Etanercept concentration and immunogenicity do not influence the response to Etanercept in patients with juvenile idiopathic arthritis

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ABSTRACT.

Objective: To investigate the relationship of clinical response of Juvenile Idiopathic Arthritis (JIA) to etanercept (ETN) with ETN levels, and the presence of anti-drug antibodies to ETN (ADAb).

Methods: Prospective study of JIA patients under 18 years old. Clinical and pharmacological data were collected at two visits. JIA clinical inactivity and activity were assessed according to the Wallace criteria and to the Juvenile Arthritis Disease Activity Score (JADAS). ETN and ADAb serum levels assessments were determined using ELISA-based assays.

Results: 126 patients were enrolled. The median duration of ETN treatment at inclusion was 569 days (range 53-2,340). ADAb were undetectable (< 10 ng/ml) in 171/218 (78%) samples and were > 25 ng/mL in 2/218 samples. No significant relationship between ETN concentration and the clinical inactivity status and JIA activity was found using either univariate logistic regression or multiple logistic regression analysis, adjusted on one individual descriptors, time since diagnosis, time of sampling, use of corticosteroids or methotrexate and classification of JIA. No correlation was found between the remission status and the detection of ADAb.

Conclusion: This study did not demonstrate any correlation between JIA activity and circulating ETN levels in a large population of patients with JIA previously treated with ETN for at least 1.5 months. As described for adults, our study confirms that ETN is marginally immunogenic in pediatric patients. These results do not support the clinical usefulness of a monitoring of ADAb or ETN concentrations for the management of this group of JIA patients if they fail to achieve clinical inactive disease.

Etanercept (ETN) is a dimeric fusion protein consisting of two extracellular portions of the TNF receptor 2 (TNFR2) linked to the Fc portion of human IgG1 (TNFR2-Fc). It is a competitive inhibitor of TNF-α binding to its membrane-bound receptors. Etanercept has revolutionized the treatment of patients suffering from severe juvenile idiopathic arthritis (JIA) since at least 70% of patients in all categories except rheumatoid factor (RF)-positive polyarthritis and systemic JIA achieved ACR Pedi 30 and at least 40% of patients in all categories achieved ACR70 [1]. Unfortunately, approximately 10–22% of biologic naive JIA patients discontinue ETN within 12 months because of primary inefficacy or loss of response [2]. The mechanisms underlying these response failures are unclear. In adult patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS), the results of studies that have investigated the relationship between serum ETN levels and clinical response are conflicting [3-7]. In children, ETN is prescribed in a fixed dose of 0.8 mg/Kg (without exceeding 50 mg) weekly, without taking differences in pharmacokinetics into account, and no study has investigated the possible relationship between ETN circulating level or immunogenicity and disease activity in a population of JIA patients. In the present study, we aim for the first time to investigate the possible relationship of clinical response of JIA with ETN levels and with the presence of anti-drug antibodies to ETN (ADAb) in a large prospective cohort.

PATIENTS AND METHODS

Study design and participants

This prospective multicenter study involved seven French pediatric rheumatology centers. Inclusion criteria were all the seven types of JIA according to the ILAR criteria, age ≤ 18 years, treatment with at least two injections of ETN (allowing to achieve a steady state). All patients received ETN at a dosing and frequency based on the physician judgement. Patients were treated with ETN and concomitant non-steroidal anti-inflammatory drugs (NSAID) and/or disease modifying anti-rheumatic drug (DMARD) therapy, or with ETN monotherapy. Exclusion criteria were any contraindication to administration of anti-TNF-α therapy. Serum samples were collected at two regular visits spaced from a maximal interval of 2 years. The date and hour of the last ETN injection before the visit were precisely recorded. The protocol was approved by the ethics committee “Comité pour la protection des Personnes Ile de France IV”. The study is registered at

Clinical Trials.gov under number NCT02030613. All patients (or their parents) gave written informed consent.

Clinical response

JIA clinical inactivity was assessed according to the Wallace criteria [8] at both visits of the study. Clinically inactive disease was defined by i) no joints with active arthritis, and ii) no fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to JIA and iii) no active uveitis and iv) erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) level within normal limits in the laboratory where tested (if both are tested, both must be normal and v) Physician's global assessment of disease activity score < 10/100 on a visual analogue scale. JIA activity was assessed according to the Juvenile Arthritis Disease Activity Score 10 (JADAS10) [9], ranging from 0 (no activity) to 40 (high activity) with 4 items scored between 0 and 10: the number of active joints score (with 10 as the higher score in patients with 10 or more active joints), the physician assessment of disease activity on a visual analog scale (VAS), the patient or parent assessment of disease activity on a VAS and ESR. The cutoff score for classifying a patient as having 1) inactive disease was 1, 2) minimal disease activity was 2 for oligoarticular JIA and 3.8 for polyarticular JIA, 3) high disease activity was 4.2 and 10.5 in oligoarthritis and polyarthritis, respectively. Children with systemic arthritis, RF-positive polyarthritis, RF-negative polyarthritis, or extended oligoarthritis were included in the polyarthritis group.

Measurements of ETN concentration and ADAb

Serum level of ETN (Cm, µg/mL) and the presence of ADAb (ng/mL) were determined using the Lisa-Tracker® Duo Etanercept (Theradiag, Marne-la-Vallée, France) kit. In this assay, indirect and bridging-type ELISA format are used for free drug determination and detection of ADAb, respectively. According to manufacturer, no interference with serum components such as cryoglobulins, RFs, autoantibodies, heterophilic antibodies, IgG and/or IgM, C1q and high levels of triglycerides or bilirubin is observed with this immunoassay. Limits of detection of the assay were 0.2 µg/mL and 10 ng/mL for ETN and ADAb, respectively. Intra and inter-assay coefficient of variation values of this ELISA are <15%. Etanercept Cm and ADAb level were measured at both visits for each patient.

Statistical analysis

Population characteristics: they were analyzed with descriptive statistics. Medians (range) were calculated for continuous data and percentages were used for discrete data. For the comparison of values between the two visits, the samples of patients who dropped out of the study before the second visit were not considered.

Effect-concentration relationship.

The effect-concentration relationship was analyzed using the lme4 package [10] with the R program (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org) which enables to perform statistical analyses for repeated measures. Continuous and categorical variables were analysed and compared, respectively, by an analysis of variance and a generalized linear mixed-effects models with a logit link (using the lmer or glmer functions with the patient as a grouping factor to estimate the random-effects term η). p values <0.05 were considered significant. Logistic regression analyses were specifically performed to assess association between clinical inactivity status (according to Wallace criteria) and ETN concentration and/or dose per kg per week plus other plausible factors that can influence the remission status (age, size descriptors, time since diagnosis, sampling time groups, ADAb, co-treatments for JIA and ILAR classification). Sampling times were categorized into 4 groups, 0 to3, >3 to 7, >7 to 14 and >14 days. The sampling times groups were added as a factor to take into account their contribution to the concentration level effect

RESULTS

Patient characteristics

One hundred and twenty-six patients (96 females, 30 males) were included. Two patients potentially eligible declined participation. A total of 22 patients (17%) dropped out of the study before the second visit, due to treatment failure (14 patients), loss of follow-up (7 patients) or refusal (1 patient). They were 6 enrolled patients that did not provide samples Demographics features are shown in Table 1. Thus, 98 patients had at least one available sample. The median time elapsed between the two visits was 366 days (range, 302-712). At enrollment, the median age was 10.5 years (range, 2 to 17), the median JIA duration

was 4.6 years (range, 0.16 to 16.3) and the median duration of ETN treatment was 569 days (range, 53 to 2340). JIA subtypes were persistent oligoarticular JIA (17 patients), extended oligoarticular JIA (32 patients), polyarticular JIA (52 patients), enthesitis-related JIA (20 patients); psoriatic JIA (2 patients); systemic JIA (2 patients) and undetermined subtype (1 patient). ETN was associated with methotrexate in 36 patients, corticosteroids in 3 patients or non-steroidal anti-inflammatory drugs in 18 patients. There were no significant demographics differences between patients who remained in the trial and those who dropped out due to treatment failure with regard to age, size descriptors, time since diagnosis, ADAb, co-treatments for JIA and ILAR classification.

Clinical response and ETN levels

A total of 218 samples were available for this study. Extensive sample hemolysis did not allow the dosage in 5 samples. 170/218 of the samples (78 %) occurred within the seven days post-dose. ETN concentration ranged from 0.15 to 20.5 µg/mL, and were significantly different between the two visits (Table 2). It did not significantly differ between patients who remained in the trial and those who dropped out due to treatment failure. The percentages of clinically inactive disease were high and did not significantly differ between visits (Table 2). Univariate logistic regression showed no significant relationship between ETN concentration and either the inactive disease percentage (Wallace criteria) or disease activity (JADAS10) ($p>0.9$) (Figure 1), the number of arthritis ($p>0.6$), CRP level ($p>0.2$), ESR ($p>0.1$), and CHAQ ($p>0.6$). Thereafter, ETN concentration and/or dose per kg per week and other potential confounders (age, size descriptors, time since diagnosis, sampling time groups, ADAb, co-treatments for JIA and ILAR classification) that can influence the disease's clinical inactivity status (according to the Wallace criteria) were tested along with. Whatever the analysis, "concentration + dosage + covariates or concentration + covariates" or "drug per kg per week + covariates", there was no significant and positive effect of either concentration or dosage. In patients who were clinically inactive, the lowest ETN concentration ranges, 0.15 to 0.43 µg/mL, seemed efficient to sustain a response. There was no significant parallel increases between ETN concentrations and the inactive disease percentages (Figure 2).

To better understand these results, an analysis of variance for ETN and ADAb levels between participants who remained in the trial and those who dropped out because of poor response was conducted at time-point 1. ADAb levels were not significantly different between the two groups (7.22±3.60 *versus* 6.47±3.98 ng/mL). Similarly, ETN levels were not significantly different between the two groups (4.37±3.18 *versus* 3.09±3.38 µg/mL). In both cases, the clinical inactivity status was not a significant factor.

No severe adverse events occurred.

ADAb against ETN

Two hundred and eighteen samples were available for ADAb testing. Six samples were not analyzable due to hemolysis or other technical issues. For both visits, ADAb concentration was below the positivity threshold of the assay of 10 ng/mL in 171/224 samples (76%), and was high (> 25 ng/mL) in only two patients (Table 2). The number of positive ADAb patients did not significantly differ between patients who remained in the trial and those who dropped out due to treatment failure. Of note, JADAS10 score and the number of patients who received concomitant methotrexate were not significantly different between ADAb positive and ADAb negative patients (Figure 3). .Conversely, median ETN concentration was significantly lower in the ADAb positive patients than in the ADAb negative patients (2.1 *versus* 3.8 µg/mL ; p=0.02)

DISCUSSION

This large prospective study of 126 patients with JIA did not show any significant relationship between the level of ETN and the clinical inactivity status and the disease activity. One explanation for this absence of relationship may be that all patients were treated by ETN for at least 53 days (mean duration of treatment: 569 days) and consequently: i) the percentage of patients clinically inactive was already high, > 70%, at the beginning of the study ii) patients with a primary complete failure to ETN were probably not enrolled in our study since improvement may occur in JIA as early as two weeks after the beginning of ETN [11]. However, it is clear that the inactive disease rate was not different whatever the ETN concentration. The lower rates of remission in the highest concentration ranges could reasonably result from a dose

escalation in patients that failed to achieve clinically inactive disease. In responders, the lowest ETN concentration range seem efficient to sustain a response, questioning the paradigm of the concentration-response relationship for these biological TNF inhibitors, at least in the concentration range we observed in this study. In adult patients with RA and AS, the studies, which have investigated the relationship between serum trough ETN levels and clinical response resulted in conflicting results. Two studies have shown that intensification of treatment with ETN did not increase the response rate in patients with RA and SA [3, 4]. In addition, circulating ETN levels were found to be similar in responders and non-responders in AS [5]. Conversely, one study demonstrated that a low circulating ETN level was a predictor of non-response in RA [6], and another one that disease activity and inflammation were associated with ETN levels in patients with AS at 24 weeks of treatment [7].

A main reason for the loss of efficacy to some anti-TNF-α drugs, especially monoclonal antibodies (mAb) adalimumab or infliximab, is subtherapeutic drug-levels due to ADAb formation in an important proportion of patients. Conversely, ETN, which is a fusion molecule, seems not to be or only marginally immunogenic since ADAb against ETA have been detected in 0-18 % of patients treated for RA, AS, Crohn's disease or psoriasis [12]. When detectable, ETN ADAb were always non-neutralizing and did not appear to affect trough serum ETN level, clinical efficacy or tolerability in patients with RA [13, 14]. However, ETN concentration was significantly lower in ADAb positive patients than in ADAb negative patients, which had no clinical consequences in our cohort. Indeed, neither ETN concentration or ADAb positivity correlated with JIA activity or remission. Different immunoassays have been developed to measure ADAb serum concentrations. They have led to important variations between studies in the evaluation of the prevalence of ADAb. In the present study, a bridging-type ELISA test to detect ADAb was used. Bridging ELISAs and radioimmuno-assays (RIA) have been the most commonly utilized for ADAb detection in patients treated by TNF-α inhibitors. As compared to RIA testing, ELISA-based assays are less expensive and simpler tests and despite limitations remain a valid option for implementation in clinical settings. In both assays, high titer ADAb response is associated with undetectable drug levels [14]. Known limitations of bridging ELISAs-based testing such as interference of free circulating drug or lack of detection of functionally monovalent IgG4 ADAb have to be taken into account while analyzing and

interpreting results. In our study ADAb concentrations were high (> 25 ng/mL) in only two patients after a median duration of ETN treatment of 569 days. These findings are consistent with previous reports analyzing ETN immunogenicity in adult patients and confirm the low immunogenicity of ETN in pediatric patients. Notably, none of the ADAb positive samples correlated with lack of clinical response. The differences in immunogenicity between ETN and anti-TNF-α mAb might be explained, at least in part, by the lower number of potentially immunogenic domains of ETN as compared to anti-TNF-α mAb. ETN is a dimeric fusion protein comprised of two TNFR2 linked to the Fc portion of IgG1. Only the junction between these domains can contain immunogenic epitopes. In contrast, anti-TNF-α mAb have multiple epitopes within the Fab variable region against which an immune response can be directed [12].

Our study did not support the clinical usefulness of a monitoring of ADAb or ETN concentrations for the management of JIA patients who were previously treated with ETN and who did not achieve a clinical inactive disease. This group of JIA patients may have either a loss of response to ETN, or a partial response to ETN from the beginning of ETN. In previous studies, the response to ETN treatment in JIA was associated only with baseline clinical and biological characteristics of the patient and /or disease: a potential better response was associated with a younger age at disease onset, a shorter disease duration, a lower disability scores at therapy initiation, a higher ESR, an absence of wrist involvement, a history of acute anterior uveitis, an absence of concomitant steroid use, and a smaller number of previously used DMARDs [14-16]. The usefulness of common single-nucleotide polymorphism (SNP) data for predicting anti- TNF-α treatment efficacy has not yet been studied in JIA, and no biomarkers have been identified to predict anti-TNF treatment efficacy in children, beside aforementioned clinical factors. Thus, further studies are required to identify biomarkers associated with a response to ETN.

This study has several limitations. First, no patient has been included at the initiation of ETN treatment. We have chosen to include all the patients that had received at least two injections of ETN, and not only those who started ETN, in order to study a large number of patients, owing to the fact that JIA is a rare disease, requiring ETN in a minority of patients. Unfortunately, no patients were included at the initiation of ETN, which was theoretically possible. Thus, our study did not take into consideration the case of primary failure to ETN. Second, since ETN is a drug administered at home there might have been some

variation in the real dose received by the patient. However, this study includes a large, prospective cohort. This allowed us to test ETN immunogenicity and the relationship between circulating ETN level and disease activity in a subpopulation of JIA patients.

In conclusion, our study did not demonstrate any correlation between JIA clinical inactivity status or JIA activity and either circulating ETN levels or immunogenicity of ETN in a large population of patients with JIA receiving ETN for at least 1.5 months. Thus, these results do not support the clinical usefulness of a routine monitoring of ADAb presence or trough ETN levels in patients who did not completely remitted. Further studies are needed to assess if these results could be extrapolated to JIA patients with primary failure to ETN.

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Contributors. Study concept and design: BBM, SU. Acquisition of data: EH, LC. Analysis and interpretation of the data: BBM, RK, SHBA, SU. Clinical revision and drafting of the manuscript for important intellectual content: BBM, RK, IL, CP, AC, SP, IM, PQ, JMT, AB, SHBA, SU. Obtained funding: BBM. Study supervision: BBM. Final approval: BBM, RK, IL, CP, AC, SP, IM, PQ, EH, LC, JMT AB, SHBA, SU

Competing Interests. BBM, PQ have participated as co-investigators to clinical trials from Pfizer. PQ invited to congress and participation to symposium for Pfizer. None of the other authors had conflicts of interest

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Figure 1. Relationship between ETN concentration and either the disease's clinical inactivity status (Wallace criteria) (A) or disease activity (JADAS10) (B)

Figure 1A

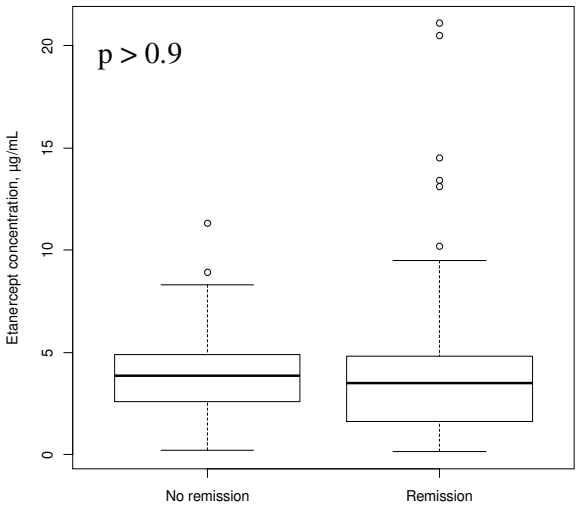

Figure 1B

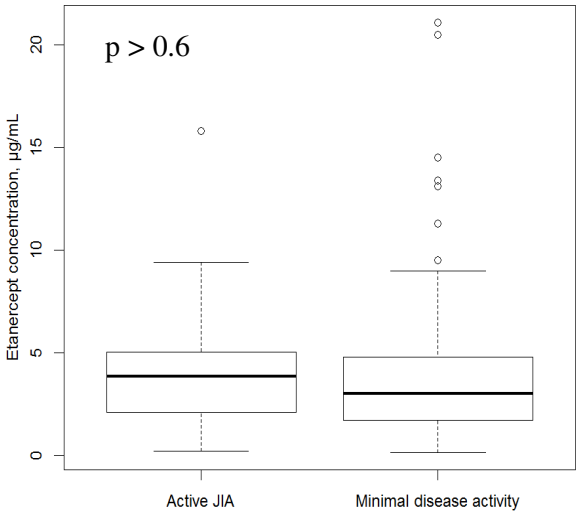

Figure 2. Etanercept concentration according to the inactive disease percentage (Wallace criteria). The lower and upper segments limited by the dashed lines denote the 95% confidence interval for each concentration interval.

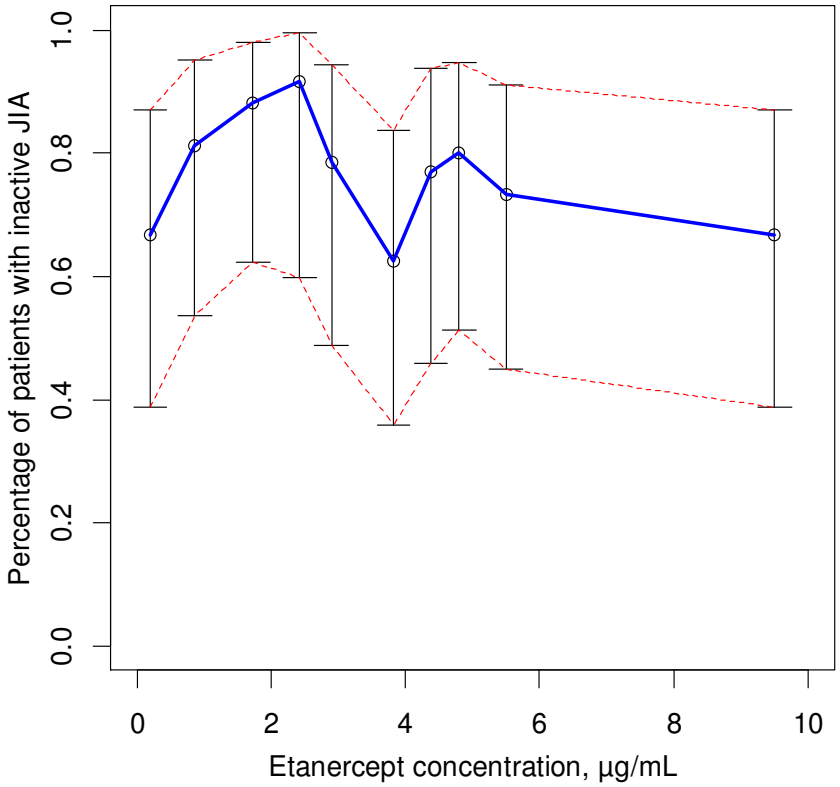

Figure 3. Relationship between ADAb values and JADAS10.

JADAS10 did not significantly differ between ADAb positive and ADA negative patients (p=0.75)

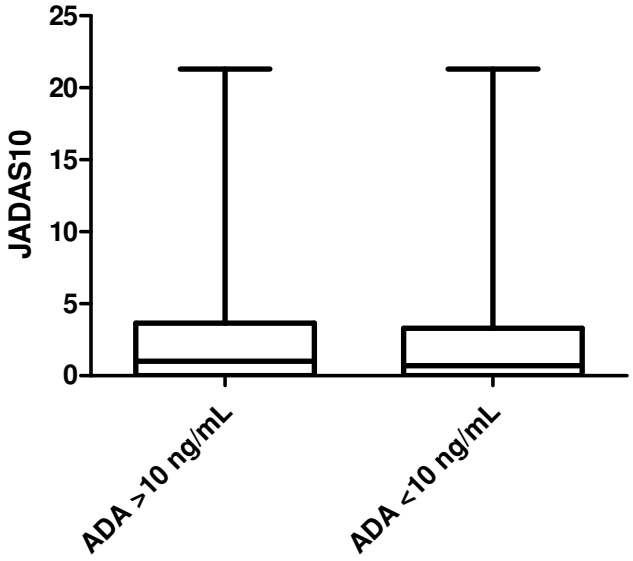

Table 1. Baseline demographic features of 126 patients

Baseline demographics	
Age, years (median, range)	10.5 (2-17)
Female (%)	96 (77)
JIA Subtype (%)	
Oligoarticular JIA	17(13.7)
Extended oligoarticular JIA	32 (25.8)
Polyarticular	52 (41.9)
Enthesitis	20 (16.1)
Psoriasis	2 (1.6)
Systemic JIA	2 (1.6)
Undetermined	1 (0.8)
JIA duration, years (median, range)	4.62 (0.16-16.3)
Concomitant treatments (%)	36
MTX	3
Corticosteroids	18
NSAIDs	
Patients with clinically inactive disease (%) (95% confidence interval)	72 (61-81)
JADAS-10 score (Median, range)	1 (0-21)

Table 2. Remission status, ETN dosage, ETN concentration andADAb concentrations, at visit 1 (inclusion) and visit 2 (366 days (range, 302-712) later)

	Visit 1	Visit 2	p
Number of patients	126	104	
Patients with clinically inactive disease (%) (95% confidence interval)	72 (61-81)	75 (64-84)	0,78
JADAS-10 score (Median, range)	1 (0-21)	0,7 (0-16)	0.29
Dose of ETN (mg/kg/week) (Median, range)	0,75 (0,11-1,79)	0,71 (0,12-2,25)	< 0,01
ETN concentration (µg/mL) (Median, range) (number of patients tested)	3,63 (0,15-21.1) (n=120)	2.50 (0.20-11.3) (n=98)	< 0.01
Number of patients with undetectable ADAb(< 10ng/mL) (%)	102/121 (84)	69/103 (67)	< 0,01
Number of patients with ADAb> 25 ng/mL (%) (number of patients tested)	0 (0) (n=120)	2/103 (2) (n=98)	< 0,001

p denotes the p values obtained using tests for repeated measures from the lme4 R package.

p values <0.05 were considered significant.