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Original Article

Cystatin C−Adiponectin Complex in Plasma Associates with Coronary Plaque Instability

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***Aim*: Adiponectin (APN) is an adipocyte-derived bioactive molecule with antiatherogenic properties. We previously reported that cystatin C (CysC) abolished the anti-atherogenic effects of APN. We aimed to elucidate the clinical significance of CysC−APN complex in patients with coronary artery disease (CAD).**

***Methods*: We enrolled 43 stable CAD male patients to examine the relationship between CysC−APN complex and coronary plaque characteristics. Serum was immunoprecipitated by the anti-APN antibody and immunoblotted by the anti-CysC antibody to demonstrate the presence of CysC−APN complexes *in vivo*. To confirm their binding *in vitro*, HEK293T cell lysates overexpressing myc-APN and FLAG-CysC were immunoprecipitated with an anti-myc or anti-FLAG antibody, followed by immunoblotting with an anti-APN or anti-CysC antibody.**

***Results*: CysC was identified as a specific co-immunoprecipitant with APN by the anti-APN antibody in human serum. *In vitro*, FLAG-CysC was co-immunoprecipitated with myc-APN by the anti-myc antibody and myc-APN was co-immunoprecipitated with FLAG-CysC by the anti-FLAG antibody. Among CAD patients, serum CysC−APN complex levels negatively correlated with fibrotic components of coronary plaques and positively correlated with either necrotic or lipidic plus necrotic components. Plaque burden negatively correlated with serum APN levels but not serum CysC−APN complex levels. Serum CysC levels had no association with plaque characteristics. In multivariate analysis, CysC−APN complex levels were identified as the strongest negative factor for fibrotic components and the strongest positive factor for both necrotic and lipidic plus necrotic components.**

***Conclusion*: Measuring serum CysC−APN complex levels is helpful for evaluating coronary plaque instability in CAD patients.**

Key words: Adiponectin, Cystatin C, Atherosclerosis, Plaque, Coronary artery disease

Introduction

Coronary artery disease (CAD) is one of the leading causes of death in many countries. The global status report on noncommunicable diseases 2014 demonstrated that 17.5 million people died of CAD in 2012 and that the number of deaths due to CAD is predicted to increase to 22.2 million in 2030[1]. Although a reduction in blood pressure and serum low-density lipoprotein-cholesterol (LDL-C) and glucose levels improves the prognosis of cardiovascular diseases[2-4], patients with well-controlled these parameters still have a high risk of cardiovascular events. Acute coronary syndrome (ACS) is induced by the rupture of an atherosclerotic plaque and subsequent luminal thrombosis[5], and a vulnerable plaque is characterized by its large necrotic core and fibrous cap thinning[6, 7]. Therefore, the accurate evaluation of a coronary plaque is important for identifying high-risk patients having a vulnerable plaque.

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Intravascular ultrasound (IVUS) is a useful modality for intracoronary imaging[8], and the iMap®-IVUS system (Boston Scientific, Marlborough, MA) is a developed version using a 40 MHz radiofrequency. Previous studies have validated that iMap®-IVUS can accurately evaluate lesion components both *in vitro* and *in vivo*[9]. This system classifies coronary plaque composition into four subtypes, fibrotic, lipidic, necrotic, and calcified, and evaluates plaque vulnerability. However, the detection of a vulnerable plaque using IVUS is invasive. A reliable and simple screening method to select candidates whose coronary arteries should be examined in detail with IVUS is urgently needed.

Visceral fat obesity is a common feature in CAD patients. We found adiponectin (APN), the most abundant adipocyte-derived secretory protein from human visceral fat tissues, and plasma APN levels negatively correlate with human visceral fat mass[10]. High plasma levels of APN are associated with insulin sensitivity in the healthy population[11], and hypoadiponectinemia is an independent risk factor for diabetes, hypertension, and CAD[12-14]. Many clinical and experimental studies have revealed that APN has beneficial effects on the cardiovascular system[15, 16] and that low plasma APN levels are associated with the presence of a vulnerable plaque in stable CAD male patients[17]. However, the mechanisms by which APN affects the incidence of cardiovascular events in stable CAD patients are still unknown.

We have demonstrated that proteins interacting with APN (e.g., platelet-derived growth factor-BB, calreticulin, cystatin C (CysC), E-selectin ligand-1, and Mac-2 binding protein) modulate APN-mediated vasculoprotective effects[18-22]. CysC is a cysteine protease inhibitor that is constantly produced by human cells in general and excreted into the bloodstream[23]. Serum CysC levels are a well-known marker of renal function and are suggested to be superior to serum creatinine levels[24, 25], and a combination of CysC and creatinine is more accurate than each separately[26, 27]. Elevated serum CysC levels indicate an increasing risk for cardiovascular events in subjects regardless of renal dysfunction[28, 29]. We have previously reported that CysC reduces the clearance of plasma APN, leading to the inhibition of APN-mediated vasculoprotective effects[19], and a recent report has demonstrated that serum CysC levels might be linked to carotid plaque size and instability[30]. However, the clinical significance of the CysC−APN complex on plaque vulnerability is still unknown.

Aim

We aimed to demonstrate the CysC−APN interaction thoroughly both *in vivo* and *in vitro* and to clarify its clinical significance on coronary plaque instability in stable CAD patients with relatively normal renal function.

Methods

Immunoprecipitation and Immunoblotting

Transfection, immunoprecipitation, and immunoblotting were performed as described previously[20, 22]. Briefly, 100 μg mouse anti-human APN monoclonal antibody (ANOC9121; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) or mouse control immunoglobulin G (IgG) was coupled to 5 mg tosylactivated Dynabeads® (M-280; Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. After washing, approximately 0.4 mg of these magnetic beads was used for immunoprecipitation of human serum containing 200 ng APN. For *in vitro* analysis, expression vectors of myc-APN and FLAG-CysC[31] (kindly provided by Dr. John Hulleman) were co-transfected into HEK293T cells with Lipofectamine 2000 (Life Technologies, Carlsbad, CA), according to the manufacture's protocol. The cell lysates were used for immunoprecipitation with anti-c-myc magnetic beads (Thermo Fisher Scientific, Waltham, MA) or anti-FLAG M2 magnetic beads (Sigma-Aldrich, St. Louis, MO). After gel electrophoresis and transfer to the nitrocellulose membranes (BioRad, Hercules, CA), the membranes were sequentially incubated with a biotinylated mouse anti-human APN antibody (100 ng/mL for *in vivo* or 200 ng/mL for *in vitro* analysis) and an HRP-conjugated streptavidin (Thermo Fisher Scientific, Waltham, MA), or with an anti-human CysC monoclonal antibody (Abcam, Cambridge, UK) and an HRP-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). The secondary antibody was detected by enhanced chemiluminescence systems (Thermo Fisher Scientific, Waltham, MA). The CysC band intensity measured with Image J (National Institutes of Health, Bethesda, MD) was used to identify CysC−APN complex levels.

Subjects

The enrolled subjects in this study were 43 male patients with stable angina who underwent selective percutaneous coronary intervention and IVUS at the Department of Cardiology in Hyogo Prefectural Nishinomiya Hospital, Japan. Patients who were aged >85 years, had renal dysfunction (serum creatinine >1.5 mg/dL) or malignant diseases, or whose target lesions were chronic total occlusion or in-stent restenosis were excluded from this study. This study was conducted according to the Declaration of Helsinki, and was approved by the ethics committees of both Hyogo

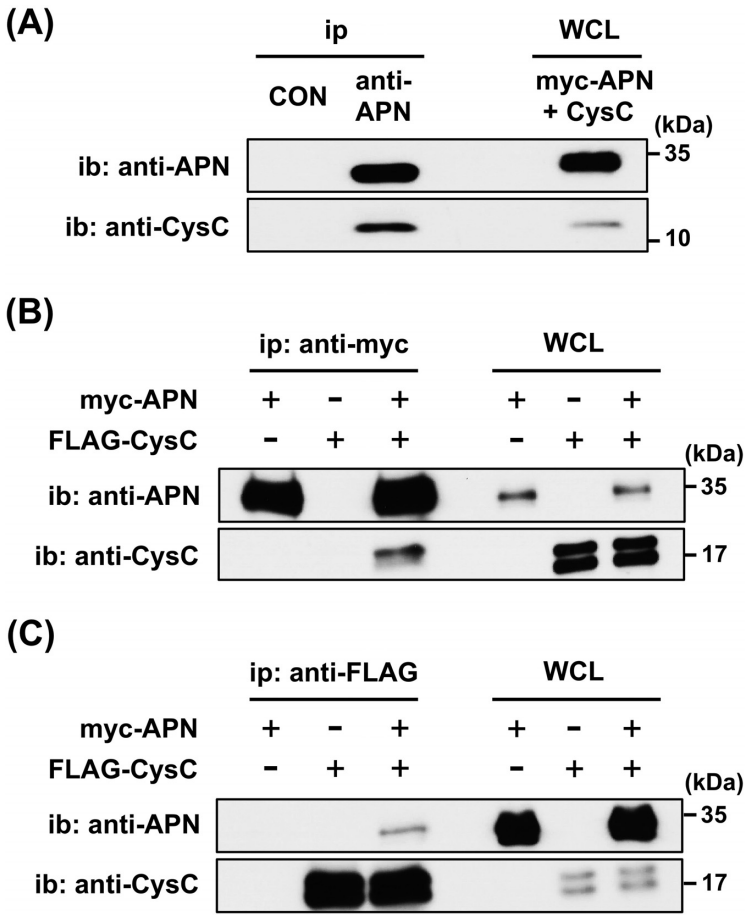

Fig. 1. CysC−APN complex in serum and *in vitro*.

(A) Human serum was immunoprecipitated by the anti-APN antibody, followed by immunoblotting with either the anti-APN or anti-CysC antibody. Whole cell lysate (WCL) of HEK293T cells overexpressing both myc-tagged APN and CysC was used as a positive control. Experiments were repeated more than five times, and representative data are shown.

(B and C) Cell lysate expressing myc-APN and FLAG-CysC was used for the immunoprecipitation and immunoblotting. FLAG-CysC was co-immunoprecipitated with myc-APN by the anti-myc antibody (B), and myc-APN was co-immunoprecipitated with FLAG-CysC by the anti-FLAG antibody (C).

CON: control IgG, WCL: whole cell lysate, ip: immunoprecipitation, ib: immunoblotting. Experiments were repeated more than three times, and representative data are shown.

Prefectural Nishinomiya Hospital and Osaka University Graduate School of Medicine. Written informed consent was obtained from each patient.

Measurement of Serum Parameters

Fasting serum biochemical markers were measured in commercial chemical laboratories. APN levels were measured by using a total APN enzyme-linked immunosorbent assay (ELISA) kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions.

Evaluation of Plaques in Coronary Arteries by iMap®-IVUS

IVUS was performed with a 40 MHz catheter (Atlantis™ SR Pro Coronary Imaging Catheter, Boston Scientific, Marlborough, MA) and the evaluation of the plaque characteristics were performed as described previously[32]. The plaques were classified into four types according to the tissue components, and the percentage of each component in the entire plaque was calculated as "components volume (mm^3)/plaque volume (mm^3) × 100" and named as fibrotic, lipidic, necrotic, or calcified components, respectively.

Table 1. Clinical characteristics of the enrolled patients

	mean ± SD	range
Age (year)	69.3 ± 9.5	37-83
BMI (kg/m^2)	25.1 ± 3.8	19.9-34.4
Systolic blood pressure (mmHg)	133 ± 18	92-170
Diastolic blood pressure (mmHg)	78 ± 10	60-103
Fasting blood sugar (mg/dL)	111 ± 26	77-202
IRI (μU/mL)	8.5 ± 5.5	1.6-23.2
HOMA-R	2.3 ± 1.6	0.42-7.5
HbA1c (NGSP) (%)	6.5 ± 0.8	5.2-8.5
Total cholesterol (mg/dL)	175 ± 35	109-267
Triglyceride (mg/dL)	127 ± 61	50-361
HDL-C (mg/dL)	42.3 ± 9.4	26-68
LDL-C (mg/dL)	108 ± 32	50-200
hsCRP (mg/dL)	0.21 ± 0.29	0.01-1.16
serum creatinine (mg/dL)	0.89 ± 0.22	0.48-1.40
eGFR (mL/min/1.73 m^2)	70.6 ± 20.9	38.2-127.9
CysC (mg/L)	1.14 ± 0.30	0.63-1.77
APN (μg/mL)	9.9 ± 5.0	3.5-28.1

Data are expressed as mean ± standard deviation (SD).
IRI: immunoreactive insulin, hsCRP: high-sensitive C-reactive protein.

Table 2. Medication of the patients

	number (%)
Statins	24 (55.8%)
Anti-platelet agents	35 (81.4%)
Anti-hypertensive agents	29 (67.4%)
Anti-diabetic agents	18 (41.9%)
Oral hypoglycemic agents (OHA) alone	12 (27.9%)
Insulin alone	4 (9.3%)
OHA + Insulin	2 (4.7%)

Table 3. Characteristics of coronary plaques

	mean ± SD	range
Plaque burden (%)	64.3 ± 5.0	55.1-78.2
Fibrotic components (%)	54.1 ± 12.7	29.2-86.6
Lipidic components (%)	10.3 ± 2.2	4.5-14.8
Necrotic components (%)	32.1 ± 11.0	8.6-55.4
Calcified components (%)	4.0 ± 3.0	0.6-11.8
Lipidic plus Necrotic components (%)	42.4 ± 12.3	13.1-67.6

Data are expressed as mean ± SD.

Statistical Analysis

Statistical analysis was performed with JMP Pro version 11.2.1 (SAS Institute Inc., Cary, NC). Spearman's correlation coefficient and multiple regression analysis were used to evaluate the association between plaque and clinical characteristics. Statistical significance was defined as $p < 0.05$.

Results

CysC Binds to APN *in vivo* and *in vitro*

To investigate the CysC−APN complex *in vivo*, human serum was used for immunoprecipitation with the anti-APN antibody or control IgG. Immunoblotting with the anti-APN or anti-CysC antibody demonstrated that CysC was specifically co-immunoprecipitated with APN (**Fig. 1A**), indicating the presence of the CysC−APN complex in the human serum.

To confirm this CysC−APN interaction *in vitro*, HEK293T cell lysates overexpressing myc-APN and FLAG-CysC were immunoprecipitated with the anti-myc antibodies, followed by immunoblot analysis using the anti-APN or anti-CysC antibody. FLAG-CysC was specifically co-immunoprecipitated with myc-APN by the anti-myc antibody (**Fig. 1B**). In addition, the same cell lysates immunoprecipitated with the anti-FLAG antibody demonstrated that myc-APN was also the specific co-immunoprecipitant with FLAG-CysC (**Fig. 1C**).

Patient Characteristics

The clinical characteristics of the 43 male CAD patients are listed in **Table 1**. All patients were diagnosed with stable angina and received medication, including statins (56%), anti-platelet agents (81%), anti-hypertensive agents (67%), and anti-diabetic agents (42%) (**Table 2**). Due to medical treatment, fasting blood sugar (FBS) levels were well controlled (HbA1c: 6.5% ± 0.8%). However, blood pressure and serum LDL-C levels were not sufficiently controlled (blood pressure: 133/78 mmHg, LDL-C: 108 ± 32 mg/dL). The average age and body mass index (BMI) were 69.3 ± 9.5 years and 25.1 ± 3.8 kg/m^2, respectively.

Table 3 shows the coronary plaque characteristics of the CAD patients evaluated by iMap®-IVUS. At the culprit lesion, the plaque burden and the percentage contribution of each component to the entire plaque were calculated. The average plaque burden was 64.3% ± 5.0%.

CysC−APN Complex is Associated with Coronary Plaque Instability

To clarify the clinical significance of the CysC−APN complex with plaque vulnerability, serum CysC−APN complex levels in CAD patients were assessed by immunoprecipitation−immunoblot analysis. The association between plaque characteristics and serum APN, CysC, or CysC−APN complex levels are shown in **Table 4**. Among them, serum CysC−APN complex

Table 4. Correlation between plaque characteristics and APN, CysC, or CysC−APN complex

	APN		CysC		CysC−APN complex	
	r	*p*	*r*	*p*	*r*	*p*
Plaque burden (%)	−0.317	0.039	−0.064	0.682	−0.020	0.899
Fibrotic components (%)	0.123	0.431	−0.002	0.990	−0.384	0.011
Lipidic components (%)	−0.114	0.467	0.140	0.370	0.171	0.273
Necrotic components (%)	−0.171	0.273	−0.064	0.682	0.406	0.007
Calcified components (%)	0.107	0.497	0.058	0.712	0.034	0.830
Lipidic plus Necrotic components (%)	−0.179	0.251	−0.002	0.990	0.417	0.005

The relationships of the parameters were investigated by Spearman's correlation coefficient.

levels negatively correlated with fibrotic components ($r=-0.384$, $p=0.011$) and positively correlated with necrotic ($r=0.406$, $p=0.007$) and lipidic plus necrotic ($r=0.417$, $p=0.005$) components (**Fig. 2A−D**). The plaque burden negatively correlated with serum APN levels ($r=-0.317$, $p=0.039$) but not with serum CysC−APN complex levels (**Fig. 2E and F**). Serum CysC levels correlated with neither the plaque burden nor any component.

Factors Affecting Plaque Burden and Components

We next performed univariate and multivariate analyses to determine the factors affecting the plaque burden and components (**Table 5**). Univariate analysis showed that the plaque burden was positively correlated with BMI ($r=0.474$, $p=0.002$) and negatively correlated with systolic blood pressure ($r=-0.313$, $p=0.041$) and APN. Fibrotic components were negatively correlated with CysC−APN complex levels and FBS ($r=-0.301$, $p=0.050$). Necrotic components and lipidic plus necrotic components positively correlated with FBS (necrotic: $r=0.352$, $p=0.021$, lipidic plus necrotic: $r=0.313$, $p=0.041$) and CysC−APN complex levels. Lipidic components were positively correlated with immunoreactive insulin ($r=0.442$, $p=0.004$) (data not shown). However, none of the lipid parameters were significantly correlated with either plaque burden or any plaque component. In the multivariate analysis, BMI was identified as the most important positive factor for plaque burden ($\beta=0.369$, $p=0.018$). CysC−APN complex levels were identified as the strongest negative factor for fibrotic components ($\beta=-0.285$, $p=0.069$) and the strongest positive factor for necrotic ($\beta=0.276$, $p=0.076$) and lipidic plus necrotic ($\beta=0.287$, $p=0.067$) components.

Discussion

In this study, we investigated CysC−APN binding both *in vivo* and *in vitro* and found that serum CysC−APN complex levels negatively correlated with stable fibrotic coronary plaque components and positively correlated with unstable necrotic or lipidic plus necrotic coronary plaque components in CAD patients.

We have demonstrated the CysC−APN interaction by ELISA using recombinant APN and human immunoglobulin-conjugated CysC[33]. We also investigated the effects of CysC on the functions of APN using mice and cultured cells[19]. In mice, CysC injections reduced the clearance rate of plasma APN, leading to elevated plasma APN levels. In cultured human umbilical vein endothelial cells, CysC eliminated the suppressive effect of APN on the adhesion molecules mRNA expression induced by tumor necrosis factor-α. In the present study, we demonstrated that the CysC−APN complex is present in human serum. We further confirmed this interaction *in vitro* by immunoprecipitation and immunoblot analysis using epitope-tagged recombinant proteins, which is more convincing than ELISA.

CysC belongs to the endogenous inhibitors of the cathepsin family proteins[23], which degrade elastin and collagen, the major matrix components of the vascular wall. Previous reports showing reduced CysC levels in human atherosclerotic lesions[34] and accelerated atherosclerosis in CysC-deficient mice[35-37] have suggested the anti-atherogenic roles of intracellular CysC. Plasma CysC levels are strongly associated with an increased risk of CAD, particularly for secondary CAD events[28, 29, 38]. Wen *et al.* have reported the relationship between serum CysC levels and the presence of a carotid plaque[30]. Therefore, serum CysC and intracellular CysC should have different functions in atherosclerotic mechanisms, i.e., serum CysC may cause a reduction in serum APN clearance and suppress the vasculoprotective effects of APN through the formation of the CysC−APN complex, which can explain the paradox that hyperadiponectinemia is associated with increased CAD risk in patients with renal impairment[39].

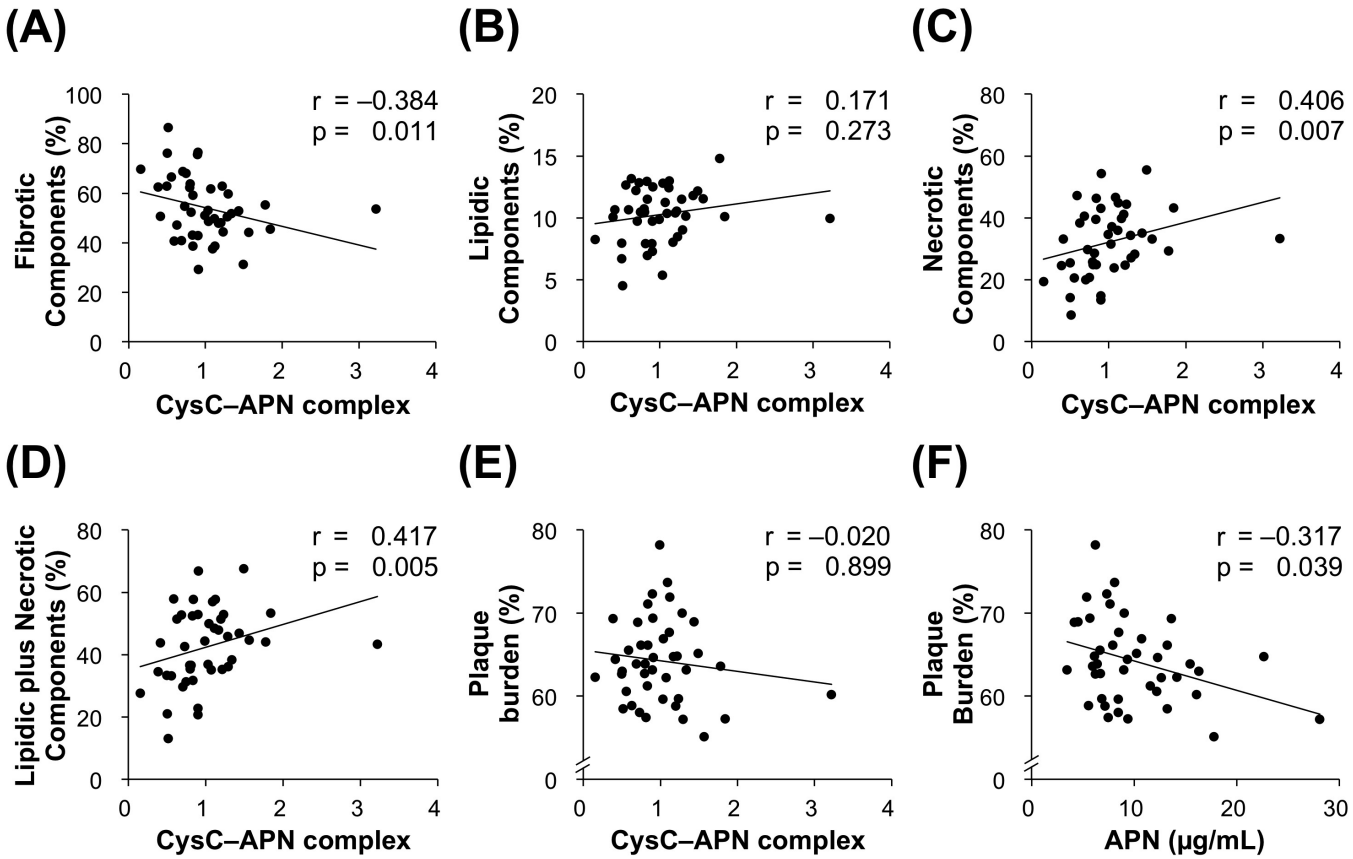

Fig. 2. CysC−APN complex levels correlate with coronary plaque instability.

(A−D) Serum CysC−APN complex levels negatively correlated with fibrotic components and positively correlated with both necrotic and lipidic plus necrotic components (n=43).
(E and F) Plaque burden was negatively correlated with serum APN levels, but not with serum CysC−APN complex levels (n=43).

In this study, we evaluated coronary plaque components using iMAP®-IVUS in male CAD patients with a relatively normal renal function. Currently, three radiofrequency signal-based IVUS (RF-IVUS) systems are available, allowing us to obtain more detailed information on plaque components. Virtual histology IVUS (VH-IVUS) was the first released RF-IVUS system, and then, integrated backscatter IVUS (IB-IVUS) and iMap®-IVUS were launched. Although these three modalities provide similar results on plaque phenotypes, there are some differences in their classifications. Yamada *et al*. reported that the "lipid pool" assessed by IB-IVUS was recognized as a "necrotic" component by iMap®-IVUS and that "fibrosis" and "calcification" evaluated by IB-IVUS correlated well with "fibrotic" and "calcified" by iMap®-IVUS, respectively[40]. It is advantageous to use iMap®-IVUS not only for identifying vulnerable plaques[41] but also for distinguishing between ACS and non-ACS patients because more lipidic and necrotic components and less fibrotic components are found in ACS[42].

Serum CysC−APN complex levels, but not serum APN or CysC levels, were significantly negatively correlated with fibrotic components and positively correlated with necrotic or lipidic plus necrotic components in this study. On the other hand, serum APN levels negatively correlated with only plaque burden, which is in agreement with our previous observation[32]. We have shown that serum APN levels are reduced in obese patients and that hypoadiponectinemia might be an independent risk factor for CAD[15]. Because BMI was the strongest factor affecting plaque burden in the multiple regression analysis (**Table 5**), hypoadiponectinemia due to obesity might accelerate the progression of a coronary plaque. Sawada *et al*. have reported that low plasma APN levels are associated with the presence of a vulnerable plaque in stable CAD male patients[17]; Otake *et al*. have demonstrated that low serum APN levels are linked to an increased necrotic core in ACS patients but not in stable angina patients[43]. Because CysC-bound APN is supposed to be a biologically inactive APN, serum CysC−APN

Table 5. Correlation between plaque characteristics and clinical parameters

	Plaque burden				Fibrotic components			
	Univariate		Multivariate		Univariate		Multivariate	
	r	*p*	*β*	*p*	*r*	*p*	*β*	*p*
Age	−0.050	0.751			−0.081	0.606		
BMI	0.474	0.002	0.369	0.018	−0.115	0.468		
Systolic blood pressure	−0.313	0.041	−0.194	0.168	−0.056	0.720		
Diastolic blood pressure	−0.072	0.645			−0.055	0.728		
Fasting blood sugar	0.029	0.854			−0.301	0.050	−0.085	0.581
IRI	0.111	0.491			−0.248	0.117		
HOMA-R	0.110	0.495			−0.261	0.100		
HbA1c (NGSP)	−0.073	0.643			−0.069	0.663		
Total cholesterol	0.005	0.976			−0.117	0.462		
Triglyceride	0.164	0.293			−0.249	0.107		
HDL-C	−0.290	0.060			0.012	0.940		
LDL-C	0.158	0.317			−0.030	0.851		
hsCRP	0.138	0.398			0.154	0.343		
serum creatinine	−0.075	0.633			0.073	0.641		
eGFR	0.071	0.652			−0.069	0.661		
CysC	−0.064	0.682			−0.002	0.990		
APN	−0.317	0.039	−0.201	0.190	0.123	0.431		
CysC−APN complex	−0.020	0.899			−0.384	0.011	−0.285	0.069
	Necrotic components				Lipidic plus Necrotic components			
	Univariate		Multivariate		Univariate		Multivariate	
	r	*p*	*β*	*p*	*r*	*p*	*β*	*p*
Age	0.027	0.865			0.040	0.797		
BMI	0.152	0.337			0.169	0.286		
Systolic blood pressure	0.053	0.737			0.032	0.838		
Diastolic blood pressure	0.051	0.746			0.031	0.842		
Fasting blood sugar	0.352	0.021	0.120	0.433	0.313	0.041	0.009	0.572
IRI	0.230	0.149			0.292	0.064		
HOMA-R	0.262	0.098			0.305	0.053		
HbA1c (NGSP)	0.099	0.529			0.054	0.732		
Total cholesterol	0.147	0.355			0.136	0.389		
Triglyceride	0.287	0.062			0.277	0.072		
HDL-C	0.028	0.860			−0.011	0.942		
LDL-C	0.039	0.809			0.045	0.779		
hsCRP	−0.196	0.226			−0.145	0.373		
serum creatinine	−0.150	0.339			−0.121	0.438		
eGFR	0.148	0.343			0.122	0.436		
CysC	−0.064	0.682			−0.002	0.990		
APN	−0.171	0.273			−0.179	0.251		
CysC−APN complex	0.406	0.007	0.276	0.076	0.417	0.005	0.287	0.067

The relationships among parameters were investigated by Spearman's correlation coefficient (univariate) and multiple regression analysis (multivariate).

complex levels might correlate with plaque vulnerability more sensitively than serum APN levels in stable CAD male patients. Hence, we propose that serum CysC−APN complex levels are a novel biomarker of coronary plaque instability.

Dyslipidemia is a well-established risk factor for ACS. Nasu *et al*. have reported a positive relationship between serum LDL-C levels and plaque vulnerability

in stable angina patients without any lipid-lowering treatment[44]. In the present study, however, there was no obvious correlation between any lipid parameter and plaque burden or plaque components. Systolic blood pressure, also known as a positive risk factor for atherosclerosis, is negatively correlated with plaque burden. These unexpected results might be due to the medical treatments the enrolled patients were receiving. As shown in **Table 1**, 56% and 67% of the patients had already been treated with statins and anti-hypertensive agents, respectively. However, serum CysC−APN complex levels were still predictors for coronary plaque instability in these patients. We found it useful to measure serum CysC−APN complex levels in fully medicated stable CAD patients. A simple and reliable system for measuring serum CysC−APN complex levels would be a non-invasive method to predict plaque vulnerability and would be useful for the treatment and prevention of ACS.

This study has some limitations. We conducted a cross-sectional, single-center study, with a relatively small number of patients. The patients had already been treated with medication at enrollment. Further larger case-control or prospective studies including female patients are needed to obtain additional information on the clinical significance of serum CysC−APN complex levels.

Conclusion

Serum CysC−APN levels can be a useful biomarker for predicting coronary plaque instability in stable CAD male patients.

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Conflict of Interest

None declared.

Author Contributions

A.M., H.Y., K.K., S.O., and N.M. performed research; T.M. performed clinical work; H.Y. and S.K. designed research; A.M., H.Y., and S.K. wrote the manuscript.

Abbreviations

APN, adiponectin; CAD, coronary artery disease; CysC, cystatin C; IVUS, intravascular ultrasound

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