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Iron level and vulnerable coronary atherosclerotic plaques

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WHAT'S NEW

Study aimed to fill in pathophysiological link between the "iron hypothesis" and coronary artery disease. We showed that higher iron was associated with low attenuation plaque presence. Patients with higher iron had higher fibro-fatty and necrotic core plaque components, therefore higher iron was associated with vulnerable coronary plaque composition.

ABSTRACT

Background: Preliminary research indicates that higher iron is associated with worse outcomes in patients with coronary artery disease.

Aims: The study aimed to investigate the relationship between iron and the type and composition of coronary plaques.

Methods: In patients with ≥1 coronary stenosis ≥50% on computed tomography angiography, iron level, the presence of high-risk plaque features: low-attenuation plaque (LAP), napkin-ring sign, positive remodeling and spotty calcium, type and plaque composition (calcified/fibrous/fibro-fatty/necrotic core) were evaluated. Fibro-fatty and necrotic core were analyzed together as vulnerable component.

Results: The study included 300 patients (191 men, 62.2 [23.0] years). 2184 plaques were found (1201 calcified, 584 non-calcified and 397 mixed). high-risk plaque were in 208 patients (107 LAP, 54 napkin-ring sign, 354 positive remodeling, and 281 spotty calcium). Patients with LAP had higher iron (P <0.0001). In univariable regression, LAP predictors were iron (odds ratio [OR], 1.02; 95% CI, 1.00–1.03; P <0.0001), male sex (OR, 2.50, 95% CI, 1.40–4.46; P = 0.002) and hemoglobin (OR, 1.23; 95% CI, 1.01–1.49; P = 0.04). In multivariable regression, only iron and male gender were LAP predictors (OR, 1.02; 95% CI, 1.01–1.03; P = 0.0002, and OR, 2.41; 95% CI, 1.24–4.70; P = 0.009, respectively). The iron threshold for LAP prediction was >98 μg/dl (AUC 0.665; P <0.001) what correspond with OR 3.2 (95% CI, 1.80–5.80; P = 0.0001) for LAP prediction.

Higher iron correlated with more fibro-fatty (P = 0.01) and necrotic core (P = 0.02), with no relation to calcified and fibrous. Higher iron were positively correlated with the percentage of vulnerable plaque component (P = 0.004).

Conclusions: Higher iron level was associated with LAP presence and greater vulnerable component of coronary atherosclerotic plaques.

Key words: atherosclerosis, coronary artery disease, iron, low attenuation plaque, vulnerable plaque

INTRODUCTION

In 1981, Sullivan proposed iron as a cardiovascular risk factor, hypothesizing that the lower body iron stores were associated with a lower incidence of cardiovascular disease (CVD) in premenopausal women compared to men and postmenopausal women [1]. The “iron hypothesis” was based on observations on myocardial failure in iron storage diseases, accumulation of stored iron with age in men, and accumulation of stored iron in women after

menopause to the levels found in men [1]. Further research indicated higher iron levels to be associated with enhanced oxidative stress, higher SYNTAX (SYNergy between PCI with TAXUS™ and Cardiac Surgery) score, more severe atherosclerosis, and aggravated atherosclerosis progression in the aorta in a mouse model [2–4]. Moreover, Salonen et al. [5] showed that men with high serum ferritin levels had a 2.2-fold higher risk of myocardial infarction (MI). However, there are divergent results from studies on the role of iron in CVD [6].

Reactive oxygen species (ROS) mediated changes to lipoproteins have a crucial role in atherogenesis. Preliminary research indicated that iron participates in a redox reaction (Fenton's reaction) through the transfer of electrons between the Fe^{3+} and Fe^{2+} and catalyzes the formation of ROS which play a crucial role in the oxidation of low-density lipoproteins (LDLs) [7, 8]. Hypothetically, the above mechanism may contribute to the development of atherosclerosis, including its vulnerable components formation, plausibly linking the increased iron levels with adverse cardiovascular outcomes. However, the potential association between iron and coronary plaque morphology is unknown.

Therefore, the study aims to investigate the relationship between free blood iron level and the type and composition of atherosclerotic plaques in coronary arteries as evaluated on computed tomography coronary angiography (CTCA).

MATERIAL AND METHODS

Study population

This is a prospective single-center study with enrolled 300 consecutive patients with newly diagnosed coronary artery disease (CAD) on CTCA (Figure 1). The presented analysis was carried out as a part of a grant funded by the National Science Centre (grant number 2016/21/N/NZ5/01450, funded to AO). Detailed grant design has been published previously [9]. Among the examined patients in National Institute of Cardiology, active screening was conducted in the CT lab between March 2017 and October 2019, and then consecutive patients meeting the inclusion criteria were offered the opportunity to participate in the study. Patients were referred to the CTCA in accordance with the ESC recommendations in force at the time of recruitment [10, 11]. The inclusion criteria were: signed informed consent; age over 18 years; ≥1 plaque with ≥50% narrowing of the coronary artery lumen with a reference diameter >2.0 mm in CTCA. The exclusion criteria were: poor quality of CTCA; the presence of artificial heart valves or pacemakers; previous coronary revascularization; previous MI,

and recipient of red blood cells concentrate or iron supplementation during the last four months before the CTCA. The study did not include patients with advanced renal impairment (estimated glomerular filtration ratio <30 ml/min/1.73 m^2) and heart failure with reduced ejection fraction (HFrEF). All patients provided informed consent and the study received Ethics Committee approval.

Laboratory tests

All patients underwent laboratory tests directly after CTCA including complete blood count, non-transferrin bound iron level, and high sensitive C-reactive protein (hsCRP). The reference iron values were 33–193 µg/dl, and the reference hemoglobin (Hb) levels were 11.2–15.7 mg/dl in women and 13.7–17.5 mg/dl in men. The reference hsCRP values was <0.5 mg/dl.

CTCA performance and analysis

Computed tomography coronary angiography was performed with a dedicated CT scanner (Somatom Force; Siemens, Germany). Sublingual nitrates (0.8 mg) were administered. If the heart rate was ≥70 beats/min, an intravenous bolus of metoprolol (increasing doses at 2.5 mg intervals, up to a maximum dose of 20 mg) was given if necessary. From 60 to 70 ml of contrast agent (iohexol) was injected intravenously at 6 ml/s. An electrocardiogram-gated retrospective or prospective acquisition protocol was used in all patients when possible. Scan data were reconstructed routinely in mid to end-diastole (60%–70% of R–R interval). Data sets that contained motion artifacts were individually optimized by changing the reconstruction window. CTCA analyses were performed in our core lab by an experienced reader (at least 10 years with CTCA) fully blinded to clinical and laboratory data, who evaluated all arteries with a reference diameter of above 2 mm for the presence of coronary stenoses. QAngioCT analysis was performed by a blinded reader professionally trained in QAngioCT analysis.

In the study population, we assessed the presence of high-risk plaque (HRP) features: low-attenuation plaque (LAP), napkin-ring sign, positive remodeling and spotty calcium (Syngo, Siemens), type of plaque (calcified, mixed, non-calcified) (Syngo, Siemens) and their composition provided as a percentage value of each plaque component per patient (calcified, fibrous, fibro-fatty, necrotic core) (QAngioCT, Medis) (Figure 2) [12–14].

In noncalcified plaques, readers placed 3 random region-of-interest measurements in the noncalcified low CT attenuation area of the plaque. LAP was defined as the mean CT

number within these 3 regions of interest <30 Hounsfield units (HU). The napkin ring sign was defined as a ring-like peripheral higher attenuation of the non-calcified portion of the coronary plaque. Spotty calcification was defined as the presence of calcification <3 mm in any direction within a plaque. The threshold of 1.1 was used to define positive remodeling [15, 16].

The atheroma composition was distinguished based on tissue attenuation ranges in HU: dense calcium (>351 HU), fibrous plaque (151 to 350 HU), fibrofatty plaque (31 to 150 HU), and necrotic core (–30 HU to 30 HU) (Figure 2) [15, 17]. The combination of fibro-fatty and necrotic core components were analyzed together as vulnerable plaque component, and the combination of fibrous and dense calcium as stable plaque component. All coronary stenoses were categorized as: minimal <25%, mild 25%–49%, moderate 50%–69%, severe 70%–100%.

Statistical analysis

Continuous data with normal distribution are presented as means (standard deviation). Non-normally distributed variables are presented as medians with interquartile ranges. The categorical variables are presented as frequencies and percentages. The differences between patients were determined with Student's t-test (normal distribution) or the U Mann–Whitney test (non-normal distribution), as appropriate. The differences between the qualitative variables were determined using the χ^2 test or Fisher's exact test, as appropriate. Correlation analysis, univariate logistic regression, multivariable regression analyses and receiver operating characteristic analysis were used as appropriate. In multivariable regression, we included all factors which presented at least a trend association in univariate regression (P <0.10). Additionally, normalized parameter of iron/Hb was used for subanalysis to exclude the influence of anemia on the results. P <0.05 was considered statistically significant. All analyses were performed using MedCalc Software (version 13.2.2, Ostend, Belgium).

RESULTS

The study included 300 patients (191 men [63.7%], 62.2 [23.0] years). The baseline study group characteristics are provided in Table 1. CASC was 565.2 (454.4–633.7). Dose length product for CTCA was 431.8 (413.7–458.9) mGy × cm.

The median iron level was 87.5 (80.8–100.0) µg/dl for women and 100.9 (96.7–106.0) µg/dl for men (P = 0.01). According to the reference values, 5 (1.7%) patients had iron level

under reference values (3 men [60%]) and 4 (1.3%) patients had iron level above reference values (4 men [100%]). The median Hb level was 13.5 (13.2–13.7) mg/dl in women and 14.7 (14.5–15.0) mg/dl in men (*P* <0.0001). According to the reference values, 34 (11.3%) patients had anemia (30 men [88.2%]). The median hsCRP level was 0.14 (0.13–0.16) mg/dl, with no differences between men and women (*P* = 0.2).

On CTCA 2184 coronary plaques were found, from which 1201 (55%) were calcified, 584 (27%) were non-calcified and 397 (18%) were mixed. From all analyzed plaques, 864 (39.6%) caused minimal stenosis, 613 (28.0%) caused mild stenosis, 500 (22.9%) caused moderate stenosis, and 207 (9.5%) caused severe stenosis. HRP features were found in 208 (69.3%) patients, including 107 low-attenuation plaques in 85 (28.3%) patients, 54 plaques with napkin-ring sign in 50 (16.7%) patients, 354 plaques with a positive remodeling in 168 (56.0%) patients, and 281 plaques with spotty calcium in 151 (50.3%) patients. 52 (17.3%) patients had only 1 HRP feature, and 156 (52.0%) patients had ≥2 HRP features.

Iron level correlated with LAP presence (*P* <0.0001) and with the increasing number of LAP per patient (*P* = 0.005). Patients with LAP had higher iron levels (111.0 vs. 91.7 µg/dl; *P* <0.0001). There was no association between iron level and napkin-ring sign, positive remodeling and spotty calcium (*P* = 0.6; *P* = 0.5; *P* = 0.9, respectively).

In univariable regression analysis, the predictors of LAP were iron level (odds ratio [OR], 1.02; 95% confidence interval [CI], 1.00–1.03; *P* <0.0001), male gender (OR, 2.50; 95% CI, 1.40–4.46; *P* = 0.002) and Hb level (OR, 1.23; 95% CI, 1.01–1.49; *P* = 0.04) (Supplementary material, *Table S1*). The inflammation parameters (hsCRP and white blood cell) were not LAP predictors (*P* = 0.4; *P* = 0.9, respectively). In multivariable regression analysis, only iron level and male sex were predictors of LAP (OR, 1.02; 95% CI, 1.01–1.03; *P* = 0.0002, and OR, 2.41; 95% CI, 1.24–4.70; *P* = 0.009, respectively) (Supplementary material, *Table S1*). In additional multivariable regression analysis, normalized parameter iron/Hb level and male gender were predictors of LAP (OR, 1.28; 95% CI, 1.12–1.46; *P* = 0.0003, and OR, 2.48; 95% CI, 1.36–4.49; *P* = 0.003, respectively). Multivariable regression analysis performed separately for both sexes showed that iron level was independently associated with LAP in both men (*P* = 0.003) and women (*P* = 0.03).

Additionally performed receiver operating characteristic curve analysis revaled the iron threshold for LAP prediction >98 µg/dl (area aunder the curve 0.665; *P* <0.001). In univariable regression analysis, iron >98 µg/dl was LAP predictor (OR, 3.2; 95% CI, 1.87–5.56; *P* <0.0001). In multivariable regression analysis model, iron >98 µg/dl and male gender

were predictors of LAP (OR, 3.2; 95% CI, 1.80–5.80; *P* = 0.0001, and OR, 2.41; 95% CI, 1.25–4.74; *P* = 0.009, respectively).

Higher iron levels correlated with more fibro-fatty (r = 0.2; *P* = 0.01) and necrotic core (r=0.1; *P* = 0.02); and not with calcified (r = –0.1; *P* = 0.23) nor fibrous (r = –0.03; *P* = 0.82) plaque components (Figure 3). Higher iron levels were positively correlated with the percentage of vulnerable plaque component (*P* = 0.004) and inversely correlated with stable plaque component (*P* = 0.004).

DISCUSSION

Our study for the first time showed a significant association between iron and the presence of LAP and an increasing quantity of vulnerable coronary plaque including fibro-fatty component and necrotic core [18].

The role of iron in the pathogenesis of atherosclerosis has been investigated for more than 40 years, when Sullivan proposed iron as a cardiovascular risk factor, suggesting that it might explain the lower incidence of CVD in premenopausal women [1]. Salonen et al. [5] showed that men with high serum ferritin levels had a 2.2-fold higher risk of MI. Later, Bagheri et al. [3] showed that patients with increased serum iron levels had more severe atherosclerosis. Also, Gustaffson et al. [19] proved that the higher iron concentrations in carotid plaques positively correlated with plaque vulnerability for rupture. A more recent study by Vinchi et al. [4] investigated the susceptibility to atherosclerosis in a mouse model (ApoE–/– FPNwt/C326S), which developed the disease in the context of elevated iron level. The authors showed that iron overload aggravated atherosclerosis in the aorta suggesting that elevated iron levels lead to atherosclerosis progression [4]. However, none of the studies investigated the connection between circulating iron and coronary atherosclerosis structure, including coronary plaque type, the presence of HRP features, and plaque composition. The role of iron stores and iron deffciency in patients with CAD cannot yet be answered with certainty, because there are conflicting reports published [6, 18]. Recent study which investigated the association between iron level and all-cause mortality in patients with acute coronary syndromes showed that patients with lower iron level had worse prognosis [20]. However, study participants were mostly male (75%) with great proportion of ST-elevation MI, therefore results may be influenced by post-MI heart failure development. Moreover, the influence of anemia on the ouitcomes was not excluded [20]. It is possible that patients with

CVD may be negatively affected by both iron deficiency and higher iron levels through different metabolic pathways and mechanisms.

In a SCOT-HEART, large multicenter study, an increased burden of low-attenuation plaque was the principal predictor of an increased number of coronary events, above and beyond other established classic markers of cardiovascular risk, including coronary artery stenosis severity and calcium score [21]. Also, a review by Nerlekar et al. [14] showed that the presence of HRP features is an independent predictor of major adverse cardiac events [16]. It has been shown that 88% of ruptured coronary plaques in patients with acute coronary syndrome had a low attenuation (P <0.0001) [22]. The reliable differentiation between lipid-rich and fibrotic lesions made solely based on CT attenuation until some time ago was not possible [12]. New automated plaque quantification software tools, with scan-specific adaptive attenuation threshold settings, can potentially overcome some of the method limitations and improve CT-based plaque component quantification [23, 24]. Factors causing vulnerable atherosclerotic plaque components growth and LAP formation may comprise potential targets for new anti-atherosclerotic therapies.

The results of our study may be plausibly explained within the context of known pathophysiological and biochemical processes contributing to atherogenesis, as presented in Figure 4. Iron is a redox-active metal. Increased serum iron levels catalyze the formation of ROS and promote LDL oxidation. Oxidized LDLs trigger endothelial activation and induce macrophage recruitment [7]. Moreover, oxidized LDLs are taken up by the LDL receptor (ScR, Scavenger receptor) on pro-inflammatory M1 macrophages leading to their development into foam cells [7, 8, 25–27]. Foam cell infiltration and necrotic core expansion are key events in atherogenesis and vulnerable plaque formation [7]. Necrosis of foam cells leads to further plaque growth and finally to the formation of a necrotic core. Moreover, foam cells may upregulate proteolytic enzymes leading to plaque rupture [28]. Multiple macrophage phenotypes have been detected in atherosclerotic lesions, including macrophages with M1, M2, or M(Hb) phenotypes [29]. Classically activated M1 macrophages promote inflammation and are characterized by high expression of pro-atherogenic cytokines including tumor necrosis factor alpha, interleukin(IL)-6, and IL-1 [30]. M1 macrophages are the predominant phenotype found in advanced, unstable, atherosclerotic plaques, which have a large lipid-rich necrotic core [31]. Moreover, role of the local intraplaque hemorrhage with local iron deposition and oxidation contributing to individual plaque development has not been clearly established yet.

Taking into account the abovementioned data, our study provides a mechanistic link between the current knowledge of iron homeostasis, clinical manifestation of CAD and higher iron levels. More studies investigating this issue are needed to fully explain the influence of the observed phenomenon.

Limitations

The limitations of our study were single-center analysis and lack of full iron metabolism assessment (including the levels of hepcidin, ferritin, and transferrin etc.) or wider spectrum of inflammatory parameters. Further studies with a wider range of investigated iron homeostasis parameters are needed. However, iron measurement is cheap and easily accessible, even for primary care physicians, which may be of practical importance in the context of new biomarkers of increased risk of vulnerable plaque.

Depending on the duration of lipid-lowering therapy and the degree of sustained level of LDL cholesterol, some patients may have already remodelled plaque, including the plaque components. However, the prestented association beween iron ald LAP is significant despite it. Our study population reflect „real-life" population of patients who underwent CTCA in one of the leading cardiology centers in Poland.

Although, there were only 2 women in the age under 50 and 7 women in the age between 50 and 55, the subanalysis including pre- and post- menopausal podgroups were impossible.

This paper did not analyze association between iron and cardiovascular outcomes during long-term follow up, therefore the association between increased iron and LAP does not allow to draw the conclusion that higher iron levels is associated with cardiovascular events in CAD patients.

CONCLUSIONS

Higher iron levels are associated with low-attenuation plaque presence and a greater proportion of vulnerable (fibrofatty and necrotic core) component of atherosclerotic plaques in patients with CAD.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

Conflict of interest: None declared.

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Table 1. Baseline patients characteristics

Parameter	**Value (n = 300)**
Clinical characteristics:	
Age, years	62 (23)
Sex (male)	191 (64%)
BMI, kg/m^2	27.6 (27.0–28.5)
CAD risk factors:	
Hypertension	257 (86%)
Dyslipidemia[a]	203 (68%)
Diabetes	74 (25%)
Family history of CAD	51 (17%)
Smoking — current	46 (15%)
Smoking — in the past	42 (14%)
Medications:	
Beta-blocker	207 (69%)
Statin	180 (60%)
Other lipid lowering drugs	23 (8%)
ACEi	99 (33%)
ARB	89 (30%)
Calcium antagonist	106 (35%)
Diuretic — any	98 (33%)
Metformin	55 (18%)
Acetylsalicylic acid	143 (48%)

Values are mean (SD), n (%) or median [IQR]

[a]Dyslipidemia diagnosis in the medical records at the day of CTCA

Abbreviations: ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CASC, coronary artery calcium score; CTCA, computed tomography coronary angiography; IQR, interquartile range; SD, standard deviation

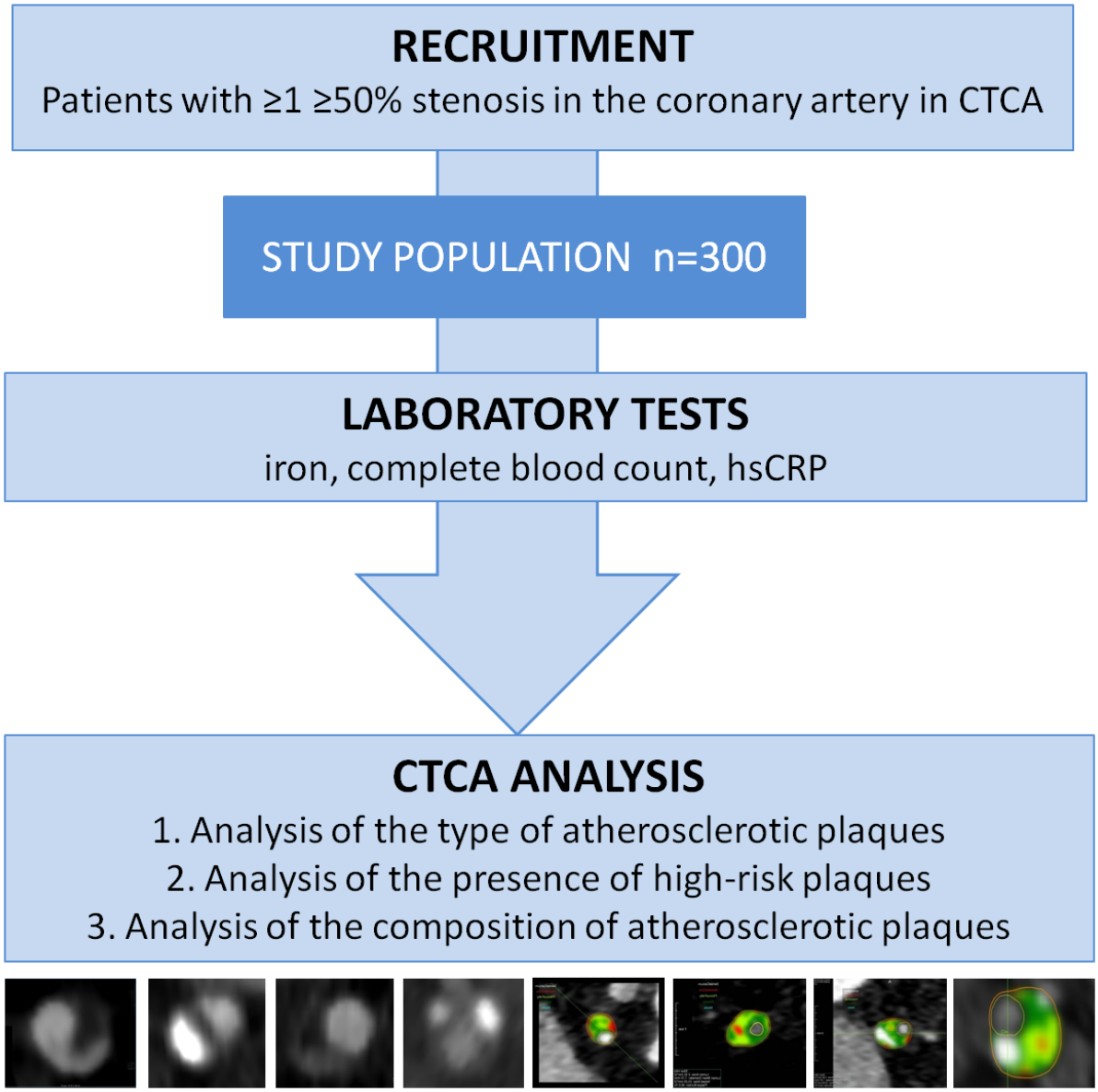

Figure 1. The study flowchart

Abbreviations: CTCA computed tomography coronary angiography; hsCRP, high-sensitivity C-reactive protein

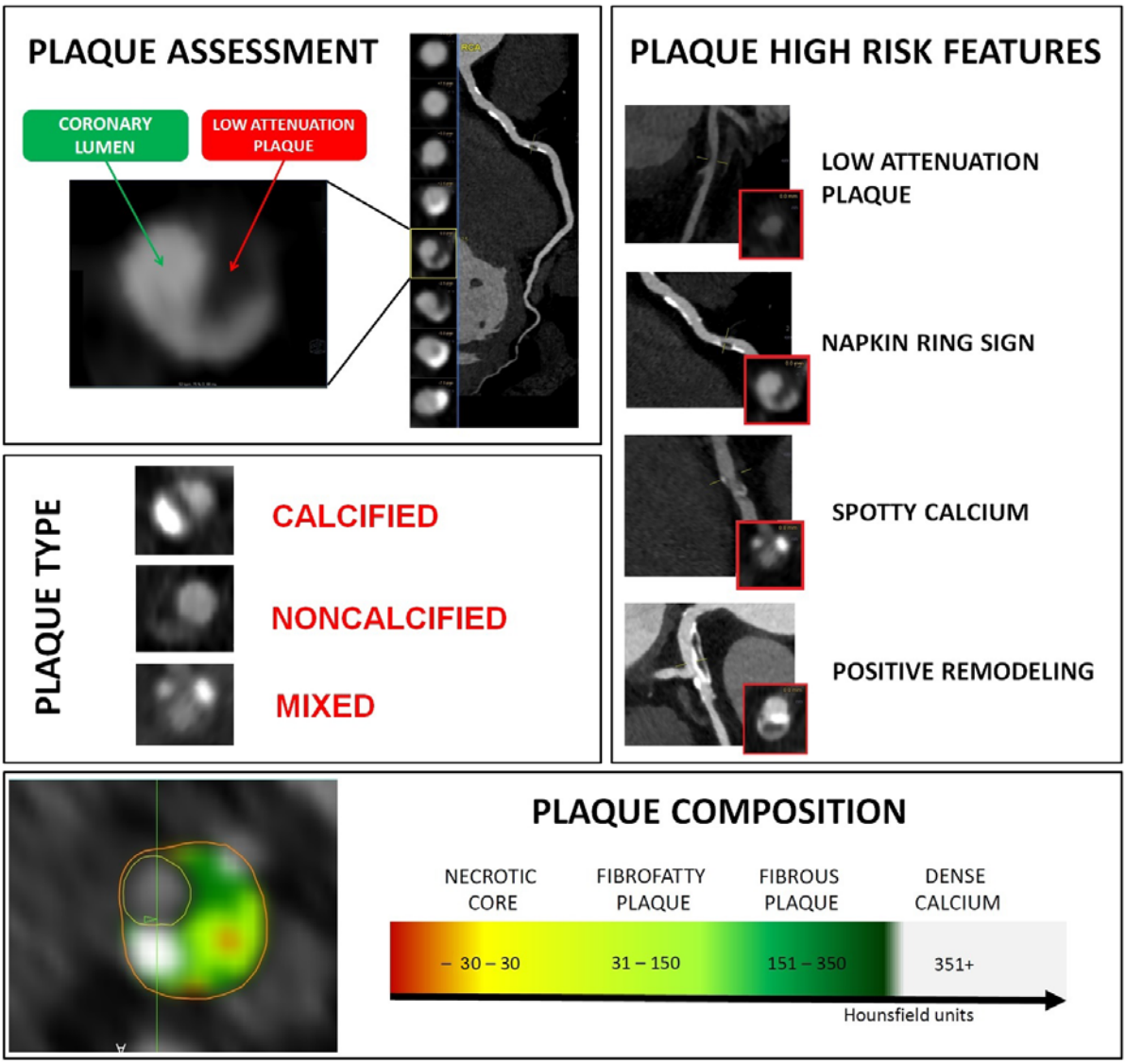

Figure 2. Advanced coronary plaque analysis by computed tomography coronary angiography including: coronary plaque type (calcified, non-calcified, mixed), presence of high-risk plaque features (low attenuation plaque, napkin ring sign, spotty calcium, positive remodeling) and plaque composition (fibro-fatty, necrotic core, fibrous and dense calcium)

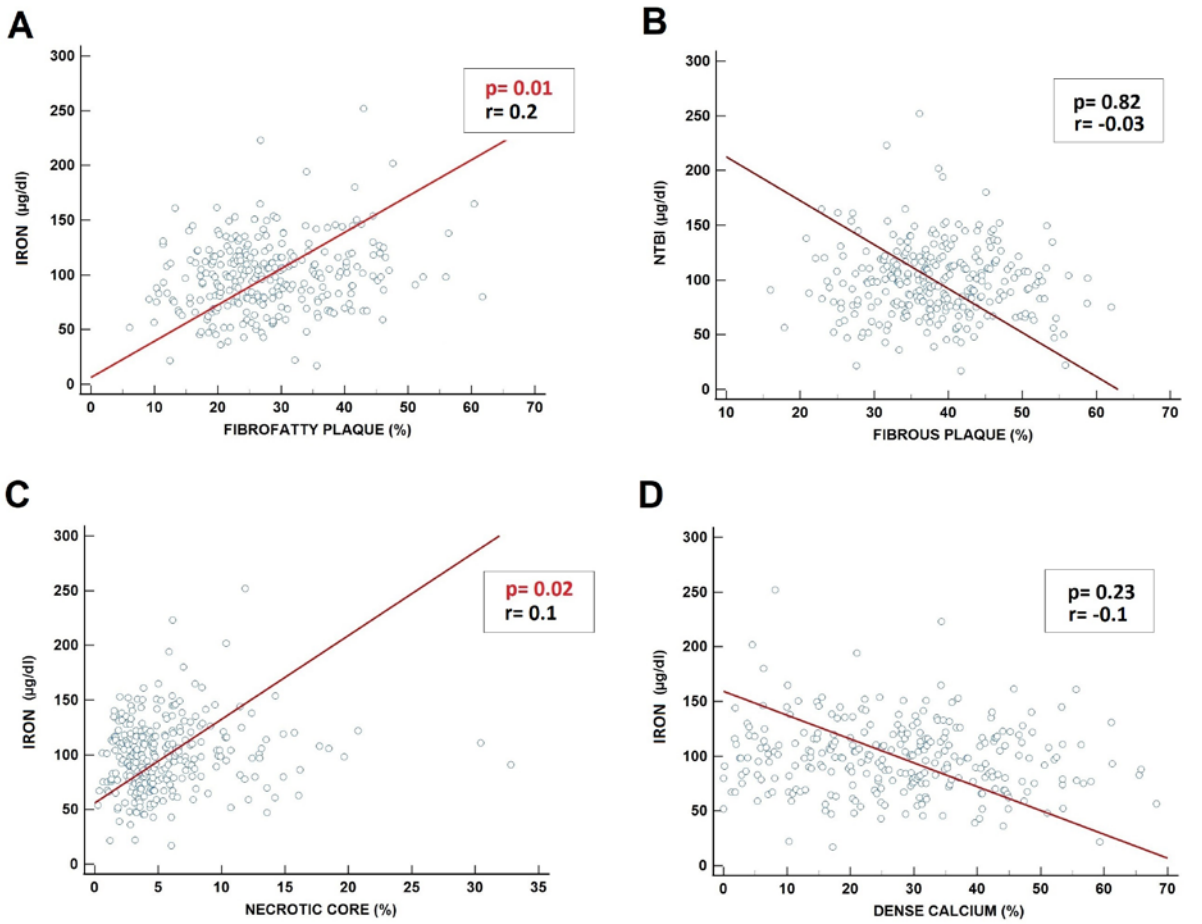

Figure 3. A–D. The association between iron level and main plaque components in quantitative plaque analysis (fibro-fatty, necrotic core, fibrous and dense calcium)

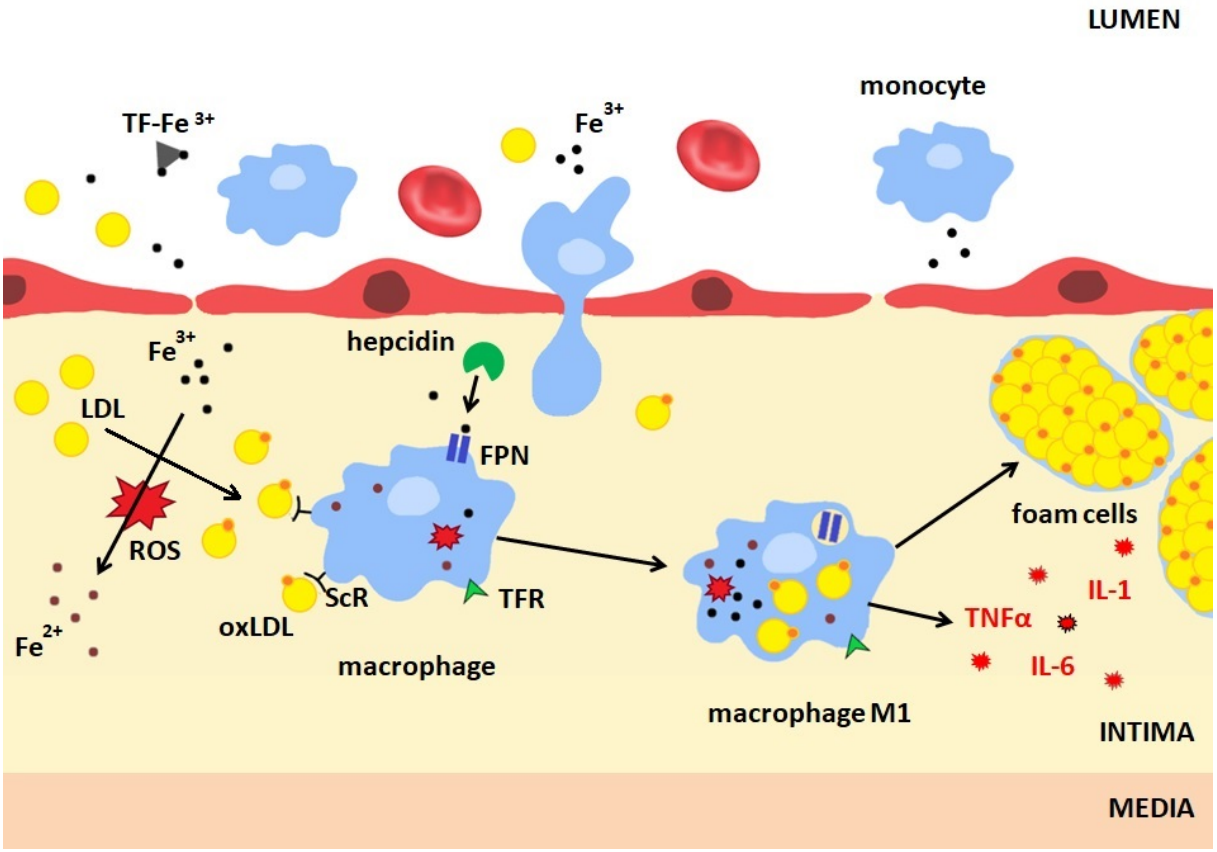

Figure 4. A. The possible explanation of role of iron (presented as $Fe^{3+)}$, in the pathogenesis of low-attenuation plaque (LAP) formation

Abbreviations: Fe, iron; FPN, ferroportin; IL-1, interleukin 1; IL-6, interleukin 6; LDL, low density lipoprotein; oxLDL, oxidized low density lipoprotein; ROS, reactive oxygen species; ScR, Scavenger receptor; TF-Fe, transferrin–iron complex; TFR, transferrin receptor; TNF-α, tumor necrosis factor α