

# Interleukin-6 and soluble Interleukin-6 receptor in peritoneal fluid and serum of patients with endometriosis

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*Summary:* OBJECTIVE - Interleukin-6 (IL-6) and soluble Interleukin-6-receptor (sIL-6R) concentrations were investigated in patients with endometriosis and other benign gynecologic diseases.

METHODS - During laparoscopy or laparotomy peritoneal fluid and serum were collected from 29 patients with endometriosis, 31 patients with benign ovarian masses and 4 patients with chronic inflammation or adhesions. Interleukin-6 (IL-6) concentrations were determined by Elisa-technique.

RESULTS - Patients with endometriosis stage IV revealed slightly higher IL-6 concentrations in peritoneal fluid when compared to patients with stage I to III disease and ovarian masses/chronic inflammation. IL-6 serum concentrations were higher in cases of stage I and II when compared to stage III and IV and ovarian masses/chronic inflammation. Patients with endometriosis revealed significantly higher sIL-6 receptor concentrations in peritoneal fluid and serum as compared to patients with ovarian cysts and chronic inflammation.

CONCLUSION - IL-6 and soluble IL-6 receptor may be considered to be involved in endometriosis. However, the patho-physiologic mechanism must be the subject of further investigation.

*Key words:* Interleukin-6; Soluble Interleukin-6 receptor; Endometriosis; Interleukin-6 in endometriosis.

## INTRODUCTION

Peritoneal fluid and its cellular and humoral constituents are considered to be involved in the pathophysiology of pelvic

inflammatory disease, endometriosis and infertility <sup>(1)</sup>.

Interleukin-6 (IL-6) is a cytokine produced by various cell types including peritoneal macrophages <sup>(2)</sup> and human endometrial <sup>(3)</sup> as well as endometriotic stromal cells <sup>(4)</sup>.

IL-6 is suggested to be an important mediator in acute phase reaction, inflammation and angiogenesis during folliculogenesis and formation of decidua <sup>(5)</sup>. Oosterlynck *et al.* <sup>(6)</sup> considered angiogenic activity important in the growth and progression of endometriotic lesions. As a consequence, a role of IL-6 in the endometriosis process may be assumed. In this study IL-6 and soluble Interleukin-6

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receptor concentrations in peritoneal fluid and serum were measured in patients with endometriosis and in those with other benign gynecologic diseases.

## MATERIALS AND METHODS

### *Patients*

Peritoneal fluid and serum were obtained from 64 patients during laparoscopy or laparotomy for benign gynecologic disease. The mean age of the patients was 33.9 ( $\pm 12$ ) years ranging from 18 to 72 years. Thirty-one patients had benign ovarian masses. Chronic inflammation or adhesions were found in 4 cases. Endometriosis was diagnosed in 29 patients. The revised American Fertility Society Classification was used for staging of endometriosis. Four patients had endometriosis stage I, 9 stage II, 8 stage III and 8 stage IV.

Protein content, Interleukin-6 and Interleukin-6-receptor. Peritoneal fluid was centrifuged for sedimentation of cellular material. The supernatants and the serum were stored at  $-80^{\circ}\text{C}$  until measurements were performed. Interleukin-6 and soluble Interleukin-6 receptor concentrations were determined by the quantitative "sandwich" enzyme immunoassay technique using commercial kits (IL-6: R&D Systems, Minneapolis, USA; IL-6-Receptor: Biosource, Camarillo, USA). The Bio-Rad-Protein Assay Dye Reagent (BIO-RAD, Richmond, USA) was used for measurement of protein content of peritoneal fluid. Interleukin-6 and soluble Interleukin-6 receptor concentrations in peritoneal fluid were referred to total protein concentrations.

### *Statistical analysis*

Statistical evaluation of the data was performed by the Wilcoxon test. Medians were used for statistical description as the data, tested by Kolmogoroff-Smirnoff-test, were not normally distributed.

## RESULTS

### *Interleukin-6*

Patients with endometriosis stage IV revealed slightly higher IL-6 concentration in peritoneal fluid when compared to patients with stage I to III disease and ovarian masses/chronic inflammation. However, the differences were not statistically significant. In contrast IL-6 serum

concentrations were higher in cases of endometriosis stage I and II when compared to stage III and IV or patients with benign ovarian masses/chronic inflammation. Patients having ovarian masses or chronic inflammation/adhesions showed similar IL-6 values. IL-6 concentrations in peritoneal fluid were not correlated to serum values.

### *Soluble Interleukin-6 receptor*

As the commercial sIL-6R test kit was available sIL-6 receptor concentration was measured in 24 nonselected consecutive patients. Patients with endometriosis revealed significantly higher sIL-6 receptor concentrations in peritoneal fluid and serum as compared to patients with ovarian cysts or chronic inflammation ( $p < 0.05$ ). The peritoneal fluid of stage I and II disease revealed higher amounts of sIL-6 receptor than those of stage III and IV, but this was not statistically significant. Patients with ovarian masses and chronic inflammation/adhesions showed similar sIL-6 receptor values.

## DISCUSSION

Immunological mechanisms are postulated as being involved in endometriotic disease. Several Authors have reported decreased natural killer cell activity in patients with endometriosis (<sup>7, 8, 9</sup>). According to the transplantation theory of Sampson it is assumed that defective immunological mechanism has facilitated outgrowth of endometriotic implants (<sup>10</sup>).

IL-6 is suggested as a mediator in angiogenesis, which may be important during progression of endometriotic lesions. Angiogenic activity of peritoneal fluid was enhanced in patients with endometriosis (<sup>6</sup>). Tabibzadeh *et al.* demonstrated estradiol regulated IL-6 secretion by human endometrial stromal cells (<sup>3</sup>). The Authors suggested an effect of IL-6 on the proliferation of overlying epithelium

Table 1. — *Interleukin-6 concentrations in serum and peritoneal fluid.*

INTERLEUKIN-6					
		Serum pg/ml		Peritoneal fluid pg/mg protein	
		Median	Range	Median	Range
<i>Endometriosis</i>					
Stage I . . . . .	n. = 4	1.3	1 - 16.1	0.6	0.4 - 1
Stage II . . . . .	n. = 9	0.9*	0 - 7.1	0.6	0 - 100
Stage III . . . . .	n. = 8	0*	0 - 1	0.7	0 - 5.9
Stage IV . . . . .	n. = 8	0	0 - 6.7	1.2	0.3 - 17.3
Total . . . . .	n. = 29	0.3	0 - 16.1	0.7	0 - 100
Ovarian masses/ chronic inflammation/ adhesions . . . . .	n. = 35	0.005	0 - 8.1	0.6	0 - 41.3

\* p = 0.0165.

in a hormonally modulated way. In contrast IL-6 secretion by endometriotic stromal cells was not influenced by estradiol or progesterone<sup>(4)</sup>. An autocrine regulation of IL-6 in development of endometriotic lesions may be supposed as anti-IL-6 inhibited endometriotic cell proliferation in vitro<sup>(4)</sup>.

In agreement with our results Boutten *et al.* reported that IL-6 concentrations in peritoneal fluid of patients with stage I and II disease were not significantly different when compared to endometriosis-

free women<sup>(2)</sup>. In our population slightly enhanced IL-6 values were found in the peritoneal fluid of patients with stage IV disease, which were not included in Boutten's study. However, Boutten *et al.*<sup>(2)</sup> demonstrated significantly enhanced IL-6 secretion by peritoneal macrophages of endometriosis patients.

Whether the IL-6 detected in the peritoneal fluid of patients with endometriosis originated from endometriosis implants themselves or from activated peritoneal macrophages could not be decided by this

Table 2. — *Soluble Interleukin-6-receptor concentrations in serum and peritoneal fluid.*

INTERLEUKIN-6-RECEPTOR					
		Serum pg/ml		Peritoneal fluid pg/mg protein	
		Median	Range	Median	Range
<i>Endometriosis</i>					
Stage I . . . . .	n. = 1	1171	—	2.7	—
Stage II . . . . .	n. = 7	111	83 - 1231	2.1	0.9 - 4.8
Stage III . . . . .	n. = 2	569	88.3 - 1050	0.9	—
Stage IV . . . . .	n. = 6	144	8.4 - 211	1.2	0 - 2.9
Total . . . . .	n. = 16	188*	8.4 - 1231	1.9**	0 - 4.8
Ovarian masses/ chronic inflammation/ adhesions . . . . .	n. = 8	30*	8.9 - 34.8	0.3**	0 - 1

\* p = 0.0346; \*\* p = 0.0282.

study. As patients with benign ovarian masses had IL-6 concentrations comparable to patients with mild endometriosis IL-6 production by peritoneal macrophages may be assumed.

In general, soluble cytokine receptors are supposed to be natural antagonists of cytokine<sup>(11)</sup>. Nevertheless, there is strong evidence that soluble IL-6 receptor mediates and potentiates cytokine action<sup>(12, 13, 14)</sup>. Moreover, the biological effects of IL-6 may be mediated to cells not expressing membranous IL-6 receptor<sup>(14, 15)</sup>. In the present study patients with endometriosis showed significantly enhanced sIL-6 receptor concentrations in peritoneal fluid and serum. The sIL-6R is generated by the shedding of the membrane IL-6 receptor<sup>(14)</sup>. It may be speculated that proteases secreted by endometriotic cells cause enhanced shedding of membrane IL-6 receptor. In contrast to IL-6 peritoneal fluid of patients with stage I and II disease revealed higher sIL-6 receptor levels when compared to stage III and IV. Due to the small number of observations these correlations require further confirmation. sIL-6R may be masked in stage III/IV endometriosis, revealing higher IL-6 concentrations by bioactive IL-6-sIL-6R complexes.

In summary, IL-6 and soluble IL-6 receptor, may be considered to be involved in endometriosis. Direct effects on endometriotic cell growth as well as indirect effects e.g. on angiogenesis are discussed. At present, there is no detailed knowledge concerning the pathophysiologic mechanism of IL-6 and soluble IL-6 receptor action in endometriosis. This will have to be subject of further investigation.

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