A DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED CLINICAL TRIAL OF THE SAFETY AND EFFICACY OF ANAKINRA IN PATIENTS WITH HIDRADENITIS SUPPURATIVA

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SYNOPSIS

Aim of this double-blind, randomized, controlled clinical trial is to compare the safety and the efficacy of anakinra over placebo for the management of patients with hidradenitis suppurativa (HS) of Hurley II and Hurley III disease stage. Patients will be evaluated on subsequent follow-up visits. Two scores will be applied: disease activity as assessed in the protocol by the investigator; and Sartorius score. Primary efficacy endpoint will be the comparisons of visual analogue scores, of disease activity, of Sartorius score and of dermatology life quality index between the two groups of treatment over follow-up.

BACKGROUND AND SCOPE

Hidradenitis suppurativa (HS) is a chronic devastating skin disorder affecting areas rich in apocrine glands. Nodules appear in the affected areas; they progressively become swollen and rupture with the release of pus. This process occurs repeatedly leading to sinus tract formation and scars\(^1\). This disease course creates a frustrating situation for the patients but also for physicians. Traditional treatments comprise short-courses of antibiotics and surgical excision. However, relapse is the rule so that HS leads to severe impairment of the quality of life. The Dermatology Quality Life Index (DQLI) for HS is 8.9 being higher than any other skin disorder\(^2\).

This devastating disorder has often been neglected and considered a rare situation. However, HS seems to indiscriminately affect the global population. Although the exact epidemiology is largely unknown, the point-prevalence is reported to range between 1% and 4%. A recent large epidemiological survey in France reports 0.97% disease prevalence\(^3\).

The exact pathophysiology of HS is unknown. Smoking, dietary habits and genetic predisposition have all been linked with HS\(^4\),\(^5\). However, a recent survey by our group in 56 patients, disclosed a severe derangement of the monocyte function and of subsequent antigen processing in these patients. The percentage of NK cells was increased and that of CD4-lymphocytes decreased compared to healthy controls probably implying the existence of an autoimmune
predilection for the disorder. We have previously demonstrated defective lipopolysaccharide (LPS)-induced production of the pro-inflammatory cytokines, tumour necrosis factor-alpha (TNFα) and interleukin (IL)-6 by blood monocytes of patients with HS.

As a consequence, a hypothesis for the implication of some autoimmune of autoinflammatory mechanism in the pathogenesis of HS was started to be created over the last years. The hypothesis is further reinforced by positive results from the administration of TNF antagonists in prospective studies with limited number of patients, one of these was conducted by our study group. Subcutaneous treatment with 50mg etanercept once weekly for 12 weeks in 10 patients, reduced patients’ suffering, attenuated local signs of inflammation and retarded disease relapse.

Anakinra is a recombinant interleukin-1 (IL-1) receptor antagonist (IL-1Ra). Anakinra blocks the biologic activity of naturally occurring IL-1, including inflammation and cartilage degradation associated with rheumatoid arthritis, by competitively inhibiting the binding of IL-1 to the interleukin-1 type receptor, which is expressed in many tissues and organs. IL-1 is produced in response to inflammatory stimuli and mediates various physiologic responses, including inflammatory and immunologic reactions. The biological properties of anakinra and the existing clinical and laboratory data favoring a derangement of the immune response in HS, prompted to investigate whether anakinra would be efficient in the management of patients with HS.

STUDY PROTOCOL

Study population

This is a prospective, randomized, placebo-controlled study. Patients will be screened from those who are currently are under follow-up in the Outpatient Department of Immunology of Infectious Diseases of ATTIKON University Hospital. The protocol of the study will be approved by the Ethics Committee of the hospital and by the National Organization for Medicines (Competent Authority of Greece).
Inclusion criteria are:

- written informed consent provided by the patient;
- age above 18 years;
- diagnosis of HS; and
- HS of Hurley II or III stage disease

Exclusion criteria are:

- history of systemic lupus erythematosus, of rheumatoid arthritis of of seronegative inflammatory arthritis;
- any prior administration of any type of anti-TNF therapy over the last six months;
- administration of any live (attenuated) vaccine over the last 4 weeks;
- history of recurrent vein thrombosis or embolism compatible with antiphospholipid syndrome;
- any present or smoldering infection;
- hepatic dysfunction defined as any value of transaminases, of γ-glutamyl transpeptidase or of bilirubin> 2 x upper normal limit;
- history of haematological or solid tumor malignancy, arterial hypertension, liver cirrhosis, HIV infection, and hepatitis virus B or C infection
- history of episodes mimicking demyelinating disorders or a definite diagnosis of multiple sclerosis
- any creatinine value above 1.5 mg/dl
- intake of corticosteroids defined as daily intake of prednisone or equivalent more than 1mg/kg for the last three weeks;
- neutropenia defined as <1000 neutrophils/mm$^3$; and
- pregnancy or lactation

Diagnosis of HS is based on the following criteria, set by the 2$^{nd}$ Conference of the HS foundation in San Francisco$^{13}$:

- Disease onset after puberty.
- Involvement of at least two areas of the skin rich in apocrine glands. These areas might be: left or right axillae; left or right inframammary areas; left or
right femoral-inguinal fold; left or right gluteal area. Any other affected areas may be taken into account on the follow-up of the patients but they are not considered as inclusion criterion

- History of recurrent drainage of pus from the affected areas.

Clinical characteristics of patients will be recorded comprising demographics, age since disease onset and involved areas.

Hurley II disease stage is defined as recurrent abscesses with sinus tracts and scarring involving single or multiple widely separated lesions. Hurley II disease stage is defined as diffuse or almost diffuse involvement or multiple interconnected tracts and abscesses.

Patient screening

Once a patient is considered eligible for the study the following procedures will be done:

- Skin tuberculin test; any diameter below 5mm is considered negative
- Chest X-ray;
- Serology for human immunodeficiency virus (HIV), for hepatitis B virus (HBV) and for hepatitis C virus (HCV); and
- Liver biochemistry

Once the above screening is within normal the patient may be enrolled in the study

Randomization

Patients will be randomly assigned to receive either placebo or anakinra subcutaneously. The allocated sequence will be generated by an independent biostatistician. Anakinra will be supplied in single use pre-filed glass syringes with 27-gauge needles. Anakinra syringe will contain 100mg of anakinra at a volume of 0.67 ml. Placebo syringes will contain 0.67ml of sterile water for injection.
Injections will be performed subcutaneously at a dose regimen of once daily for 12 weeks.

Patients’ visits

Patients’ visits will be done at week 0, at week 4, at week 8, at week 12, at week 16, at week 20 and at week 24. At every visit patients should deliver the empty syringes to ascertain administration. At every visit the following procedures will be done (APPENDIX I):

- Patients will be asked to provide an assessment of the severity of their disease using the visual analogue scale (VAS) in mm. They will be told that 0 represents no disease activity and 100 the worst disease activity they ever felt. Patients will be asked to provide one score for their overall impression about their disease (APPENDIX II) and another score about the physical pain they feel (APPENDIX III)

- The investigators will ask the patient to provide the frequency of the exacerbation of his disease and the pain felt at the affected sites. Patients will be encouraged to visit the study site on each exacerbation so that this is confirmed by the investigators.

- Patients will be given the DLQI score and they will be asked to fill it out (APPENDIX IV). This will be done only at week 0, at week 12 and at week 24

- The investigators will count the following from each individually affected area and will take a photo of that area: the number of fistulas; the number of nodules or abscesses; the number of scars; their impression about the degree of inflammation scored from 0 to 3 as follows: 0, absent, 1, mild; 2, moderate; 3, intense; the two largest dimensions of each lesion in mm (APPENDIX V)

- Ten ml of heparinized venous blood will be sampled from every patient after venipuncture of one forearm vein under aseptic conditions. This will be done only at week 0, at week 12 and at week 24
Based on the above the following two scores will be assessed at each visit:

- **Disease activity.** This is defined as the sum of scores of all affected areas of each patient. Each area will be evaluated by the following formula: (multiplication of the two largest diameters in each affected area in mm) x (the degree of inflammation of each lesion).

- **The Sartorius score**\(^{14}\). This is the sum of separate scoring for each affected area as follows:
  - Three points per anatomical region involved (left and/or right axilla, groin, gluteal, inframammary or other region)
  - Eight points for each ulcer; four points for each fistula; 2 for each nodule or abscess and 1 for each scar
  - Two points when the longest distance between two relevant lesions in each affected area is <5 cm; 4 points when it is 5-10 cm; and 8 points when it is >10 cm
  - Six points when there is no clear separation of lesions from adjacent normal skin and zero points when there is

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**Laboratory investigation**

Peripheral blood mononuclear cells (PBMCs) will be isolated after gradient centrifugation of the collected venous blood over Ficoll Hypaque. PBMCs will be washed three times with ice-cold PBS (phosphate-buffered saline, pH: 7.2) and counted in a Neubauer chamber with trypan-blue exclusion of dead cells. They are going then to be distributed into wells of a 96-well plate of a 200 μl final volume at a density of 2.5 x 10^6 cells/ml and incubated with RPMI 1640 enriched with 10% fetal bovine serum and 2mM of glutamine in the presence of 100 U/ml of penicillin G and 0.1 mg/ml of streptomycin. All experiments will be run in duplicate. PBMCs will be left unstimulated or they will be stimulated with one of the following stimuli:

- 1 ng/ml lipopolysaccharide (LPS) of the strain *Escherichia coli* O155:B5 which is a Toll-like receptor 4 (TLR4);
• 10μg/ml of purified and pyrogen-free crystals of monosodium urate (MSU) which is a NLRP3 inflammasome agonist as already defined\textsuperscript{15};
• the combination of LPS and MSU

Experiments will be performed in the absence/presence of 5pmol/l of capsase-1 inhibitor. Plates will be incubated for 24 hours at 37\textdegree{}C in 5% CO\textsubscript{2}. After centrifugation, supernatants will be collected and stored at -70\textdegree{}C until assayed. Concentrations of TNF\textalpha{}, IL-1\beta{} and IL-6 will be determined in duplicate by an enzyme immunoassay.

STUDY OBJECTIVES

Primary endpoint
The safety and efficacy of anakinra in patients with HS of Hurley II and III stage disease. This will be defined by the differences of VAS, of severity scores and of DLQI between the two study groups over visits.

Secondary endpoint
• The effect of anakinra in the ex vivo function of PBMCs of patients with HS. This will be defined by the differences of cytokines produced by PBMCs between the two study groups over visits.
• The effect of anakinra on the time to new exacerbation

POWER OF THE STUDY
The study is powered for the primary endpoint aiming to disclose a significant difference between the two study arms at a 5% level using the disease activity score already used by the investigators in one former trial\textsuperscript{9}. According to that calculation, to achieve this with 80% power, 10 patients should be assigned into each arm.

STATISTICAL ANALYSIS
Patients will be divided into responders as those with decrease of the disease severity score from the baseline and into non-responders as those
without decrease of the disease severity score from the baseline. Comparisons will be done by the Fischer's exact test. Comparison of VAS, of severity scores, of DLQI and of released cytokines from PBMCs of patients over study visits will be done by non-parametric statistics. The time to new exacerbation will be compared between the two groups by the log-rank test. Any value of p below 0.05 will be considered significant.

ADVERSE EVENTS

Adverse events (AEs) and Serious Adverse Events (SAEs) will be collected from baseline until the last patient’s evaluation. Investigators should monitor subjects for adverse events and are responsible for recording ALL adverse events and serious adverse events occurring to a patient during the trial.

An adverse event is any undesirable medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The time relationship is defined from the moment the AE occurs during therapeutic treatment until 30 days or 5 half-lives after treatment discontinuation. The adverse event may be a sign, a symptom, or an abnormal laboratory finding.

Serious adverse events (SAEs) must be reported to within 24 days. If an adverse event meets any of the following criteria, it is considered SAE:

- **Death**

- **Life-threatening situation** - The subject was at risk of death at the time of the adverse event/experience. It does not refer to the hypothetical risk of death if the AE were more severe or were to progress.

- **Inpatient hospitalization** or prolongation of existing hospitalization.

- **Persistent or significant disability/incapacity** - Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.

- **Congenital anomaly/birth defects** - Any structural abnormality in subject’s offspring that occurs after intrauterine exposure to treatment.
• **Important medical events/experiences** that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, **they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above**, i.e., death, a life-threatening adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Examples of such medical events/experiences include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

• **Spontaneous and elective abortions** experienced by study subject.

  A **non-serious adverse event** is any untoward medical occurrence in a patient or subject who is administered a pharmaceutical product, and which does not necessarily have a causal relationship with this treatment. A non-serious adverse event is one that does not meet the definition of a serious adverse event given.

**Grading of severity**

The severity of the adverse events shall be graded as:

- **Mild** - the adverse event is transient and well tolerated by the patient
- **Moderate** – The adverse events causes discomfort and affects the usual activities of the patient.
- **Severe** – The adverse events affects the usual activities of the patient to an important degree and may cause disability or be life-threatening.

**Relationship to the drug**

The investigator will use the following definitions to assess the relationship of the adverse event to study drug:

- **Probably Related**: The adverse event has a strong time relationship to the drug or relapses if re-induced, and another aetiology is improbable or clearly less probable.
- **Possibly Related**: The adverse event has a strong time relationship to the drug and an alternative aetiology is as probable or less probable.

- **Probably not Related**: The adverse event has a slight or no time relationship to the drug and/or there is a more probable alternative aetiology.

- **Unrelated**: The adverse event is due to an underlying or concomitant disease or to another pharmaceutical product and is not related to the drug (no time relationship and a much more probable alternative aetiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternate etiology must be provided by the investigator. Please note that a severe adverse event/experience is not necessarily serious, as the term severe is a measure of intensity while a serious adverse event is determined based on the aforementioned regulatory criteria. Individual unblinding thought to be necessary for the management of an adverse event will be documented in the subject Case Report Form.
REFERENCES


Version 1 (10 June 2011)


APPENDIX I. Follow-up of patients. The performed tests are indicated.

<table>
<thead>
<tr>
<th>Visits</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>DQLI</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>x</td>
</tr>
<tr>
<td>Physical examination</td>
<td>x</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>VAS</td>
<td>x</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Photo</td>
<td>x</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Blood sampling</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

DQLI: Dermatology Quality of Life Index
VAS: Visual Analogue Scale

APPENDIX II. Visual analogue scale for the impression the patient has for his disease.

Patient's code:
Number of visit:

```
0 1 2 3 4 5 6 7 8 9 10
```

APPENDIX III. Visual analogue scale for the physical pain the patient feels due to his disease.

Patient's code:
Number of visit:

```
0 1 2 3 4 5 6 7 8 9 10
```
**APPENDIX IV.** The Dermatology Quality of Life Index (DQLI). Each question is scored from 0 (absence) to 3 (intense problem)

<table>
<thead>
<tr>
<th>Question</th>
<th>Score</th>
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<tbody>
<tr>
<td>1. How itchy, sore, painful or stinging has your skin condition been?</td>
<td></td>
</tr>
<tr>
<td>2. How embarrassed or self-conscious have you been because of your skin?</td>
<td></td>
</tr>
<tr>
<td>3. How much has your skin interfered with you going shopping or looking after your home or garden?</td>
<td></td>
</tr>
<tr>
<td>4. How much has your skin influenced the clothes you wear?</td>
<td></td>
</tr>
<tr>
<td>5. How much has your skin affected your social or leisure activities?</td>
<td></td>
</tr>
<tr>
<td>6. How much has your skin made it difficult for you to do any sport?</td>
<td></td>
</tr>
<tr>
<td>7. Has your skin prevented you from working or studying?</td>
<td></td>
</tr>
<tr>
<td>8. How much has your skin created problems with your partner or any of your close friends or relatives?</td>
<td></td>
</tr>
<tr>
<td>9. How much has your skin caused any sexual difficulties?</td>
<td></td>
</tr>
<tr>
<td>10. How much of a problem has the treatment for your skin been?</td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX V.** Follow-up data of each visit to be included in the Case Report Form (CRF).

<table>
<thead>
<tr>
<th>Code of patient:</th>
<th>Visit number:</th>
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<tbody>
<tr>
<td></td>
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<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Affected area</th>
<th>Left axilla</th>
<th>Right axilla</th>
<th>Left inframammary</th>
<th>Right inframammary</th>
<th>Left femo-inguinal</th>
<th>Right femo-inguinal</th>
<th>Other (indicate)</th>
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<tbody>
<tr>
<td>Dimensions (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of lesions:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fistulas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodules</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Scars</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Degree of inflammation</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>(0= absent, 1= mild, 2= moderate, 3= intense)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Have lesions clear borders with surrounding skin</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Disease activity of that</td>
<td></td>
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Version 1 (10 June 2011)
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<tbody>
<tr>
<td>Sartorius score of that area</td>
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<tr>
<td>Total disease activity</td>
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<tr>
<td>Total Sartorius score</td>
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