RESEARCH ARTICLE

No predictive value of serum interleukin-6 and transforming growth factor-β1 in identifying patients with a first restenosis, recurrent restenosis or a history of restenosis

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ABSTRACT. *Background*. The efficacy of percutaneous coronary intervention (PCI) is limited by the need for repeat revascularization resulting from restenosis. The restenosis rate after treatment for in-stent restenosis (recurrent restenosis) is high (> 30%). Numerous studies have suggested the predictive value of interleukin 6 (IL-6) and transforming growth factor β1 (TGF-β1). *Methods*. We sought to determine whether serum levels of IL-6 and TGF-β1 could help identify individuals with recurrent restenosis. Thirty seven patients with a history of stent implantation were enrolled and divided into three groups: (1) patients with a current, first restenosis (n = 9); (2) patients with current restenosis and at least one prior restenosis (recurrent restenosis) (n = 11), and (3) patients with a history of restenosis, but without current restenosis (n = 17). *Results*. The baseline profile was similar in all three groups. The median (25^{th}-75^{th} percentile) concentrations of IL-6 were: group 1 – 2.8 (1.4-5.5); group 2 – 2.6 (0.6-8.6); group 3 – 2.4 (0.9-4.7) p = 0.69 and TGF-β1: group 1 – 3.6 (0.2-14.4); group 2 – 4.2 (1.8-57.6); group 3 – 6.6 (2.8-30.0) p = 0.57. Moreover we found no correlation, either between diameter stenosis and IL-6 (R = 0.10; p = 0.38) or TGF-β1 (R = 0.10; p = 0.57). Both IL-6 (AUC 0.59 p = 0.4 and AUC 0.51 p = 0.9) and TGF-β1 (AUC 0.64 p = 0.2 and AUC 0.50 p = 0.9) failed to provide significant results in receiver-operating characteristic analysis. *Conclusion*. We report that there is no association between the severity of diameter stenosis (restenosis) and IL-6 or TGF-β1 concentrations. Our findings might suggest that levels of IL-6 and TGF-β1 have no predictive value for identifying patients with recurrent restenosis.

Keywords: interleukin-6, transforming growth factor β1, recurrent restenosis

Percutaneous coronary intervention (PCI) with stent implantation has revolutionized the management of coronary artery disease (CAD) [1]. The efficacy of PCI is limited by the need for repeat revascularization resulting from restenosis. Bare-metal stents lead to in-stent restenosis (ISR) in 20-30% of cases [2], and the restenosis rate after treatment for an initial ISR – recurrent restenosis – is over 30%.

Numerous studies have suggested that ISR is initiated by inflammatory cells, which participate in an intense reaction caused by stent placement. Stent implantation leads to the release of a variety of cytokines that are responsible for rapid restenosis.

Recent studies have suggested a potential modulating ability for two cytokines: interleukin-6 (IL-6) and transforming growth factor-β1 (TGF-β1). IL-6, being one of the essential inflammatory mediators, plays a key role in the initiation of the inflammatory reaction [3, 4]. Acting locally on the vessel wall, it stimulates the production of C-reactive protein. TGF-β1 coordinates regenerative processes in the body [4, 5]. It enhances atherogenesis by increasing the synthesis of extracellular matrix (ECM). However, it also modulates immune reactions by inhibiting the inflammatory response, reducing the migration and proliferation of smooth muscle cells (SMCs) *in vitro*, thereby producing anti-atherogenic effects [6].

We aimed to investigate the value of serum IL-6 and TGF-β1 concentrations as predictors of restenosis in a population of patients who had previously undergone percutaneous coronary intervention. We sought to determine whether serum levels of IL-6 and TGF-β1 could help to identify individuals with recurrent ISR, who constitute a high-risk group, potentially optimizing the use of coronary stenting in the treatment of CAD.

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METHODS

The approval of the local bioethics committee was obtained. All patients gave written, informed consent and the study conformed to the Declaration of Helsinki. Patients with a history of stent implantation who underwent coronary angiography from January 2006 to July 2008 were entered into the study. Coronary angiography was performed because of the clinical presentation or as a result of exercise testing. Thirty seven patients were enrolled and divided into three groups:
– group 1: patients with a current, first restenosis (n = 9);
– group 2: patients with current restenosis and at least one prior restenosis (recurrent restenosis) (n = 11);
– group 3: patients with a history of restenosis, but without current restenosis (n = 17).
The main exclusion criteria were: coexisting autoimmune disorders, acute infectious diseases, chronic inflammatory diseases, renal failure (creatinine serum concentration > 1.5 mg/dL), known malignant diseases and lack of patient consent to participate. Other exclusion criteria are listed in our previous publication [7].
Angiographic restenosis was defined by a binary approach and the commonly used cut-off of ≥ 50% diameter stenosis. The choice of ISR treatment modality was at the operators' discretion.
Serum concentrations of IL-6 and TGF-β1 were measured with commercial kit enzyme-linked immunosorbent assays (ELISA) (RTOTOD Systems, USA) in duplicates. Measurements for each patient were made with the same kit to avoid inter-kit variability.
For the IL-6 measurements, the intra-assay coefficient of variation (% CV) was 4.2%, the inter-assay % CV was 6.4%, and the sensitivity of the ELISA was: < 0.7 pg/mL. For the TGF-β1 measurements, the intra-assay % CV was 7.3%, the inter-assay % CV was 1.8%, and the sensitivity of the ELISA was: < 7.0 pg/mL. Patients did not receive any medication mentioned in the exclusion criteria prior to the study.

STATISTICAL ANALYSIS

Quantitative data are presented as means ± standard deviations (SD), medians, and lower and upper quartiles. Qualitative data are presented as frequencies. The Shapiro-Wilk test was used to determine whether random samples came from normal distribution. The Chi-square test with Yates' correction was used to compare categorical variables. Because of the sample size, the Kruskal-Wallis analysis of variance (ANOVA) test was used to compare continuous variables between groups. Receiver-operating characteristic (ROC) curves were estimated for both IL-6 and TGF-β1 concentrations. The areas under the ROC curves (AUC) for IL-6 and TGF-β1 were compared by a nonparametric test. A ROC analysis was planned to identify possible cut-offs to predict restenosis. A value of $p < 0.05$ was considered to be significant.

RESULTS

The baseline profile was similar in all three groups *(table 1)*. Only the rate of prior myocardial infarction was lower in group 2 (63.4%) compared to group 1 (77.8%) and group 3 (70.6%) (p = 0.01). A longer hospital stay was observed in patients with first (7.2 ± 2.9 days) and recurrent restenosis (6.2 ± 2.4 days), compared to patients with a history of restenosis (4.5 ± 1.4 days) (p = 0.006). Other clinical and angiographic parameters are summarized in *table 2*. Interestingly, the recurrent restenosis group had a lower prevalence of multivessel CAD (36.4%) compared to the other two groups (p = 0.01). In addition, group 2 was more likely to have had more severe angina at admission (Canadian Cardiovascular Society angina class III or IV) (81.82%) compared to groups 1 (66.70%) and 3 (41.17%) (p = 0.05). Laboratory test results were similar in all three groups *(table 3)*. Median concentrations of IL-6 and TGF-β1 were similar irrespective of restenosis status *(figure 1)*. There was no relationship between IL-6 and TGF-β1 concentrations and the percentage of diameter stenosis *(figure 2)*. IL-6 [group 1 *versus* group 3 – AUC 0.64 (95% CI 0.43-0.81) p = 0.22; group 2 *versus* group 3 – AUC 0.54 (95% CI 0.34-0.72) p = 0.74] and TGF-β1 [group 1 *versus* group 3 – AUC 0.61 (95% CI 0.39-0.81) p = 0.34; group 2 *versus* group 3 – AUC 0.53 (95% CI 0.31-0.74) p = 0.8] failed to provide significant results in ROC analysis.

DISCUSSION

ISR is rapidly becoming the major drawback of repeated PCI procedures with multiple stent implantations. Bauters *et al.* reported a second restenosis rate of 39% [8] while Jeong *et al.* found a much higher rate of 51.4% [9].

Table 1
Patients' baseline characteristics

	Group 1 n = 9	Group 2 n = 11	Group 3 n = 17	p
Age, years (mean ± SD)	64.6 ± 10.2	58.7 ± 12.8	61.6 ± 8.4	0.52
Gender, males n (%)	6 (66.7%)	7 (63.4%)	14 (82.3%)	0.5
Systemic hypertension n (%)	6 (66.7%)	6 (54.5%)	14 (82.3%)	0.28
Hyperlipidemia n (%)	6 (66.7%)	7 (63.4%)	18 (88.2%)	0.18
Diabetes mellitus n (%)	4 (44.4%)	3 (27.3%)	6 (35.3%)	0.5
Prior myocardial infarction n (%)	9 (77.8%)	7 (63.4%)	12 (70.6%)	0.01
Prior CABG n (%)	2 (22.2%)	2 (18.2%)	1 (5.9%)	0.4

Table 2
Clinical and angiographic characteristics

	Group 1 n = 9	Group 2 n = 11	Group 3 n = 17	p
Hospital stay, days (mean ± SD)	7.2 ± 2.9	6.2 ± 2.4	4.5 ± 1.4	0.006
% diameter stenosis (restenosis) (mean ± SD)	84.4 ± 13.5	82.2 ± 29.0	8.2 ± 13.3	< 0.0001
Multivessel CAD	7 (77.8%)	4 (36.4%)	14 (82.4%)	0.01
CAD severity				
- CCS 2	3 (33.33%)	2 (18.18%)	10 (58.82%)	0.05
- CCS 3	6 (66.67%)	6 (54.55%)	6 (35.29%)	
- CCS 4	0 (0.0%)	3 (27.27%)	1 (5.88%)	
LVEF (%) (mean ± SD)	43.5 ± 12.8	50.5 ± 12.4	41.3 ± 10.6	0.05
METs (mean ± SD)	8.0 ± 2.4	7.2 ± 2.5	5.6 ± 1.7	0.16
BMI (mean ± SD)	26.7 ± 2.6	26.4 ± 3.4	28.2 ± 4.6	0.4

Table 3
Laboratory findings

	Group 1 n = 9	Group 2 n = 11	Group 3 n = 17	p
Leucocytes (10^3/mm^3)	6.5 ± 1.8	7.1 ± 2.3	7.6 ± 2.0	0.4
Erythrocytes (10^6/mm^3)	4.6 ± 0.4	4.5 ± 0.4	4.3 ± 0.5	0.3
Hemoglobin (mmol/L)	8.4 ± 1.2	8.5 ± 0.8	8.5 ± 1.0	0.9
Hematocrit (%)	41 ± 5	42 ± 4	41 ± 4	0.7
Platelets (10^3/mm^3)	196 ± 91	192 ± 42	182 ± 64	0.8
Fasting glucose (mmol/L)	5.4 ± 0.3	6.2 ± 2.1	5.8 ± 2.1	0.7
Total cholesterol (mmol/L)	5.0 ± 1.1	4.4 ± 1.1	4.7 ± 0.8	0.5
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	0.9
LDL cholesterol (mmol/L)	3.2 ± 1.2	2.6 ± 1.1	2.8 ± 0.8	0.6
Triglycerides (mmol/L)	1.3 ± 0.4	1.0 ± 0.5	1.3 ± 0.5	0.3
Creatinine (μmol/L)	85.6 ± 9.7	77.9 ± 16.9	80.7 ± 15.5	0.5

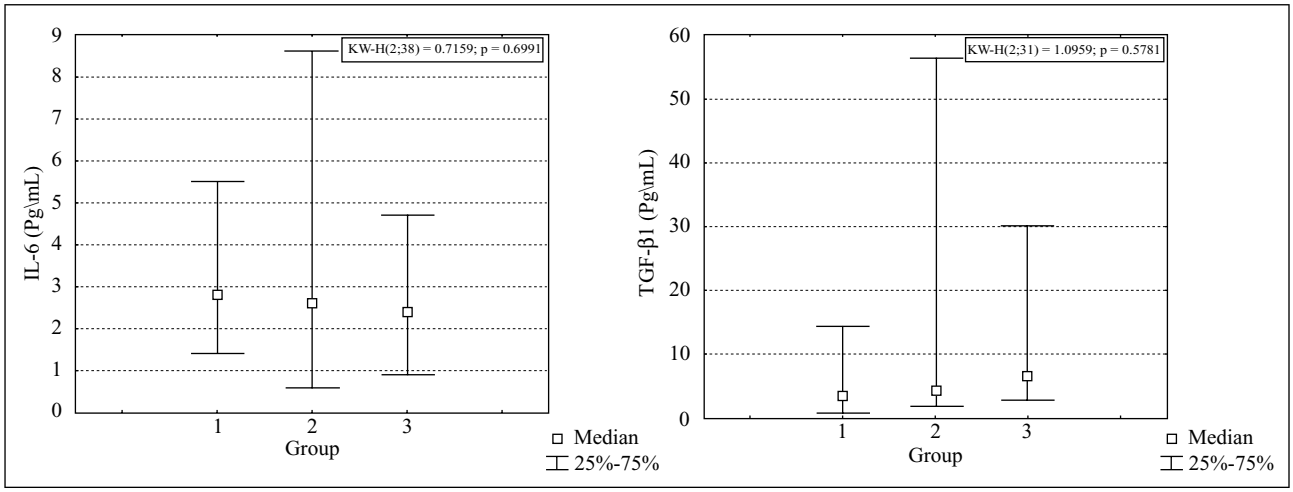

Figure 1
Median concentrations of IL-6 and TGF-β1(KW-H – Kruskal-Wallis ANOVA test).

Intimal hyperplasia is the predominant mechanism involved in the development of ISR. A number of studies have shown that the inflammatory process plays a pivotal role in the proliferation of the neointima, leading subsequently to ISR [10]. During the weeks or months after coronary intervention, the stent is covered with neointima, a structure composed of immunopositive SMCs, surrounded by proteoglycan matrix [11, 12].

Newman *et al.* [13] demonstrated an increased concentration of IL-6 in patients with acute myocardial infarction and Biasucci *et al.* [14] found similar results in patients with unstable angina. Ikeda *et al.* [15] measured IL-6 concentrations in patients with acute coronary syndromes (ACS), and found a higher rate of restenosis in patients with elevated IL-6 concentrations. This strongly suggested that IL-6 is not only a marker of inflammation,

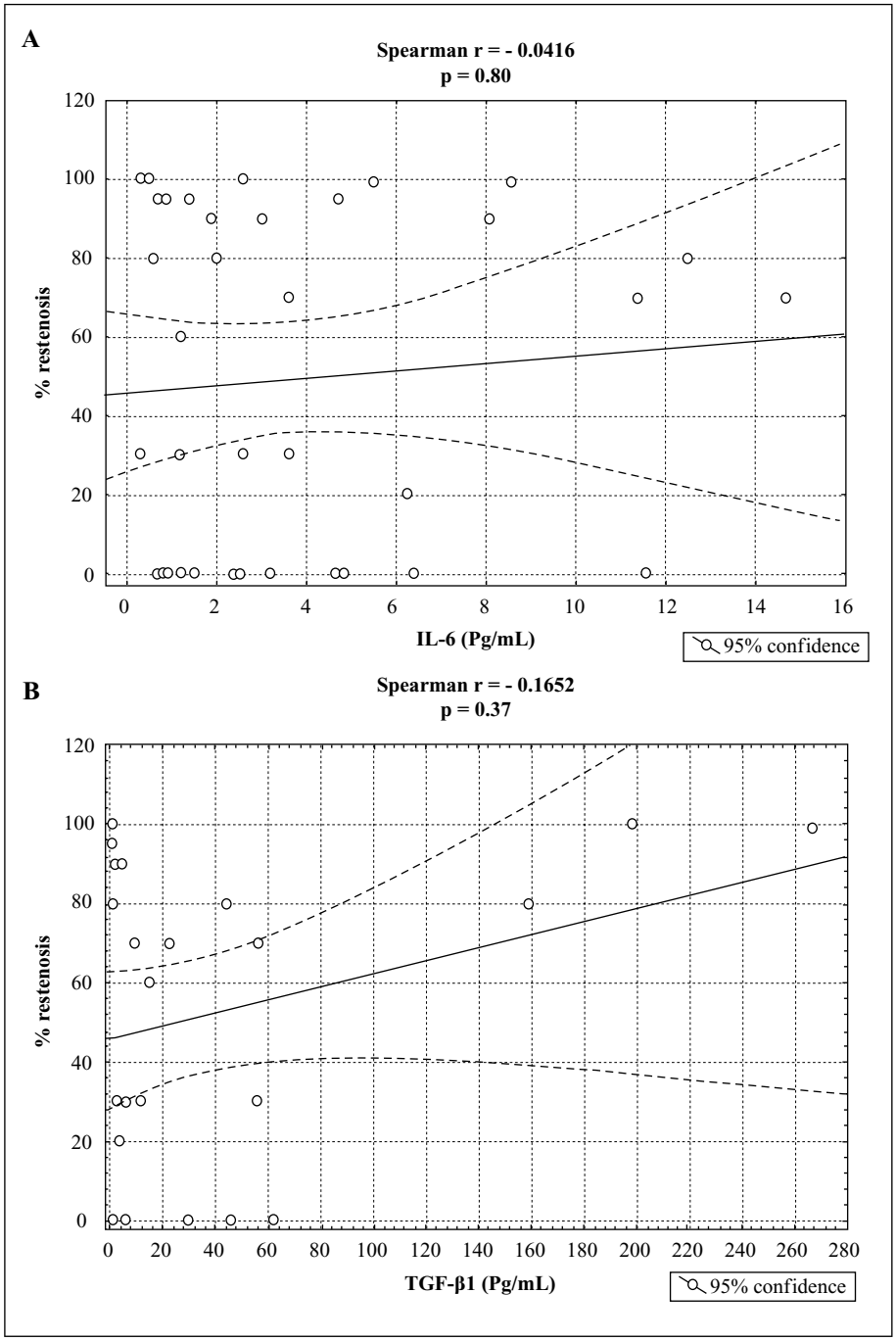

Figure 2
Correlation between IL-6 concentration and percentage of diameter stenosis (**A**), and TGF-β1 concentration and percentage of diameter stenosis (**B**).

but also potentially a marker of late restenosis after PCI [15]. Hojo *et al.* [16] reported increased IL-6 concentrations in the coronary sinus, regardless of the therapeutic technique, and elevated IL-6 levels correlated significantly with the rate of late restenosis. The authors argued that the development of an excessive and prolonged inflammatory reaction to PCI correlated with the highest concentration of IL-6 and led to restenosis in susceptible patients in a short time [16]. The value of high serum concentrations of IL-6 in predicting restenosis has also been reported by Suzuki *et al.* [17].

However, we studied 37 patients who had had a previous PCI and found the concentration of IL-6 to be similar in all three groups, irrespective of their restenosis status (initial restenosis, recurrent restenosis, or history of restenosis). We also found no correlation between IL-6 concentrations and the severity of the stenosis. ROC curve analysis confirmed the low diagnostic value of IL-6. IL-6 concentrations did not distinguish patients with a history of restenosis from those with an actual restenosis, first or recurrent, nor could we find any reports in the literature of an association between IL-6 concentration and recurrent restenosis. In view of earlier results demonstrating that cytokine concentrations might serve as the indicator of ISR (*i.e.* first restenosis), one has to keep in mind that this statement might no longer be true in the case of recurrent restenosis.

Our findings agree with those of Segev *et al.* who reported that increased IL-6 concentrations did not predict late restenosis [18]. Gomma *et al.* obtained similar results [19]. They investigated whether measurements of inflammatory markers could predict late ISR, and found no relation between levels of inflammatory markers and ISR rates six months after stent deployment.

Neointimal hyperplasia (intimal thickening) is the most important contributor to ISR. TGF-β1 has been reported to stimulate ECM protein expression and vascular SMC proliferation and migration, leading eventually to luminal narrowing. Attenuation of TGF-β1 activity has been shown to diminish intimal thickening and reduce ISR [20, 21]. Nikol *et al.* [22] reported elevated concentrations of TGF-β1 in the SMCs of coronary arteries. Moreover, they demonstrated that restenotic lesions, after PCI, showed higher concentrations of TGF-β1 than primary lesions. Chung *et al.* [23] examined human restenotic lesions after stenting and found increased levels of TGF-β1. Overall, it appears that the mechanisms induced by TGF-β1 lead to vascular remodeling and fibrosis that, under some circumstances, are reparative (plaque stabilization), but under others are harmful (neointimal hyperplasia in restenosis).

In our study, we found no differences in the concentrations of TGF-β1 in patients with a current initial restenosis or recurrent restenosis, or a history of past restenosis. The concentration of TGF-β1 was higher in patients with only a history of restenosis, possibly reflecting anti-inflammatory and vasoprotective effects for TGF-β1, but this was not statistically significant. Furthermore, in this subset of patients we found no correlation between the severity of the stenosis and the concentration of TGF-β1, nor did the analysis of the area under the ROC curve produce any significant results.

Disclosures. *No commercial association to disclose.*

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