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A high serum concentration of interIeukin-6 is predictive of relapse in quiescent Crohn's disease

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Background/aims: Relapses of Crohn's disease are difficult to predict. We assessed the value of serum level of interleukin-6, tumour necrosis factor alpha (TNF-α) and soluble TNF receptors as predictors of relapse in quiescent Crohn's disease.

Patients/methods: Thirty-six patients with inactive Crohn's disease, treated or not, were included. Various clinical and biological parameters, including interleukin-6, TNF-α and soluble TNF receptors serum levels were measured at inclusion in the study and the patients were followed clinically for 1 year. The relapse was defined as a Crohn's Disease Activity Index (CDAI) greater than 150 with an increase greater than 100 compared to the inclusion value. We analysed the ability of these parameters to predict relapse in parallel to clinical characteristics and other laboratory parameters.

Results: Among the 32 variables tested, interleukin-6 serum level had the greatest ability to predict the time-to-relapse, with 17-fold chance of relapse over a 1-year period for patients with an interleukin-6 serum level greater than 20 pg/ml than for patients with a lower level ($P < 0.001$). A high serum level of the soluble TNF receptors pS5 and p75 also had significant predictive value, in contrast to TNF-α serum levels. An interleukin-6 serum level greater than 20 pg/ml and either an acid α-1-glycoprotein level greater than 1.1 g/l or a soluble interleukin-2 receptor serum level greater than 95 pM/l were risk factors selected by a stepwise multivariate analysis. In both models a good prognosis group was defined by the absence of the two risk factors, a bad prognostic group by the presence of the two risk factors and an intermediate in between. With both models, the good prognosis group included 17 patients who experienced no relapse over the 1-year follow-up, whereas all patients (seven with the first model and six with the second) in the bad prognosis group had a relapse during the follow-up. Looking specifically at two homogeneous subgroups including either naturally/5-aminosalicylic acid (5-ASA) quiescent or corticoid quiescent patients, a very good predictive value for interleukin-6 serum concentration was also found.

Conclusion: Interleukin-6 serum level alone or in association with other biological parameters such as acid α-1-glycoprotein or the soluble interleukin-2 receptor serum level may be useful for predicting the course of the disease in patients with quiescent Crohn's disease.

Keywords : Crohn's disease ; interleukin-6 ; soluble interleukin-2 receptor ; tumour necrosis factor alpha ; soluble tumour necrosis factor receptor

Introduction

Crohn's disease is a chronic relapsing disease of unknown aetiology. Clinical relapses are difficult to predict. Some clinical features [1] and inflammatory markers [2,3] have been proposed along with more invasive tests such as the measure of intestinal permeability [4]. Since the immune system is implicated in the pathogenesis of the disease [5], immune parameters could be useful in monitoring the disease. We have recendy shown that soluble interleukin-2 receptor serum level is a good predictor of relapse in patients with inactive Crohn's disease, treated or not [6]. In addition to lymphocytes, macrophages are activated in Crohn's disease [7]. Interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) are proinflammatory cytokines mainly produced by activated

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macrophages [8,9]. The production of these cytokines by lamina propria mononuclear cells is increased in Crohn's disease [10,11] and their serum level has been shown to be also increased and to be higher in active than in inactive disease [12-15]. Soluble TNF receptors (sTNFR) p55 and p75 are shed during expression of TNF receptor by various cells [16]. They can modulate TNF activity [17]. The serum level of these soluble receptors has been reported to be increased in Crohn's disease [15]. The value of these parameters in predicting clinical relapse of Crohn's disease has never been studied so fir.

We determined the value of IL-6, TNF-α and the sTNFR p55 and p75 serum levels in predicting clinical relapse in 36 quiescent Crohn's disease patients, treated or not and followed prospectively over 1 year. Their role was analysed in conjunction with the predictive value of sIL-2R serum level and of various classical inflammatory and clinical parameters.

Patients and methods

Patients

Thirty-six consecutive patients, treated or not, with inactive Crohn's disease, defined by a Crohn's Disease Activity Index (CDAI) [18] less than 150, were included in the study. There was no patient with post-resection remission. The sample was partly similar to the one studied in a previous work [6]. Indeed, among the 36 patients, 13 were new patients, 6 were already studied but at another rime of their clinical history and 17 were the same as in the previous work. Various clinical characteristics and laboratory parameters were determined at inclusion.

Study design

The patients were then followed clinically for 1 year. A clinical examination was done every 3 months, or earlier if symptoms recurred. A relapse was defined as a CDAI greater than 150 with an increase greater than 100 compared to the CDAI at inclusion.

Laboratory tests

The total white cell count and differential, total neutrophil and lymphocyte counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen and acid α-1-glycoprotein were determined using routine procedures. Serum samples were stored at -20°C until assay of cytokines. Serum level of sIL-2R was measured using a commercial double-antibody sandwich enzyme-linked immunosorbent assay detecting the α-chain (p55) of the receptor (Boehringer Manheim Biochemical). Serum levels of IL-6, TNF-α, sTNFR p55 and sTNFR p75 were measured using a commercial enzyme amplified sensitivity immunoassay based on an oligoclonal system, in which several monoclonal antibodies, directed against distinct epitopes of the cytokine or the receptor measured, are used (Medgenix).

Statistics

Parameters described in Tables 1 and 2 were analysed for their ability to predict relapse. The continuous variables were categorized using either usual limits (such as 50 and 100 for the CDAI), previously established limits, such as 95 pM/l for sIL-2R [6] or limits selected in the following way: each variable was first divided into four successive categories with approximately nine patients per category at approximately the 25th, 50th and 75th percentiles. If the relative relapse rates (ratio of the estimates of the hazard of relapse, i.e. observed number of relapses to the expected number of relapses in each category assuming no variation of the relapse rate across the categories) [19] in two or more adjacent categories were not substantially different, these categories were grouped together [20]. If no dear pattern was observed, the median was taken as a cut point The value of each independent clinical or laboratory parameter in predicting the time to relapse was tested by using the proportional hazard model [21] providing an estimate of the relative risk of relapse (RR: mean, 95% confidence interval). Time-to-relapse curves were estimated by the Kaplan-Meier method [22]. Stepwise multivariate analysis with the Cox model [21] was used to select the best combination of parameters for prediction of time to relapse, and to provide different prognostic groups. The predictive value of the parameters selected on the whole population were also assessed in two more homogeneous subgroups of patients using the logrank test [23]. The first one, called naturally/5-aminosalicylic acid (5-ASA) quiescent, was composed of 14 patients in remission for more than 4 months and under no treatment except 5-ASA at a dosage of 2g/day or less. The second, called corticoid quiescent, contained eight patients in remission for less than 6 months but still treated with corticosteroids.

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Table 1. *Characteristics of the 36 patients at inclusion.*

	Median	Range
Age (years)	30	16-62
Duration of disease (years)	7	1-36
Time since last flare-up (month)	5	1-112
CDAI	70	0-148
	n	%
Familial inflammatory bowel disease	4	11
Previous surgery	15	42
Site of the disease		
Ileal disease	*7*	19
Colonic disease	8	22
Multiple localization	21	58
Anal lesions	11	31
Treatment*		
Steroids	11	31
5-ASA	19	53
Azathioprine.	3	8
Total patenteral nutrition	2	6
Ciprofloxacin	1	3
Metronidazole	1	3
No treatment	6	17

* Seven patients received several drugs.

Table 2. *Laboratory tests at inclusion.*

	Mean ± SE	Normal range
Leucocyte count ($x10^9$/l)	9.3 ± 3.7	4.3-10.6
Neutrophil count ($x10^9$/l)	6.5 ± 3.0	1.8-7.7
Relative neutrophilia (%)	69 ± 10	41-83
Lymphocyte count ($x10^9$/l)	1.9 ± 1.0	1.0-4.8
Relative lymphocytosis (%)	20 ± 9	11-53
ESR (mm/h)	15 ± 12	0-10
C-reactive protein (mg/l)	12 ± 10	0-6
Acid α-1-glycoprotein (g/l)	1.2 ± 0.4	0.4-0.9
Fibrinogen (g/l)	3.7 ± 1.0	1.9-3.7
sIL-2R (pM/l)	96 ± 27	50-100
TNF-a (pg/ml)	12 ± 5	0-20
sTNFR p55 (ng/ml)	2.0 ± 0.6	0.3-2.9
sTNFR p75 (ng/ml)	4.6 ± 1.1	1.9-8.5
IL-6 (pg/ml)	10.2 ± 12.3	0-8.5

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Table 3. *Parameters significantly related to the time to relapse: relative risk (RR) of relapse (mean, 95% confidence interval (95% Cl)).*

Parameter	No. relapse/ no. patients	*P* value	RR Mean (95% Cl)
IL-6 (pg/ml)			
<20	3/27	<0.001	1
≥20	8/9		17.2 (4.3-69)
Acid α-1-glycoprotein (g/l)			
≤1.1	1/19	<0.001	1
>1.1	10/17		15.7 (2.0-124)
Relative lymphocytosis (%)			
>20	1/18	0.002	1
≤20	10/18		12.9 (1.7-101)
sIL-2R (pM/l)			
≤95	2/20	0.002	1
>95.	9/16		7.8 (1.7-36)
sTNFR p55 (ng/ml)			
<2	1/17	0.004	1
≥2	10/19		11.4 (1.5-89)
C-reactive protein (mg/I)			
≤8	2/17	0.029	1
>8	9/19		4.7 (1.0-22)
Fibrinogen (g/l)			
≤3.7	3/19	0.038	1
>3.7	8/17		3.7 (1.0-14)
Anal lesions			
No	5/25	0.023	1
Yes	6/11		3.7 (1.1-12)
sTNFR p75 (ng/ml)			
<4.3	3/18	0.040	1
≥4.3	8/18		1.6 (1.0-2.6)

Results

Characteristics of the patients at inclusion are shown in Table 1.

The results of the laboratory tests done at inclusion are shown in Table 2.

Over one year, 11 out of 36 patients experienced a relapse. The mean time to relapse was 5 months (range: 1-10 months).

Parameters with a significant value in predicting the time to relapse are shown in Table 3. A high IL-6 serum level, acid α-1-glycoρrotein, sIL-2R serum level, sTNFR ρ55 serum level, CRP, fibrinogen and sTNFR p75 serum level as well as a low relative blood lymphocytosis and the presence of anal lesions had a significant relapse predictive value. The steroid treatment and a high level of blood neutrophils (> 6 x 10^9/l) just failed to reach significance with a relative risk around 3. The estimated time-to-relapse curves over a one year period depending on the IL-6 serum level at inclusion are shown in Fig. 1.

The stepwise multivariate analysis with the Cox model selected two grossly equivalent models each containing two factors: in the first one, relapse risk factors were an IL-6 serum level greater than 20 pg/ml and an acid α-1-glycoprotein greater than 1.1 g/l; in the second one they were an IL-6 serum level greater than 20 pg/ml and a sIL-2R serum level greater than 95 pM/l. In the first model, none of the 17 patients (0%) without any risk factor had a relapse over 1 year, versus 4 out of 12 patients (33%) presenting one risk factor, and 7 out of 7 patients (100%) having the two risk factors. In the second model, none of the 17 patients (0%) without a risk factor experienced a relapse over 1 year, compared with 5 out of 13 patients (38%) having one risk factor and 6 out of the 6 patients (100%) having the two risk factors. The estimated time-to-relapse curves over a 1-year period depending on the number of unfavourable prognostic factors at inclusion in this second model are shown in Fig.

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2.

The prognostic values of IL-6 serum concentration, acid α-1-glycoprotein, relative lymphocytosis and sIL-2R serum concentration in the two homogeneous subgroups are given in Table 4. In the naturally quiescent subgroup, the models selected by the multivariate analysis on the whole sample were still efficient, including IL-6 serum level and acid α-1-glycoprotein ($P = 0.002$) or sIL-2R serum level ($P = 0.002$). However, all the patients were already correctly classified in that subgroup using only the IL-6 serum level. In the corticoid quiescent subgroup, the model with IL-6 and sIL-2R serum levels was still significantly effective ($P = 0.014$), in contrast to the model with IL-6 serum level and acid α-1-glycoprotein ($P = 0.076$).

Table 4. *Predictive value of the best parameters in two clinically homogeneous subgroups of patients.*

	No. relapse/no. patients		P	
Subgroups*	A	B	A	B
Parameters IL-6				
<20pg/ml	0/11	1/5	<0.001	0.025
≥20pg/ml	3/3	3/3		
Acid α-1-glycoprotein				
≤1.1g/l	1/11	0/1	0.011	0.377
>1.1g/l	2/3	4/7		
slL-2R				
≤95pM/l	1/10	1/4	0.09	0.603
>95pM/l	2/4	3/4		
Relative lymphocytosis				
>20%	0/9	1/3	0.068	0.103
≤20%	3/5	3/5		

*Subgroup A: 14 patients in remission for more than 4 months and under no treatment or 5-ASA; B: 8 patients in remission for less than 6 months and still treated with steroids.

Fig. 1. *Time-to-relapse curve (Kaplan-Meier) depending on the concentration of IL-6 in the serum at inclusion in the study.*

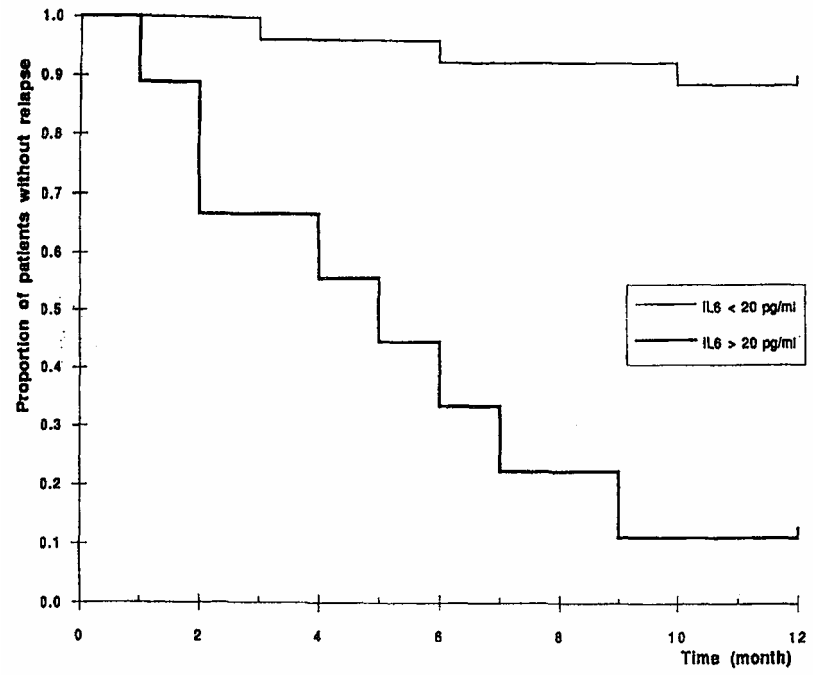
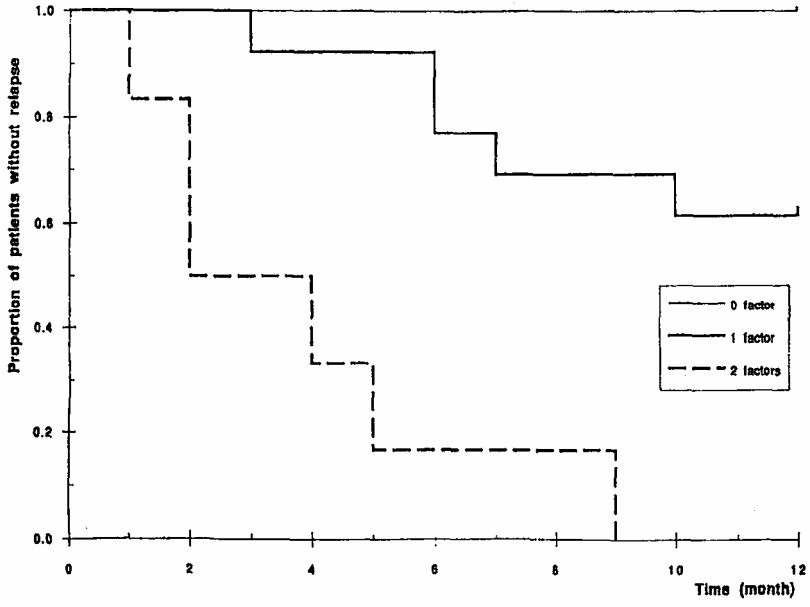


Fig. 2. *Time-to-relapse curve (Kaplan-Meier) depending on the number of risk factors (IL-6 serum level >20pg/ml; sIL-2R serum level > 95pM/l) at inclusion in the study.*



Discussion

Relapses are very frequent in Crohn's disease and remain difficult to predict. The relapse risk is not homogeneously distributed among patients. The present data show that IL-6 serum level, alone or in association with acid α-1-glycoprotein or sIL-2R serum level, is a good predictor of relapse in quiescent Crohn's disease.

Patients with inactive Crohn's disease were defined as patients having a CDAI less than 150. Our group of patients was heterogeneous as far as treatment, characteristics or past history of the disease were concerned. We first tried to identify in the whole sample prognosis factors that were applicable to any type of quiescent Crohn's disease. Then we checked these factors in more homogeneous subgroups of patients, such as patients in remission for more than 4 months without treatment or only with 5-ASA less than 2g/day and patients more recendy put into remission and still on steroids. This second subgroup may actually have included some subjects with persistent activity of the disease despite a CDAI value lower than 150 [24].

The analysed parameters were on the one hand classical clinical characteristics and inflammatory markers previously studied [1-3,6], and on the other hand serum levels of two proinflammatory cytokines mainly produced by macrophages (IL-6, TNF-α) which had never been studied before. IL-6 and TNF-α are very powerful cytokines produced early in the development of mucosal inflammation [10,11]. We also studied the two sTNFRs that have been shown to bind TNF-α and to modulate its in-vitro and in-vivo activity [17].

All these clinical or laboratory parameters were assessed at inclusion and the patients prospectively followed over 1 year. The evolutions of the various laboratory parameters over time were not available, although this would have been interesting in order to confirm the ability of their variation prior to relapse to predict the event.

The prognostic value of the parameters determined at inclusion were evaluated retrospectively, but no systematic research of the most effective cut-off value was performed. Our results should nevertheless be confirmed by a fully prospective study using the cut-off levels of the various parameters determined in this study. We used proportional hazard model analysis to evaluate the prognostic value of the different parameters we studied. Although this method has not always been used in previous studies [2,3], it is the most appropriate statistical approach of the time-to-relapse curve analysis.

Our results show a good ability of several laboratory parameters to predict relapse. The best of all was IL-6 serum level with a relative risk of relapse of 17 over 1 year for patients with an IL-6 serum level greater than 20 pg/ml. This had never been showed before. Our study also showed for the first time a significant predictive value for sTNFR p55 and p75 serum levels. In contrast, it did not show any predictive value for TNF-α serum level.

This discrepancy between IL-6 and TNF-α may be explained by different stimuli of production, different producing cells, mainly macrophages for TNF-α versus T-lymphocytes, macrophages and possibly intestinal epithelial cells for IL-6 [8,9,25] or different metabolization or binding to soluble receptors. The discrepancy between TNF-α and its soluble receptors is more surprising because their production or expression is stimulated by similar factors [16]. However, the TNF receptors are expressed on a much greater number of cell types and their half-life is longer [17]. Besides these cytokines, other immune, inflammatory or clinical parameters already studied [2,3,6] also had good predictive ability in the present work: acid α-1-glycoprotein, C-reactive protein, fibrinogen, relative lymphocytosis, sIL-2R serum level and the presence of anal lesions. These various individual parameters may have different predictive value in different subgroups of patients. Looking at more homogeneous subgroups, characterized either by a remission longer than 4 months and no treatment or 5-ASA no more than 2 g/day, or by a remission shorter than 6 months and steroid treatment, we found that the IL-6 serum concentration was still a good predictor of relapse in each subgroup. The value of the other parameters tested in those subgroups was less striking but the size of the subgroups was probably too small to draw any conclusion.

Because most of these parameters are correlated [6,15], it was important to complete these results by a stepwise multivariate analysis. On the whole sample, two equivalent models were selected, both including an IL-6 serum level greater than or equal to 20pg/ml associated with either an acid α-1-glycoprotein greater than 1.1 g/l or a sIL-2R serum level greater than 95 pM/l. These two models allowed us to divide our sample into three subgroups with different risk of relapse. These subgroups were nearly the same with the two models, showing that models with different parameters may well lead to similar classifications of patients due to the complex relationships between those parameters. Among these three subgroups, the one without any risk factor (n = 17) was maybe the most relevant because it gave a very clear-cut information: 0% relapse over 1 year. In the naturally/5-ASA quiescent and in the corticoid quiescent patient groups, the model including IL-6 and sIL-2R serum levels was the only one still effective, indicating that these parameters may be effective in predicting relapses in various clinical conditions.

Some previous studies had already shown some good predictive value of a variety of inflammatory and immune parameters. An initial study carried out in quiescent untreated patients showed a good predictive value for acid α-1-glycoprotein and CRP [2], which is in accordance with our results. The same group confirmed those data more recently [3]. This last study suggested also the ability of some clinical characteristic to predict relapse in Crohn's disease but did not directly compare them with laboratory parameters. More recently, we suggested the potential role of sIL-2R serum level, a relative lymphopenia and an increased neutrophil count in predicting relapse in quiescent Crohn's disease [6]. Similar results were found in the present study carried out on a partly different sample (more than 50% of the sample being different).

Nevertheless, some variations from that study to the one presented here were observed. Indeed, the cut-off values for some parameters varied due to the procedure used on two partly different samples (for instance, the median of relative lymphocytosis was different). The ability of the neutrophil count to predict relapse was only borderline significant in the present study whereas the acid α-1-glycoprotein parameter was more effective. Actually, as suggested by the subgroups analysis, the predictive value of some parameters may vary in different clinical conditions and the proportion of corticoid quiescent patients was higher in our previous study [6]. Another study evaluated the predictive value of a variety of clinical characteristics, and found an age less than 25 years, an interval since first symptoms greater than 5 years, an interval since previous relapse less than 6 months and colonic involvement to be predictive of relapse [1]. That study used proportional hazard model analysis and was carried out on a very large sample. However, these clinical characteristics were not directly compared to various inflammatory and immune parameters, except ESR. The absence of predictive value for such clinical characteristics in our study may be due either to the smaller size of our sample or to its heterogeneity. Our results suggest, however, that immune and inflammatory parameters such as IL-6 serum concentration and acid α-1-glycoprotein either have a better predictive value or remain applicable in different clinical conditions.

In conclusion, IL-6 serum level in combination with acid α-1-glycoprotein or sIL-2R serum level allows the division of patients with inactive Crohn's disease into subgroups with different risks of relapse. This division may be useful for clinical trials evaluating the efficacy of maintenance therapy or for prolonging treatment in patients in remission after an acute flare-up.

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