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Impact of 719 Trp>Arg polymorphism of KIF 6 gene on coronary artery disease, contrast induced nephropathy and modulation of statin therapy effectiveness

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Part 1 Introduction

Coronary artery disease (CAD) is a multifactorial and complex condition resulting from the interaction between genes and environmental factors. In the last decades several studies have tried to identify major cardiovascular risk factors, therapies and preventive measures in order to fight against this common and severe disease. Statin treatment plays a central role in the prevention of cardiovascular disease, in fact, in addition to their impact on cholesterol levels, statins have shown multiple non lipid-lowering pleiotropic effects such as anti-oxidant, anti-inflammatory and anti-thrombotic properties with enhancement of endothelial nitric oxide production and reducing of endothelin secretion (1-3). Therefore, they are highly recommended both as chronic therapy in patients with CAD, but also with a loading dose in the early phase of acute coronary syndrome to stabilize the plaque and reduce acute complication, especially if a percutaneous coronary intervention (PCI) is performed. Genetics plays an important role in determining the inherent CAD vulnerability and in affecting the response and potentially the beneficial effects of statin therapy. Contrast-induced nephropathy (CIN) is a common complication of procedures that foresee the use of contrast media and is known as the third leading cause of hospital-acquired acute kidney injury, accounting for 11% of all cases (4). In last decades several therapies for the prevention of CIN, such as different hydration and alkalinization measures (5,6), N-acetylcysteine (NAC) (7), Fenoldopam, hemodyalisis and hemofiltration (8,9) have been explored in randomized clinical trial with conflicting results. Due to the important pleiotropics effect of statins, a large number of studies have assessed their role for the prevention of CIN. Recent studies (10,11) showed a protective effect of statin therapy in

patients treated before coronary angiography/PCI, while other studies suggested that statin therapy is not useful for the prevention of CIN (12,13). Kinesis-Like Protein 6 (KIF 6) is an omodimeric protein expressed in coronary arteries and other vascular tissues, that is involved in cellular microtubular transport (14). The impact of KIF 6 gene on cardiovascular risk modulation has been investigated since 2007 due to the presence of a single nucleotide polymorphism (non synonym replacement A>G) related with the replacement of Trp 719 with arginine (Arg). Several prospective trials and meta-analysis (15-17) assessed the association between this genetic variant (expressed in 60% of European population) and a significant increase of cardiovascular risk, anyway these results were not confirmed in a large study (Heart Protection study that involved more than 18000 patients (18). Several mechanism such as a modification in the particles binding capacity (19), a modulation in the endothelial cells progenitors growth (20) and an increased expression of KIF 6 in the population with the risk allele (21) has been proposed to explain this association. Particular attention in last years has been focused on the role of the Trp719ARg polymorphism in the modulation of response to statin treatment. Several clinical trials showed a significant association between anti-inflammatory, metabolics and vasoprotective effects of statin therapy and a reduction in cardiovascular events in the population with the risk allele (22,23). Moreover these protective effects with a significant reduction (about 13%) of LDL-cholesterol level and risk of cardiovascular events of statin therapy has been recently confirmed by two meta-analysis (24,25).

Therefore, the aim of the current thesis was to identify new prognostic factor for cardiovascular risk assessment and the risk of contrast induced nephropathy, with a special

focus on the association between of KIF-6 polymorphism (Trp719Arg polymorphism) and the prevalence and extent of CAD, the occurrence of CIN and modulation of the protective effects of statin therapy.

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Part 2

Cardiovascular risk assessment

Chapter 2

Impact of sex on uric acid levels and its relationship with the extent of coronary artery disease: A single-centre study

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Abstract

Background. Serum uric acid (SUA) elevation has been largely addressed in the past as a possible risk factor for cardiovascular disease. However, uric acid has not clearly emerged as independent risk factor for coronary artery disease. Several studies in literature have assessed sex-related differences in the association between elevated SUA levels and cardiovascular events with conflicting results. Therefore, aim of the current study was to evaluate the relationship between uric acid levels and the extent of coronary artery disease in male and female patients undergoing coronary angiography.

Methods. Our population is represented by 3520 consecutive patients undergoing coronary angiography from March 2007 to October 2012. Patients were divided according to Tertiles of SUA (Males, Group 1, $\leq$ 5.5mg/dL-0.33mmol/mol, n=762, Group 2, 5.5-6.8mg/dL–0.33-0.40mmol/mol, n=829 and Group 3 $\geq$ 6.8mg/dL-0.40mmol/mol, n=851), (Females, Group 1, $\leq$ 4.8mg/dL-0.28mmol/mol, n=349, Group 2, 4.8-6.3mg/dL–0.28-0.37mmol/mol, n=359 and Group 3 $\geq$ 6.3mg/dL-0.37mmol/mol, n=370). Fasting samples were collected for uric acid levels assessment. Coronary disease was defined for at least 1 vessel stenosis > 50% as

evaluated by QCA. Severe coronary disease was defined as three-vessel disease and/or left main disease.

Results. Among 3520 patients, we identified 2442 men (69.4%) and 1078 women (30.6%). Males had higher levels of uric acid than women (6.33 $\pm$ 1.7 vs 5.8 $\pm$ 1.9 - $p<0.001$). The association between elevated uric acid ($\geq$ 7 mg/dl or 0.42 mmol/l) and male gender was confirmed after correction for baseline confounding factors (Adjusted OR=1.28 [1.01-1.62], $p=0.004$). Males displayed a significantly higher prevalence and extent of CAD ($p<0.001$) and more complex coronary lesions ($p<0.001$). However, no significant relationship was observed between uric acid and CAD (Adjusted OR [95%CI] = 0.90 [0.76-1.06], $p = 0.22$) or severe CAD (Adjusted OR [95%CI] = 0.89 [0.79-1.01], $p = 0.08$). Among females, higher SUA levels were significantly associated with higher prevalence of severe CAD ($p<0.001$) (Adjusted OR [95%CI] = 1.29 [1.03-1.62], $p = 0.03$).

Conclusion. Our study showed that uric acid levels are significantly higher in men. However, high uric acid levels are associated with severe CAD only in women. Future large studies are certainly needed to confirm our findings and to evaluate the effects of SUA lowering therapies on cardiovascular prevention and outcome, especially in women.

Keywords: uric acid, sex, coronary atherosclerosis

Introduction

Coronary artery disease (CAD) still represents the leading cause of death in developed countries (1,2). The improvement in pharmacological therapies and percutaneous revascularization procedures have greatly contributed to the relevant reduction in mortality observed in the last decades for coronary artery disease, particularly in the setting of acute myocardial infarction (3-5), however, the results are still unsatisfactory in high-risk subgroups such as patients with diabetes or with impaired renal function (6,7). Therefore, large interests have been focused on the identification of new risk factors for CAD and its prevention.

Serum uric acid (SUA), a degradation metabolite of purines, has been addressed in the past as a possible risk factor for cardiovascular disease (8,9) on the basis that hyperuricemia could induce atherosclerosis progression increasing oxidative stress and endothelial dysfunction (10,11). However, in the last decades several studies have assessed the role of SUA as a risk factor for CAD with conflicting results. We previously found no association between uric acid and the extent of CAD (12). However, even though it is well know that SUA levels are lower in women than in men (13), some, but not all studies, have reported an association between SUA, cardiovascular events and CAD related mortality only in women. Plausible explanation for such a mechanism is still lacking (14,15).

Therefore, aim of the current study was to evaluate the relationship between uric acid levels and the extent of coronary artery disease in male and female patients undergoing coronary angiography.

Methods

Our population is represented by 3520 consecutive patients undergoing coronary angiography at Catheterization Laboratory of AOU "Maggiore della Carità", Novara, from March 2007 to October 2012.

All demographic and clinical data were collected after obtaining written informed consent from the patient and included in a dedicated database, in adherence to rules for protection of human subjects. No exclusion criteria were applied. Hypertension was defined as systolic pressure > 140 mmHg and/or diastolic pressure > 90 mmHg or if the individual was taking an antihypertensive medication. The diagnosis of diabetes was based on previous history of diabetes treated with or without drug therapies, fasting glycaemia > 126 mg/dL, random glycaemia > 200 mg/dL or HbA1c > 6.5%. ACS was defined as an elevation of cardiac biomarkers beyond the upper limit of normal (ULN) (respectively 0,04 µg/l for Troponin I and 5,00 µg/l for CK-MB) due to angiographically documented critical coronary stenosis (>70%). Hyperuricemia was defined as SUA levels >7mg/dL or 0.42mmol/mol.

Biochemical measurements

Blood samples were drawn at admission in patients undergoing elective (following a fasting period of 12 h) or urgent coronary angiography. Glucose, creatinine, uric acid, blood cells count and lipid profile were determined by standard methods. Cardiac biomerakers (Troponin I and CK-MB) were assessed by sandwich immunoassay with direct chemiluminescence.

Coronary angiography

Coronary angiography was routinely performed, preferring a radial approach, using 6-French right and left heart catheters. Quantitative coronary angiography was performed by

experienced interventional cardiologists by automatic edge-detection systems (Siemens Acom Quantcor QCA, Erlangen, Germany) as previously described (16). After the visual inspection of the coronary artery, the frame of optimal clarity was selected, showing lesion at maximal narrowing and arterial silhouette in sharpest focus. After the calibration of guiding catheter, analysed arterial segment with coronary lesion was defined by moving the cursor from the proximal to the distal part of coronary artery to ensure adequate determination of reference diameter. We have measured minimal luminal diameter, reference diameter, percent diameter stenosis, and length of the lesion.

Significant coronary artery disease was defined as at least 1 coronary stenosis more than 50%. Severe coronary disease was defined as three-vessel disease and/or left main disease. For patients who had previously undergone a percutaneous coronary intervention, even though no restenosis was observed, the treated vessel was considered as significantly diseased. In previously bypassed patients, native arteries and grafts were taken into account in the evaluation of extension of artery disease (number of diseased vessels).

Statistical analysis

Continuous data were expressed as mean $\pm$ SD and categorical data as percentage. Analysis of variance and chi-square test were used for continuous and categorical variables, respectively. A trend analysis was performed across tertiles of SUA according to sex (17). The relationship between uric acid and coronary artery disease was evaluated at multivariate analysis separately in males and females after correction for baseline confounding factors that were entered in the model in block for each analysis. Results were considered

statistically significant at two-sided p< 0.05. Statistical analysis was performed using the SPSS 17.0 statistical package.

Table 1. Clinical and demographical characteristics according to sex.

Baseline Clinical Characteristics	**Female (n=1078)**	**Male (n=2442)**	**P value**
Arterial hypertension (%)	75.3	69.7	0.001
Age (Mean +-SD)	70.8+/-10.6	66.2+/-11.3	<0.001
Smokers (%)			<0.001
Active smokers	15.8	31.7	
Previous smokers	8.0	24.1	
Dyslipidemia (%)	55.3	55.9	0.73
Diabetes (%)	37.3	36.8	0.77
Family history of CAD (%)	27.8	28.9	0.51
History of MI (%)	15.7	28.5	<0.001
Previous PCI (%)	16.1	27.3	<0.001
Previous CABG (%)	6.4	14.3	<0.001
Previous Stroke (%)	4	7.9	<0.001
Renal failure (%)	39.2	23.4	<0.001
Indication for angiography			p<0.001
Stable angina or silent ischemia (%)	19.2	25.7	
Acute Coronary Syndrome (%)	60.4	57.4	
DCM or valvular disease (%)	20.4	17	
Biochemistry			
White blood cells (10^3/µl)	7.64+/-2.5	8.01+/-3.1	0.001
Platelets Count (10^5/ml)	237.3+/-72.4	206.9+/-60.3	<0.001
Haemoglobin (g/dl)	12.4+/-1.5	13.8+/-1.6	<0.001
Creatinine (mg/dl)	0.98+/-0.4	1.01+/-0.36	<0.001
Glycaemia (mg/dl)	126.3+/-54.7	124.6+/-48.8	0.35
Glycosylated Haemoglobin (%)	6.3+/-1.3	6.2+/-1.3	0.08
Total cholesterol (mg/dL)	170.1+/-43.5	160.7+/-40.5	<0.001
Tryglicerides (mg/dL)	125.9+/-67.7	137.7+/-85	<0.001
HDL cholesterol	44.7+/-13.6	39.4+/-11.6	<0.001
LDL cholesterol	104.5+/-41.6	100.5+/-46	0.01
Reactive protein C (mg/dL)	1.53+/-3.1	1.25+/-2.6	0.009
Uric Acid (mg/dl)	5.8+/-1.9	6.3+/-1.7	<0.001
Fibrinogen (mg/dL)	464.4+/-143.1	429+/-152.6	<0.001
Therapy at admission			
ACE inhibitors (%)	35.5	39.1	0.04
ARB (%)	24.5	18.5	<0.001

Nitrate (%)	35.6	35.7	0.94
Beta blockers (%)	50.8	51	0.95
Calcium antagonists (%)	20.1	20.2	0.93
Diuretics (%)	36.3	27.7	<0.001
Statins (%)	41.8	50.9	<0.001
ASA (%)	52.9	59.8	<0.001
Clopidogrel (%)	21.6	23.7	0.17

Table 2. Angiographic characteristics (per lesion) according to sex.

Variable	**Female (n=3528)**	**Male (n=8054)**	**P value**
Coronary artery disease (%)§	65.1	81.5	<0.001
Left main/trivessel disease (%)§	21.5	32	<0.001
Left main coronary (%)§	7.6	10.1	0.02
Left anterior descending coronary (%)§	45.4	56.3	<0.001
Circumflex coronary (%)§	30.7	45	<0.001
Anterolateral branch (%) §	5.5	8.8	0.002
Right coronary(%)§	35.7	50	<0.001
Complex type C lesions (%)	27.5	37.1	<0.001
Proximal vessel tortuosity (%)	3.6	3.6	0.952
Spontaneous dissection (%)	0.5	0.4	0.492
Lesion lenght (mm)	18.43 □ 11.57	20.39 □ 13.12	<0.001
Vessel diameter (mm)	2.88 □ 0.76	2.97 □ 1.24	0.014
Stenosis %(mean□SD)	85.0 □ 15.2	86.9 □ 14.7	<0.001
Calcified lesions(%)	24.2	24.2	0.976
Bifurcation (%)	22	21.5	0.654
Intracoronary Thrombus (%)	4.4	5.8	0.05
In-stent Restenosis (%)	3.4	4.5	0.067
TIMI flow			<0.001
3 (%)	76.2	68.3	
2 (%)	4.8	5	
1 (%)	2.6	3.2	
0 (%)	16.4	23.5	

Results

Among 3520 patients undergoing coronary angiography, we identified 2442 men (69.4%) and 1078 women (30.6%). Main demographic and clinical characteristics in females and males are listed in Table 1.

Males were younger (p<0.001), smokers (p<0.001) with previous history of myocardial infarction (p<0.001), previous PCI (p<0.001), CABG (p<0.001) and cerebrovascular

accident ($p<0.001$), but with lower prevalence of hypertension ($p=0.001$) and renal failure ($p<0.001$). Male sex was related to higher haemoglobin ($p<0.001$), white blood cells ($p=0.001$), creatinine ($p<0.001$) and tryglicerides ($p<0.001$), but lower platelets count ($p<0.001$), total cholesterol ($p<0.001$), HDL-cholesterol ($p<0.001$), LDL-cholesterol ($p=0.01$), reactive protein C ($p=0.009$) and fibrinogen ($p<0.001$). Males were more often on therapy with angiotensin coverting enzyme (ACE) inhibitors ($p=0.04$), statins ($p<0.001$) and ASA ($p<0.001$), but less often with angiotensin II receptor blockers (ARBs) ($p<0.001$) and diuretics ($p<0.001$) at admission. Stable angina or silent ischemia was more often related to male sex as indication for angiography ($p<0.001$). Males had higher levels of uric acid than women (6.33 ± 1.7 vs 5.8 ± 1.9 - $p<0.001$) (Figure 1).

The association between elevated uric acid ($\geq$ 7 mg/dl or 0.42 mmol/l) and male gender was confirmed after correction for baseline confounding factors (hypertension, age, smoke, renal failure, previous AMI, previous PCI, previous CABG, previous stroke, indication for angiography, platelets count, white blood cells, haemoglobin, basal creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, tryglycerides, fibrinogen, reactive C protein, therapy with ARB, ACE inhibitors, diuretics, ASA and statins); Adjusted OR=1.28[1.01-1.62], $p=0.004$). Males displayed a significantly higher prevalence and extent of CAD ($p<0.001$) and more complex coronary lesions ($p<0.001$) such as left main/three vessel disease ($p<0.001$), complex type C lesions ($p<0.001$) and the presence of intracoronary thrombus (0.05) (Table 2). Moreover males had more often a low TIMI flow grade at angiography ($p<0.001$). All angiographic characteristics according to gender are listed in Table 2.

Uric acid and CAD in women

Baseline clinical characteristics and angiographic characteristics according to Tertiles of serum uric acid in females (Group 1, $\leq$ 4.8mg/dL or 0.28mmol/mol, n = 349, Group 2, 4.8-6.3mg/dL – 0.28-0.37mmol/mol, n = 359 and Group 3 $\geq$ 6.3mg/dL or 0.37mmol/mol, n = 370) are listed in Table 3. Patients with elevated SUA were older ($p<0.001$), with higher prevalence of hypertension ($p=0.02$), diabetes ($p<0.001$) and renal failure ($p<0.001$), but with lower family history of CAD ($p=0.001$). They had higher levels of white blood cells ($p=0.01$), tryglicerides ($p=0.002$) and fibrinogen ($p<0.001$) and higher creatinine ($p<0.001$), glycaemia ($p<0.001$) and glycosilated haemoglobin ($p<0.001$) at admission, but lower haemoglobin ($p=0.05$) and HDL-cholesterol ($p<0.001$). Patients of the Third Tertile were more often in therapy with ARBs ($p=0.006$) and diuretics ($p<0.001$) at admission, but less often with Clopidogrel ($p=0.04$) and had more frequently dilated cardiomyopathy and valvular disease as indication for angiography ($p<0.001$). Women with higher SUA had a higher prevalence of severe CAD (OR [95% CI] = 1.38 [1.16-1.64], $p<0.001$; age-adjusted OR [95% CI] = 1.32 [1.11-1.57], $p = 0.002$) at coronary angiography with a more frequent involvement of left main ($p=0.01$), left anterior descending ($p=0.03$) and right coronary artery ($p=0.006$). No other differences were found in other biochemical parameters or angiographic characteristics. The significant relationship between uric acid levels and prevalence of severe CAD ($p<0.001$) (Figure 3A) was also confirmed after correction for baseline confounding factors (hypertension, age, diabetes, family history of CAD, renal failure, indication for angiography, white blood cells, haemoglobin, creatinine, glycaemia, glycosylated haemoglobin, tryglicerides, HDL cholesterol, fibrinogen, ARB, diuretics, clopidogrel) (adjusted OR [95%CI] = 1.29 [1.03-1.62], $p = 0.03$), despite no significant

relationship was observed with the prevalence of CAD (OR [95% CI] = 1.16 [0.99-1.34], p=0.10; age-adjusted OR [95% CI] =1.11 [0.95-1.28[, p = 0.22) (Figure 2A), also after correction for all baseline confounding factors (adjusted OR [95%CI] = 0.97 [0.79-1.18], p = 0.76).

Table 3. Baseline clinical and angiographic characteristics (per lesion) according to Tertile of SUA in Females.

Baseline Clinical Characteristics Females	**1° Tertile** SUA<4.8mg/dL (0.28mmol/mol) (n = 349)	**2° Tertile** SUA 4.8-6.3mg/dL (0.28-0.37mmol/mol) (n = 359)	**3° Tertile** SUA ≥ 6.3mg/dL (0.37mmol-mol) (n = 370)	**P value**
Arterial hypertension (%)	72.5	73.2	80.2	0.02
Age (Mean +-SD)	68.4±10.7	70.3±10.7	73.6±9.7	<0.001
Smokers (%)				0.57
Active smokers	19.5	14.2	13.7	
Previous smokers	6.3	9.8	7.9	
Dyslipidemia (%)	55.5	55.6	54.9	0.88
Diabetes (%)	29.5	33.1	48.8	<0.001
Family history of CAD (%)	35	25.4	23.4	0.001
History of MI (%)	14	17.9	15.3	0.67
Previous PCI (%)	14.7	16.5	17.2	0.37
Previous CABG (%)	4.9	6.4	7.9	0.10
Previous Stroke (%)	3.4	5.6	53	0.74
Renal failure (%)	18.5	31.7	65.5	<0.001
Indication for angiography				<0.001
Stable angina or silent ischemia (%)	22.2	18.5	17.2	
Acute Coronary Syndrome (%)	64.9	66.8	49.9	
DCM or valvular disease (%)	12.9	14.7	33	
Biochemistry				
White Blood Cells (10^5/ml)	7.36±2.42	7.64±2.42	7.92±2.5	0.01
Platelets Count (10^5/ml)	238.5±62.6	239.7±75.8	233.8±77.5	0.5
Haemoglobin (g/dl)	12.6±1.46	12.4±1.5	12.3±1.66	0.05
Creatinine (mg/dl)	0.83±0.32	0.92±0.34	1.2±0.45	<0.001
Glycaemia (mg/dl)	122±50.9	120.8±45	135.8±64.6	<0.001
Glycosylated Haemoglobin (%)	6.13±1.25	6.16±1.12	6.6±1.5	<0.001
Total cholesterol (mg/dL)	173.3±45	168.7±43	168.4±42.2	0.243
Tryglicerides (mg/dL)	117.2±58.8	125±62	135±79.1	0.002
HDL cholesterol (mg/dL)	47.1±14.2	45.3±13.9	41.6±12	<0.001

LDL cholesterol (mg/dL)	105.8±41.5	100.8±39	107±43.9	0.11
Reactive protein C (mg/dL)	1.34±3.05	1.56±3.5	1.68±2.7	0.35
Uric Acid (mg/dl)	3.8±3.9	5.4±5.5	7.7±8	<0.001
Fibrinogen (mg/dL)	446.8±149.3	457±132.2	488.2±144.6	<0.001
Therapy at admission				
ACE inhibitors (%)	36.3	33.9	36.3	0.98
ARB (%)	19.3	25.8	28.2	0.006
Nitrate (%)	35.3	36.7	34.7	0.85
Beta blockers (%)	51.4	49.6	51.5	0.98
Calcium antagonists (%)	19.7	20.7	19.8	0.97
Diuretics (%)	21.7	32.2	53.9	<0.001
Statins (%)	43.4	42	40.2	0.39
ASA (%)	52.2	54.9	51.8	0.9
Clopidogrel (%)	24.3	22.7	18.2	0.04
Angiographic characteristics				
Coronary artery disease (%)§	61.5	66.3	67.3	0.10
Left main/trivessel disease (%)§	17.1	18.1	29	<0.001
Left main coronary (%)§	5.5	6.8	10.3	0.01
Left anterior descending coronary (%)§	40.2	47.3	48.4	0.03
Circumflex coronary (%)§	29.2	30.5	32.2	0.39
Anterolateral branch (%) §	5	6.1	5.3	0.85
Right coronary(%)§	3	0	0	0.006
Complex type C lesions (%)	30.7	24.5	25.3	0.08
Proximal vessel tortuosity (%)	3.9	4.3	2.5	0.22
Spontaneous dissection (%)	0	1.4	0.6	0.34
Lesion lenght (mm)	19±11.8	17.9±11.3	18.4±12.1	0.44
Vessel diameter (mm)	2.86±0.62	2.88±0.56	2.86±0.6	0.86
Stenosis %(mean□SD)	85.5±14.8	84.2±16	84.7±15.1	0.44
Calcified lesions(%)	21.4	23.8	23.6	0.45
Bifurcation (%)	19.9	22.9	22.6	0.34
Chronic total occlusion (%)	11.7	9.1	13.2	0.39
Intracoronary Thrombus (%)	4.7	4.7	3.1	0.2
In-stent Restenosis (%)	3.7	2.5	3.7	0.95
TIMI flow				0.39
3 (%)	76	76.2	77.9	
2 (%)	3.9	5.6	4.2	
1 (%)	2.2	3.3	2.3	
0 (%)	17.9	14.9	15.5	

Uric acid and CAD in men

Baseline clinical characteristics and angiographic characteristics according to Tertiles of serum uric acid in males (Group 1, ≤ 5.5mg/dL or 0.33mmol/mol, n = 762, Group 2, 5.5-6.8mg/dL – 0.33-0.40mmol/mol, n = 829 and Group 3 ≥ 6.8mg/dL or 0.40mmol/mol, n = 851) are listed in Table 4. Patients with elevated SUA were older (p<0.001), with higher prevalence of hypertension (p<0.001), renal failure (p<0.001) and previous smoke (p=0.009). They had a lower incidence of previous PCI (p=0.008), but they were more often in therapy with ACE inhibitors (p=0.001), calcium antagonist (p=0.01) and diuretics (p<0.001) at admission. About baseline chemistry patients of the Third Tertile had higher level of baseline creatinine (p<0.001), glycaemia (p=0.006), glycosilated haemoglobin (p=0.02), total cholesterol (p=0.004), tryglicerides (p<0.001), reactive C protein (p=0.006) and fibrinogen (p<0.001) but lower HDL-cholesterol (p<0.001) and haemoglobin (p=0.02). Moreover they had more frequently dilated cardiomyopathy and valvular disease as indication for angiography (p<0.001). Interestingly elevated Uric acid was associated with a trend towards lower prevalence of CAD (OR [95% CI] = 0.91 [0.78-1.01], p = 0.052; age-adjusted OR [95% CI] = 0.92 [0.79-1.01 = 0.055]) (Figure 2B) that was not confirmed after correction for baseline confounding factors (hypertension, age, smoke, previous PCI, renal failure, indication for angiography, haemoglobin, basal creatinine, glycaemia, glycosylated haemoglobin, total cholesterol, tryglicerides, HDL-cholesterol, reactive protein C, fibrinogen, ACE inhibitors, calcium antagonist, diuretics, lesion length, calcified lesions, intracoronary thrombus) (Adjusted OR [95%CI] = 0.90 [0.76-1.06], p = 0.22; p gender interaction = 0.46). Similarly, no association was found between SUA level and the presence of severe CAD (Figure 3B) (OR [95% CI] = 1.01 [0.92-1.12], p = 0.76; age-

adjusted OR [95% CI] = 1.0 [0.91-1.11] = 0.96), even after correction for baseline confounding factors (adjusted OR [95%CI] = 0.89 [0.79-1.01], p = 0.08; p gender interaction = 0.002).

Table 4. Baseline clinical and angiographic characteristics (per lesion) according to Tertile of SUA in Males.

Baseline Clinical Characteristics Males	**1° Tertile** SUA<5.5mg/dL (0.33mmol/mol) (n =762)	**2° Tertile** SUA 5.5-6.8mg/dL (0.33-0.40mmol/mol) (n =829)	**3° Tertile** SUA ≥ 6.8mg/dL (0.40mmol-mol) (n =851)	**P value**
Arterial hypertension (%)	64.2	65.9	78.4	<0.001
Age (Mean ±-SD)	65.4±11.7	65.4±11.4	67.7±11.7	<0.001
Smokers (%)				0.009
Active smokers	32.6	34.3	28.3	
Previous smokers	20.1	24.1	27.6	
Dyslipidemia (%)	53.2	58.3	56.1	0.28
Diabetes (%)	37.9	34	38.6	0.71
Family history of CAD (%)	28.3	31.6	26.8	0.48
History of MI (%)	28.4	27.5	29.5	0.60
Previous PCI (%)	30.6	27	24.7	0.008
Previous CABG (%)	13.4	12.8	16.5	0.07
Previous Stroke (%)	7.6	6.3	9.8	0.10
Renal failure (%)	12.4	18.3	38.1	<0.001
Indication for angiography				<0.001
Stable angina or silent ischemia (%)	27.6	26	23.6	
Acute Coronary Syndrome (%)	60.6	60	52	
DCM or valvular disease (%)	11.8	14	24.4	
Biochemistry				
White Blood cells (10^5/ml)	7.9±3.2	7.9±3.2	8.2±3	0.22
Platelets Count (10^5/ml)	205.3±59.6	203.8±56.6	211.6±64.2	0.19
Haemoglobin (g/dl)	13.8±1.6	13.9±1.6	13.7±1.7	0.02
Creatinine (mg/dl)	0.99±0.32	1.06±0.31	1.22±0.4	<0.001
Glycaemia (mg/dl)	128.7±53.2	120.8±42.5	129.6±50.3	0.006
Glycosylated Haemoglobin (%)	6.19±1.3	6.10±1.3	6.3±1.2	0.02
Total cholesterol (mg/dL)	156.6±38.9	162.2±40.7	162.7±41.3	0.004
Tryglicerides (mg/dL)	122.6±73.5	137.1±77.7	151.7±98.3	<0.001
HDL cholesterol	40.9±12.1	39.7±11.5	38±11	<0.001
LDL cholesterol	98±45.2	101.1±45.7	102±46.8	0.21
Reactive protein C (mg/dL)	1.2±2.5	1.07±2.5	1.48±2.89	0.006
Uric Acid (mg/dl)	4.6±0.65	6±0.37	8.1±1.3	<0.001

Fibrinogen (mg/dL)	414.4±153	417±146	453.2±155.8	<0.001
Therapy at admission				
ACE inhibitors (%)	34.5	40	42.5	0.001
ARB (%)	17.3	17.4	20.7	0.07
Nitrate (%)	35.2	34.7	37.1	0.4
Beta blockers (%)	50.7	50.1	52.1	0.56
Calcium antagonists (%)	18.4	18.6	23.4	0.01
Diuretics (%)	18.9	22	39.7	<0.001
Statins (%)	52.2	52.1	48.5	0.13
ASA (%)	60.6	59.5	59.5	0.66
Clopidogrel (%)	23.3	24.5	23.4	0.99
Angiographic characteristics				
Coronary artery disease (%)§	83.4	81.9	79.6	0.052
Left main/trivessel disease (%)§	32.8	29.8	33.3	0.76
Left main coronary (%)§	9.6	9.1	11.5	0.19
Left anterior descending coronary (%)§	60.1	53.6	55.5	0.08
Circumflex coronary (%)§	46.3	45.1	43.8	0.30
Anterolateral branch (%) §	10.6	7.6	8.3	0.13
Right coronary(%)§	49.5	49	51.4	0.43
Complex type C lesions (%)	36.8	34.6	39.4	0.155
Proximal vessel tortuosity (%)	3.3	4	3.6	0.74
Spontaneous dissection (%)	0.5	0.4	0.1	0.08
Lesion length (mm)	21.6±14	19.6±12.3	20±12.8	0.001
Vessel diameter (mm)	2.94±0.6	2.92±0.62	2.95±0.62	0.36
Stenosis %(mean□SD)	86.4±15.6	87±14	87±14.7	0.52
Calcified lesions(%)	19.8	22.5	26.9	<0.001
Bifurcation (%)	22.2	21.9	20.7	0.34
Chronic total occlusion (%)	19.1	18.2	22	0.053
Intracoronary Thrombus (%)	6.8	5.3	4.4	0.005
In-stent Restenosis (%)	5.3	4.7	4.3	0.21
TIMI flow				0.138
3 (%)	69	70.4	66.6	
2 (%)	4.9	4.7	4.8	
1 (%)	3.1	2.9	3.5	
0 (%)	23	21.9	25.1	

Figure 1. Bar graph showing the mean values of uric acid in males and females.

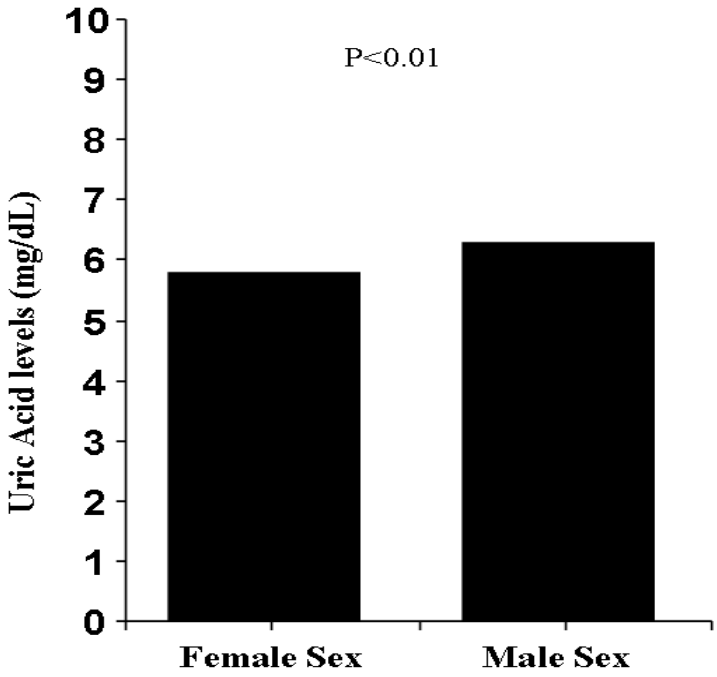


Figure 2. Bar graph showing the prevalence of coronary disease according to uric acid tertiles in females (Figure 2A) and males (Figure 2B).

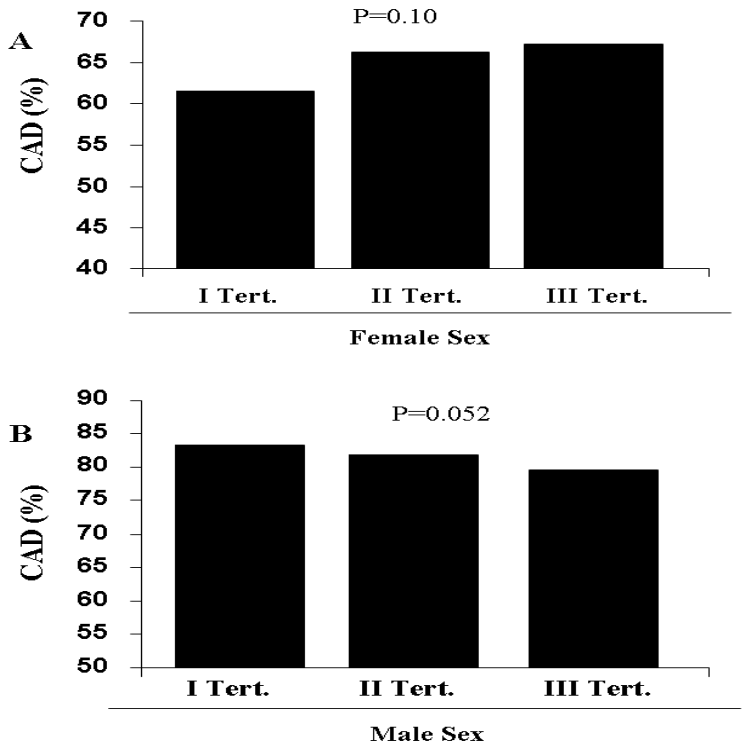
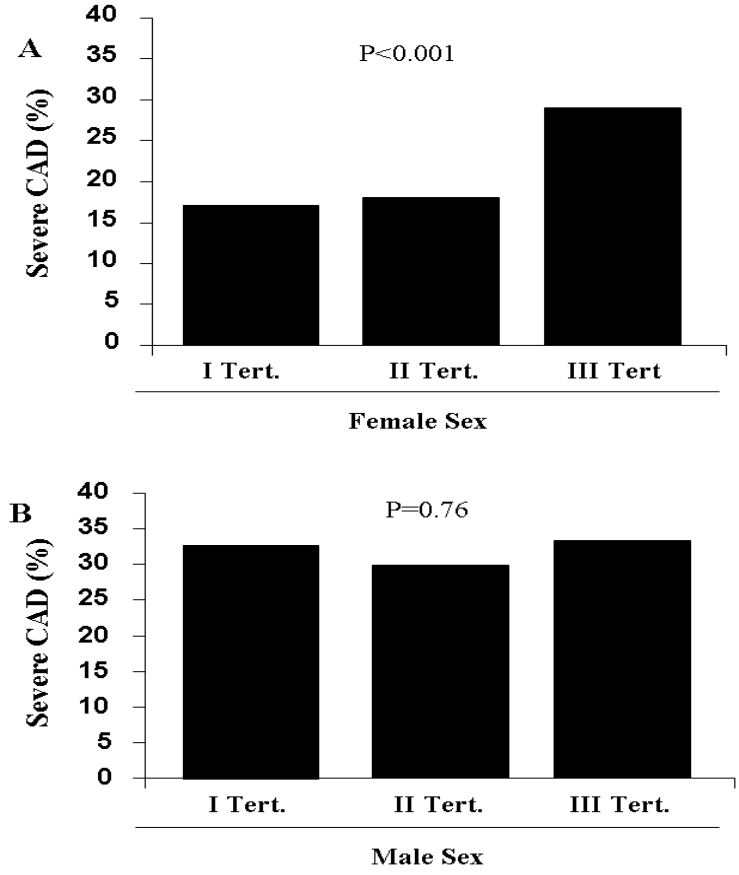


Figure 3. Bar graph showing the prevalence of severe (left main and/or trivessel) coronary disease according to uric acid tertiles in females (Figure 3A) and males (Figure 3B).



Discussion

The main findings of our study are that: 1) male sex, is independently associated with higher SUA; 2) high uric acid levels are independently associated with more severe CAD in women. Despite the great improvement in pharmacological and revascularization therapies to treat and reduce cardiovascular mortality in the last decades (3-5), especially in the acute setting, the results are still suboptimal, in particular among high-risk subgroups of patients (6, 7, 18). Therefore, large interest has been focused on the identification of new risk factors in order to prevent CAD. Uric acid represents a metabolite of the degradation pathway of purines, with renal elimination (19). Its catabolism via the xanthine oxidase has been related to increased production of reactive oxygen species and reduced availability of nitric oxide, with consequent oxidative damage (20). Therefore, elevated serum uric acid (SUA) has been addressed in literature as a potential contributor of cardiovascular risk, but the results were conflicting. In the past several studies showed an association between hyperuricemia and an increased rate of cardiovascular events and higher mortality (21,22), recently confirmed in a large study by Ndrepepa G et al. (23). On the contrary, other large studies, such as the Framingham Heart Study, (24) and the study from Panero et al, (25) concluded that uric acid was not a causal risk factor for cardiovascular disease. We previously found no association between uric acid and the extent of CAD (12). Moreover, it is know that serum uric acid levels are lower in women than in men and several studies in the past assessed the association between elevated uric acid levels and the extension of CAD, cardiovascular events and CAD related mortality in association to sex with important contradictions. Fang J et al. showed that increased serum uric acid levels are independently and significantly associated with risk of cardiovascular mortality both in women and men

(26). A recent study by Onat et al. (27) showed among more than 1500 patients that CAD risk was independently predicted by elevated SUA in nondiabetic men and was modulated by metabolic syndrome and gender. Subsequently, Tuttle et al. (15) suggested a significant association between high uric acid levels and CAD in woman but not in men and these results were also confirmed in a more recent meta-analysis by Kim et al. (28) in which among 402,997 patients, subgroup analyses showed no significant association between hyperuricemia and CAD incidence/mortality in men, but an increased risk for CAD mortality in women (RR 1.67, 95% CI 1.30-2.04). Moreover, a recent study by Ndrepepa et al. (29) showed between 13,273 patients a stronger association between hyperuricemia and an increased risk of mortality in both sex, with a stronger association in women. In line with most of the studies, we found that despite the higher uric acid levels observed in men a significant association between uric acid and the severity of CAD was observed only in women. In our population women were older, with higher prevalence of the most important cardiovascular risk factors and this finding can probably explain the strong association between severe CAD and SUA in the female population. Anyway, this association remained significant even after correction for all baseline confounders, underlying the presence of an independent correlation between elevated SUA and CAD. Therefore, the complex gender-interaction in the relationship between uric acid and CAD can not only be explained by the major cardiovascular risk factors in women, such as diabetes, hypertension and chronic renal failure (30). A recent study (31) has shown a significant gender-difference in the association between uric acid and MPV, with larger platelets observed in the presence of increased uric acid only among women. In fact, MPV has recently been shown to affect platelet reactivity (32,33) and may therefore contribute to explain our findings. Future large

studies are certainly needed to confirm our findings, to provide physiopathologic insights on the observed gender interaction and to evaluate gender-specific benefits from SUA lowering therapies on cardiovascular prevention and cardiovascular outcome. In fact, so far weak benefits have been demonstrated and therefore their use is, till now, very modest in the subset of asymptomatic patients (34-36).

Limitations

Our study is not able to provide data about long term effect of SUA on cardiovascular outcome. Our study has a cross sectional design, therefore we have no data are regarding the follow up of this high risk subgroup of patients. Finally the use of intravascular imaging, as with IVUS technique, would have improved the definition of CAD, especially in patients with complex eccentric plaques and with negative remodelling, which has been more often demonstrated in older patients, as our patients with higher SUA (37).

Conclusion

Our study showed that even though uric acid levels are significantly higher in men, high uric acid levels are associated with severe CAD only in women. Future large studies are certainly needed to confirm our findings and to evaluate the effects of SUA lowering therapies on cardiovascular prevention and outcome, especially among women.

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Chapter 3

Neutrophil to Lymphocyte Ratio and the extent of coronary artery disease: results from a large cohort study

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Abstract

The neutrophil to lymphocyte ratio (NLR), an inflammatory biomarker, may be of predictive and prognostic value for cardiovascular (CV) events. We evaluated the relationship of NLR with the prevalence and extent of coronary artery disease (CAD) in consecutive patients undergoing elective or urgent coronary angiography. Our population (n=3738 patients) was divided into NLR quartiles. Higher NLR was associated with ageing and established CV risk factors, previous percutaneous coronary revascularization, acute presentation and more complex pharmacological therapy. NLR was related with platelet count, WBC count, creatinine, glycemia, uric acid and C reactive protein (all p=0.001) levels, but inversely related with hemoglobin (p<0.001), total cholesterol (p=0.005) and triglycerides (p<0.001) levels. NLR was associated with multivessel disease (p<0.001), anterior descending, right coronary arteries (p<0.001) or circumflex branch lesions (p=0.01), percentage stenosis (p<0.001), coronary calcification (p<0.001) and intracoronary thrombus (p<0.001) but inversely with instent restenosis (p<0.001) and Thrombolysis-In Myocardial-Infarction flow (p=0.04). NLR was directly related with the prevalence of CAD (p=0.001) and severe CAD (p<0.001). In patients undergoing coronary angiography, the NLR is independently associated with the prevalence and severity of CAD.

Keywords: white blood cells, neutrophils, lymphocytes, coronary artery disease; coronary angiography

Introduction

Atherosclerosis is an inflammatory process leading to vascular wall degeneration as a response to various risk factors.[1] Considerable interest has been given to the field of cardiovascular (CV) disease prevention and major advances have been achieved in mechanical reperfusion and antithrombotic therapies [2-6], especially in acute myocardial infarction. However, the outcome is still unsatisfactory in a relatively large proportion of patients [7-9], thus shifting the focus on the identification of new indicators, better allowing to stratify the risk of CV events. [10-12] Several inflammatory biomarkers, such as high sensitivity C-reactive protein (hsCRP), interleukin-6 and lipoprotein associated phospholipase A2, have been associated with coronary artery disease (CAD) [13-15]. The WBC count has emerged as one of the easiest to obtain, cheapest and moreover, best predictive indicators of CV risk [16.] In fact, leukocytes play a crucial role in the progression of atherosclerosis and in destabilization and rupture of a plaque, leading to thrombotic events. [17] More recently, attention has shifted to leukocyte subtypes and to the Neutrophil to Lymphocyte Ratio (NLR), potentially being more accurate and stable than absolute blood cell counts, especially in patients with acute presentation, where it has been associated with clinical outcome and procedural results after percutaneous revascularization. [18, 19] However, few reports have evaluated the relationship between NLR and the prevalence and extent of CAD; this was the aim of the present study.

Methods

Our population consisted of consecutive patients undergoing elective or urgent coronary angiography between April 2007 and December 2013 at the Ospedale "Maggiore della Carità", Novara, Italy. Informed consent was obtained from all patients before angiography. The study was approved by our local Ethical Committee. All demographic and clinical data were prospectively collected in a dedicated database. Hypertension was defined as systolic pressure >140 mmHg and/or diastolic pressure >90 mmHg or if the individual was taking antihypertensive medication. Diabetes mellitus was defined as previous diagnosis, specific treatment (oral drug or insulin), fasting glycemia >126 mg/dL or glycosylated hemoglobin (HbA1c) >6.5%. [20] Chronic renal failure was considered as a history of renal failure or an admission glomerular filtrate (GFR) <60 ml/min/1.73m^2 as defined by Modifying Diet in Renal Disease (MDRD) formula. Non-ST-elevation myocardial infarction was defined as chest pain lasting more than 5 min, associated with elevation of cardiac biomarkers beyond the upper limit of normal (ULN) (0.04 µg/l for Troponin I and 5.00 ng/ml for creatine-kinase-MB, respectively), with or without ECG changes.

Biochemical measurements

Blood samples were drawn at admission in patients undergoing (following a fasting period of 12 h) or urgent coronary angiography. Glucose, creatinine, HbA1c and lipid profile were determined by standard methods.

The WBC counts were measured in a blood sample collected in Ethylenediaminetetraacetic acid (EDTA) (7.2 mg) tubes. These blood samples were analyzed within 2 h of venipuncture using an automatic blood counter (Sysmex XE-2100, Sysmex Corporation, Kobe, Japan). [21]

Coronary angiography

Coronary angiography (Siemens AXIOM ARTIS *d*TC, Erlangen, Germany) was routinely performed by the Judkins technique using 6-French right and left heart catheters. Quantitative coronary angiography was performed, by an automatic edge-detection systems (Siemens Acom Quantcor QCA, Erlangen, Germany) as previously described. [22] The measured parameters were minimal luminal diameter, reference diameter, percent diameter stenosis and length of the lesion. Significant CAD was defined as at least 1 coronary stenosis >50%. Severe CAD was defined as 3-vessel disease and/or left main disease. In cases who had previously undergone percutaneous coronary intervention (PCI), even if no restenosis was observed, the treated vessel was counted as significantly diseased. In previously bypassed patients, native arteries and grafts were taken into account in the evaluation of the extent of artery disease (number of diseased vessels).

Statistical analysis

Statistical analysis was performed using SPSS 15.0 statistical package. Continuous data were expressed as mean ± SD and categorical data as percentage. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively. Patients were grouped according to quartiles of NLR. Multiple logistic regression analysis was performed to evaluate the relationship between NLR and CAD, even in higher risk subsets of patients, after correction for baseline confounding factors that were entered in the model in block. A two-sided p value < 0.05 was considered significant.

Table I. Clinical and demographic characteristics according to NLR quartiles.

Baseline Characteristics	**I quart**	**II quart**	**III quart**	**IV quart**	**P value**
Age (mean +/- SD)	64.3□11.2	66.9□10.9	68.7□10.9	70.4□11.4	<0.001
Male Sex (%)	67.5	69.9	70.8	69	0.49
Dyslipidemia (%)	62.1	59.5	54.6	45.4	<0.001
Diabetes mellitus (%)	37.1	33.5	36.9	39.5	0.13
Renal failure (%)	9.6	14.6	17.8	24.1	<0.001
Smokers (%)					<0.001
Active smokers	30.9	26.9	25.5	23.2	
Previous smoker	19.2	20.8	17.1	16	
Hypertension (%)	69.4	71.4	72.6	71.5	0.26
History of MI (%)	25.1	24.9	23.5	24	0.44
Previous PCI (%)	27.8	26.3	22	18.9	<0.001
Previous CABG (%)	10.4	13	11	12.4	0.38
Biochemistry					
Platelet Count (10^3/µl)	212□58	214□57	217□67	224□79	0.001
Hemoglobin (g/dl)	13.7□1.6	13.6□1.6	13.4□1.8	12.9□1.9	<0.001
White blood cell count	7.1□2.7	7.2□2	7.8□2.2	9.5□3.1	<0.001
Creatinine (mg/dl)	0.99□0.3	1.04□0.3	1.08□0.4	1.13□0.5	<0.001
Glycaemia (mg/dl)	121□46	121□45	125□50	137□61	<0.001
Glycosylated hemoglobin (%)	6.3□1.4	6.2□1.3	6.2□1.2	6.2□1.4	0.73
Total cholesterol (mg/dL)	167□41	165□42	163□42	160□42	0.005
Triglycerides (mg/dL)	140□86	140□85	134□82	115□61	<0.001
HDL cholesterol (mg/dl)	41□12	41□12	41□12	41□13	0.82
Uric acid (mg/dl)	6.0□1.6	6.1□1.7	6.2□1.8	6.3□2	0.001
C-reactive protein (mg/dl)	1.1□0.04	2.0□0.07	2.5□0.8	4.1□0.14	<0.001
Indication to angiography					<0.001
Stable angina (%)	31.8	27.9	22.4	11.7	
Acute coronary syndrome (%)	53	53.2	58.8	69	
Arrhythmias/Valvulopathy/LV	15.2	18.9	18.8	19.3	
Therapy at admission					
ACE inhibitors (%)	37.7	36.3	38.9	37.7	0.74
ARB (%)	19	22.4	21.7	17.6	0.43
Beta-blockers (%)	55.6	53.9	50.3	42.1	<0.001
Calcium antagonists (%)	19.2	21.1	21.7	17.2	0.53
Nitrates (%)	34.6	36.2	36.7	33.4	0.67
Diuretics (%)	25.5	28.2	33.4	32.4	<0.001
Statins (%)	53.2	50.5	48.7	38.7	<0.001
ASA (%)	62.7	62.1	56.9	47.8	<0.001

Clopidogrel (%)	23.4	23.4	24.8	19.2	0.08

Results

Our population consisted of 3738 patients undergoing coronary angiography. They were divided according to quartiles values of NLR (< 1.8; 1.8-2.49; 2.5-3.69; ≥3.7). The main clinical and demographical features of included patients are displayed in Table 1. Higher NLR values were associated to ageing (p<0.001), dyslipidemia (p<0.001), renal failure (p<0.001), smoking (p<0.001), previous PCI (p<0.001), acute presentation (p<0.001), therapy at admission with beta-blockers, diuretics, statins and acetylsalicylic acid (p<0.001, respectively). NLR was directly related with the platelet count (p=0.001), WBC count, creatinine, glycemia (p<0.001), uric acid (p=0.001) and C-reactive protein (p<0.001) levels, while inversely with hemoglobin levels (p<0.001), total cholesterol (p=0.005) and triglycerides (p<0.001) levels. Table 2 shows main angiographic features according to NLR quartiles.

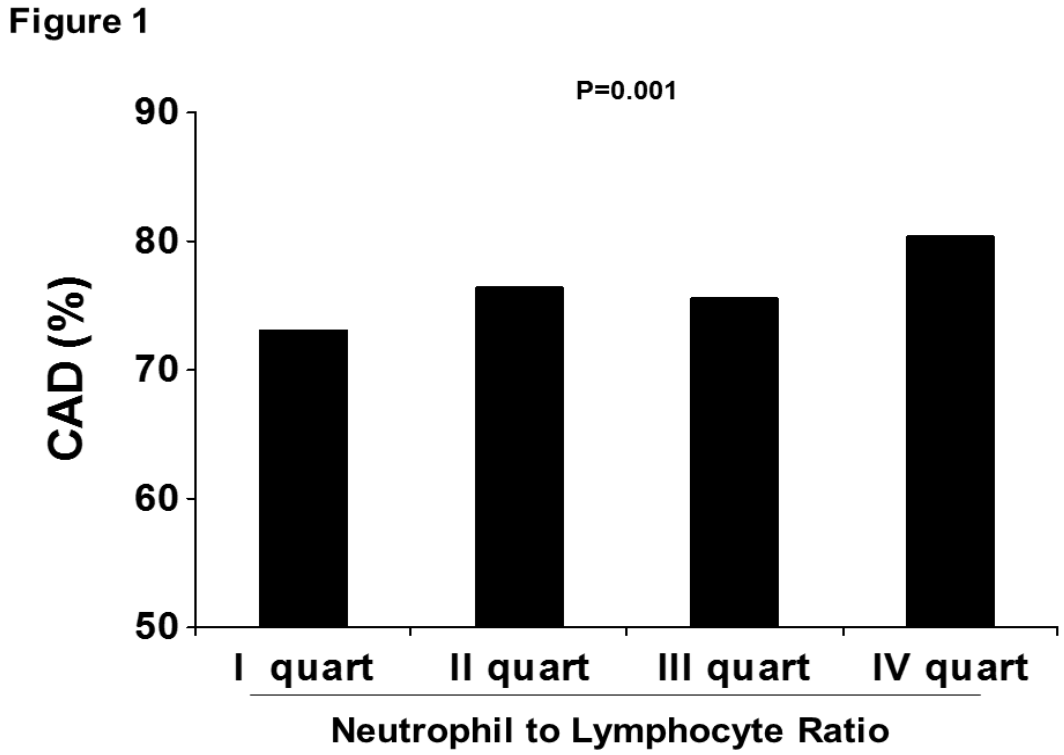
Table 2. Angiographic characteristics (per lesion) according to Neutrophil to Lymphocyte Ratio.

Variable	**I quart (n= 1368)**	**II quart (n=1468)**	**III quart (n=1444)**	**IV quart (n=1626)**	**P value**
Multivessel (%)§	40.9	45.6	45.9	51.4	<0.001
Left main coronary artery (%)§	9	8.3	9.8	9.9	0.33
Left anterior descending coronary artery (%)§	48	53.4	52.8	57.9	<0.001
Circumflex coronary artery (%)§	36.7	40.1	39.4	44.9	0.01
Right coronary artery (%)§	40	44.8	43.9	50.7	<0.001
Type C lesions (%)	34.4	34.1	33.7	36.6	0.25
Lesion length, mm (mean □□SD)	19.8□13	19.9□12.8	19.9□12.7	19.9□12.8	0.99
Vessel diameter, mm (mean	2.9□1	3□1.3	2.9□0.7	3□1	0.94
Stenosis % (mean □□SD)	85.2□15.7	85.7□15.7	85.7□14.3	87.9□13.9	<0.001
Calcified lesions (%)	18.1	22.2	24.9	28.1	<0.001
Bifurcation (%)	21.8	21.2	23.6	20.7	0.77

Intracoronary Thrombus (%)	3.7	2.9	5.6	9.1	<0.001
Chronic occlusion (%)	18.3	18.8	17.1	16.7	0.07
In-stent Restenosis (%)	6.3	5.4	3.7	2.5	<0.001
TIMI flow					0.04
3	71.9	70.6	72.4	67.6	
2	4.7	4.6	5.1	5.1	
1	2.7	3	3.1	3.5	
0	20.7	21.8	19.3	23.8	

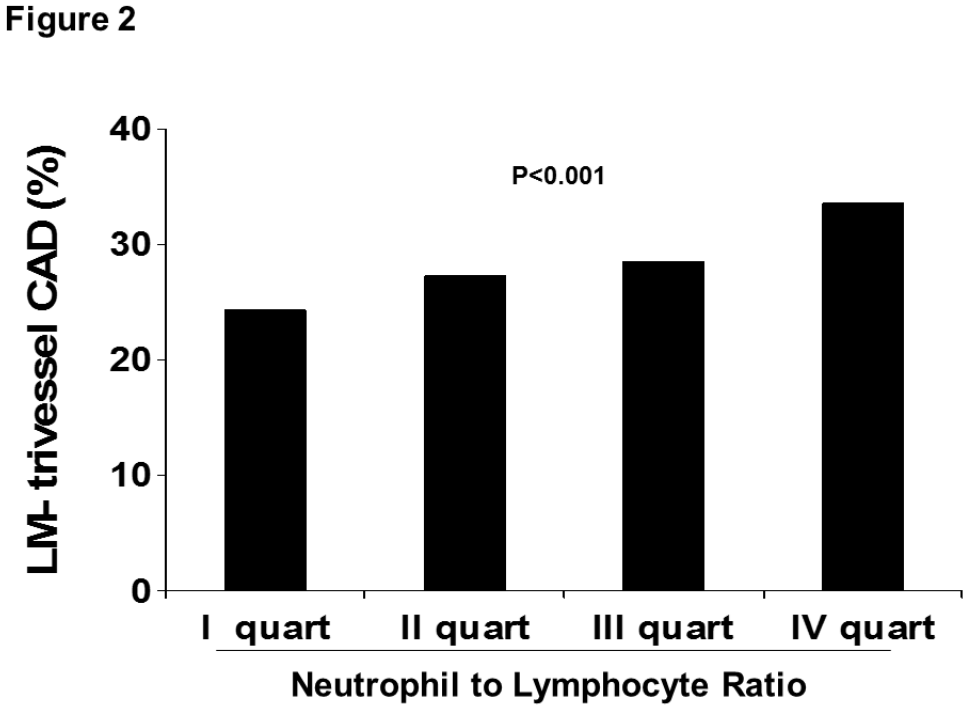
Higher NLR was associated with the extent of multivessel coronary disease ($p<0.001$) and with lesion location ($p<0.001$). NLR was related with the percentage of stenosis ($p<0.001$), the presence of coronary calcification ($p<0.001$), intracoronary thrombus ($p<0.001$) and inversely with instent restenosis ($p<0.001$) and TIMI flow ($p=0.04$).

Figure 1. Bar graphs showing the relationship between Neutrophil to lymphocyte ratio (NLR) quartiles values and the prevalence of coronary artery disease (CAD).



As shown in Figure 1, NLR was directly related with the prevalence of CAD (73.1 vs 76.4 vs 75.6 vs 80.4, p=0.001; OR [95%CI] = 1.12 [1.05-1.2], p=0.001) and with the prevalence of severe CAD (24.3 vs 27.3 vs 28.5 vs 33.6%, p<0.001; OR [95%CI] = 1.16 [1.08-1.23], p<0.001) (Figure 2).

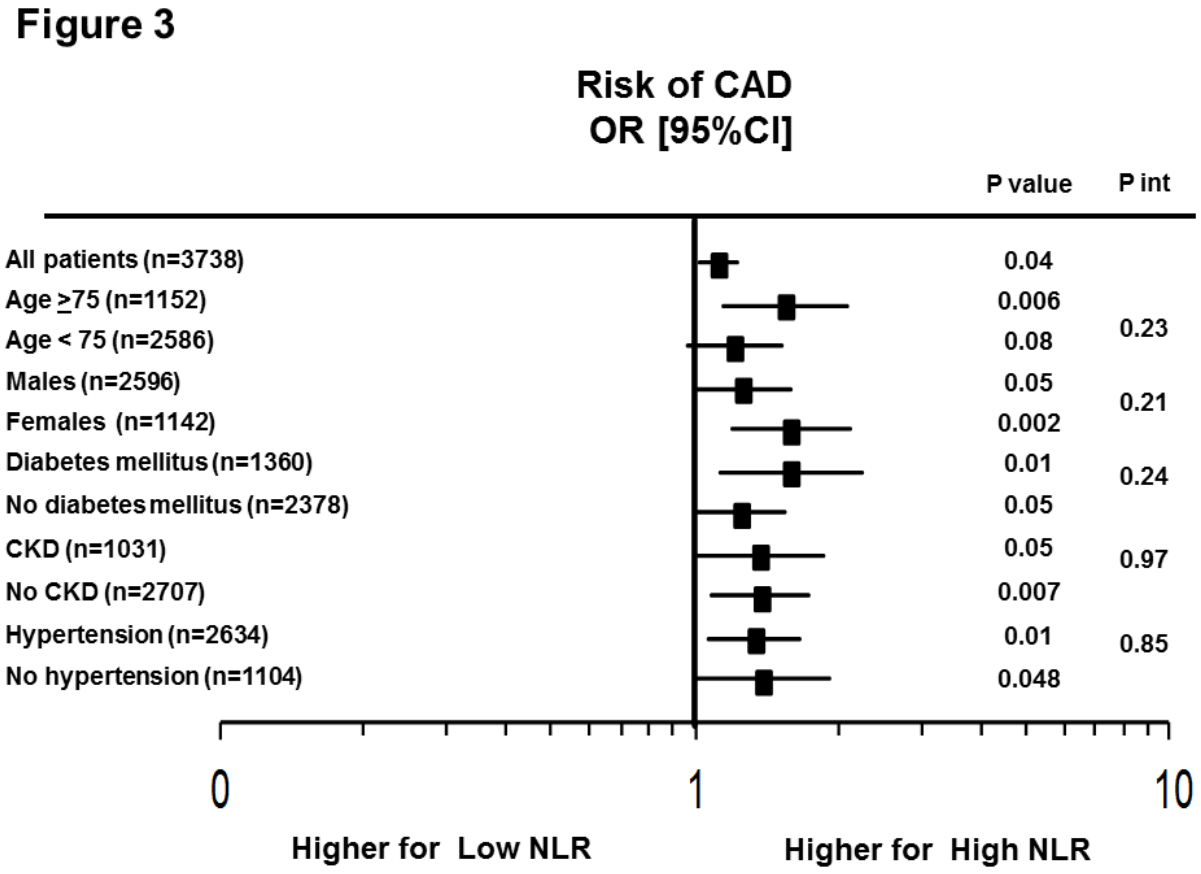
Figure 2. Bar graph showing the relationship between Neutrophil to lymphocyte ratio (NLR) quartiles values and the prevalence of severe coronary artery disease (CAD)



Results were confirmed after correction for baseline differences for both prevalence of CAD (adjusted OR [95%CI] = 1.11 [1.01-1.22], p=0.04) and severe CAD (adjusted OR [95%CI] = 1.12 [1.03-1.23], p=0.007). Similar results were confirmed in the majority of high risk subsets of patients when considering high (4th quartile) vs all lower NLR quartiles. Data for each subgroup are shown in Figure 3, including elderly (≥75 years, OR = 1.55 [1.14-2.11],

p=0.006) or young patients (<75 years old, OR=1.23 [0.97-1.54], p=0.08, p interaction=0.23), males (OR=1.26 [1-1.60], p=0.05) and females (OR=1.59 [1.19-2.14], p=0.002, p interaction=0.21), according to diabetes (diabetics: OR=1.59 [1.13-2.25], p=0.01 and non-diabetics: OR=1.25 [1.001-1.56], p=0.05, p interaction=0.24), renal function (renal failure: OR=1.37 [1-1.88], p=0.05; normal function: OR=1.38 [1.09-1.74], p=0.007, p interaction=0.97), hypertension (hypertension: OR=1.34 [1.07-1.67], p=0.01, no hypertension: OR= 1.39 [1.002-1.92], p=0.048, p interaction =0.85).

Figure 3 Forest plot showing the risk of coronary artery disease (CAD) for Neutrophil to lymphocyte ratio (NLR) in IVth quartile vs lower values in higher risk subsets of patients (CKD = chronic kidney disease).



Discussion

This is the largest single-center cohort study conducted so far that evaluated the relationship between the NLR and angiographically defined CAD. Our main finding is that higher NLR values are associated with the extent and severity of coronary lesions, independently from the main established CV risk factors and in the majority of higher risk subsets of patients. Pharmacological and technical innovations have changed the approach to patients with CAD, extending the indication for PCI to more complex subsets of patients, especially those with acute coronary syndromes (ACS), reducing the rate of repeated target vessel revascularization and increasing event-free survival. [23-29] Indeed, potent antiplatelet agents, new anti-ischemic drugs and high-dose statins have contributed to reduce the progression of atherosclerosis and CV disease, although a considerable residual risk of acute events remains. [30-32] Therefore, there is a need to identify new markers to allow better risk stratification and also as potential pharmacological targets. Special attention has been dedicated to inflammatory biomarkers, with contrasting results. [33] Indeed, inflammation plays a central role in the pathogenesis of atherosclerosis and an elevation of inflammatory markers, indicates the activation of vascular damage. [34] However, a modest potential of predicting CV events has been reported in patients without established CAD, even with complex combinations of indicators of acute response and endothelial dysfunction. [17, 35] Moreover, initial attempts to target selective inhibition of cytokines in CV prevention has resulted in inconsistent results. [36,37] The WBC count represents a cheap, widely available and early indicator of the inflammatory response and initial studies showed that an elevation of total WBC was associated with increased mortality and worse outcomes after acute

myocardial infarction (MI). [38,39] Thereafter, with the recognition of a more relevant role for immune rather than inflammatory response, in the pathogenesis of CAD, attention has been addressed to leukocyte subtypes and especially the NLR, combining the effects of the non-specific inflammatory response, mediated by neutrophils, and the subsequent regulatory immune response, involving lymphocytes. [40] Neutrophils have been claimed for every step leading to acute coronary events and can release pro-oxidant and pro-thrombotic substances, leading to endothelial damage and platelet aggregation. A low lymphocyte count has also been associated with worse prognosis in patients with CAD and unstable angina [41], as certain subset of lymphocytes, have been shown to play an inhibitory role in atherosclerosis, possibly by controlling and regulating the inflammatory response. [40] An increased NLR has been related with arterial stiffness and with indirect indicators of atherosclerosis, such as coronary calcium score and carotid intima-media thickness. Moreover, this hematological index has been associated with thrombus formation in ACS [42], where it has been suggested to influence short and long- outcome, especially in patients with ST elevation MI (STEMI) undergoing primary PCI. [43, 44] In particular, in a large cohort of STEMI patients, a NLR >6.97 was associated with increased in-hospital and long-term CV mortality [45], and similar results were identified in a Korean population. [46] Recent studies, in addition, have reported a prognostic role of NLR in patients undergoing elective PCI or surgical coronary revascularization [22,47] and NLR was associated with worse outcome at 3 years follow-up independently from the therapeutic strategy selected for CAD. [48] In addition, the authors [48] suggested a more advanced obstructive CAD (OR = 2.45, CI 95% 1.76, 3.42, $p < 0.001$) in patients with higher NLR. However, only few small studies have evaluated the relationship between NLR and angiographic findings, thus not providing conclusive results. We present

the results from the largest single center cohort of patients undergoing coronary angiography. We identified a relationship between NLR and main established CV risk factors, thus confirming the hypothesis that the vascular damage induced by ageing, hypercholesterolemia, smoking, hyperglycemia could be mediated by the immune response. Moreover, NLR showed a direct association with acute presentation and with inflammatory biomarkers, as C-reactive protein. In our patients, the NLR was independently associated with the prevalence and extent of CAD (with results being confirmed after correction for potential confounders) and with higher complexity of the coronary plaque, including calcified lesions, intracoronary thrombus and stenosis. Results, in addition, were confirmed also in the majority of high-risk subsets of patients. Our findings confirm the results reported by Kaya et al, in 186 patients undergoing coronary angiography, where NLR was related with the complexity of CAD assessed by SYNTAX score. [49] Similar conclusion was reached by Altun et al [50], in a Scandinavian cohort of 287 ACS patients and by Açar et al [51] in 283 patients evaluated by Coronary Computed Tomography, where NLR values in the third tertile increased the risk of CAD and the severity of critical luminal stenosis. Therefore, the present study provides a deeper insight in the stratification tools for assessing the risk of CAD and in the role of immune response in the pathogenesis of CAD, suggesting the hypothesis of immune-modulation as a potential pharmacological target for the prevention of CV disease. In fact, Wang et al. demonstrated a positive effect of diet and exercise on NLR. [52] Limitations of the study are mainly those inherent to any prospective but observational study. In fact, we did not collect follow-up data, especially in patients undergoing coronary angioplasty, and thus cannot provide data on the role of NLR on the progression of CAD, and especially on the risk of adverse events, as stent thrombosis or

restenosis after PCI. It is known that inflammation plays a key role in in-stent restenosis process.[53] However, we have found an inverse paradoxical association between NLR and in-stent restenosis. However, our data should be interpreted with caution. In fact, evaluation of in-stent restenosis was not the aim of our study, and we took note of significant in-stent restenosis but not of segments with successful stent results, with a subsequent evident bias. In addition, patients with higher NLR were more often on statins and that could potentially explain the lower rate of restenosis and lower levels of cholesterol in patients with high NLR. This does not certainly allow to generate any hypothesis from present findings. However, more dedicated studies would certainly be helpful in clarifying these issues. In addition, the use of intracoronary ultrasound or virtual histology would have improved our results by providing more accurate information on the coronary atherosclerotic plaque, as in fact previous reports have related NLR to coronary ectasia [54] The present study demonstrates that in patients undergoing coronary angiography, higher NLR values emerge as independent predictors of the prevalence and extent of CAD.

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Part 3

Contrast induced nephropathy

Chapter 4

Pre-diabetes and the risk of Contrast Induced Nephropathy in patients undergoing coronary angiography or percutaneous intervention.

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Abstract

Background. Contrast Induced Nephropathy (CIN) is a complication of coronary angiography/percutaneous intervention (PCI). It is known that diabetes is an independent risk factor for CIN, but we have no data regarding the association between CIN and glycemic levels in patients without diabetes. Aim of our study was to evaluate whether high level of glycated-haemoglobin in patients without diabetes is associated with an increased risk of CIN.

Methods. 1831 patients without diabetes, undergoing elective/urgent coronary angiography/angioplasty were divided according to quartiles of baseline glycated-haemoglobin. CIN was defined as an absolute ≥0.5mg/dl or a relative ≥25% increase in creatinine level at 24-48h after the procedure.

Results. Patients with elevated glycated-haemoglobin were older, with hypertension, previous history of AMI, PCI and CABG. They had higher gycaemia, fasting-glycaemia and

triglycerides but lower HDL-cholesterol. Patients with higher glycated-haemoglobin were more often on therapy with statins, diuretics and calcium-antagonist at admission, had higher basal, 24 and 48h creatinine and lower creatinine clearance. They had the highest incidence of PCI and contrast volume-eGFR rate. CIN occurred in 10.6% of patients with a linear association with glycated-haemoglobin (p=0.001). No relationship was found between glycaemia/fasting glycaemia at admission and CIN. At multivariate analysis age>70years (AdjustedOR[95%CI]=1.70[1.18-2.42],p=0.004) and glycated-haemoglobin (AdjustedOR[95%CI]=1.63[1.12-2.36],p=0.01) were independently associated with the risk of CIN. The subgroups analysis showed similar results.

Conclusion. This is the first study showing that among patients without diabetes undergoing coronary angiography/PCI elevated glycated-haemoglobin but not glucose levels is independently associated with the risk of CIN.

Keywords: Glycated haemoglobin; contrast induced nephropathy

Introduction

Contrast Induced Nephropathy (CIN) is one of the major complications of procedures that foresee the use of contrast media and is associated with a poor long-term clinical outcome (1,2). It is commonly defined as an acute deterioration in renal function characterized by a significant increase in serum creatinine levels, usually more than 0.5mg/dl or 25% of baseline levels, within 24-48h after exposure to a contrast agent compared to baseline serum creatinine values. In particular in patients undergoing diagnostic and/or therapeutic coronary angiography it has shown to occur in up to 20-25% depending on the presence of known risk factors, such as chronic kidney disease, diabetes mellitus, the dose of contrast medium (3,4). Due to the improvement in stent technology and antithrombotic therapies (5-10), a yearly growing number of patients is now revascularized percutaneously, with a larger proportion of high-risk patients, including those with impaired renal function. In patients with chronic kidney disease, with an estimated glomerular filtration rate (eGFR) <60ml/min, there is a loss of nephron units with a poor residual renal function more vulnerable to renal insults. The pathogenesis of contrast induced acute kidney injury is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species (11). The use of contrast media superimposed acute vasoconstriction, caused by the release of adenosine and endothelin, with the reduction in renal blood flow to the outer medulla, consequent medullary hypoxia, ischemic injury and death of renal tubular cells (12). Hyperglycemia leads to increased endothelin and angiotensin levels, causing intrarenal vasoconstriction; it also modifies the regulation of intrarenal blood flow increasing the medullary lactate level, reducing pH and oxygen delivery and increasing reactive oxygen species and oxidative

stress (13-14). It is known that diabetes mellitus is an independent risk factor for contrast induced nephropathy (15-16), because of altered renal oxygen supply and enhanced reactive oxygen species generation, but we have no data regarding the association between glycemic levels in patients without diabetes and the risk of contrast induced nephropathy (17). Therefore, the aim of the current study was to evaluate whether high level of glycated haemoglobin in patients without diabetes undergoing elective coronary angiography, percutaneous intervention or urgent coronary angioplasty, is associated with an increased risk of contrast induced acute kidney injury.

Material and Methods

Our population is represented by 1831 consecutive patients undergoing coronary angiography and/or angioplasty at Catheterization Laboratory of AOU "Maggiore della Carità", Novara, from January 2007 to September 2011, who were included in our registry on coronary artery disease, as previously described (18), and did not meet the diagnostic criteria for diabetes mellitus, defined as previous diagnosis, specific treatment administration (oral or insulin), fasting glycemia > 126 mg/dL or HbA^{1c} > 6.5% - 47mmol/mol (19)**.** Informed consent was obtained by all patients before angiography. The study was approved by our local ethical committee. All demographic and clinical data were prospectively collected in a dedicated database. Hypertension was defined as systolic pressure > 140 mm Hg and/or diastolic pressure was > 90 mm Hg or if the individual was taking antihypertensive medications. Metabolic syndrome was defined according to the guidelines of the International Diabetes Federation by the presence of three or more of these characteristics: obesity on the basis of waist circumference (males >94 cm, females >80

cm), tryglycerides >150mg/dL, low HDL-cholesterol (M: <40 mg/dL; F: <50 mg/dL), hypertension (>130/>85 mmHg) and fasting glycaemia >110mg/dL (20). All patients were hydrated with saline solution 1ml/kg/h 12h before and after the procedure or with saline solution 0,5ml/kg/h (ejection fraction ≤ 40%) or with sodium bicarbonate (154 mEq/l in dextrose and water received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure) for primary PCI. Acute kidney injury was defined as an absolute ≥ 0.5mg/dl or a relative ≥ 25% increase in the serum creatinine level at 24 or 48h after the procedure.

Coronary angiography was routinely performed by the Judkins technique using 6-French right and left heart catheters through the femoral or radial approach. Quantitative coronary angiography (Siemens Acom Quantcor QCA, Erlangen, Germany) was performed by two experienced cardiologists who had no knowledge of the patients' clinical information (21). Significant coronary artery disease was defined as at least 1 coronary stenosis more than 50%. Severe multivessel disease was defined as three-vessel disease and/or left main disease. Coronary angioplasty was performed with standard techniques (22). Use of stents, type of stents and stent implantation techniques, as much as the use of directional or rotational atherectomy, IVUS, glycoprotein IIb-IIIa inhibitors, was left at the discretion of the operators. The contrast medium used was non-ionic, iso-osmolar (Optiray, Visipaque, Ultravist). All patients received, according to guidelines, high-dose bolus of clopidogrel (600 mg) at the time of hospitalization or before angioplasty.

Biochemical measurements

Blood samples were drawn at admission in patients undergoing elective (following a fasting period of 12 h) or urgent coronary angiography. Glucose, glycated haemoglobin, creatinine

and haemochrome were determined by standard methods. Creatinine was measured at 12, 24 and 48 hours after the procedure or longer in case of development of contrast induced nephropathy.

Statistical analysis

Statistical analysis was performed with the SPSS 15.0 statistical package. Continuous data were expressed as mean ± SD and categorical data as percentage. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively. A trend analysis was performed as previously described (23). Multiple logistic regression analysis was performed to evaluate the relationship between glycated haemoglobin levels and CIN, after correction for baseline confounding factors (clinical and demographic variables with a p value < 0.2 at univariate analysis), that were entered in the model in block.

Table 1. Baseline Clinical Characteristics according to quartiles of glycated haemoglobin.

Variable	I Quartile HbA^{1c} < 5.4% (36 mmol/mol) (n = 326)	II Quartile HbA^{1c} 5.4-5.7% (36-39 mmol/mol) (n = 270)	III Quartile HbA^{1c} 5.7-6% (39-42 mmol/mol) (n = 379)	IV Quartile HbA^{1c} > 6% (42 mmol/mol) (n = 349)	p-value
Demographic and clinical characteristics					
Age (M-SD)	64.2±13.04	67.2±10.9	68.22±10.74	70±10.42	<0.001
Sex (M %)	70.9	68.1	69.4	70.2	0.935
Hypertension (%)	60.2	67.8	72.9	68.3	0.01
Smokers (%)					0.229
Active smokers (%)	27.7	21.6	23.9	25	
Previous smokers (%)	16	22	19.5	13.6	
Hypercolesterolemia (%)	47.7	60.4	55.4	55	0.142
Family history of CAD (%)	31.5	35.9	30	28	0.22
Previous AMI (%)	17.6	21.9	26.4	28.9	<0.001
Previous PCI (%)	14.5	21.5	20.3	21.6	0.035
Previous CABG (%)	7.7	12.2	8.7	13.8	0.045
Previous Stroke (%)	7.1	5.6	6.3	5.2	0.39
Indication for					0.28

angiography					
Stable angina or silent ischemia (%)	18.7	24.1	22.2	21.7	
Acute Coronary Syndrome (%)	58	57.8	49.6	49.7	
DCM or valvular disease (%)	23.3	18.1	28.2	28.6	
Baseline Chemistry					
White blood cells (10^3/µl) (M-SD)	7.49±2.87	7.28±2.32	7.75±2.18	7.96±3.61	0.007
Red blood cells (10^6/µl) (M-SD)	4.55±0.6	4.61±0.52	4.57±0.66	4.55±0.53	0.85
Haemoglobin (M-SD) (g/dl)	13.5±1.79	13.66±1.57	13.49±1.61	13.43±1.6	0.35
Platelet (M-SD) (10^5/ml)	214±65.5	209.2±59.4	215±64.8	217.9±62.9	0.25
Fibrinogen (M-SD) (mg/dL)	445.7±153.8	434±120.5	449.7±145.8	459.6±151.9	0.11
Glycaemia at admission (M-SD) (mg/dL)	105±17.3	106.9±19.9	109.8±19.2	112.1±21.4	<0.001
Glycated Haemoglobin (M-SD) (%)	5.17±0.27	5.56±0.05	5.78±0.08	6.14±0.13	<0.001
Fasting Glycaemia (M-SD) (mg/dL)	101.8±13.8	102.9±15.5	106.5±15.2	108.9±18.6	<0.001
Basal Creatinine (M-SD) (mg/dL)	1 ± 0.27	1.03 ± 0.29	1.09 ± 0.37	1.13 ± 0.4	<0.001
24h Creatinine (M-SD (mg/dL)	1.02 ± 0.32	1.02 ± 0.3	1.08 ± 0.38	1.14 ± 0.4	<0.001
48h creatinine (M-SD) (mg/dL)	0.82 ± 0.57	0.91 ± 0.55	0.96 ± 0.6	1.04 ± 0.65	<0.001
Baseline Creatinine Clearance (M-SD) (ml/min)	80.2±32.8	75.5±29.2	74.3±31.2	68.3±30.8	<0.001
Creatinine Clearance <60 (%)	32.8	37.8	37.5	48.9	< 0.001
Total Cholesterol (M-SD) (mg/dL)	160.7±38.2	165.7±41.4	162.5±37.2	162.3±39.4	0.86
HDL-Cholesterol (M-SD) (mg/dL)	41.3±13.2	41.7±11.2	40.1±11	39.9±12.7	0.04
Triglycerides (M-SD) (mg/dL)	130.1±76.5	131.8±73	140.3±72.2	144.1±69.2	0.005
LDL-Cholesterol (M-SD) (mg/dL)	94.1±33.2	98±35.7	94.9±32	95.4±35.4	0.92
Theraphy at admission					
ACE I (%)	34.4	38.5	36.9	38.9	0.30
ARB (%)	15	21.1	17.2	19.4	0.30
Statins (%)	40.2	46.7	49.9	47.4	0.03
Nitrate (%)	28.5	38.5	39.8	33.7	0.13

Beta-Blockers (%)	46	52.2	47.8	53.4	0.13
ASA (%)	50.9	64.1	58.8	59.7	0.06
Clopidogrel (%)	30	35.7	29.1	25.6	0.11
Calcium Antagonist (%)	16.6	17.8	20.6	22	0.04
Diuretics (%)	19.3	21.5	27.7	32.9	<0.001
Procedural characteristics					
PCI (%)	41.2	45.7	50.5	50.9	0.006
Contrast Volume (M-SD)	212.4±147.9	225.1±148.5	229.4±156.2	229.8±139.8	0.12
Contrast volume/eGFR	3.25±3.34	3.44±2.97	3.66±2.98	4.09±3.32	<0.001
Hydration Protocol					0.158
SS 1 ml/kg/h (%)	78.2	78.1	76.8	70.3	
SS 0.5 ml/kg/h (%)	8.9	7.8	10.3	13.4	
Sodium Bicarbonate (%)	12.9	14.1	12.9	16.3	

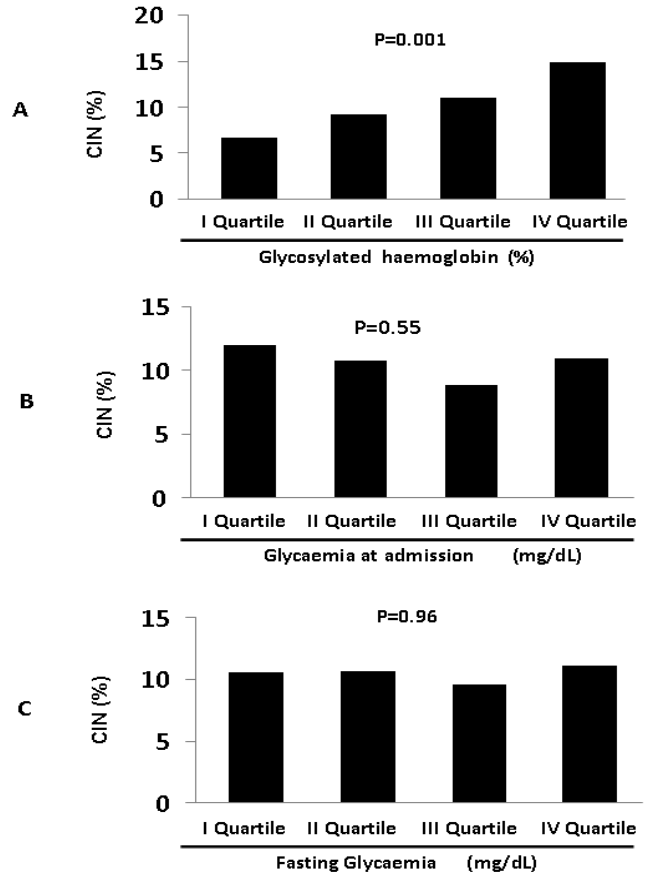
M = Mean; SD = Standard Deviation; CAD: coronary artery disease; AMI: acute myocardial infarction; PCI: percutaneous coronary intervention; CABG: coronary artery by-pass graft surgery; DCM: dilated cardiomyopathy; HDL: high-density lipoprotein; LDL: low density lipoprotein; ACE I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; ASA: acetylsalicylic acid; SS: saline solution.

Results

Our population is represented by consecutive 1831 patients without diabetes. A total of 507 patients were excluded because of end stage renal failure requiring dialysis or because basal glycated haemoglobin levels were not available. Therefore our final study population was represented by 1324 patients. Patients were divided according to quartiles of baseline glycated haemoglobin (Group 1, < 5.4% - 36mmol/mol, n = 326; Group 2, 5.4-5.7% - 36-39mmol/mol, n = 270; Group 3, 5.7-6% 39-42mmol/mol, n = 379; Group 4, > 6 – 42mmol/mol, n = 349). The baseline patients' clinical and demographic characteristics, indication for angiography, procedural main characteristics, baseline chemistry and admission therapy, according to the quartiles of glycated haemoglobin levels, are listed in Table 1. Patients with elevated glycated-haemoglobin levels were older (p<0.001), with history of hypertension (p=0.01) and previous acute myocardial infarction (p<0.001),

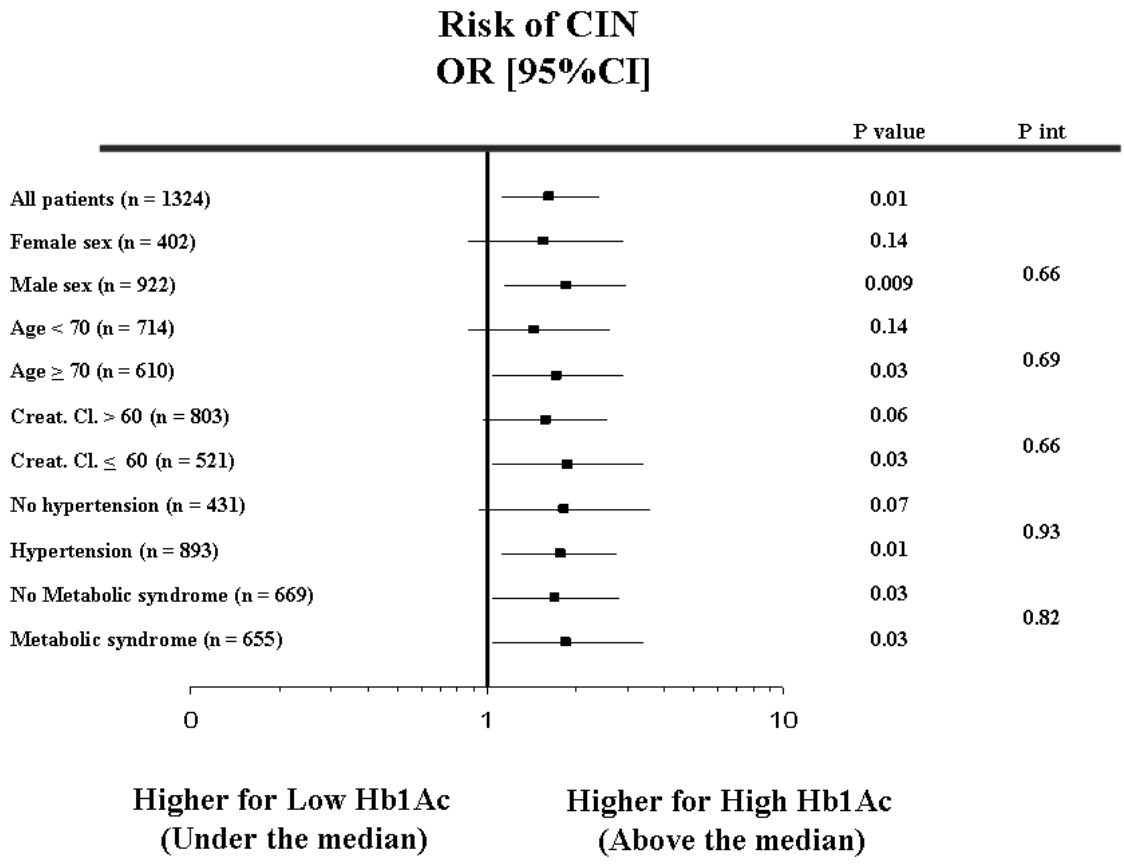
previous percutaneous coronary intervention (p=0.035) and previous coronary artery by-pass graft (CABG) (p=0.045). They had higher levels of white blood cells (p=0.007), higher glycaemia (p<0.001), glycated haemoglobin (p<0.001) and fasting glycaemia (p<0.001), higher tryglicerides (p=0.005) and lower HDL-cholesterol (p=0.04). In addition, patients with higher levels of glycated haemoglobin were more often on therapy with statins (p=0.03), diuretics (p<0.001) and calcium antagonist (p=0.04) at admission. Patients with elevated glycated haemoglobin had higher basal creatinine (p<0.001), lower creatinine clearance (p<0.001) and higher creatinine values also at 24 hours (p<0.001) and 48 hours (p<0.001) after coronary angiography or percutaneous intervention. About procedural characteristics patients of the fourth quartiles had the highest incidence of percutaneous intervention (p=0.006) and the highest contrast volume-eGFR rate (p<0.001). No other differences were found in biochemical parameters, clinical characteristics, therapy or procedural characteristics. Contrast induced nephropathy occurred in 141 patients (10.6%) with a significant linear association with glycated haemoglobin (6.7% in Group 1 versus 9.3% in Group 2, 11.1% in Group 3 and 14.9% in Group 4) (p=0.001) (Figure 1A), whereas no relationship was found between glycaemia at admission (p = 0.55) (Figure 1B) or fasting glycaemia (p=0.96) (Figure 1C) and the risk of CIN.

Figure 1. Bar graph showing the relationship between the development of contrast induced nephropathy and: glycated haemoglobin levels (A), glycaemia at admission (B) and fasting glycaemia (C).



We identify other 3 variables associated with the risk of CIN: Age > 70 years (OR [95% CI] = 1.78 [1.25-2.53], p = 0.001), Female gender (OR 1,38 [95%CI] = [1,01-1,91], p = 0.047), use of diuretics (OR [95% CI] = 1.51 [1.04-2.20], p = 0.03). However, at multivariate analysis only age > 70 years (Adjusted OR [95% CI] = 1.70 [1.18-2.42], p = 0.004) and glycated haemoglobin (Above the 2 quartile) (Adjusted OR [95% CI] = 1.63 [1.12-2.36], p = 0.01) were independently associated with the risk of CIN. At subgroup analysis we showed the association between the risk of contrast induced nephropathy and major risk factors among our population (Above the 2 quartile).

Figure 2. Relationship between glycated haemoglobin levels and the risk of contrast induced nephropathy (CIN) in major subgroups of patients according to age, gender, hypertension, metabolic syndrome and baseline renal failure.



Data for each subgroup are shown in Figure 2, according to gender (Female gender: adjusted OR= 1.56 [0.86-2.84], p=0.14; Male gender: adjusted OR= 1.85 [1.16-2.95], p=0.009, P int 0.66), age (age < 70: adjusted OR = 1.45 [0.87-2.58], p=0.14; age> 70: adjusted OR= 1.74 [1.04-2.90], p=0.03, P int 0.69), creatinine clearance (creatinine clearance >60: adjusted OR = 1.59 [0.97-2.56], p=0.06; creatinine clearance < 60: adjusted OR= 1.89 [1.05-3.40], p=0.03, P int 0.66), history of hypertension (no-hypertension: adjusted OR = 1.83 [0.95-3.56], p=0.07; hypertension: adjusted OR= 1.77 [1.13-2.75], p=0.01, P int 0.93) and the presence of metabolic syndrome (no metabolic syndrome:

adjusted OR = 1.71 [1.05-2.79], p=0.03; metabolic syndrome: adjusted OR= 1.87 [1.05-3.33], p=0.03, P int 0.82).

Discussion

Our study shows that in patients without diabetes undergoing coronary angiography or percutaneous interventions, glycated haemoglobin, but not fasting glycaemia, is independently associated with an increased risk of contrast induced nephropathy. Coronary artery disease is still the first cause of mortality in developed countries. However, a larger application of revascularization procedures, especially in the setting of acute myocardial infarction (24-27), has contributed to the relevant reduction in mortality observed in the last decades. Due to the improvement in stent technology and antithrombotic therapies (5-10), a yearly growing number of patients undergo percutaneous revascularization, with a larger proportion of high-risk patients, including those with impaired renal function.

In this kind of patients there is a loss of nephron units with a residual renal function more vulnerable to external insults. The development of contrast induced acute kidney injury is associates with increased mortality and morbidity costs, in fact, although usually transient, the impairment of renal function may be permanent in some cases with progression to chronic renal failure and the necessity of a temporary or permanent dialysis (28). Proposed pathophysiologic mechanism through which contrast administration may potentiates renal injury include oxidative stress, free radical damage and endothelial dysfunction (29). The use of contrast media superimposed acute vasoconstriction, caused by the release of adenosine and endothelin, with the reduction in renal blood flow to the outer medulla, consequent medullary hypoxia, ischemic injury and death of renal tubular cells (12).

Prevention is the key to reduce the incidence of contrast induced nephropathy and it begins with the identification of the high risk patient coupled with appropriate periprocedural management (hydration and administration with acetylcysteine) (30-31). From the current literature we know that diabetes is an independent risk factor for contrast induced nephropathy (15-16) because of intensified hypoxic and oxidative stress following the administration of contrast media. In a “diabetic kidney” there is an enhanced tubular transport activity, oxygen consumption, and generation of reactive oxygen species; moreover there is a reduction of renal blood flow caused by an altered regulation of vascular tone particularly due to defective nitrovasodilation, enhanced endothelin production, and a particular hyperresponsiveness to adenosine-related vasoconstriction. In addition, micro- and macrovascular diseases and chronic tubulo-interstitial changes further compromise regional oxygen delivery, and renal antioxidant capacity might be hampered (17).

A condition of hyperglycemia, also in a patient without diabetes, leads to increased endothelin and angiotensin levels, causing intrarenal vasoconstriction and modifies the regulation of intrarenal blood flow increasing the medullary lactate level, reducing pH and oxygen delivery and increasing reactive oxygen species and oxidative stress (13-14). In the literature there are a lot of studies regarding acute-stress hyperglycemia and the increased risk of contrast induced nephropathy in ST-segment elevation myocardial infarction (STEMI) patients. Marenzi et al (32) showed that in a group of 780 STEMI patients undergoing primary percutaneous coronary intervention acute hyperglycaemia is an independent predictor of contrast induce nephropathy and increases in-hospital mortality (38%). Similar impact of hyperglycaemia on the risk of contrast induced nephropathy was observed in the study by Stolker et al (33) and by Naruse et al (34) among patients

undergoing emergency coronary angiography. However, no study has so far investigated the relationship between glycated haemoglobin (that is a more stable parameter and therefore better representative of chronic glycemic control) and contrast induced nephropathy in patients without diabetes. In our population of patients we demonstrated a significant relationship between glycated haemoglobin levels and the contrast induced nephropathy percentage that was confirmed at multivariate analysis after correction for baseline confounding factors. Interestingly, patients of the third and fourth quartiles had glycated haemoglobin levels suggesting for a pre-diabetic condition. As diabetes is one of the most important risk factor for the development of contrast induced nephropathy, this observation reinforced our findings and supports the importance of a correct management of this high risk subgroup of patients. On the other side, no relationship was found between fasting glycaemia and the occurrence of contrast induced nephropathy. Several factors may explain the absence of any relationship between glycaemia and contrast induced nephropathy. First of all only a minority of patients underwent primary angioplasty (therefore angiography and angioplasty soon after admission). In addition, while acutely elevated glycaemia may also be a surrogate marker of impaired haemodynamic instability, glycated haemoglobin represents a better reliable marker of chronic glycaemic control. Furthermore, we excluded patients with diabetes and therefore those patients expected to have higher fasting glucose levels or glucose at admission. In fact, it is well known that diabetes is associated with increased risk of contrast induced nephropathy. Finally, HbA^{1c} could be considered a good marker for glycated proteins, which plays a contributory role in atherosclerosis (35, 36) not only in patients with diabetes, but also in subjects without diabetes (37). This is supported

by the findings that even subjects that don't meet diagnostic criteria for diabetes with coronary artery disease have increased levels of HbA^{1c} (38).

Future large studies are certainly needed to confirm our findings and to evaluate the beneficial effects of additional strategies to prevent contrast induced nephropathy in this high-risk subgroup of patients.

Limitations

Even though the occurrence of contrast induced nephropathy is commonly evaluated at 48 hours, it may appear even later than this time threshold. Patients with elevated glycated haemoglobin had a higher baseline risk profile that may contribute to explain the potential association between such a parameter and the risk of CIN. In fact, even though glycated haemoglobin resulted to be an indipendet predictor of CIN, statistics can not completely overcome the observed differences in baseline characteristics between the groups. We observed a similar absolute reduction in creatinine values between baseline and 48 hours in the three groups. In fact, we have to realize that hydratation contributed to reduce overall values of creatinine, despite the occurrence of CIN in a minority of patients (10 to 15%). Therefore, the overall difference in change in creatinine levels among the groups should not be considered in the evaluation of association between Glycated haemoglobin and CIN. A larger population would have certainly improved our findings. Finally, we were not able to provide data on the progression of kidney failure at follow-up, being this disease chronically progressive.

Conclusions

This is the first study showing that among patients without diabetes undergoing coronary angiography or percutaneous interventions elevated glycated haemoglobin but not glucose levels is independently associated with the risk of contrast induced nephropathy.

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Chapter 5

Elevated Homocysteine and the risk of contrast-induced nephropathy: a cohort study

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Abstract

Contrast-induced nephropathy (CIN) is a common complication in patients with impaired kidney function undergoing coronary angiography/angioplasty. We evaluated whether elevated homocysteine (known to be associated with free radical generation and oxidative stress) increases the risk of CIN. Patients (n=876) with creatinine clearance <60 ml/min undergoing coronary angiography or percutaneous coronary intervention (PCI) were divided into tertiles of homocysteine levels. CIN was defined as ≥0.5 mg/dl or ≥25% creatinine increase 24-48h post-PCI. A significant relationship was observed between homocysteine levels and the risk of CIN (p=0.033), confirmed after correction for baseline confounding factors (adjusted odds ratio (OR) [95%CI] =1.68[1.09-2.59], p=0.019). This association was also significant applying the new definition of Contrast-Induced Acute Kidney Injury (11.9% Group 1, 10.4% Group 2 and 22.8% Group 3; p<0.001), adjusted OR [95% CI]

=1.96 [1.3-2.95], p=0.001). Future studies are needed to confirm our findings and to define the role of homocysteine in CIN.

Keywords: Contrast-induced nephropathy, percutaneous coronary intervention, homocysteine, creatinine, kidney function

Introduction

Due to the improvement in pharmacological therapies and mechanical devices[1-5], a growing number of patients are now revascularized percutaneously, with a larger proportion of high-risk patients, including those with impaired renal function. Therefore, considerable interest has focused on contrast-induced nephropathy (CIN), a common complication associated with using contrast media, especially in patients undergoing diagnostic and/or therapeutic coronary angiography, where it may occur in up to 20-25% of patients. The development of CIN after coronary angiography or angioplasty is associated with poor long-term clinical outcome[6]. Therefore, it is important to identify patients at risk to develop this iatrogenic complication and manage them appropriately. The pathogenesis of this condition is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species[7,8]. The incidence of CIN is related with the presence of risk factors, such as patient-related characteristics (i.e. age >75 years, diabetes mellitus, chronic congestive heart failure, or admission with acute pulmonary edema, hypotension, anemia and chronic kidney disease) and procedure-related characteristics (i.e. the use of elective intra-aortic balloon pump (IABP) or increased volumes of contrast media)[9]. Homocysteine (Hcy) may be a risk factor for atherothrombosis, mainly related to endothelial dysfunction, decreased bioavailability of nitric oxide and generation of free radicals and oxidative stress[10-14], mechanisms that are also involved in the occurrence of CIN. It follows that a potential link between elevated plasma Hcy levels and the risk of CIN may be hypothesized but has not been extensively evaluated. Therefore, we evaluated the association between elevated Hcy and the risk of CIN among patients undergoing coronary angiography and/or angioplasty.

Methods

Our population is represented by 1188 consecutive patients with chronic kidney disease (CKD; defined as estimated glomerular filtration rate (eGFR) of 60 mL/min or less, as calculated by the Cockroft-Gault formula) undergoing coronary angiography and/or angioplasty at the Catheterization Laboratory of AOU Maggiore della Carità, Novara, from January 2007 to September 2011, who were included in our registry on coronary artery disease (CAD). Informed consent was obtained from all patients before angiography. All demographic and clinical data were prospectively collected in a dedicated database. Hypertension was defined as systolic pressure > 140 mmHg and/or diastolic pressure was > 90 mmHg or if the individual was taking antihypertensive medications. The diagnosis of diabetes was based on previous history of diabetes treated with or without drug therapies. All patients were hydrated with saline solution 1 ml/kg/h 12h before and after the procedure or with saline solution 0.5 ml/kg/h (ejection fraction ≤ 40%) or with sodium bicarbonate (154 mEq/l in dextrose and water received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure) for emergency percutaneous coronary intervention (PCI). Metformin was discontinued at admission, as were diuretics and angiotensin converting enzyme (ACE) inhibitors, with the only exception of patients with heart failure. CIN was defined as an absolute ≥ 0.5 mg/dl or a relative ≥ 25% increase in serum creatinine level at 24 or 48h after the procedure. We additionally evaluated CIN according to the new Contrast Induced Acute Kidney Injury (CI-AKI) definition[15]. The Mehran risk score for prediction of CIN was calculated as previously described[9]

Biochemical measurements

Blood samples were drawn at admission in elective patients (following a fasting period of

12h) or urgent coronary angiography[16]. Glucose, creatinine, hemoglobin, white and red blood cells, uric acid and lipid profile were determined by standard methods. Plasma Hcy was measured using a competitive immunoassay with direct chemiluminescence detection. All the assays were performed on Siemens ADVIA Centaur using ADVIA Centaur HCY Ready Pack. The lower detection limit was 0.5 µmol/L. The upper limit for method linearity without dilution was 65 µmol/L. The analytical variability was 7%. Creatinine was measured at 12, 24 and 48 h after the procedure or longer in case of development of CIN.

Coronary angiography

Coronary angiography was routinely performed by the Judkins technique using 6-French right and left heart catheters through the femoral or radial approach. Quantitative coronary angiography (Siemens Acom Quantcor QCA, Erlangen, Germany) was performed by two experienced cardiologists who had no knowledge of the patients' clinical information. Significant CAD was defined as at least 1 coronary stenosis >50%. Severe multivessel disease was defined as 3-vessel disease and/or left main disease[17]. The contrast medium used was non-ionic, iso-osmolar (Optiray-Ioversolo, 350 mg/ml, Visipaque-Iodixanolo, 320 mg l/ml or Ultravist-Iopromide, 370 mg/ml).

Statistical analysis

Statistical analysis was performed using the SPSS 15.0 statistical package. Continuous data were expressed as mean $\pm$ SD and categorical data as percentage. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively. A trend analysis was performed as previously described[18]. Multiple logistic regression analysis was performed to evaluate the relationship between Hcy and CIN, after correction for baseline

confounding factors (clinical and demographic variables with a two-sided $p < 0.05$), that were entered in the model in block.

Table 1. Baseline Clinical Characteristics.

Variable	**Group 1** Homocysteine < 15.3 μmol/L	**Group 2** Homocysteine 15.3.21.7 μmol/L	**Group 3** Homocysteine > 21.7 μmol/L	P
Demographic and clinical characteristics				
Age (years) (Mean ± SD)	75.2 ± 7	75.3 ± 7.8	76.3 ± 6.6	0.059
Male Sex (%)	45.8	57.6	65.7	<0.001
Hypertension (%)	74	78.1	81.5	0.031
Smoking (%)	10.8	16.1	17.2	0.058
Hypercholesterolemia (%)	55.7	54.7	50	0.17
Diabetes (%)	39.3	36.1	39.5	0.93
Family history of CAD (%)	27.8	17.2	19.3	0.015
Previous AMI (%)	27.4	27.7	33.3	0.11
Previous PCI (%)	17.5	23.2	21.8	0.22
Previous CABG (%)	17.2	17.6	19.5	0.46
Previous CVA (%)	8.8	12.6	12.5	0.16
Indication for angiography				0.019
Stable angina or silent ischemia (%)	8.7	11.4	9.2	
Acute Coronary Syndrome (%)	75.8	62.4	63.7	
DCM or valvular disease (%)	15.5	26.2	27.1	
Baseline Chemistry				
White blood cell count (10^5/ml) (Mean ± SD)	8.0 ± 4	7.9 ± 3.7	7.5 ± 2.1	0.13
Red blood cell count (10^6/μl) (Mean ± SD)	4.3 ± 0.5	4.4 ± 0.6	4.3 ± 0.5	0.45
Hemoglobin levels (g/dL) (Mean ± SD)	12.6 ± 1.5	12.7 ± 1.7	12.6 ± 1.9	0.65
Platelet count (10^5/ml) (Mean ± SD)	215 ± 68	217 ± 65	214 ± 72	0.92
Fibrinogen (mg/dL) (Mean ± SD)	473 ± 156	487 ± 156	492 ± 143	0.14
Glycemia (mg/dL) (Mean ± SD)	136 ± 62	123 ± 42	130 ± 59	0.15
Glycosylated Hemoglobin (%)(Mean ± SD)	6.3 ± 1.2	6.3 ± 1.1	6.3 ± 1.3	0.92
Basal creatinine (mg/dL) (Mean ± SD)	1.1 ± 0.33	1.3 ± 0.3	1.5 ± 0.5	<0.001
eGFR (ml/min) (MDRD)	58.6 ± 14.8	53.9 ± 13.9	47.9 ± 15.4	<0.001
eGFR (ml/min) (MDRD) 24h	63.5 ± 27.7	55.6 ± 14.9	51.5 ± 17.6	<0.001
eGFR (ml/min) (MDRD) 48h	55.7 ± 21.6	51 ± 15.6	45.5 ± 19.1	<0.001
Total-cholesterol (mg/dL) (Mean ±	156 ± 38	157 ± 38	157 ± 40	0.70

SD)				
HDL-Cholesterol (mg/dL) (Mean ± SD)	40 ± 11	40 ± 13	39 ± 13	0.20
Triglycerides (mg/dL) (Mean ± SD)	129 ± 66	138 ± 79	145 ± 76	0.012
LDL-Cholesterol (mg/dL) (Mean ± SD)	90 ± 33	91 ± 32	91 ± 33	0.76
Treatment at admission				
ACE Inhibitor (%)	57.1	60.8	56.8	0.91
Angiotensin-receptor blockers (%)	21.3	22.8	26.4	0.15
Statins (%)	51.3	51.3	40.6	<0.001
Nitrate (%)	47.3	44	47.5	0.93
Beta-blockers (%)	48	51	49.2	0.79
Aspirin (%)	55.6	61.1	57.8	0.62
Clopidogrel (%)	42.4	41.2	30.1	0.014
Calcium Antagonist (%)	20.9	26.5	27.4	0.076
Diuretics (%)	35.4	45.3	51.2	<0.001
Procedural characteristics				
Coronary Angioplasty	45.4%	42.9%	43.3%	0.63
Contrast Volume (ml) (Mean ± SD)	216 ± 145	206 ± 136	218 ± 147	0.59
Contrast Volume (CA) (ml) (Mean ± SD)	138.8 ± 66.2	147.6 ± 86.6	141.3 ± 86.8	0.60
Contrast Volume (PCI) (ml) (Mean ± SD)	322.9 ± 158	269 ± 139.4	327.6 ± 152	0.50
Mehran Risk Score	11 ± 4	10.8 ± 4.5	12.2 ± 4.2	<0.001
Risk of CIN	24.2 ± 14.6	24.5 ± 16.1	28.4 ± 16.6	0.002

CAD = Coronary artery disease; AMI = Acute Myocardial Infarction; PTCA = Percutaneous Transluminal Coronary Angioplasty; CABG: Coronary Artery Bypass Grafting; CVA = Cerebrovascular Accident; DCM = Dilated Cardiomyopathy; ACE = angiotensin converting enzyme; LDL = low density lipoprotein; HDL = high density lipoprotein; eGFR = estimated glomerular filtration rate; CA = coronary angiography; PCI = percutaneous coronary intervention

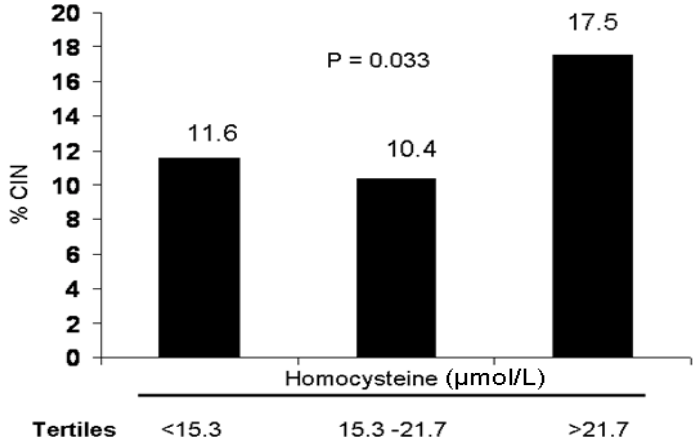
Results

Our initial population included 1188 consecutive patients with CKD. A total of 312 were excluded because of the following reasons: 136 patients because serial serum creatinine measurements (at baseline, 24 or 48h) were not available; 49 patients for end stage renal failure requiring dialysis and 127 patients because of unavailability of serum Hcy levels. Therefore, our final study population included 876 patients.

Baseline clinical characteristics, indication for angiography, procedural characteristics, baseline chemistry and admission therapy, according to tertiles (Group 1, <15.3; Group 2, 15.3-21.7, Group 3 >21.7 μmol/l) of plasma Hcy levels, are listed in Table 1.

Patients with higher Hcy levels were predominantly males ($p < 0.01$), with higher prevalence of hypertension ($p = 0.031$), higher baseline creatinine ($p < 0.01$), lower eGFR at baseline ($p < 0.01$) and higher triglycerides ($p < 0.01$), but with lower prevalence of family history of CAD ($p = 0.015$). In addition, elevated Hcy was more often associated with dilated cardiomyopathy/valvular disease as indication for angiography ($p = 0.019$) and diuretics at admission ($p < 0.001$), but less often were on statins ($p < 0.001$) and clopidogrel ($p = 0.014$) at admission. No differences were found in any other biochemical parameters, clinical characteristics, treatment or procedural characteristics, and contrast agent volume (both in case of coronary angiography alone or followed by PCI). Of the 116 out of the 876 patients developed CIN (13.2%); the incidence of CIN was significantly different among groups (17.5% in Group 3 *vs* 10.4% in Group 2 and 11.6% in Group 1; p=0.033) (Figure 1).

Figure 1. Bar graph showing the relationship between homocysteine and the risk of contrast-induced nephropathy.



The association between elevated Hcy levels (third tertile) and the occurrence of CIN was confirmed by multivariate analysis after correction for baseline confounding factor (gender, hypertension, family history of CAD, basal creatinine, creatinine clearance, triglycerides, statins, clopidogrel and diuretics, dilated cardiomyopathy or valvular disease as indication for angiography and Mehran Risk Score) (adjusted OR [95% CI] = 1.68 [1.09-2.59], p = 0.019). The association between elevated Hcy levels and the occurrence of CIN was confirmed also when applying the new definition of CI-AKI according to current K-DIGO (Kidney Disease Improving Global Outcomes) guidelines (11.9% Group 1, 10.4% Group 2 and 22.8% Group 3; p < 0.001), and after correction for baseline confounding factors (adjusted OR [95% CI] = 1.96 [1.3-2.95], p = 0.001).

Discussion

Our main finding is that elevated Hcy is independently associated with the risk of CIN among patients with impaired renal function undergoing coronary angiography and/or angioplasty. Due to the great improvement in pharmacological therapy and interventional procedures, most of the patients with CAD are currently revascularization percutaneously, especially in the setting of acute coronary syndromes [19-23], with a growing proportion of high-risk patients, including those with impaired renal function[5]. Considerable interest has focused on the risk of CIN, which is a common complication among patients undergoing coronary angiography and/or angioplasty. In fact, the higher complexity of patients currently undergoing angioplasty implies the presence of more clinical risk factors for such a complications, in addition to use of larger amount of contrast media[24]. Several studies have described an occurrence of CIN in up to 20-25% of interventions[9]. Advanced age,

diabetes, poor renal function, use of diuretics, oral antidiabetic therapies, cardiogenic shock and the amount of contrast media, are some of the recognized risk factors for such a complications[25]. In 2004 Mehran et al[9] proposed a CIN risk stratification score based on 8 readily available variables to better identify patients at high risk of CIN. In fact, the identification of subjects at high-risk for CIN and the identification of new pre-treatment strategies beyond hydration, administration with acetylcysteine[26-27], is of extreme importance in order to reduce the risk of this complication and to further improve clinical outcome of patients undergoing such a procedure. The pathogenesis of CIN is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species. In patients with CKD, identified by an eGFR <60 ml/min/1.73m^2 there is a loss of nephron units and residual renal function is vulnerable to decline. Contrast agents may trigger the release of endothelin and adenosine from endothelial cells, increasing vasoconstriction, and decrease the release of prostaglandins, preventing vasodilatation, thus decreasing oxygen in the outer medulla. In fact, adenosine can have a vasoconstrictor effect in the afferent arteriole, unlike the vasodilatation it causes in larger arterioles located in the coronary and peripheral vasculature[28,29]. Hcy is metabolized by the kidney, so its plasma levels are correlated with renal function (Hcy levels are 2 to 4-fold higher in patients with CKD)[10-14]. Experimental studies have shown that hyperhomocysteinemia is associated with elevated risk of atherosclerosis and vascular damage, mediated by endothelial dysfunction, decreased bioavailability of nitric oxide, generation of free radicals and oxidative stress[11]. On the basis of these assumptions an association between hyperhomocysteinemia and the occurrence of CIN may be conceivable. So far only one study [30] has investigated the relationship between

Hcy and the risk of CIN. In a population of 572 patients undergoing PCI, the incidence of CIN was significantly greater in patients in the third Hcy tertile (24.2 *vs* 4.7% in the first and 7.3% in the second tertile, $p < 0.001$). In our study, the largest so far, we included 872 patients, undergoing coronary angiography and/or intervention. Differently from Seung et al[30], we included patients undergoing coronary angiography and/or angioplasty, but focused only on patients with baseline impaired renal function. Like Seung et al[30], we divided our population into Hcy tertiles and found a significantly higher occurrence of CIN in the third tertiles (> 21.7 μmol/L) as compared with patients in the second (15.3-21.7 μmol/L) and first tertile (< 15.3 μmol/L) (17.5, 10.4 and 11.6%, $p = 0.033$). The relationship between elevated Hcy and the risk of CIN was confirmed at multivariate analysis after correction for baseline confounding factors. The association between elevated Hcy levels and the occurrence of CIN was similarly observed when applying the new CI-AKI definition according to current K-DIGO guidelines[15]. The association was confirmed at multivariate analysis after correction for baseline confounding factors, including the Merhan Risk Score. Future large studies are needed to confirm our findings. Lowering Hcy levels has not been proven to reduce cardiovascular events[31]. However, due to safety and low costs of vitamin-based Hcy-lowering therapy[32,33], future randomized trials are needed to evaluate whether in an elective context, Hcy-lowering therapy (e.g. started in the week before the procedure) may lower the risk of CIN among patients with elevated Hcy and impaired renal function undergoing coronary angiography and/or angioplasty.

Limitations

Our population is represented by patients with impaired renal function (creatinine clearance $\leq$ 60 ml/min). CKD is the most important risk factor for the development of CIN, and the vast majority of trials focus on this kind of population for the evaluation preventive strategies, like hydration and N-acetylcysteine administration. This decision also allowed us to have a more homogeneous population. Patients of the Third Tertile were more often on statins at admission and several studies in literature evaluated the protective effects of statin[34-36] therapy on the risk of CIN. However our results were confirmed after correction for all baseline confounding factors, including pretreatment with statins. Even though the occurrence of CIN is commonly evaluated at 48 h, it may appear even later than this time limit. Furthermore, we were not able to provide data on the progression of kidney failure at follow-up, although this disease may be chronically progressive. Finally, hydration and N-acetylcysteine has been shown to reduce Hcy levels[37]. However, all patients in the 3 groups underwent adequate hydration protocols and therefore this would not have affected our results.

Conclusions

This is the first large study showing that elevated Hcy is a risk factor for CIN and therefore more attention should be paid to these patients in order to provide optimal hydration and minimize contrast volume. Future randomized trials are needed to address whether in an elective context, lowering Hcy levels with vitamins may reduce the risk of CIN among patients with elevated Hcy.

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Chapter 6

Uric acid levels and the risk of Contrast Induced Nephropathy in patients undergoing coronary angiography or PCI

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Abstract

Background. Contrast Induced Nephropathy (CIN) is a common complication of procedures that foresee the use of contrast media that seems to be mediated by oxidative stress and reactive oxygen species generation. Hyperuricemia is characterized by inhibited nitric oxide system and enhanced synthesis of reactive oxygen species. However, few studies have so far investigated the association between hyperuricemia and CIN that is therefore the aim of the current study among patients undergoing coronary angiography or percutaneous intervention.

Methods. We analyzed a total of 1950 patients with Creatinine clearance <90ml/min) undergoing elective or urgent coronary angiography and/or angioplasty. Patients were divided according to tertiles of baseline uric acid (Group1, ≤ 5.5mg/dL n= 653; Group2, 5.6-7.0 mg/dL, n=654; Group 3, ≥ 7.0 mg/dL, n=643). CIN was defined as an absolute ≥

0,5mg/dl or a relative ≥ 25% increase in the serum creatinine level at 24 or 48h after the procedure.

Results. Patients with higher uric acid levels were older, previous smokers, with higher prevalence of hypertension and diabetes, but with lower family history of CAD. They had more often history of a previous CABG and baseline renal dysfunction. Patients of the third Tertile had also higher levels of white blood cells, higher triglycerides and lower HDL-cholesterol and higher percentage of dilated cardiomyopathy/valvular disease as indication for angiography and consequently a lower prevalence of PCI. Patients with higher SUA were more often on therapy with ACE inhibitors and diuretics, but less often with statins, nitrate, ASA and Clopidogrel at admission. The occurrence of CIN was observed in 251 patients (12.9%), and was significantly associated with uric acid levels (12.3% in Group 1, 10.4% in Group 2 and 16.0% in Group 3; p=0.04). Similar results were observed when the analysis was performed according to each tertiles values in both male and female gender. The association between elevated uric acid (≥ 7 mg/dl) and CIN was confirmed by multivariate analysis after correction for baseline confounding (Adjusted OR [95%CI] = 1.42 [1.04-1.93], p=0.026). Similar results were observed across major subgroups of high-risk patients, such as patients with diabetes, female gender, renal failure, hypertension, and elderly.

Conclusions. This is the first large study showing that among patients undergoing coronary angiography or percutaneous interventions elevated uric acid level is independently associated with an increased risk of CIN.

Keywords: Uric acid; contrast induced nephropathy

Introduction

Contrast Induced Nephropathy (CIN) is one of the most common complications of procedures that foresee the use of contrast media (1,2) and is commonly defined as an increase in serum creatinine levels, usually more than 0,5mg/dl or 25% of baseline levels, within 24-48h after contrast exposure. The development of CIN after coronary angiography or angioplasty is associated with a poor long-term clinical outcome and in particular patients who developed acute renal failure and required dialysis after PCI have a 40% in-hospital mortality and about an 80% 2-year mortality rate (3). In patients undergoing diagnostic and/or therapeutic coronary angiography CIN has shown to occur in up to 20-25% depending on the presence of known risk factors, such as chronic kidney disease, diabetes mellitus, accompanying hypotension, high dose of contrast medium, congestive heart failure, advanced age and anemia (4,5). In recent years, due to the improvement in device technology (6-8) and antithrombotic therapies (9-11), a yearly growing number of patients is now revascularized percutaneously, especially for acute myocardial infarction, with a larger proportion of high-risk patients, including those with impaired renal function. The pathogenesis of CIN is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species (12). The use of contrast media superimposed acute vasoconstriction, caused by the release of adenosine and endothelin, with the reduction in renal blood flow to the outer

medulla, consequent medullary hypoxia, ischemic injury and death of renal tubular cells (13).

Serum uric acid (SUA), a degradation metabolite of purines, has been extensively addressed in the past years as a possible risk factor for cardiovascular disease (14).

Hyperuricemia is characterized by inhibited nitric oxide system, activation of the local rennin-angiotensin system, pro-inflammatory and proliferative actions and enhanced synthesis of reactive oxygen species with increased oxidative stress and consequent renal dysfunction (15,16). These effects of hyperuricemia should be more evident in patients with chronic kidney disease, in which there is a loss of nephron units with a poor residual renal function more vulnerable to external insults. So far few studies have investigated the role of uric acid in the occurrence of CIN. Therefore, the aim of the current study was to evaluate whether high level of serum uric acid in patients undergoing elective coronary angiography, percutaneous intervention or urgent PCI, is associated with an increased risk of CIN.

Material and Methods

Our population is represented by consecutive 1950 patients with estimated glomerular filtration rate (GFR) of 89 mL/min or less, as calculated by applying the Cockroft-Gault formula) (17) undergoing coronary angiography and/or angioplasty at Catheterization Laboratory of AOU Maggiore della Carità, Novara, from January 2007 to September 2011, who were included in our registry on coronary artery disease protected by password. Informed consent was obtained by all patients before angiography. Hypertension was defined as systolic pressure > 140 mm Hg and/or diastolic pressure > 90 mm Hg or if the individual was taking antihypertensive medications. All patients with creatinine clearance

<60ml/min were hydrated with saline solution 1ml/kg/h 12h before and after the procedure or with saline solution 0.5ml/kg/h, if ejection fraction ≤ 40% or with sodium bicarbonate (154 mEq/l in dextrose and water received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure) for emergency PCI. CIN was defined as an absolute ≥ 0.5mg/dl or a relative ≥ 25% increase in the serum creatinine level at 24 or 48h after the procedure.

Biochemical measurements

Blood samples were drawn at admission in patients undergoing elective (following a fasting period of 12 h) or urgent coronary angiography. Glucose, creatinine, uric acid, blood cells count and lipid profile were determined by standard methods.

Creatinine was measured at 12, 24 and 48 hours after the procedure or longer in case of development of CIN.

Coronary angiography

Coronary angiography was routinely performed by the Judkins technique using 6-French right and left heart catheters through the femoral or radial approach. Quantitative coronary angiography (Siemens Acom Quantcor QCA, Erlangen, Germany) was performed by two experienced cardiologists who had no knowledge of the patients' clinical information. Significant coronary artery disease was defined as at least 1 coronary stenosis more than 50%. The contrast medium used was non-ionic, iso-osmolar (Optiray-Ioversolo, 350mg/ml, Visipaque-Iodixanolo, 320mg l/ml, Ultravist-Iopromide, 370mg/ml).

Statistical analysis

Statistical analysis was performed with the SPSS 15.0 statistical package. Continuous data were expressed as mean $\pm$ SD and categorical data as percentage. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively. A trend analysis was performed as previously described (18). Multiple logistic regression analysis was performed to evaluate the relationship between serum uric acid levels and CIN, after correction for baseline confounding factors (clinical and demographic variables with a p value < 0.05), that were entered in the model in block.

Results

We analyzed a total of 2025 patients with chronic kidney disease undergoing coronary angiography and/or angioplasty. A total of 75 patients were excluded because of end stage renal failure requiring dialysis or because baseline uric acid levels were not available. Therefore our final study population was represented by 1950 patients. Patients were divided according to tertiles of baseline serum uric acid (Group1, $\leq$ 5.5mg/dL n = 653; Group2, 5.6-7.0 mg/dL, n = 654; Group 3, $\geq$ 7.0 mg/dL, n = 643). Patient's baseline clinical and demographic characteristics, indication for angiography, procedural main characteristics, baseline chemistry and admission therapy, according to the tertile of serum uric acid levels, are listed in Table 1.

Table 1. Baseline Clinical Characteristics according to uric acid levels

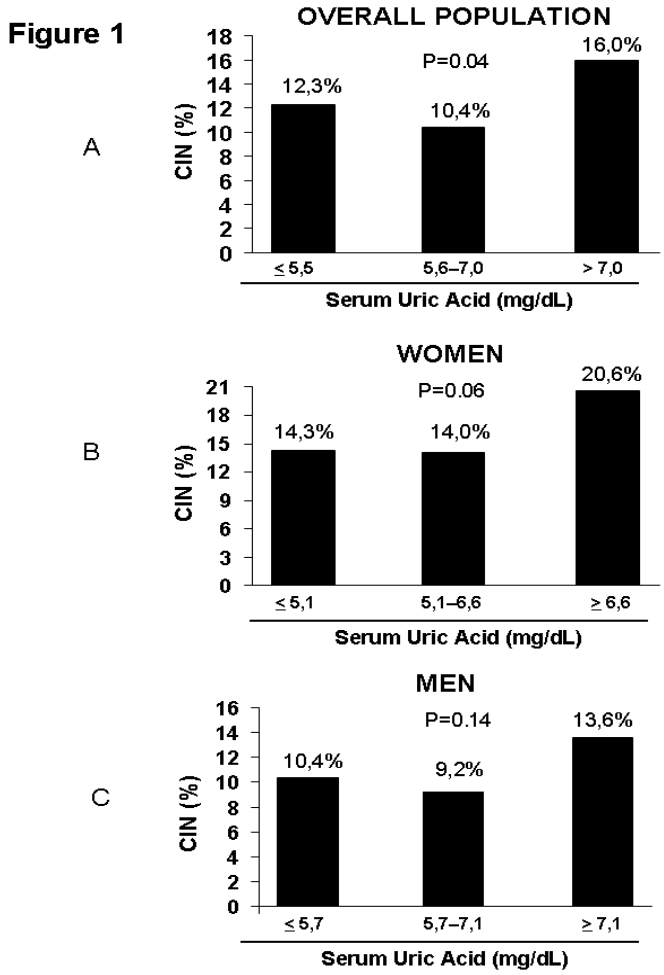
Variable	1° Tertile ≤ 5.5 mg/dL (n = 653)	2° Tertile 5.6-7.0 mg/dL (n = 654)	3° Tertile ≥7 mg/dL (n = 643)	p-value
Demographic and clinical characteristics				
Age (M-SD)	71.6+/-9.1	72+/-8.7	72.8+/-8.4	0.01
Age > 75 (%)	42.6	41.3	48.2	0.04
Hypertension (%)	72	73.1	82.1	<0.001
Smokers (%)				0.03
Active smokers (%)	35.2	33.3	32.3	
Previous smokers (%)	49.6	57.4	57.5	
Hypercolesterolemia (%)				
Diabetes (%)	31.9	37.8	42.9	<0.001
Family history of CAD (%)	28.2	25.7	22.6	0.02
Previous AMI (%)	26.2	26.8	27.2	0.67
Previous PCI (%)	21.9	24.2	20.4	0.51
Previous CABG (%)	13.6	14.4	18	0.03
Previous CVA (%)	8.7	8.4	9.6	0.57
Cr. Clearance<60ml/min (%)	45.8	50.3	68.4	<0.001
Indication for angiography				<0.001
Stable angina or silent ischemia (%)	28	29.5	20.7	
Acute Coronary Syndrome (%)	55.9	52.9	48.5	
DCM or valvular disease (%)	16.1	17.6	30.8	
Baseline Chemistry				
White blood cells (M-SD)	7.4+/-3.1	7.9+/-4	8+/-2.7	<0.001
Red blood cells (M-SD)	4.4+/-0.5	4.5+/-0.5	4.5+/-0.6	0.46
Haemoglobin (M-SD)	13.1+/-1.6	13.2+/-1.6	13.1+/-1.9	0.58
Platelet (M-SD)	216.2+/-62.7	214.1+/-72	218.9+/-72.2	0.5
Glycaemia at admission (M-SD)	127.2+/-49	123.07+/-42	131.8+/-59	0.1
Total Cholesterol (M-SD)	158.7+/-39.9	159.3+/-39.7	159.2+/-39.1	0.84
HDL-Cholesterol (M-SD)	43.4+/-13.4	40.4+/-12.2	38.6+/-13	<0.001
Triglycerides (M-SD)	126.6+/-62.8	138.8+/-77.8	154.1+/-101.7	<0.001
LDL-Cholesterol (M-SD)	90.5+/-35.2	91.7+/-33.3	91.3+/-32.4	0.7
Theraphy at admission				
ACE I (%)	36.6	42	42.6	0.03
ARB (%)	21.7	20.9	22.9	0.63
Statins (%)	52.8	52.4	44.2	0.002
Nitrate (%)	44	41	36.9	0.009
Beta-Blockers (%)	53.8	52.3	50.7	0.27
ASA (%)	62	61.6	53.8	0.003

Clopidogrel (%)	24.7	24.2	20.1	0.05
Ticlopidine (%)	9.5	6.9	8.4	0.47
Calcium Antagonist (%)	21.7	20.8	26.1	0.06
Diuretics (%)	24.7	32.6	51.8	<0.001
Allopurinol (%)	5.5	4.7	5.4	0.95
Procedural characteristics				
PCI (%)	49.2	49.8	42	0.01
Contrast Agent (M-SD)	229+/-144	231+/-143.4	219.4+/-147.9	0.25

M = Mean; SD = Standard Deviation; CAD = coronary artery disease; AMI = acute myocardial infarction; PCI = percutaneous coronary intervention; CABG = coronary artery by-pass graft; CVA = cardiovascular accident; DCM= dilated cardiomyopathy; HDL = high density lipoprotein; LDL = low density lipoprotein; ACE = angiotensin converting enzyme; ARB = angiotensin II receptor blockers; ASA = acetylsalicylic acid

Patients with higher uric acid levels were older (p=0.01), more often previous smokers (p=0.03), with higher prevalence of hypertension (p<0.001) and diabetes (p<0.001), but with lower family history of CAD (p=0.02). They had more often history of previous CABG (p=0.03) and baseline renal dysfunction (Creatinine clearance <60ml/min, p<0.001). Patients of the third Tertile had also higher levels of white blood cells (p<0.001), higher triglycerides (p<0.001) and lower HDL-cholesterol (p<0.001) and higher percentage of dilated cardiomyopathy/valvular disease as indication for angiography (p<0.001) and consequently a lower prevalence of PCI (p=0.01). Patients with higher SUA were more often on therapy with ACE inhibitors (p=0.03) and diuretics (p<0.001), but less often with statins (p=0.002), nitrate (p=0.009), ASA (p=0.003) and Clopidogrel (p=0.05) at admission. No differences were found in any other biochemical parameters, clinical characteristics, therapy (in particular regarding use of uric acid lowering therapy) or procedural characteristics, and contrast agent volume (both in case of coronary angiography alone or followed by PCI).

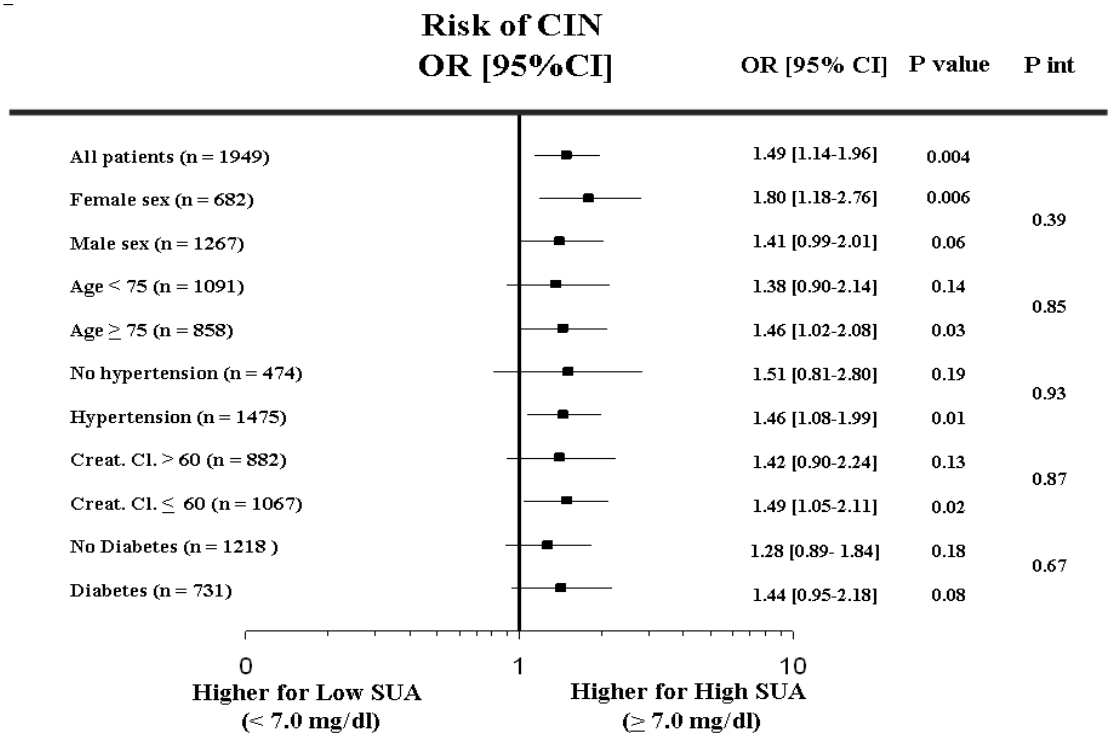
Figure 1. Bar graph showing the relationship between uric acid level and the risk of contrast-induced nephropathy in all patients (Graph A), in women (Graph B) and men (Graph C).



CIN was observed in 251 patients (12.9%), and was significantly associated with uric acid levels (12.3% in Group 1, 10.4% in Group 2 and 16.0% in Group 3; p=0.04, Figure 1A). Similar results were observed when the analysis was performed according to each tertiles values in both female and male gender (Figures 1B-1C). The association between elevated uric acid ($\geq$ 7 mg/dl) and CIN was confirmed by multivariate analysis after correction for baseline confounding factors (age, hypertension, smoking, diabetes, previous CABG, creatinine clearance <60ml/min, family history of CAD, white blood cells, HDL-cholesterol, triglycerides, ACE inhibitors, ASA, Clopidogrel, Statins, Nitrate and Diuretics, dilated cardiomyopathy or valvular disease as indication for angiography) (Adjusted OR

[95%CI] = 1.42 [1.04-1.93], p=0.026). In fact, as shown in Figure 2, consistent results were observed across major subgroups of high-risk patients, such as patients with diabetes, renal failure, hypertension, age>75.

Figure 2. Relationship between glycated haemoglobin levels and the risk of contrast induced nephropathy (CIN) in major subgroups of patients according to gender, age, hypertension, baseline renal failure and diabetes.



Discussion

The main finding of our study is that in patients undergoing coronary angiography or percutaneous interventions, serum uric acid level is independently associated with an increased risk of CIN. Coronary artery disease is still the first cause of mortality in developed countries. However, a larger application of revascularization procedures, especially in the setting of acute myocardial infarction (19, 20), has contributed to the relevant reduction in mortality observed in the last decades. Due to the improvement in stent

technology (6-8), a yearly growing number of patients undergo percutaneous revascularization, with a larger proportion of high-risk patients, including those with impaired renal function. The development of CIN is associates with increased mortality and morbidity rate and is a costly complication (3). Several mechanism have been suggested as etiologic factors for CIN, such as chronic kidney disease, diabetes mellitus, accompanying hypotension, high dose of contrast medium, congestive heart failure, advanced age and anemia (4, 5). The pathophysiology of CIN is complex and multifactorial (21). Injury starts with a critical illness affecting the kidneys, then a contrast agent causes direct cytotoxicity to renal tubular cells because of water solubility. The use of contrast media superimposed acute vasoconstriction, caused by the release of adenosine and endothelin, with the reduction in renal blood flow to the outer medulla, consequent medullary hypoxia, ischemic injury and death of renal tubular cells (12). Prevention is the key to reduce the incidence of CIN and it begins with the identification of the high risk patient coupled with appropriate periprocedural management (hydratation and administration with acetylcysteine) (22). Serum uric acid is the final product of purine metabolism, so hyperuricemia may occur because of decreased excretion, increased production or a combination of these two mechanism (23). Hyperuricemia is characterized by inhibited nitric oxide system, activation of the local rennin-angiotensin system, pro-inflammatory and proliferative actions and enhanced synthesis of reactive oxygen species with increased oxidative stress and consequent renal dysfunction (16). These effects should be more evident in patients with chronic kidney disease, in which there is a loss of nephron units with a residual renal function more vulnerable to external insults. Several studies in literature have shown that elevated uric acid level is associated with cardiovascular events, stroke and with the

development of chronic kidney disease in type II diabetes mellitus (24). On the other side uric acid has antioxidant properties, which are both direct and indirect: it acts by promoting superoxide dismutase activity, by removing peroxynitrite and O_2 and by strengthening the antioxidant action of ascorbate with a reduction of lipid peroxidation. It was demonstrated that subjects with atherosclerosis had higher serum antioxidant capacity than matched controls (25). Furthermore, uric acid seems to restore endothelial function in type 1 diabetes (2). In fact, we previously found no association between uric acid and the extent of CAD (26). On the basis of this assumptions it might be supposed that the role of uric acid in the development of CIN could potentially represent an association with a complex clinical condition. Few studies has so far investigated the role of hyperuricemia as a predictor of CIN. Two studies in the past evaluated the relationship between contrast agents and uric acid, showing that contrast agents have a uricosuric effect caused by enhanced renal tubular secretion of uric acid with possible nephrotoxic effect (27,28).

Toprak et al. (29) showed that, among 266 patients undergoing coronary angiography, there was a significantly higher incidence of CIN in the hyperuricemic group (15.1%) vs normouricemic group (2.9%). Elevated SUA has been reported in literature as an independent predictor of CIN in STEMI patients who underwent primary PCI (15). Two recent studies, (14, 30), the first retrospective and the second prospective, showed that STEMI patient (744 and 835, respectively) who underwent primary PCI and developed CIN (incidence 12.5% and 9.6%respectively) had higher level of uric acid in comparison to patients who did not develop CIN. This is the largest study so far conducted to investigate the potential association between elevated uric acid and the occurrence of CIN. Our population was represented by patients with impaired renal function that is known as the

most important risk factor for the development of CIN. We found an occurrence of CIN of 12.9%, with a significant correlation with uric acid levels, that was confirmed at multivariate analysis after correction for all baseline confounding factors. Similar results were observed when the analysis was performed according to each tertiles values in both female and male gender and across major subgroups of high-risk patients, such as patients with diabetes, renal failure, hypertension, age>75. Future studies are certainly needed to confirm our findings and to evaluate the beneficial effects of uric acid reduction and additional strategies to prevent CIN in this high-risk subgroup of patients.

Limitations

Even though the occurrence of CIN is commonly evaluated at 48 hours, it may appear even later than this time threshold. Furthermore, we were not able to provide data on the progression of kidney failure at follow-up, being this disease chronically progressive.

Conclusions

This is the first large study showing that among patients undergoing coronary angiography or percutaneous interventions elevated uric acid level is independently associated with the risk of CIN.

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Chapter 7

The role of Statins in the prevention of Contrast Induced Nephropathy: A Meta-analysis of 8 randomized trials

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Abstract

Background. Contrast Induced Nephropathy (CIN) is a common complication of coronary angiography/angioplasty. Prevention is the key to reduce the incidence of CIN and it begins with appropriate pre-procedural management. Statins have been shown to possess pleiotropic effects (anti-oxidant, anti-inflammatory and anti-thrombotic properties) and their effects on CIN were assessed in several studies with conflicting results. Aim of this meta-analysis is to evaluate the efficacy of short-term statins for the prevention of CIN in patients undergoing coronary angiography/percutaneous interventions.

Methods. We performed formal searches of PubMed, EMBASE, Cochrane central register of controlled trials and major international scientific session abstracts from January 1990 to January 2014 of trials which compares short-term statins versus Placebo for the prevention of CIN in patients undergoing coronary angiography/angioplasty. Data regarding study design, statin dose, inclusion/exclusion criteria, number of patients, and clinical outcome was extracted by 2 investigators.

Results. Eight trials were included, with a total of 4734 patients. CIN occurred in 79/2358 patients (3.3%) treated with statins versus 153/2376 patients (6.4%) of the placebo group (OR[95%CI]=0.50[0.38-0.66],p<0.00001;p_{het}=0.39). Benefits were both observed with high-dose short-term statins (OR[95%CI]=0.44[0.30-0.65],p<0.0001;p_{het}=0.16) and low-dose statins, (OR[95%CI]=0.58[0.39-0.88],p=0.010;p_{het}=0.90). By meta-regression analysis, no significant relationship was observed between benefits from statin therapy and patient's risk profile (p=0.26), LDL cholesterol (p = 0.4), contrast volume (p = 0.94) or diabetes rate (p = 0.38).

Conclusions. This meta-analysis showed that among patients undergoing coronary angiography/percutaneous intervention the use of short-term statin reduces the incidence of CIN, and therefore is highly recommended even in patients with low LDL-cholesterol levels.

Keywords: Statins; contrast induced nephropathy; prevention

INTRODUCTION

Contrast-induced nephropathy (CIN) is a common complication of procedures that foresee the use of contrast media and is defined as an increase in serum creatinine levels more than 0,5mg/dl or 25% of baseline levels, within 24-48 hours after the procedure (1,2). CIN is the third leading cause of hospital-acquired acute renal injury, accounting for 11% of all cases (3) and in patients undergoing diagnostic and/or therapeutic coronary angiography, it has shown to occur in up to 20-25% depending on the presence of known risk factors, such as chronic renal insufficiency, diabetes mellitus and the dose of contrast medium (4,5). Due to the improvement in stent technology (6-10), a yearly growing proportion of patients is now revascularized percutaneously, with a larger proportion of high-risk patients, including those with impaired renal function that is the most important risk factor for the development of CIN. Therefore, large interest has been focused in the last years on this iatrogenic complication. Several therapies for the prevention of CIN, such as different hydration and alkalinization measures (11-12), N-acetylcysteine (NAC) (13), Fenoldopam, hemodyalisis and hemofiltration (14-16) have been explored in randomized clinical trials (17-18), leading to conflicting results. The pathogenesis of CIN is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species (1), therefore, pharmacological prophylactic strategies based on antioxidant properties have received considerable attention.

Statins have been shown to possess endothelial protective properties potentially preventing contrast induced nephropathy (19-20). A large number of studies in the last decades have evaluated the role of statins for the prevention of CIN in patients undergoing coronary angiography or percutaneous interventions with conflicting results (21-25). Therefore, aim

of the current meta-analysis is to assess the effectiveness of short term statin treatment for the prevention of CIN in patients undergoing coronary angiography or percutaneous interventions.

METHODS

Eligibility and search strategy. We conducted a search of PubMed, EMBASE, Cochrane central register of controlled trials and major international scientific session abstracts from January 1990 to January 2014 regarding the role of Statin for the prevention of CIN in patients undergoing coronary angiography or percutaneous interventions. Furthermore, oral presentations and/or expert slide presentations were included, searched on EuroPCR (www.europcr.com), ACC (www.acc.org), AHA (www.aha.org), and ESC (www.escardio.org) websites from January 2000 to January 2014. The following key words were used: “statin and CIN”, “acute kidney injury”, “contrast induced nephropathy”, “Atorvastatin”, “Rosuvastatin”, “Simvastatin”, “Pravastatin”, “acute coronary syndrome”, “coronary angiography”, “percutaneous interventions”. We included randomized controlled trials which compared a preventive strategy with short-term statin versus placebo for the prevention of contrast induced nephropathy in patients undergoing coronary angiography or percutaneous interventions. Studies comparing high-dose statins versus low-dose statins were excluded. We did not restrict eligibility according to kidney function.

Data extraction and validity assessment. Data were independently abstracted by two investigators, with a good agreement (κ statistic = 0.89). Disagreements were resolved by consensus. Data were managed according to the intention-to-treat principle.

Outcome measures. Primary endpoint was the development of CIN, defined as an increase of serum creatinine >0,5mg/dL or >25% within 48 to 120 hours after the exposure to contrast medium.

Data analysis. Statistical analysis was performed using the Review Manager 4.27 freeware package, SPSS 17 statistical package. Odds ratio (OR) and 95% confidence intervals (95% CI) were used as summary statistics. The pooled odds ratio was calculated by using a fixed effect model (The DerSimonian and Laird method). The Breslow-Day test was used to examine the statistical evidence of heterogeneity across the studies ($p<0.1$). Potential publication bias was examined by constructing a "funnel plot", in which sample size was plotted against odds ratios. The study quality was evaluated by two investigators according to a score, modified from Jadad et al. (26) and Biondi-Zoccai et al. (27), that was expressed on a ordinal scale, allocating 1 point for the presence of each of the following: 1) statement of objectives; 2) explicit inclusion and exclusion criteria; 3) description of intervention; 4) objective means of follow-up; 5) description of adverse events; 6) power analysis; 7) description of statistical methods; 8) multicenter design; 9) discussion of withdrawals; 10) details on medical therapy (e.g. hydration or NAC) during and after the procedure. A prespecified analysis was conducted according to the dosage of statins (high-dose, rosuvastatin 40 mg or Atorvastatin 80 mg, and non-high dose).

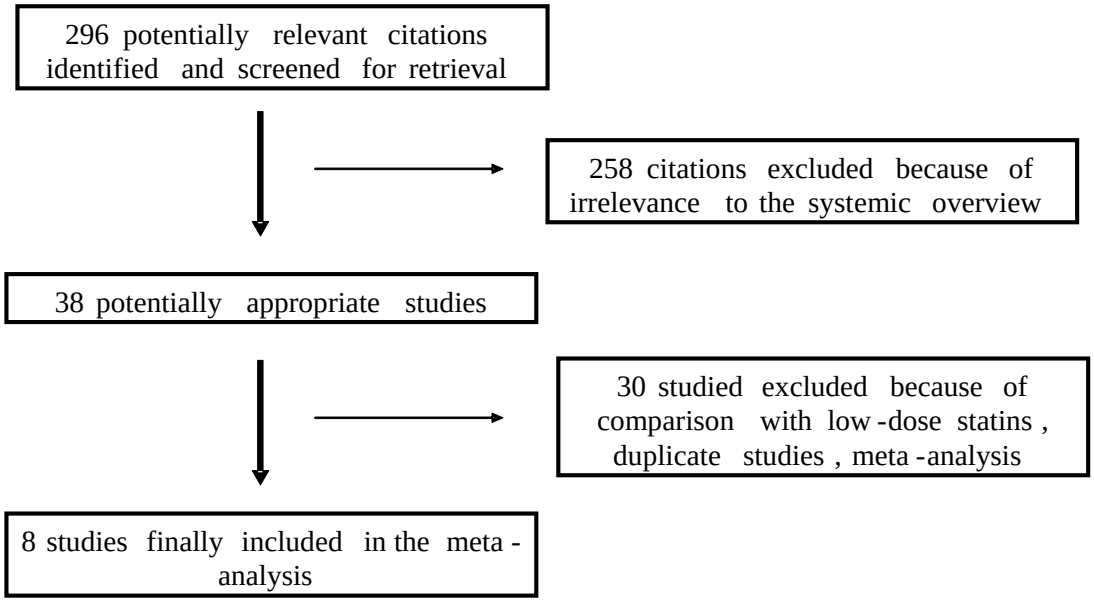
A meta-regression analysis was carried out to evaluate the relationship between benefits from statins and patient's risk profile (as evaluated by the ODDS of CIN in the control group), the amount of contrast, cholesterol LDL, and the rate of diabetes.

The study was performed in compliance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (28).

RESULTS

Eligible studies. Among 296 potentially relevant publications, 38 articles were considered of interest and reviewed in full text. Of these 30 studies were excluded because of 1) comparison between high vs with low-dose statins; 2) duplicate studies; 3) meta-analysis. Therefore a total of 8 trials (29-36) were finally included (Figure 1).

Figure 1. Flow diagram of the systematic overview process. RCT = Randomized controlled trials.



Five (29-31, 33, 35) were single center randomized trials and three (32, 34, 36) were multicenter randomized trials, with a total of 4734 patients of which 2358 (49,8%) patients randomized to receive statin treatment pre-procedure and 2376 (50,2%) to placebo. Clinical characteristics of included trials are shown in Table 1 and Table 2.

Table 1. Characteristics of included studies.

Study	N° Stat.	N° Cont.	Therapy Statins	Therapy Control	Hydration	AGE Stat.	AGE Cont	Hyper ten. (%) Stat.	Hyper ten. (%) Cont.	DM (%) Stat.	DM (%) Cont.	GEN M (%) Stat	GEN M (%) Cont.	Contr ast Stat.	Contr ast Cont.	Basal Creat	Basal Creat
Ozhan et. al	60	70	Atorvastatin 80mg (1day pre and 2days post procedure e)+NAC 600mgx2 (pre-procedure)	Placebo+ NAC 600mgx2 (pre-procedure)	SS 1000ml (6h after procedure)	54	55	25	20	15	17	62	57	97	93	0,88	0,88
Acikel et al.	80	80	Atorvastatin 40mg (3 days pre and 2days post-procedure)	-	SS 1ml/kg/h 4h pre and 24h post-procedure	59	61	60	56	24	25	64	64	105	103	0,84	0,85
Li et al.	78	83	Atorvastatin 80mg 2days pre and 2ays post-procedure+NAC 1200mg bid pre and post procedure	Placebo+NAC 1200mg bid 1day pre and 1day post procedure	SS 1ml/kg/h 12h pre and 12h post-procedure	66,3	65,4	78	83	27	29	74	77	100	103,6	0,94	0,94
PROMISS	118	118	Simvastatin 40mg (every 12h 1day pre and 1ay post procedure)	-	SS 1ml/kg/h 12h pre and 12h post-procedure	65	66,1	71	61	30	25	77	75	173,3	190,9	1,28	1,24
Toso et al.	152	152	Atorvastatin 80mg 2 days pre and 2 days post procedure+NAC 1200mg bid pre and post procedure	Placebo+NAC 1200mg bid 1day pre and 1day post procedure	SS 1ml/kg/h 12h pre and post procedure or 0,5ml/kg/h (EF≤40%)	75	76	63	59	20	22	68	61	151	164	1,2	1,18
Patti et al.	120	121	Atorvastatin 80mg (12h pre-procedure)+40mg (2h pre procedure) and 40mg (chronic)	Placebo	CsCl<60 SS 1ml/kg/h 12h pre and 24h post procedure	65	66	76	74	30	26	76	79	209	213	1,04	1,04
PRATO ACS	252	252	Rosuvastatin 40mg pre procedure and 20mg (chronic therapy)+NAC 2400mg/day	Placebo NAC 2400mg/day	SS 1ml/kg/h (0,5ml-EF<40%) 12h pre and post PCI	66,2	66,1	57	55	20	23	87	87	183	172	0,95	0,96
HAN et al	1498	1500	Rosuvastatin 10 mg 48h pre and 72 h post-procedure	Placebo	SS 1ml/kg/h 12h pre and 24h post procedure	61,4	61,4	71	73	-	-	963	991	120	110	1,08	1,08

Table 2. Characteristics of included studies.

Study	Type	Year of publication	Follow-up (H)	Definition of CIN	Inclusion Criteria	Exclusion Criteria	Quality Score
Ozhan et. Al	RCT-single centre	2010	48h	Increase of SCr>0,5mg/dL or >25% within 48h	1. CAG; 2. eGFR<70 or SCr>1,5mg/dl	1. Allergy for contrast agent	8
Acikel et al.	RCT-single centre	2010	48h	Increase of Scr>0,5mg/dL within 48h	1. CAG; 2. eGFR>60; 3. LDL<70mg/dL; 4. no cholesterol lowering medications	1. Dialysis; 2. Chronic liver disease or failure; 3. III-IV heart failure; 4. ACS; 5. Contrast exposure 3 months before CAG; 6. Active infections or systemic inflammatory disease; 7. Malignancies; 8. Hypo-hyperthyroidism; 9. Use of other antilipidemic theraphy, NAC, Theophylline, Aminophylline, FANS vitamin supplements, antibiotics or steroids	8
Li et al.	RCT-single centre	2012	72h	Increase of SCr>0,5mg/dL or >25% within 72h after PCI	STEMI patients presented within 12h of symptom onset undergoing emergency PCI	1. Current/previous (<3months) statin treatment; 2. renal or haepatic dysfunction; 3.Dialysis; 4. Prior fibrinolysis 5. Cardiogenic shock; 6. Unconsciousness; 7. Uncontroled hypertension (>200/120); 8. Stroke; 9. Recent major operation	9
PROMISS	RCT-2centres	2008	48h	Increase of Scr>0,5mg/dL or >25% within 48h	1. CAG 2. Scr≥1,1 or eGFR≤60; 3. Aged >19years	1. Pregnancy or lactation; 2. Administration of contrast media within 7days; 3. emergency CAG; 4. Acute renal failure; 5. Dialysis; 6. Allergy for contrast media; 7. Cardiogenic shock; 8. Pulmonary edema; 9. Myeloma; 10. mechanical ventilation; 11. Parenteral use of diuretics; 12. use of NAC or ascorbic acid, metformin or FANS within 48h; 13. No statin users (<30days).	10
Toso et al.	RCT-single centre	2009	120h	Increase of SCr≥0,5mg/dL within 5days	CAG and or PCI, CrCl<60ml/min	1. Current treatment with statins; 2. Controindication to statin treatment; 3. Previous contrast media administration (within 10 days); 4. Dialysis; 5. Refusal to sign informed consent	9
Patti et al.	RCT-multicentre	2011	48h	Increase of SCr>0,5mg/dL or >25% within 48h	Statins naive patients with NSTEMI-ACS and planned invasive strategies within 48h;	1. Emergency CAG; 2. Any baseline increase of liver enzymes; 3. EF<30%; 4. Hystory of liver or muscle diseases 5. Scr≥ 3mg/dL	10
PRATO ACS	RCT-single centre	2013	72h	Increase of Scr>0,5mg/dL or >25% within 72h	Statin naive NSTE-ACS patients	1. Emergency angiography; 2. Acute renal failure or dialysis; 3. Baseline SCr>3mg/dL; Controindication to statine treatment; 4. Contrast administration within 10 days	9
HAN et al	RCT-multicentre	2014	72h	Increase of Scr>0,5mg/dL or >25% within 72h	Patients with DM and CKD undergoing coronary/peripheral arterial diagnostic angiography, left ventriculography, or PCI	hypersensitivity to contrast medium or statins, type 1 DM, ketoacidosis, lactic acidosis, stage 0 or 1 CKD, stage 4 or 5 CKD, acute STEMI within 4 weeks, class IV heart failure (NYHA), hemodynamic instability, administration of contrast medium during the 2 weeks before, LDL-C <1.82 mmol/L, hepatic dysfunction, thyroid insufficiency, or renal artery stenosis.	10

Five studies (29,31,33-35) used therapy with high-dose statins (Atorvastatin 80 mg or Rosuvastatin 40 mg) while three studies (30,32,36) used low-dose statin therapy (Rosuvastatin 10mg, Simvastatin 40mg or Atorvastatin 40mg). The total duration of statin treatment before angiography ranged from 12 hours to 3 days. CIN was defined differently among the included studies. Four studies (29-30,32,34) used an increase in serum creatinine of >0,5mg/dL or >25% from baseline within 48 hours after procedure, other three studies (31, 35,36) used the same definition within 72 hours and one study (33) regarded an absolute increase in serum creatinine of 0,5 mg/dL within 5 days after contrast exposure. Three studies (32, 33, 36) included patients with Creatinine clearance < 60ml/min, one study (29) enrolled patients with creatinine clearance < 70ml/min or Serum creatinine > 1,5mg/dL, two studies (34, 35) excluded patients with serum creatinine > 3mg/dL and only one study (31) had no restrictions according to renal function. All studies enrolled patients undergoing coronary angiography or percutaneous interventions, focusing on STEMI patients in the study of Li et al (31), on NSTEMI-ACS patients in Patti at al. and in the PRATO-ACS trial (34-35) and in patients with diabetes and chronic kidney disease in the study by Han et al (36). All patients received low-osmolar or iso-osmolar contrast media and the mean contrast volume ranged from 93 to 213 ml. Peri-procedural hydration was administered in all patients except for the study of Patti et al. (34) in which hydration was administered only in patients with creatinine clearance < 60ml/min. Four studies (29, 31, 33, 35) used also N-acetylcysteine in association with hydration.

Statins and Risk of CIN

CIN was observed in 79 out of 2358 patients (3.3%) in the statin group versus 153 out of 2376 patients (6.4%) in the placebo group (OR [95%CI] = 0.50 [0.38 - 0.66], p<0.0001; p_{he} = 0.39) (Figure 2).

Figure 2. Statin vs placebo and occurrence of CIN, with odds ratios and 95% confidence intervals (CI). The size of the data markers (squares) is approximately proportional to the statistical weight of each trial.

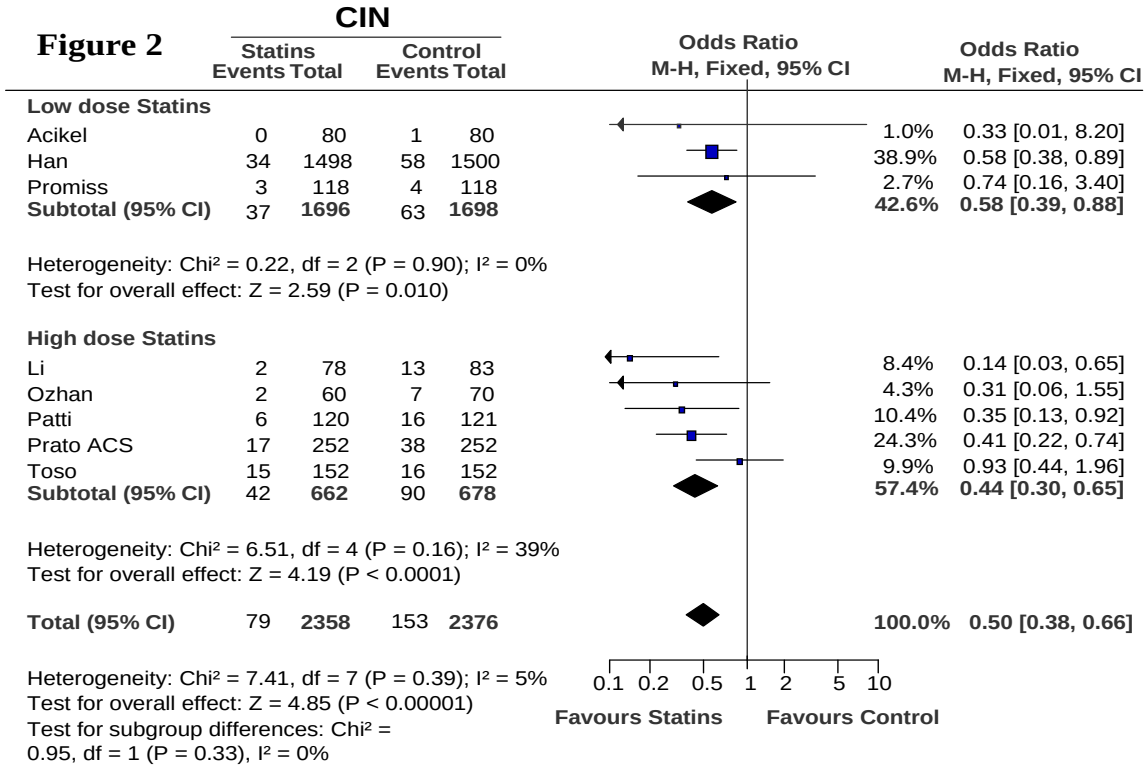
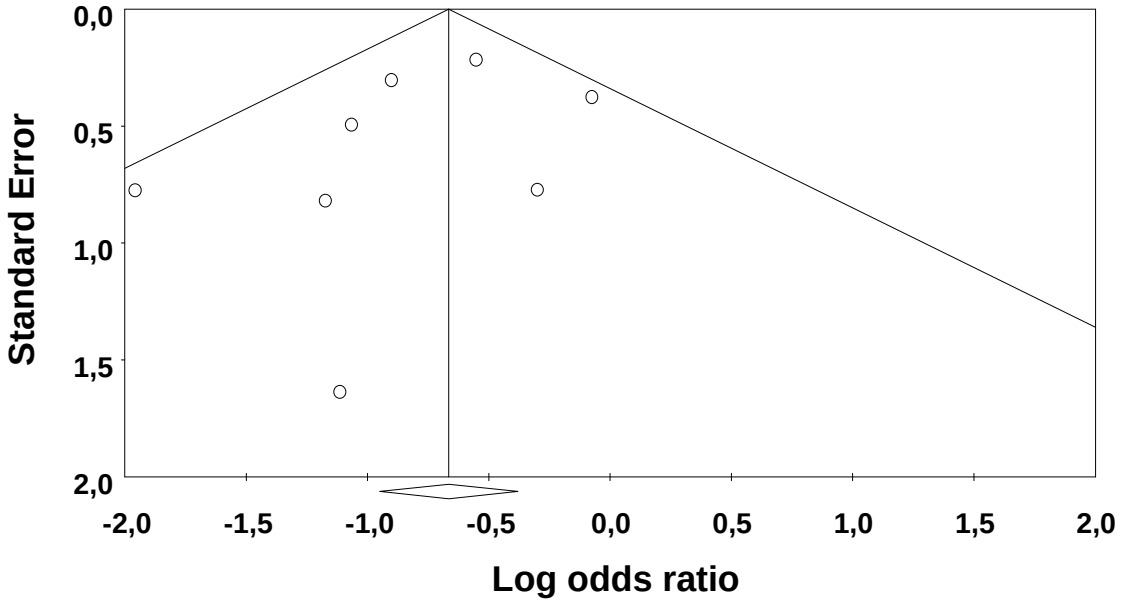


Figure 3. Funnel plot of all studies included in the meta-analysis. The Standard Error (SE) of the log Odds Ratio of each study was plotted against the Odds Ratio for CIN. No skewed distribution was observed, suggesting no publication bias.

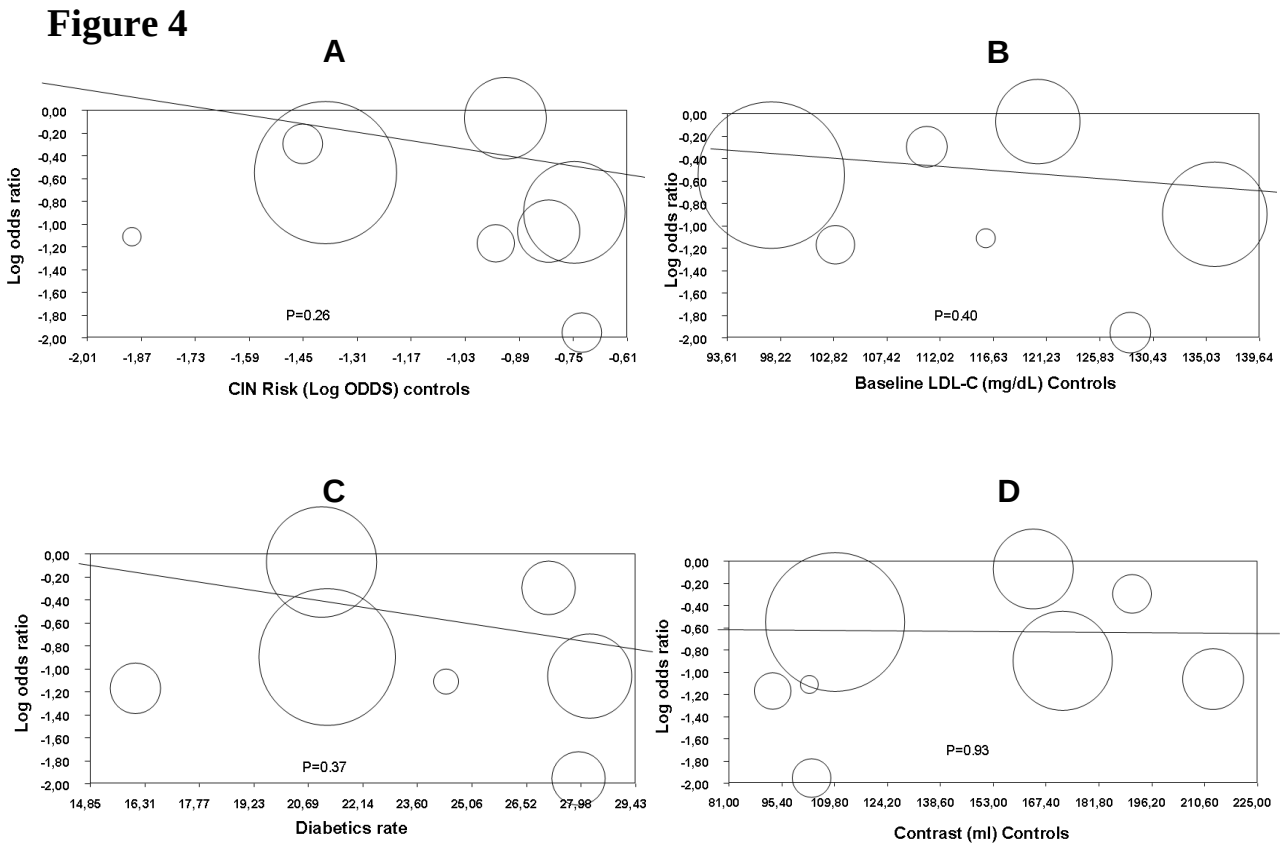


Benefits were both observed with high-dose short-term statin therapy (OR [95% CI] =0.44 [0.30-0.65], p<0.0001; p_{het} =0.16) and low-dose short-term statin therapy, (OR[95%CI]=0.58[0.39-0.88],p=0.01;p_{het}=0.90). By meta-regression analysis we did not find a relationship between benefits from statins and patient's risk profile (beta[95%CI] = -0.53[-1.47 – 0.40],p = 0.26) (Figure 4) (Table 3), contrast agent volume (beta [95% CI] = 0.0003 [-0.007 - 0.007], p = 0.94) (Figure 4) (Table 3), LDL-cholesterol (beta [95% CI] = -0.007 [-0.02 – 0.01], p = 0.40) (Figure 4) (Table 3) and diabetes rate (beta [95% CI] = -0.050 [-0.161 – 0.06], p = 0.38) (Figure 4) (Table 3).

Table 3. Meta-regression analysis evaluating the relationship between benefits from statins and patient's risk profile, rate of diabetes, amount of contrast, ejection fraction and baseline cholesterol LDL.

Plotted Variable	Beta	95% CI	p value
Contrast Volume	-0.0003	-0.008 / 0.007	0.94
Diabetes	-0.05	-0.16 / 0.061	0.38
LDL baseline	-0.008	-0.026/ 0.011	0.4
Risk Profile	-0.53	-1.47 / 0.41	0.27

Figure 4. Meta-regression analyses shows no relationship between benefits from statins in prevention of CIN (as Log odds ratio) and patients risk profile (expressed as odds of CIN of control group) (A), baseline LDL cholesterol (B), diabetes rate (C), contrast medium dose (D)



DISCUSSION

The main finding of the study is that short-term statin therapy as compared to placebo, was associated with a significant reduction in the risk of CIN and benefits were observed with both high-dose statins and low-dose statins. In the last decades, although hydration has been well recognized in literature as the most effective strategy for the prevention of CIN, the total incidence of CIN did not decrease. This may be due to the higher complexity of patients currently undergoing percutaneous revascularization, as a consequence of the great improvement in antithrombotic therapies and mechanical devices, especially in the setting of acute coronary syndromes (37-40). Several other therapies, such as alkalinization measures (11-12), the use of scavengers such as N-acetylcysteine (NAC) (13), drugs as Fenoldopam, hemodyalisis and hemofiltration (14-16) were assessed for the prevention of CIN, leading to conflicting results. Therefore, the efficacy of many other prophylactic measures is still under testing. Statins, in addition to their impact on cholesterol levels have been shown to possess multiple non lipid-lowering pleiotropic effects such as anti-oxidant, anti-inflammatory and anti-thrombotic properties with enhancement of endothelial nitric oxide production and reducing of endothelin secretion (19-20). The role of statins for the prevention of CIN was assessed also in animal models in which statins prevent ischemic nephropathy by stabilizing the endothelium and acting as free radical scavengers (41-42). As well known from literature, the pathogenesis of CIN is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species (1), therefore, pharmacological prophylactic strategies based on antioxidant properties have been considered for its prevention. The anti-inflammatory effect of statins inhibits tissue factor expression by macrophages, preventing

the activation of nuclear factor-kB and consequently reducing toxic damage on the tubular cells by pro-inflammatory cytokines and oxygen free radicals (43). Moreover, with the down-regulation of angiotensin receptors and the decreasing of endothelin-1, statin therapy may modulate kidney hypoperfusion due to contrast exposure (44). Due to these important properties, in the last decades a large number of studies have assessed the role of statins for the prevention of CIN in patients undergoing coronary angiography or percutaneous interventions, with conflicting results (21-25). The ARMYDA-CIN trial showed that a short-term, high dose Atorvastatin therapy prevents CIN and shortened hospital stay in 241 patients with acute coronary syndrome undergoing PCI (34). On the other side Toso et al. (33), showed that short term administration of high dose Atorvastatin before and after contrast exposure did not decrease CIN occurrence in patients with pre-existing chronic kidney disease (11% of patients developed CIN in the placebo group versus 10% in the atorvastatin group, p=0.86). However, the same group showed that among ACS patients high-dose Rosuvastatin was associated with a significant reduction in the risk of CIN (35). The study of Han at al. (36) is at the moment the biggest study (including almost 3000 patients with type 2 diabetes mellitus and chronic kidney disease) showing that low dose Rosuvastatin 48h pre and 72 h post coronary angiography or PCI significantly reduced the risk of CIN (p=0.02).

The role of statins for the prevention of CIN has already been assessed in several previous meta-analysis, with contrasting results. In fact, Pappy et al. (45) and Zhang T et al. (46) showed a non significant reduction in the incidence of CIN with statin treatment from the pooled estimate from the randomized trials, whereas other recent meta-analysis are in line with our results (47-49) supporting the beneficial effects of statins in the prevention of CIN.

However, it must be underscored that all of them failed to include all currently available randomized trials (35-36), and some of them included both cohort studies or randomized trials (47-48) comparing high vs low dose statins (49). This is largest meta-analysis so far conducted, including 8 randomized trials with a total of 4734 patients. We found that short-term statin therapy was associated with a significant reduction in risk of CIN in both trials with low and high-dose statins, even though some larger benefits were observed with higher doses. By meta-regression analysis we found that the benefits from statins were not affected by patients risk profile, contrast volume, rate of diabetes, LDL cholesterol or ejection fraction. These results, similar to those of other meta-analyses, encourage the use of statins for the prevention of contrast induced nephropathy, even in patients with LDL cholesterol below the recommended threshold.

LIMITATIONS

This meta-analysis was performed on limited individual patient data, as complete datasets were not available. Contrast induced nephropathy is a common complication that has shown to occur in up to 20-25% of patients depending on the presence of known risk factors, such as chronic renal insufficiency, diabetes mellitus and the dose of contrast medium. Therefore, access to individual patient data would have enabled further subgroup analyses. Studies included in this meta-analysis assessed the efficacy of different statins for varied periods of time and is possible that dose, duration and statin type may have different effects for the prevention of CIN. However, the included studies did not provide insights on the mechanisms that could explain the beneficial effects of statins in the prevention of CIN. All studies used the traditional definition of contrast induced nephropathy (CIN, defined as increase of creatinine > 0.5 mg/dl or increase by 25% as compared to baseline), whereas a

new definition (CIAKI, contrast-Induced Acute Kidney Injury) has recently been proposed (50). Finally, even if all included studies reported the incidence of CIN no trial reported on long-term mortality or the progression of kidney failure at follow-up, which are clinically relevant outcomes due to the chronic progressive nature of this disease.

CONCLUSIONS

The present meta-analysis has demonstrated that among patients undergoing coronary angiography or percutaneous intervention the use of short-term statin reduces the incidence of CIN, and therefore are highly recommended to prevent such a complication, even in patients with LDL cholesterol below the recommended threshold.

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Part 4

Impact of Trp719Arg polymorphism on patients undergoing coronary angiography or PCI

Chapter 8

KIF 6 Polymorphism and coronary artery disease

Methods

Clinical, demographic and angiographic data of consecutive patients undergoing coronary angiography and/or PCI were collected in our dedicated database protected by password. Patients with impaired renal function at baseline (creatinine clearance <60ml/min) were treated with standard hydration (1ml/kg/h of saline solution 0.9% 12h before and after the procedure or with saline solution 0,5ml/kg/h, if ejection fraction ≤40% or with sodium bicarbonate received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure). The association between KIF 6 variant and the extent of CAD was assessed after the coronary angiography by quantitative coronary angiography (QCA) analysis with "edge-detection system" (Siemens Acom Quantor QCA, Erlangen, Germany) by two experienced cardiologist who have not information about the patients' genetic profile.

Genetic analysis

A blood sample for the determination of Trp719Arg polymorphism was collected for all patients. We therefore performed DNA extraction by the use of Sigma Aldrick Gen Elute system for each patient. We set the polymerase chain reaction (PCR) with reagents concentration optimization and MgCl2 concentration curve. Amplification of the region of interest with PCR and consequent electrophorethic run on agarose gel and digestion with restriction enzyme Fok I was performed for each sample. Digestion product underwent another electrophorethic run on agarose gel and subsequent analysis with UV scan. We therefore were able to identify the different allelic patterns of our population.

Coronary angiography and QCA

Coronary angiography will be performed, preferring a radial approach, using 6-French right and left heart catheters. Quantitative coronary angiography will be performed by experienced interventional cardiologists by automatic edge-detection systems (Siemens Acom Quantcor QCA, Erlangen, Germany). After the visual inspection of the coronary artery, the frame of optimal clarity will be selected, showing lesion at maximal narrowing and arterial silhouette in sharpest focus. After the calibration of guiding catheter, analysed arterial segment with coronary lesion will be defined by moving the cursor from the proximal to the distal part of coronary artery to ensure adequate determination of reference diameter. We will measure minimal luminal diameter, reference diameter, percent diameter stenosis, and length of the lesion. Significant coronary artery disease will be defined as a stenosis more than 70%, while borderline stenosis if between 30 and 70%.

Table 1. Clinical and demographic characteristics of patients according to KIF 6 polymorphism

Variable	**KIF 6 polymorphism**		
Clinical characteristics	Trp-Trp (n = 486)	Arg carriers (n = 577)	p-value
Age (M-SD)	68.2+/-11.0	67.8+/-11.4	0.57
Male sex (%)	66.3	68.5	0.47
BMI (M-SD)	27.2+/-4.6	26.9+/-4.8	0.42
Hypertension (%)	74.6	71.8	0.29
Smokers (%)			0.03
Active smokers (%)	21.5	21.5	
Previous smokers (%)	20.9	26.3	
Hypercolesterolemia (%)	54.6	56.3	0.62
Diabetes (%)	35.3	36.9	0.61
Previous AMI (%)	23.3	23.6	0.94
Previous PCI (%)	17.5	19.8	0.38
Previous CABG (%)	10.1	11.3	0.55
Previous CVA (%)	6.0	7.1	0.46
Renal failure (%)	23.2	24.4	0.66
Ejection fraction (M-SD)	49.9+/-13.1	49.2+/-12.7	0.88
Indication for angiography			0.61
Stable angina or silent ischemia (%)	18.6	15.7	
Acute Coronary Syndrome (%)	63	66.9	
DCM or valvular disease (%)	18.4	17.4	
Baseline Chemistry			
White blood cells (M-SD)	8.1+/-2.9	7.9+/-2.7	0.23

Haemoglobin (M-SD)	13.4+/-1.6	13.3+/-1.7	0.14
Platelet (M-SD)	216.4+/-62.2	222.7+/-71	0.13
Glycaemia at admission (M-SD)	125+/-49.5	124.4+/-52	0.86
Baseline creatinine (M-SD)	1+/-0.31	1.02+/-0.39	0.23
Total Cholesterol (M-SD)	165.2+/-40.6	164.5+/-43.6	0.78
HDL-Cholesterol (M-SD)	41.6+/-12.2	41.2+/-12.6	0.57
Triglycerides (M-SD)	132.9+/-70.3	135+/-72.3	0.62
LDL-Cholesterol (M-SD)	96.7+/-34.3	95.6+/-36.1	0.60
Uric acid (M-SD)	6.2+/-1.8	6.1+/-1.8	0.41
Reactive protein C (M-SD)	1.4+/-3.0	1.4+/-2.3	0.97
Fibrinogen (M-SD)	439.3+/-133.9	438.2+/-134.6	0.89
Homocysteine (M-SD)	17.7+/-8.2	18.4+/-9.0	0.22
Theraphy at admission			
ACE I (%)	35.5	39.8	0.16
ARB (%)	23.1	18.6	0.08
Statins (%)	43.4	45.6	0.49
Nitrate (%)	28.1	31.7	0.22
Beta-Blockers (%)	47.7	47.7	1
ASA (%)	50.8	56.7	0.06
Clopidogrel (%)	15.5	20.3	0.04
Calcium Antagonist (%)	19.4	21.4	0.44
Diuretics (%)	29.5	29.9	0.95

Table 2. Angiographic characteristics of patients according to KIF 6 polymorphism

Variable	**KIF 6 polymorphism**		
Procedural characteristics	Absence (n = 486)	Presence (n = 577)	p-value
CAD detection (%)	80	80.7	0.81
Multivessel disease (%)	80.1	81.6	0.22
Left main - trivessel disease (%)	30.6	29.3	0.68
Left main (%)	10.4	9.6	0.37
Left anterior descending (%)	55.6	58.8	0.31
Left circumflex (%)	44.3	46.1	0.57
Right coronary artery (%)	50.0	51.0	0.75
Type C lesion (%)	33.7	32.4	0.53
Calcification (%)	18.4	22.7	0.02
Thrombus (%)	5.8	4.8	0.36
TIMI flow (%)			0.32
TIMI flow 0	22.1	21.3	
TIMI flow 1	3.3	2.3	
TIMI flow 2	4.6	3.7	
TIMI flow 3	69.9	72.7	
Restenosis (%)	2.7	3.4	0.43

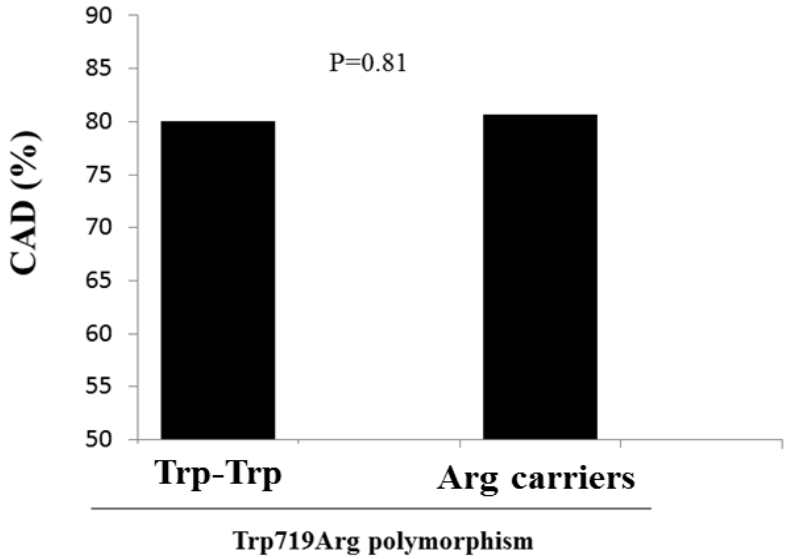
Cronic total occlusion (%)	15.8	17.7	0.28
Bifurcation (%)	20.8	22.2	0.44
Dissection (%)	0.1	0.4	0.38
Lesion lenght (M-SD)	20.3+/-12.9	20.3+/-12.1	0.93
Stenosis percentage (M-SD)	87.6+/-14.1	86.5+/-13.7	0.08
Reference diameter (M-SD)	2.97+/-0.63	2.95+/-0.61	0.37

Results

We analysed a total of 1063 patients undergoing coronary angiography and/or angioplasty. The polymorphic Arg variant of KIF 6 was found in 577 patients (heterozygotes n = 449, Homozygotes n = 128). Our population respect Hardy-Weinberg equilibrium (p = 0.118).

Patients were divided in two groups according to the presence of KIF 6 polymorphism (Group1, Trp-Trp n = 486; Group2, Arg carriers n = 577). Group 2 includes homo and heterozygous mutation. Patient's baseline clinical and demographic characteristics, indication for angiography, baseline chemistry and admission therapy, according to KIF 6 polymorphism are listed in Table 1. Main angiographic characteristics are listed in Table 2. The two groups were quite homogeneous, with only a higher prevalence of previous smokers (26.3% vs 20.9%, p = 0.03) and chronic therapy with clopidogrel at admission (20.3% vs 15.5%, p = 0.04) in Group 2. About angiographic characteristics, patients of Group 2 showed a higher prevalence of calcific plaques at coronary angiography (22.7% vs 18.4%, p = 0.02). The total prevalence of CAD in our population was 80.4% and we did not find any significant correlation between CAD and KIF 6 polymorphism (Group 1 80%, Group 2 80.7%, p = 0.81) (Figure 1).

Figure 1.



This result was confirmed by multivariate analysis after correction for baseline confounding factors (Adjusted OR [95%CI] = 0.98 [0.72-1.33], p=0.90). Similar results were also found dividing our population in three groups according to the genetic specific profile (Group 1, Trp homozygotes, n = 481; Group 2, heterozygotes, n = 449; Group 3, Arg homozygotes, n = 128) (Group 1 80%, Group 2 80.4%, Group 3 81.6%, p = 0.72). No significant differences between KIF 6 polymorphism and CAD detection were found also at subgroups analysis (Figure 2A) according to main risk factors for CAD such as diabetes (p int 0.46), renal failure (p int 0.13), statin therapy at admission (p int 0.71) and gender (p int 0.81), while the prevalence of CAD was higher in elderly patients carrying the Arg allele (87.1% vs 77.3%, p = 0.02 (p int 0.006). Absence of any interaction for main risk factors was observed for severe CAD (LM/trivessel disease) (Figure 2B).

Figure 2A

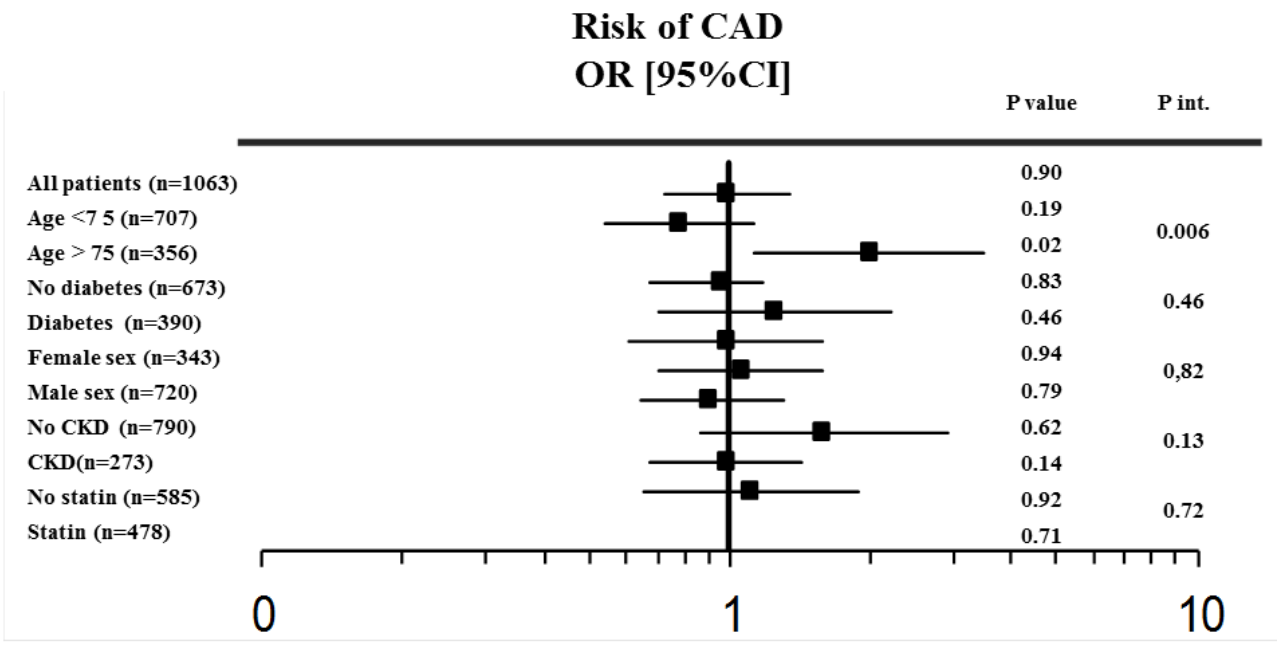
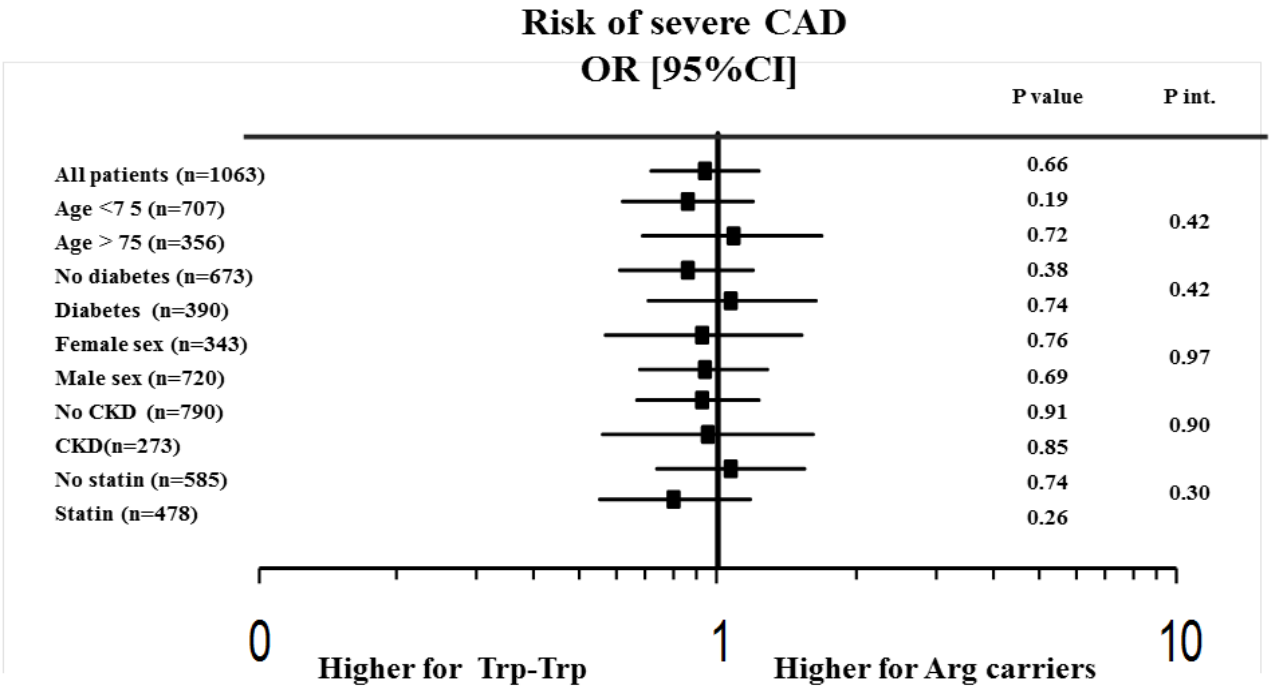


Figure 2B



Discussion and conclusion

Our analysis shows the absence of correlation between Trp719Arg polymorphism of KIF 6 and CAD in patients undergoing coronary angiography or PCI. Coronary artery disease (CAD) still represents the leading cause of death in developed countries (1,2). The improvement in pharmacological therapies and percutaneous revascularization procedures have greatly contributed to the relevant reduction in mortality observed in the last decades for coronary artery disease, particularly in the setting of acute myocardial infarction (3-5), however, the results are still unsatisfactory, especially in high-risk subgroups of patients. Therefore, large interests have been focused on the identification of new risk factors for CAD and its prevention. Statin therapy plays a central role in the setting of cardiovascular prevention and is also highly recommended both as chronic therapy in patients with CAD and with a loading dose in the early phase of acute coronary syndrome. Genetics plays an important role in determining the inherent CAD vulnerability and in determining how a person responds to statin therapy. The correlation between KIF 6 gene and an increased cardiovascular risk has been investigated since 2007 with prospective trials and meta-analysis (6-8). Anyway these results were not confirmed in the large population of the Heart Protection study that involved more than 18000 patients (9). Our study was in line with these findings, showing the absence of association between KIF 6 polymorphism and the detection of CAD at coronary angiography. Moreover, similar results were observed in our population also regarding the detection of severe CAD, such as the presence of LM/trivessel disease. Interestingly, we found a higher prevalence of plaque calcifications among polymorphic patients that could explain the presence of more complex atherosclerotic disease, without a significant increase in the percentage of lesions' severity (??) at

angiography. Several clinical trials (10,11) in last decades showed a significant association between anti-inflammatory, metabolics and vasoprotective effects of statin therapy and a reduction in cardiovascular events in the population with the risk allele. In our population we found a higher percentage of CAD among elderly patients carrying the Arg allele. Indeed the lower statin administration due to the lower adherence to preventive measures, polypharmacy and collateral effects could explain these results. In fact the percentage of severe CAD was lower (LM/trivessel disease) in Arg carriers with statin therapy at admission, suggesting a positive interaction between this genetic variant and statin therapy, although not reaching a statistically significant result. Probably, the routine use of more accurate imaging techniques, such as intravascular ultrasound (IVUS) or optical coherence tomography (OCT) would help us to better characterise coronary disease among the two groups of patients and to have more accurate results. Finally, an important limitation of our study is the absence of data about long term follow up. In future analysis we hope to be able to collect data at long term follow up in order to define the prognostic role of this genetic variant and to define any potential impact on the progression of CAD. In fact, we observed a larger prevalence of coronary calcifications, that are well-established early markers of the atherosclerotic process, in carriers of the Arg-variant and moreover, we reported a positive interaction of KIF-6 genetic status with age, suggesting potentially that a more prolonged exposure to the effects of KIF-6 Arg-allele could favor the development of CAD.

In conclusion our study showed absence of correlation between Trp719Arg polymorphism of KIF 6 and CAD in patients undergoing coronary angiography or PCI. However, a significant interaction was observed with age, being this polymorphism associated with an

increased risk of CAD only among elderly patients. Future large trials are certainly needed to further confirm our findings.

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Chapter 9

KIF 6 Polymorphism and contrast induced nephropathy

Methods

Clinical, demographic and procedural data of consecutive patients undergoing coronary angiography and/or PCI were collected in our dedicated database protected by password. Patients with impaired renal function at baseline (creatinine clearance <60ml/min) were treated with standard hydration (1ml/kg/h of saline solution 0.9% 12h before and after the procedure or with saline solution 0,5ml/kg/h, if ejection fraction ≤40% or with sodium bicarbonate received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure). Serum creatinine and creatinine clearance (calculated by applying Cockroft Gault formula) were collected at baseline, 24 and 48 hours after contrast exposure. Among these patients we assessed the incidence of CIN, defined as an absolute increase of 0.5mg/dL or a relative increase >25% in serum creatinine levels at 24 and 48h after the procedure.

Genetic analysis

A blood sample for the determination of Trp719Arg polymorphism was collected for all patients. We therefore performed DNA extraction by the use of Sigma Aldrick Gen Elute system for each patient. We set the polymerase chain reaction (PCR) with reagents concentration optimization and MgCl2 concentration curve. Amplification of the region of interest with PCR and consequent electrophorethic run on agarose gel and digestion with restriction enzyme Fok I was performed for each sample. Digestion product underwent another electrophorethic run on agarose gel and subsequent analysis with UV scan. We therefore were able to identify the different allelic patterns of our population.

Table 1. Clinical and procedural characteristics of patients according to KIF 6 polymorphism

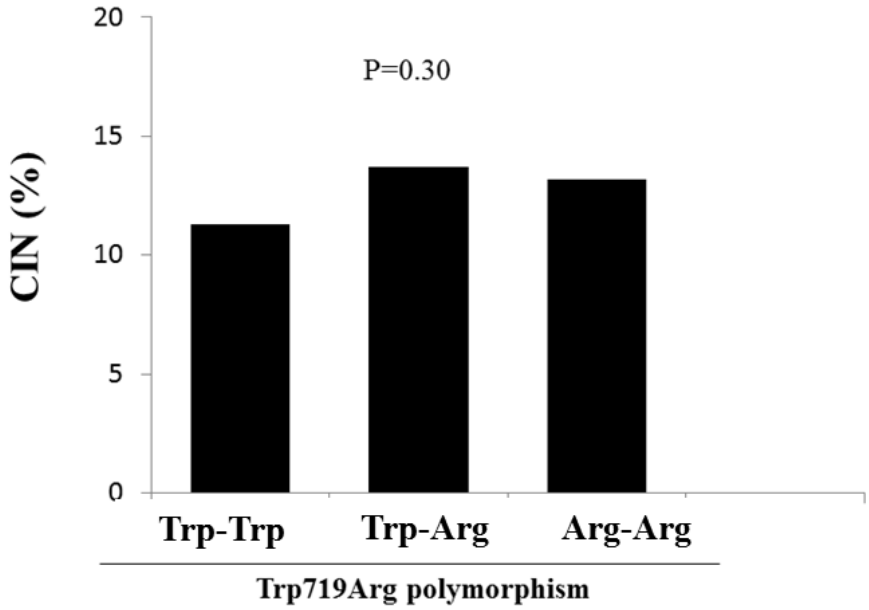
Variable	**KIF 6 polymorphism**			
Clinical characteristics	Trp-Trp (n = 584)	Trp-Arg (n = 525)	Arg-Arg (n = 144)	p-value
Age (M-SD)	68.1+/-10.7	67.8+/-11	68+/-12.3	0.90
Male sex (%)	68.2	71.6	65.3	0.94
Hypertension (%)	75.9	72.2	80.6	0.81
Smokers (%)				
Active smokers (%)	19.9	20.6	22.9	0.26
Previous smokers (%)	22.1	28.4	20.1	
Hypercolesterolemia (%)	58	56.2	66	0.31
Diabetes (%)	35.3	35.4	42.4	0.23
Family history of CAD (%)	26.6	27.4	33.3	0.17
Previous AMI (%)	27.2	24.8	27.1	0.66
Previous PCI (%)	26	28.2	28.5	0.40
Previous CABG (%)	10.9	12.8	12.5	0.40
Previous CVA (%)	6.8	6.9	6.3	0.85
Renal failure (%)	12.8	15.8	15.3	0.21
Indication for angiography				
Stable angina or silent ischemia (%)	22.2	22.3	20.2	
Acute Coronary Syndrome (%)	55.9	59.9	67.7	0.15
DCM or valvular disease (%)	22	17.8	12.1	
Baseline Chemistry				
Haemoglobin (M-SD)	13.5+/-1.6	13.3+/-1.7	12.9+/-1.6	0.03
Platelet (M-SD)	214.4+/-60.3	219.4+/-70.5	225.1+/-70.5	0.16
Glycaemia at admission (M-SD)	127+/-48.4	124.6+/-47.2	125.3+/-64.7	0.07
Baseline creatinine (M-SD)	1+/-0.28	1.05+/-0.45	1.01+/-0.36	0.08
Absolute creatinine increase (M-SD)	0.08+/-0.27	0.07+/-0.24	0.11+/-0.31	0.30
Relative creatinine increase (M-SD)	0.09+/-0.26	0.09+/-0.19	0.13+/-0.31	0.20
Creatinine clearance (M-SD)	78.8+/-31.6	78+/-34.6	79.7+/-34.2	0.83
Reactive protein C (M-SD)	1.34+/-3.02	1.33+/-2.43	1.36+/-2.46	0.99
Total Cholesterol (M-SD)	160.6+/-40.8	161.5+/-43.1	158.1+/-44.7	0.70
HDL-Cholesterol (M-SD)	41.6+/-13.4	41.2+/-13	41.5+/-13.9	0.84
Triglycerides (M-SD)	134+/-71.2	139.4+/-98.8	132.6+/-67.9	0.48
LDL-Cholesterol (M-SD)	93.3+/-36.3	93.6+/-39.9	90.8+/-38.7	0.74
Procedural characteristics				
PTCA (%)	58.6	61.3	55.2	0.89
Radial access (%)	33.2	36.2	36.1	0.32
Contrast volume (M-SD)	233.7+/-157	231.3+/-152.5	237.4+/-172.9	0.91
Theraphy at admission				
ACE I (%)	41.4	42.3	43.4	0.76
ARB (%)	26.9	20.2	19.2	0.01
Statins (%)	54.3	50.9	58.3	0.90
Nitrate (%)	35.8	39.2	36	0.58
Beta-Blockers (%)	57.7	57	46	0.04
ASA (%)	59.6	61.7	71.1	0.03
Clopidogrel (%)	20.8	26.4	29.3	0.01

Calcium Antagonist (%)	22.2	21.9	27.5	0.35
Diuretics (%)	35.2	32.1	36.8	0.82

Results

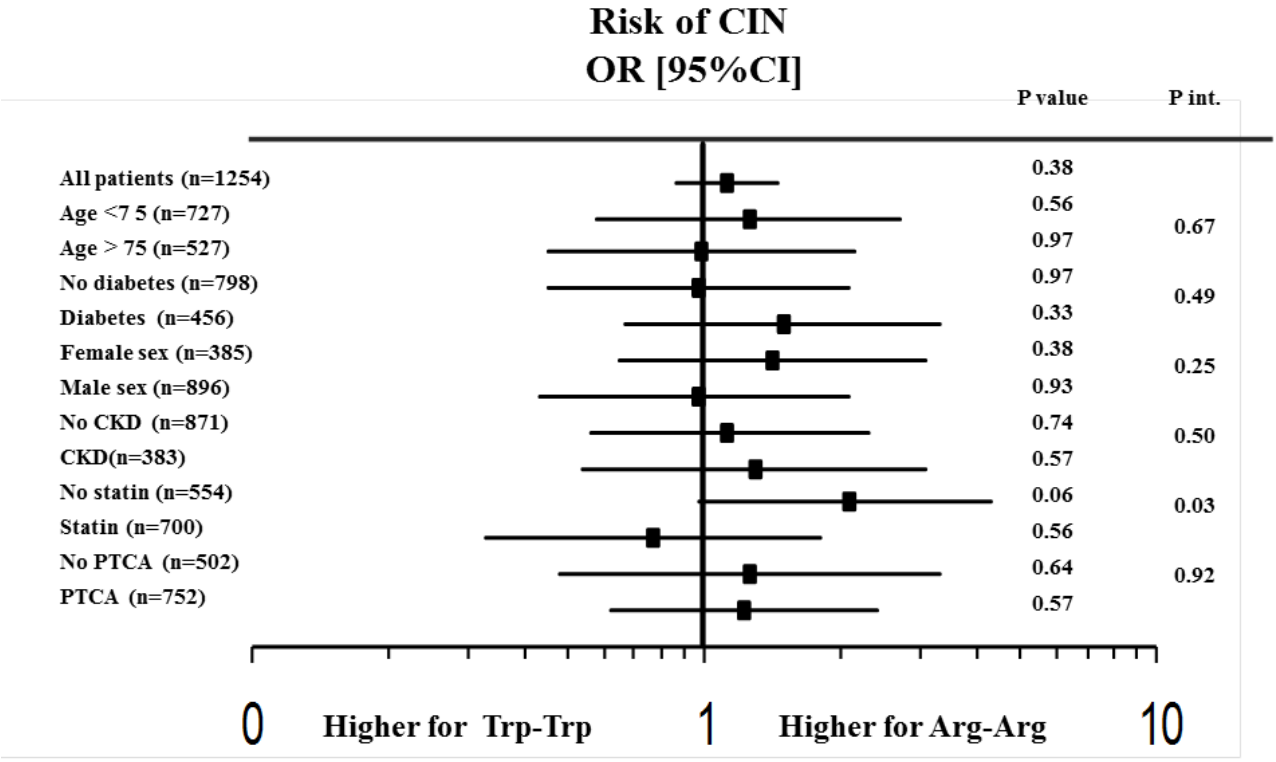
We analysed a total of 1253 patients undergoing coronary angiography and/or angioplasty. KIF 6 Arg mutation was found in 669 patients (heterozygotes n = 525, homozygotes n = 144). Our population respect Hardy-Weinberg equilibrium (p = 0.14). Patient's baseline clinical and demographic characteristics, indication for angiography, procedural characteristics, baseline chemistry and admission therapy, according to the presence of KIF 6 polymorphism are listed in Table 1. Patients without polymorphism were more often in therapy with angiotensin receptor blockers (ARB) (p = 0.01), beta blockers (p = 0.04), but less with acetylsalicylic acid (ASA) (p = 0.03) and clopidogrel (p = 0.01) at admission and they have higher haemoglobin levels at admission (p = 0.03). No other significant differences were found between the three population. The total prevalence of CIN in our population was 12.5% and we did not find any significant association between KIF 6 polymorphism and the development of CIN (Group 1 11.3%, Group 2 13.7%, Group 3 13.2% p = 0.30) (Figure 1). This result was confirmed by multivariate analysis after correction for baseline confounding factors (Adjusted OR [95%CI] = 1.12 [0.86-1.46], p=0.38).

Figure 1.



Similar results were found dividing our population in two groups according to the genetic status (Group 1 11%, Group 2 13.7%, p = 0.19). At subgroups analysis we found a higher prevalence of CIN among homozygous patients treatment "naïve" in comparison to wild-type patients (20.7% vs 11.3%, p = 0.05), while patients with statin therapy at admission showed a lower CIN development without reaching a statistical significant result (8.6% vs 13.2%, p = 0.28) (p int 0.03). No other significant differences between homozygous KIF 6 polymorphism and CIN development according to main risk factors for CIN such as diabetes (p int 0.49), renal failure (p int 0.50), gender (p int 0.25), older age (p int 0.67) and PCI (p int 0.92). (Figure 2).

Figure 2



Discussion and conclusion

The main finding of our study is the presence of a significant correlation between statin therapy modulation among KIF 6 homozygous Arg patients and the development of CIN after coronary angiography or percutaneous intervention.

Several clinical trials and meta analysis (1-4) showed a significant association between anti-inflammatory, metabolics and vasoprotective effects of statin therapy and a reduction in cardiovascular events in the population with the risk allele. Recent studies (5,6) showed a protective effect of statin therapy in patients treated before coronary angiography/PCI. Statins, in addition to their impact on cholesterol levels have been shown to possess

multiple non lipid-lowering pleiotropic effects such as anti-oxidant, anti-inflammatory and anti-thrombotic properties with enhancement of endothelial nitric oxide production and reducing of endothelin secretion (7,8). As well known from literature, the pathogenesis of CIN is the result of endothelial dysfunction, cellular toxicity from the contrast agent and tubular apoptosis resulting from hypoxic damage or reactive oxygen species (9), therefore, pharmacological prophylactic strategies based on antioxidant properties have been considered for its prevention. In our population we found that statin therapy at admission did not influence the development of CIN in overall population, while, there was a significant interaction between CIN development, Arg homozygote carriage and statins, suggesting that the nefro-protective effects of these drugs could be even more relevant in carriers of the KIF-6 variant allele. In fact, Arg homozygotes not treated with statins at the moment of angiography were at enhanced risk of CIN. These results give great effort for a systematic and early use of statin therapy in patients undergoing coronary angiography or percutaneous coronary intervention. One of the limitation of our study was that we evaluated the occurrence of CIN at 48-72 hours, but this complication may appear even later than this time threshold. Furthermore, we were not able to provide data on the progression of kidney failure at follow-up, being this disease chronically progressive. Future large studies are certainly needed to confirm our finding, in order to confirm benefits of statin treatment on the development of CIN.

In conclusion our study showed among patients undergoing coronary angiography and/or PCI that KIF 6 homozygous Arg was associated with a significant increase in the risk of CIN only among statin naive patients. Future additional studies are certainly needed to

confirm our findings and to evaluate the beneficial effects of statin therapy especially in this subset of patients.

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Chapter 10

KIF 6 and the modulation of the protective effects of statin in naïve patients

Methods

Clinical, demographic and procedural data of consecutive patients undergoing coronary angiography and/or PCI, treatment naïve for statin, were collected in our dedicated database protected by password. Patients with impaired renal function at baseline (creatinine clearance <60ml/min) were treated with standard hydration (1ml/kg/h of saline solution 0.9% 12h before and after the procedure or with saline solution 0,5ml/kg/h, if ejection fraction ≤40% or with sodium bicarbonate received 3 ml/kg for 1h before contrast exposure followed by an infusion of 1 ml/kg/h for 6h after the procedure). For each patient chemistry including total, HDL and LDL cholesterol were assessed at baseline. Each patient received statin treatment at admission according to good clinical practice. Chemistry including total, HDL and LDL cholesterol were then assessed at time 1 (at least 30 days after discharge).

Genetic analysis

A blood sample for the determination of Trp719Arg polymorphism was collected for all patients. We therefore performed DNA extraction by the use of Sigma Aldrick Gen Elute system for each patient. We set the polymerase chain reaction (PCR) with reagents concentration optimization and MgCl2 concentration curve. Amplification of the region of interest with PCR and consequent electrophorethic run on agarose gel and digestion with restriction enzyme Fok I was performed for each sample. Digestion product underwent another electrophorethic run on agarose gel and subsequent analysis with UV scan. We therefore were able to identify the different allelic patterns of our population.

Results

We analysed a total of 109 patients undergoing coronary angiography and/or angioplasty statin treatment naive. Arg-carriers were 59 patients, while 49 patients displayed wild-type genotype. Patients were divided in two groups according to the presence of KIF 6 polymorphism (Group1, Trp-Trp patients n = 49; Group 2, Arg-carriers n = 60). Group 2 includes homo and heterozygous mutation. Patient's baseline clinical and demographic characteristics, indication for angiography, baseline chemistry and admission therapy, according to the presence of KIF 6 polymorphism are listed in Table 1.

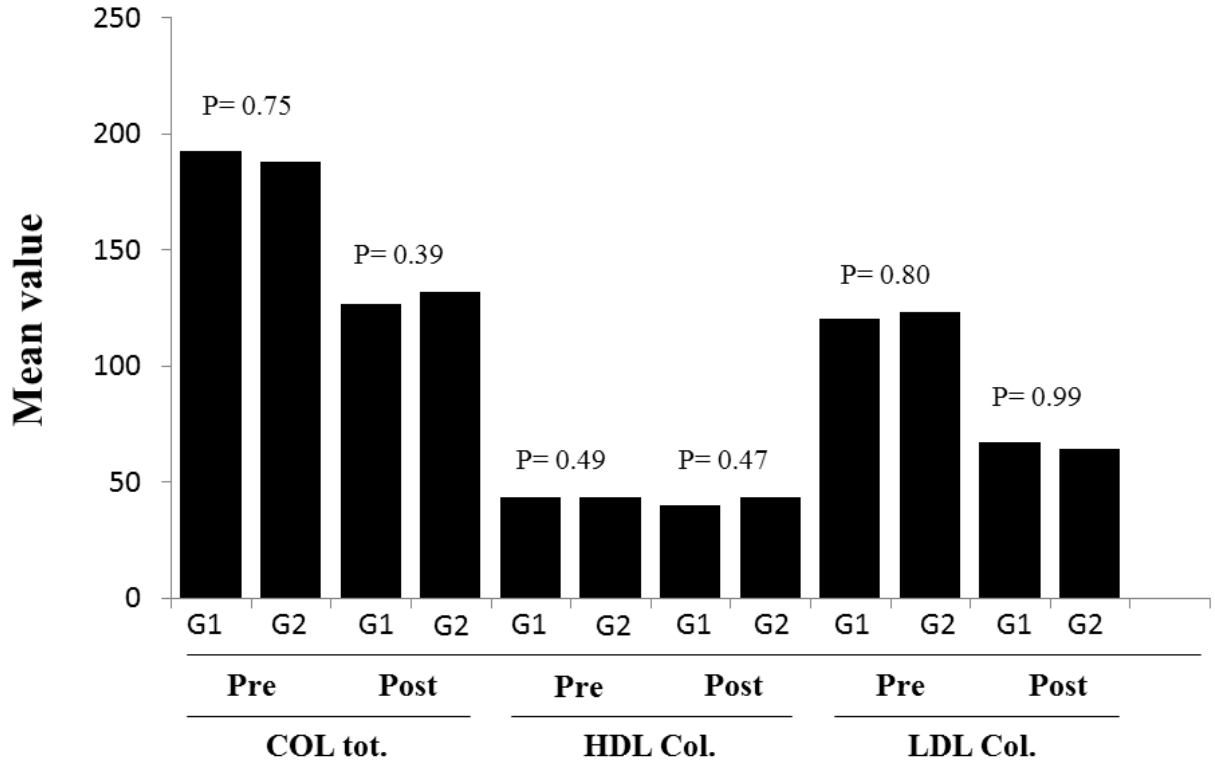
Table 1. Clinical and demographic characteristics of patients according to KIF 6 polymorphism

Variable	**KIF 6 polymorphism**		
Clinical characteristics	Trp-Trp (n = 49)	Arg carriers (n = 60)	p-value
Age>75 (%)	14.3	36.7	0.009
Age (M-SD)	64.3+/-10.8	66.4+/-12.8	0.37
Male sex (%)	76.8	74.5	0.77
BMI (M-SD)	27.3+/-3.9	26.2+/-3.9	0.17
Hypertension (%)	73.5	55	0.07
Smokers (%)			0.32
Active smokers (%)	20.4	38.3	
Previous smokers (%)	18.4	16.7	
Hypercolesterolemia (%)	42.9	56.7	0.18
Diabetes (%)	40.8	28.3	0.22
Previous AMI (%)	8.2	11.7	0.75
Previous PCI (%)	30.6	15	0.06
Previous CABG (%)	0	5	0.25
Renal failure (%)	6.1	18.3	0.08
Ejection fraction (M-SD)	52+/-10.1	52.2+/-9.5	0.93
Baseline Chemistry			
White blood cells (M-SD)	7.8+/-2.1	7.9+/-1.9	0.68
Haemoglobin (M-SD)	13.7+/-1.6	13.1+/-1.9	0.10
Platelet (M-SD)	235.4+/-70	242+/-86.6	0.67
Glycaemia at admission (M-SD)	122.6+/-36	110.8+/-27.6	0.05
Glycated haemoglobin (M-SD)	6.4+/-1.1	6.2+/-0.87	0.20
Baseline creatinine (M-SD)	0.89+/-0.15	1.04+/-0.84	0.22
Total Cholesterol (M-SD)	187.3+/-39.5	184.6+/-45.4	0.75
HDL-Cholesterol (M-SD)	43.8+/-11	42.3+/-12.2	0.44

Triglycerides (M-SD)	108.6+/-58.6	112+/-62.1	0.77
LDL-Cholesterol (M-SD)	116.7+/-35.4	118.6+/-39	0.80
Reactive protein C (M-SD)	0.71+/-0.94	0.49+/-0.81	0.44
Theraphy at admission			
ACE I (%)	63.3	58.3	0.69
ARB (%)	24.5	15	0.23
Nitrate (%)	46.9	38.3	0.43
Beta-Blockers (%)	93.9	90	0.51
ASA (%)	98	98.3	0.99
Clopidogrel (%)	62.5	90	0.10
Calcium Antagonist (%)	18.4	13.3	0.59
Diuretics (%)	24.5	23.3	0.99

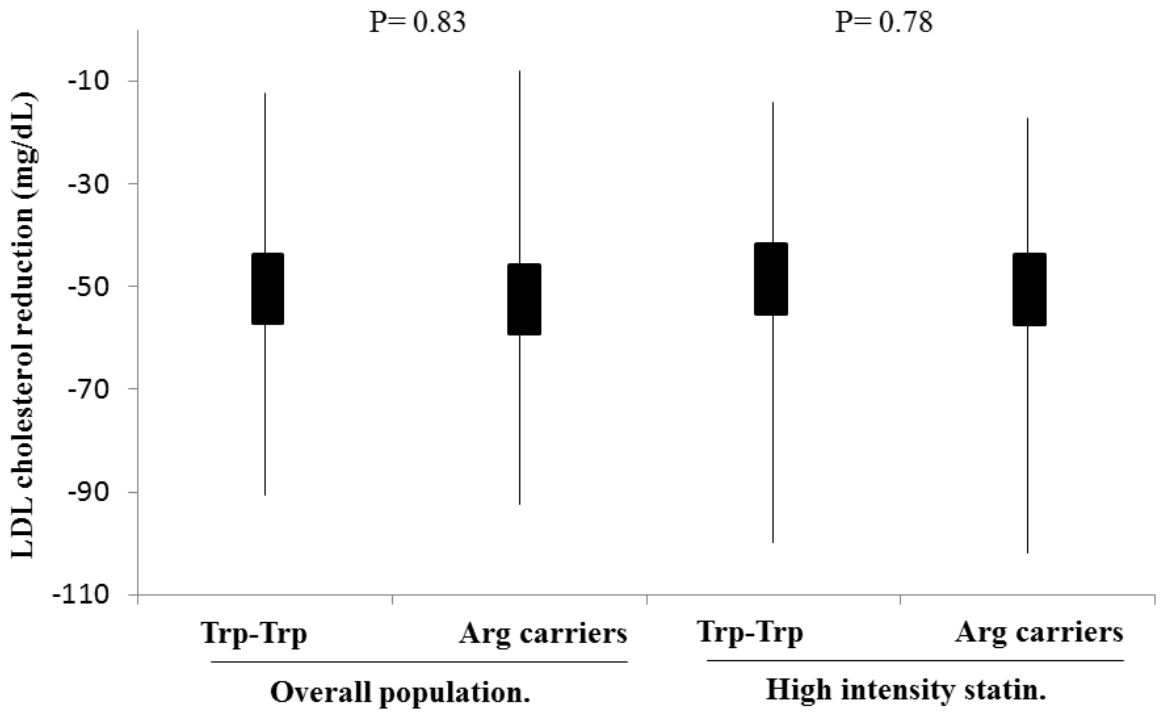
Arg carriers were more often older than 75 years old (36.7 vs 14.3, $p = 0.009$) and have lower glycaemia level at admission (110.8+/-27.6 vs 122.6+/-36). No other significant differences were found in any other biochemical parameter or clinical characteristic between the two population. All patients at admission received statin treatment. The median time from baseline to re-assessment was 86 days [IQR 45-236]. As expected, at the established follow up we found a significant reduction in total cholesterol (132.6 vs 185.4, $p < 0.001$) and LDL cholesterol (68.9 vs 117.8, $p < 0.001$) (Figure 1).

Figure 1.



KIF 6 polymorphism did not significantly influence both the absolute reduction of LDL (Group1, -51.7+/-38.8 vs Group2 -50.1+/-42.2, p = 0.83) and the relative reduction of LDL cholesterol (Group1, -39.9%+/-24.4% vs Group2 -37.3%+/-25.7%, p = 0.60). A total of 71 patients received high intensity statin therapy (Atorvastatin > 40mg, Rosuvastatin > 20mg). Among these patients 35 were wild-type and 36 Arg carriers. KIF 6 polymorphism did not affect the absolute (Group1, -56.3+/-43.3 vs Group2 -59.2+/-42.8, p = 0.78) and relative LDL (Group1, -41.%+/-27% vs Group2 -43.3%+/-21.5%, p = 0.69) decrease of LDL cholesterol also when considering the group receiving high intensity statin treatment (Figure 2).

Figure 2.



Discussion and conclusion

The main finding of our study is the absence of a correlation between KIF 6 polymorphism and LDL reduction at follow up among statin naïve patients. Statin treatment played a central role in the setting of cardiovascular prevention, in fact, in addition to their impact on cholesterol levels, statins have been shown to possess multiple non lipid-lowering pleiotropic effects such as anti-oxidant, anti-inflammatory and anti-thrombotic properties with enhancement of endothelial nitric oxide production and reducing of endothelin secretion (1-3). Therefore they are highly recommended both as chronic therapy in patients with CAD, but also with a loading dose in the early phase of acute coronary syndrome to

stabilize the plaque and reduce the incidence of any complication, especially if a percutaneous coronary intervention (PCI) is performed. Genetics plays an important role in determining the inherent CAD vulnerability and in determining how a person responds to statin therapy. Kinesis-Like Protein 6 (KIF 6) is an omodimeric protein expressed in coronary arteries and other vascular tissues, that is involved in cellular microtubular transport (14).The impact of KIF 6 gene on cardiovascular risk modulation has been investigated in several prospective trials and meta-analysis (4-6) assessed the association between this genetic variant and a significant increase of cardiovascular risk. Particular attention in last years has been focused on the role of the Trp719ARg polymorphism in the modulation of response to statin treatment. Several clinical trials showed a significant association between anti-inflammatory, metabolic and vasoprotective effects of statin therapy and a reduction in cardiovascular events in the population with the risk allele (7,8). Moreover these protective effects with a significant reduction (about 13%) of LDL-cholesterol level and risk of cardiovascular events of statin therapy has been recently confirmed by two meta-analysis (9,10). Our population was represented by consecutive patients, statin treatment naïve, undergoing coronary angiography/PCI. We found that the genetic variant did not significantly influence the response to statins, defined as the reduction in LDL cholesterol. Results were similar when restricting the analysis to high intensity statin treatment. Indeed, these results could be explained both by the small size of our sample and the use of high intensity statins in the majority of the population, that could have potentially overcame the effect of the polymorphism. Nevertheless, cholesterol reduction does not represent the only mechanism by which the statins can reduce the cardiovascular risk. Therefore, it might be argued that the positive interaction between statin

administration and KIF-6 polymorphism documented in previous trials could have been dependent by those “pleiotropic”, non-lipid dependent effects of statins.

Indeed, we did not consider other inflammatory biomarkers or their transient changes after statin administration, according to KIF 6 genetic status, in our patients. Nevertheless, no study has so far addressed the pathophysiological basis of statin-KIF 6 interplay, and in addition, the anti-atherosclerotic effects of statins include a broad spectrum of actions, not yet completely defined. Therefore, future larger studies on will help us to better define the correlation between KIF 6 polymorphism and the effects of statins.

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Summary and conclusions

Coronary artery disease is the leading cause of mortality in western countries. Therefore large efforts have been done in the last decades to identify new risk factors and new preventive strategies, with a special look at the interaction between the genetic background and environmental factors. In particular, several biomarkers involved in the inflammatory processes and in the development of the atherosclerotic plaque have been addressed, although with inconclusive results. Genetics determinants of cardiovascular risk have recently emerged as an additional tool for the stratification and prognostic evaluation of patients with cardiovascular disease. The present thesis provides a focus on several molecular aspects of the diagnosis and treatment of CAD and its complications, including contrast-induced nephropathy. In Part 2 we focused on cardiovascular risk assessment and evaluation of coronary artery disease as evaluated by coronary angiography. In Chapter 2, we found that that uric acid levels are significantly higher in men; however, high uric acid levels are associated with severe CAD only in women. In Chapter 3 we found a significant independent association between NLR and the prevalence and severity of CAD. The extensive treatment of coronary artery disease by percutaneous coronary intervention and the high-risk profile of patients currently undergoing angiography or PCI, has driven a lot of interests on the occurrence of contrast-induced nephropathy (CIN), that is a common complication of procedures that foresee the use of contrast media and is known as the third leading cause of hospital-acquired acute kidney injury. Great efforts have been done to identify new risk factors that foresee this complication and potential preventive strategies, that is the aim of Part 3. In Chapter 4, we showed that among patients without diabetes

undergoing coronary angiography/PCI elevated glycated-haemoglobin but not glucose levels is independently associated with the risk of CIN. In Chapter 5 and 6 we specially focused on Homocysteine and Uric acid, and found both of them independently associated with the occurrence of CIN. In Chapter 7, by the use of a meta-analysis approach, we showed that among patients undergoing coronary angiography/percutaneous intervention the use of short-term statin reduces the incidence of CIN.

We finally focused in Part 4 on the genetics and in particular on the KIF-6 719 Trp>Arg polymorphism, evaluating its impact on CAD, CIN and the interplay with statin therapy. In Chapter 8, we did not find a significant association between KIF 6 polymorphism and CAD detection at coronary angiography. We found a higher prevalence of calcified plaques among polymorphic patients carrying the Arg mutation. In addition, in our population we found a higher percentage of CAD among elderly patients carrying the Arg allele, that could have been explained by the more prolonged exposure of these patients to the KIF-6 mutation but also to statin under prescription, which is more common in advanced age.

In Chapter 9, we evaluated the impact of KIF6 polymorphism and contrast induced nephropathy. We found that statin therapy at admission did not influence the development of CIN, while, there was a significant interaction between CIN development, homozygous Arg allele carriage and statin therapy. In fact, Arg homozygotes not treated with statins at the moment of angiography have an increased risk of CIN, suggesting that the nephro-protective effects of these drugs could be even more relevant in carriers of the KIF-6 variant allele.

However, in Chapter 10 among naïve statin treatment patients we found that the genetic variant of KIF 6 did not significantly influence the reduction in LDL cholesterol at follow up, also in case of high intensity statin treatment administered. Therefore, it could be argued that the positive interaction between the cardioprotective effects of statins and Arg-allele of KIF 6, documented in previous studies and in our Chapter 9, in particular on the risk of CIN, could be mediated by those pleiotropic non "cholesterol" dependent effects of statins.

Indeed, the present study represents only an exploratory study on the topic, since no study has so far addressed the pathophysiological basis of statin-KIF 6 interplay, and in addition, the anti-atherosclerotic effects of statins include a broad spectrum of actions, not yet completely defined. Therefore, future large studies are certainly needed to better define the correlation between KIF 6 polymorphism and statin benefits, with a particular focus on their pleiotropic cardio and renal protective effects, and especially in higher-risk subsets of patients such as among elderly patients undergoing percutaneous coronary revascularization, in order to define the best treatment options and improve clinical outcome.