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An increase of interleukin-33 serum levels after coronary stent implantation is associated with coronary in-stent restenosis

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ABSTRACT

The study aim was to determine the predictive value of interleukin (IL)-33, a recently described member of the IL-1 family of cytokines, for the development of in-stent restenosis (ISR). IL-33 serum levels were measured in 387 consecutive patients undergoing percutaneous coronary intervention (PCI) of whom 193 had stable angina, 93 non-ST elevation myocardial infarction (NSTEMI), and 101 ST-elevation MI (STEMI), respectively. Blood was taken directly before and 24 h after stent implantation. The presence of ISR was initially evaluated by clinical means after six to eight months. When presence of myocardial ischemia was suspected, coronary angiography was performed to confirm the suspected diagnosis of ISR. Clinical ISR was present in total in 34 patients (8.8%). IL-33 was detectable in 185 patients and was below detection limit in 202 patients. In patients with decreased IL-33 ($n = 95$), unchanged or non-detectable levels ($n = 210$) or increased levels of IL-33 after PCI ($n = 82$), ISR-rate was 2.1%, 9.5% and 14.6%, respectively ($p < 0.05$). Accordingly, patients with ISR showed a significant increase of IL-33 upon PCI ($p < 0.05$). This association was independent from clinical presentation and risk factors as well as numbers and type of stents. In patients with both stable and unstable coronary artery disease, an increase of IL-33 serum levels after stent implantation is associated with a higher rate of in-stent restenosis.

1. Introduction

Restenosis after percutaneous coronary intervention (PCI) remains an unsolved clinical problem and certain patients appear to be at increased risk of developing restenotic complications. Defining the subgroups of patients at increased or decreased risk for in-stent restenosis (ISR) would be of massive utility for patient risk stratification and for the understanding of underlying molecular and cellular mechanisms [1,2].

Restenosis is a multifaceted disease and pathophysiological mechanisms involved are thought to comprise inflammation, proliferation and extracellular matrix remodeling. Inflammation seems to play a central role in the pathogenesis of ISR [1,3,4]. Both "classical" inflammation, mediated by neutrophils, monocytes and T helper type 1 (Th1) lymphocytes, and "allergic" inflammation, mainly mediated by eosinophils and T helper type 2 (Th2) lymphocytes, are implicated in restenotic reactions [2].

Different inflammatory markers for restenosis have been identified and include complement components C5a and C3a [5], tumor necrosis factor (TNF)-α [6] and interleukin (IL)-3 [7]. Circulating levels of matrix metalloproteinase (MMP)-2, MMP-9, plasminogen activator inhibitor (PAI)-1 and soluble Fas and Fas ligand were also shown as a predictive marker for ISR by our group and others [8–12].

IL-33 is the most recently described member of the IL-1 family of cytokines and is a ligand for the ST2 receptor [13]. IL-33 is expressed intracellularly predominantly by stromal cells such as endothelial and epithelial cells as well as smooth muscle cells and fibroblasts [14–16]. IL-33 is believed to be released during necrosis but kept intracellular during apoptosis where it is inactivated by caspases [17,18]. Therefore, IL-33 is recognized as a dual function cytokine that acts either intracellular to regulate gene transcription or extracellular via binding to ST2. Upon release, IL-33 was shown to be recognized by different immune and non-immune ST2-expressing cells. In such a way IL-33 integrates both

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innate and adaptive immunity in a unique fashion via activation of eosinophils, basophils, mast cells, innate lymphoid cells, and Th2 lymphocytes [16,19]. Thus, IL-33 might function as an alarmin, a danger signal belonging to the larger family of damage-associated molecular pattern (DAMP) molecules [15,20].

IL-33 is involved in the pathogenesis of different inflammatory and allergic disorders such as rheumatoid arthritis, asthma, psoriasis and ulcerative colitis [21–23]. A precise role of IL-33 in the pathogenesis of cardiovascular diseases is still not well defined. Dhillon et al. measured IL-33 levels in patients with myocardial infarction and found that elevated IL-33 was associated with increased mortality in ST-elevation myocardial infarction (STEMI) [24], but was not related to adverse events in non–ST-elevation myocardial infarction (NSTEMI) patients [25].

We propose that IL-33 may be an important player in the pathogenesis of ISR after stent implantation and therefore circulating levels of IL-33 could serve as a biomarker for the development of ISR. The aim of this study was to test whether an increase of IL-33 after PCI is associated with an increased rate of ISR.

2. Methods

2.1. Study population

Blood samples were taken from 387 consecutive patients undergoing PCI. From these patients 193 had stable angina, 93 NSTEMI, and 101 STEMI, respectively. The PCIs were performed according to standard techniques by experienced interventionalists only. Exclusion criteria were presence of autoimmune diseases, chronic infections, hepatic or renal disorders. Aspirin and unfractionated heparin were administered per standard practice. Clopidogrel therapy was started either on the day before angiography or immediately after stent implantation with 300 mg. After the procedure, patients were maintained on aspirin 100 mg indefinitely, and clopidogrel 75 mg according to guidelines of European Society of Cardiology (ESC). Other medications such as beta-blockers and angiotensin-converting-enzyme inhibitors were given as appropriate. Statin therapy was routinely administered to all patients according to international guidelines. At inclusion time new antiplatelet drugs like ticagrelor and prasugrel were not yet available. After enrollment, patients remained in the hospital for at least 48 h. Informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the ethics committee of the city of Vienna, Austria.

2.2. Blood samples

Two blood samples were taken directly before PCI (at baseline) and 24 h after PCI. Blood drawing was performed under fasting conditions whenever possible (in stable patients and 24 h after the event in patients with acute events). Venous blood was drawn from the antecubital vein with minimal tourniquet pressure into serum separator tubes. Samples were allowed to clot for 30 min (min) before centrifugation (4 °C; 3000 g for 15 min) and stored at −80 °C until use.

2.3. Laboratory measurements

IL-33 was measured with a specific enzyme-linked immunosorbent assay (ELISA; R&D Systems; Minneapolis, MN, USA). The minimum detection limit of the assay was 23.4 pg/mL. The sensitivity of the assay is expressed as minimum detectable dose (MDD) and mean MDD was 0.5 pg/mL. For calculation of the intra-assay coefficient of variability (CV) three samples of known concentration were tested twenty times on one plate and mean ± SD was assessed as 3.2 ± 1.0%. For calculation of the inter-assay CV three samples of known concentration were tested in twenty separate assays and mean ± SD was assessed as 5.3 ± 0.5%. Laboratory determinations were performed by investigators that were blinded to clinical characteristics and patients' outcome.

2.4. Angiographic definitions

Maximal lumen stenosis was measured within the stent and within the 5-mm proximal and distal edges of the stent. All measurements were performed by the same investigator that was blinded to all laboratory results.

2.5. End points

All patients were reevaluated clinically for recurrent anginal symptoms after six to eight months. Patients with clinical signs of restenosis underwent re-angiography. The primary end point of the study was the need for target lesion revascularization due to restenosis in the presence of symptoms or objective signs of ischemia during the follow-up.

2.6. Statistical analysis

Sample size calculation was based on the hypothesis that IL-33 increase is associated with at least a 15% higher restenosis rate as compared to patients with IL-33 decrease. Sample size calculation revealed that we would need at least 75 patients per group to detect a difference with a power of 80% and significance level (two-tailed) of 0.05 [26]. As IL-33 was not detectable in approximately 50% of patients we increased the sample size accordingly. Continuous variables are expressed as mean ± SD or as median, interquartile range. Categorical variables are summarized as counts and percentages and were compared by the chi-square or by Fisher exact test. Continuous variables were compared using Student's *t*-test when normally distributed and by Mann–Whitney-*U* test when not normally distributed. Spearman correlation was used to determine the correlation between level of IL-33 and cardiovascular risk factors. Multivariate analysis was performed with the logistic regression model in which restenosis was used as dependent variable and potentially confounding baseline variables were used as independent variables. Baseline variables were selected for the model if they (a) had either a clinically plausible relation with the outcome or (b) appeared to be imbalanced between patients with and without restenosis indicated by a p-value < 0.20. A value of $p < 0.05$ (two-tailed) was considered statistically significant. All statistical analyses were performed with the statistical software package SPSS version 18.0 (SPSS, Inc., Chicago, Illinois).

3. Results

3.1. Patient characteristics

BMS were used in 283 and DES were used in 104 patients. Clinical ISR was present in total in 34 patients (8.8%; 7 DES and 27 BMS). Target lesion revascularization was performed in all 34 patients. Baseline demographic data are shown in Table 1. Patients with and without restenosis at follow-up showed no significant differences in baseline clinical characteristics and cardiovascular risk factors. However, patients in the restenosis group tended to have a higher prevalence of hypertension, family history of CAD, hyperlipidaemia and peripheral artery occlusive disease (PAOD) (Table 1). There were no significant differences in baseline angiographic characteristics (Table 2).