deformability (Tables 3, 4). These findings indicate that impaired deformability mainly arises from erythrocyte membrane properties which may be altered by coronary risk factors such as hypertension, dyslipidemia, diabetes in addition to smoking.[10]

Erythrocyte membrane lipid components have profound influence on membrane fluidity and hemorheologic functions.[1] The present study demonstrated the association of impaired deformability with elevated serum triglyceride as in our previous study,[7] suggesting altered erythrocyte membrane integrity and lipid composition in hypertriglyceridemia.[12] Reportedly, our recent study clarified that diabetic erythrocytes show the impaired filterability and this impairment is enhanced by other risk factor such as smoking causing potent oxidative stress.[9,10] In this study, HbA1c is inversely proportional to the filterability (Table 3) and a marginal contributor to the impaired filterability (Table 4). Tsuda[13] demonstrated the increased erythrocyte membrane rigidity in hypertensive patients by electron spin resonance study. Therefore, the main cause of the impaired erythrocyte filterability is attributed to the erythrocyte membrane abnormalities in diabetic, hypertensive or dyslipidemic patients. Previous studies using this filtration technique (Fig. 1) clarified that deformability is markedly impaired in human erythrocytes exposed to acute oxidant stress.[14-16] Relative to such severe oxidant stress in vitro, mild oxidant stress in vivo as in above common diseases also causes persistent erythrocyte membrane lipid peroxidation and disturbs deformability.[9,17]

Erythrocyte Deformability in AF

Development of nonvalvular AF is known to be accelerated by risk factors common to those of CAD, because $CHADS_2$ and CHA_2DS_2-VASc scores predict not only the risk of ischemic stroke but also the new onset of AF.[18] Actually, more than half of enrolled patients are diabetic (69%), and one third of them are current smokers (37%) in the present study (Table 2). Moreover, major coexisting diseases are CKD (43%), hypertension (33%), dyslipidemia (32%) and CAD (15%). In this meaning, patients' profile of this study is similar to the Fushimi AF Registry, which is a Japanese domestic survey of AF patients conducted in urban community.[19]

AF is a common arrhythmia in clinical practice, and characterized by abnormal hemorheology in left atrium (LA) such as elevated hematocrit value and fibrinogen concentration.[20, 21] Stagnant blood within the enlarged fibrillating LA appendage shows procoagulative state under erythrocyte hyperaggregability and elevated platelet reactivity.[22,23] Activated platelets release prostaglandin E2, which enhances calcium entry into erythrocytes leading to the impairment of erythrocyte deformability.[24,25] AF is associated with oxidant stress via increased LA wall superoxide production or angiotensin II type 1 receptor-mediated pathway.[26,27] Such local LA environment oxidizes erythrocytes and impairs the deformability.[15,16] Erythrocyte dynamics are improved by medication such as statin,[28] aspirin,[29] Ca antagonists[15,25] and eicosapentaenoic acid.[30] These agents were prescribed to AF patients associated with CAD for coronary risk reduction and rate control of AF. Such therapeutic effects may have attenuated the impairment of the erythrocyte deformability in AF patients in this study.

It is questionable whether AF is an independent risk of ACS.[31] Reportedly, total cholesterol content of erythrocyte membrane is increased in the patients with ACS,[32] which is ameliorated by statin therapy.[33,34] Increased cholesterol content reduces the erythrocyte membrane fluidity and impairs the deformability leading to erythrocytes aggregation and coronary flow slowing.[35,36] This study excluded patients with ACS, and the answer for this question awaits future cohort investigating erythrocyte deformability in ACS patients with AF and that in ACS patients without AF.

Limitations

This study has several limitations. First limitation is small sample of patients (n = 320) treated at the discretion of attending physicians. Nonvalvular AF with few episodes of paroxysms may

Table 5: Cross Table of Atrial Fibrillation and Coronary Risk Factors

	Hypertension (+)	Dyslipidemia (+)	Diabetes mellitus (+)	Current Smoking (+)
Atrial fibrillation (+) n = 33	24	20	18	16
Atrial fibrillation (–) n = 287	83	83	202	103
N = 320	n = 107	n = 103	n = 220	n = 119
p	< 0.001	< 0.001	0.063	0.156

n, number of subjects in a specific group; N, total number of subjects

Table 6: Effects of Coronary Risk Accumulation on Erythrocyte Filterability

Coronary Risk Factors	Erythrocyte Filterability (%)
no risk factor	88.8 ± 0.3
Hypertension	87.8 ± 0.3
Hypertension + Dyslipidemia	87.4 ± 0.4
Hypertension + Dyslipidemia + Diabetes mellitus	86.9 ± 0.7
Hypertension + Dyslipidemia + Diabetes mellitus + Smoking	86.3 ± 0.9
Hypertension + Dyslipidemia + Diabetes mellitus + Smoking + Atrial fibrillation	86.1 ± 1.0

Multiple comparison of the erythrocyte filterability during the stepwise accumulation of coronary risk factors showed highly significance ($p < 0.001$).

have been dismissed in this study population. Second limitation is a cross-sectional nature of study design. Coronary risk factors were considerably accumulated in the AF patients group in that average CHA_2DS_2-VASc score was 3.30 ± 0.24. However, unlike in the other study,[37] impaired erythrocyte deformability in AF patients could not be correlated to the ischemic stroke in this study partly due to intensive anticoagulation. Finally, erythrocyte filterability shows small intergroup differences and intragroup deviations (Tables 2 and 6). This is due in part to the aforementioned medication, but in other part due to high sensitivity and reproducibility of our methodology. Filtertability of erythrocytes obtained from healthy volunteers (positive control) was 88.8 ± 0.3% (Table 6), and that of erythrocytes exposed to severe oxidant stress was declined to 20% (negative control) leading to filter occlusion or hemolysis.[15] Therefore, our data presented in this study reflects mildly pathological human erythrocyte behaviors leading to impaired but not occluded microcirculation in vivo under treatments.

Conclusions:

This study investigated the intact human erythrocyte deformability in relation to coronary risk factors of actual cardiac patients. Multiple regression analysis showed that nonvalvular AF is unexpectedly and mostly attributable to the impaired erythrocyte deformability among other coronary risk factors, reflecting nonvalvular AF as an outcome of multiple coronary risk accumulations. Therefore, this small sample study should be validated by a future cohort to validate the important hemorheorologic role of erythrocytes playing in prothrombotic state leading to ischemic stroke associated with AF.

Acknowledgments

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