Investigation of the safety of medicinal products administered for the management of patients with angioedema caused by C1-inhibitor deficiency

PhD Thesis

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1. INTRODUCTION

1.1 Hereditary angioedema with C1-inhibitor deficiency

Hereditary angioedema with C1-inhibitor deficiency (C1-INH-HAE) is a rare disorder (with an estimated prevalence of 1/10,000 to 1/50,000) of autosomal dominant inheritance. The disease is characterized by recurrent, acute episodes of subcutaneous and/or submucosal angioedema. Subcutaneous edema – not accompanied by pruritus, pain, or cutaneous erythema – may appear on the extremities, the face, and neck, as well as on the torso, and in the genital region. Moreover, subcutaneous edema may involve the upper airways and/or the gastrointestinal tract. Edema of the tongue, pharynx, or the larynx causes airway obstruction, which may lead to suffocation within a short time. In the gastrointestinal tract, the characteristic symptoms of edema formation include colicky abdominal pain, nausea, vomiting, watery diarrhea and rarely, circulatory collapse. These symptoms may be mistaken for the manifestations of an acute abdominal 'catastrophe' and therefore, C1-INH-HAE patients are often subjected to unnecessary surgery during acute abdominal episodes of angioedema. A red, map-like pattern – known as erythema marginatum – may appear on the skin as a prodromal symptom of acute episodes.

1.2 Angioedema resulting from acquired C1-INH deficiency

The estimated prevalence of angioedema resulting from acquired C1-inhibitor deficiency (C1-INH-AAE) is 1 to 500,000; however, more patients are possibly affected in reality. In the majority of cases, disease symptoms first occur after 40 years of age, and the family history of the patient is negative. Although edema formation involves the face, the oral cavity, and the upper airways in the first place, it may occur in any body region. The pathological background of this phenomenon is the consumption of the classical pathway components of the complement system. Two types of C-INH-AAE are distinguished: type I C1-INH-AAE most commonly accompanies lymphoproliferative disorders (e.g. lymphoma). The lymphoproliferative disease itself is the direct cause of the consumption of C1 and of the C1-inhibitor. Type II C1-INH-AAE is characterized by the presence of autoantibodies against C1-INH, the neutralizing effect of which results in C1-inhibitor deficiency.

1.3 Known adverse effects of the treatments administered in C1-INH-HAE

Experience with the pharmacotherapy of C1-INH-HAE dates back farthest, and most of the relevant experience has been obtained with attenuated androgens (AAs: methyltestosterone, danazol, stanozolole, and oxandrolone). The mode of the action of AAs is still unknown; these agents are thought to increase the serum level of the C1-INH protein by stimulating hepatic synthesis, as well as to enhance the expression of C1-INH mRNA in peripheral blood

mononuclear cells. Even nowadays, AAs are widely used for prophylaxis in C1-INH-HAE as effective, and inexpensive medicinal products for oral use. However, their administration may be associated with a number of adverse effects (AEs). Well known AEs of the AAs include the pro-atherogenic change of the lipid profile, hepatotoxicity, virilization in female patients, behavioral abnormalities, glucose intolerance, insulin resistance, and hypogonadism.

AAAs may also influence hematological parameters: these agents may cause erythrocytosis and polyglobulia by stimulating erythropoesis. The clinical data on the hematological changes related to treatment with AAs were recorded, in the first place, during danazol treatment of patients with endometriosis, benign breast tumors, Fanconi anemia, of idiopathic thrombocytopenic purpura (ITP).

However, the results of these studies cannot be fully extrapolated to patients with C1-INH-HAE. In particular, both the duration of drug therapy (long-term over years in C1-INH-HAE *vs*. 2 to 6 months in endometriosis, for example), and the administered doses are different (33 to 200 mg in C1-INH-HAE vs. 400 to 600 mg in endometriosis, and 50 to 800 mg in ITP). So far, two publications mentioned erythrocytosis and polyglobulia – and another paper identified polyglobulia – as possible adverse effects of AAs; however, long-term follow-up studies have not been conducted in this subject.

Growth retardation in children due to the premature closure of epiphyseal growth plates is also considered an additional, known side effect of AAs. As a preventive measure, the international guidelines on C1-INH-HAE do not recommend treatment with AAs in pediatric patients until they reach Tanner V stage of puberty. In our center, we have been using danazol (Danoval® – Krka d.d., Novo mesto, Slovenia; Danatrol® – Sanofi, Paris, France) for prophylaxis in children. However, owing to the expansion of therapeutic options during the past decade, we use AAs increasingly less often for prophylaxis in pediatric patients under 18 years of age. In the meanwhile, human plasma-derived C1-INH (pdC1-INH – Berinert, Marburg, Germany) has become available – without limitations on patient age – for the management of any HAE episode, regardless of the location and severity of edema formation. In C1-INH-HAE, no surveys have been conducted so far on growth retardation resulting from the premature of epiphyseal growth plates. Therefore, the aim of our study was to investigate the long-term effect of AAs on the growth of children with C1-INH-HAE. According to experience from clinical studies and from clinical practice, pdC1-INH for intravenous use has proven a safe and effective remedy both for the acute management and for the prophylaxis of C1-INH-HAE. Notwithstanding this, several reports have been published on thromboembolic events following the off-label administration of high doses of pdC1-INH to neonates. Therefore, we investigated the risk factor for thromboembolism, as well as the true prevalence of thromboembolic events

among pdC1-INH-treated patients undergoing regular follow-up care at the National Angioedema Reference Center.

2. OBJECTIVES

2.1 Appraisal of the safety of medicinal product administered in the management of patients with hereditary angioedema caused by C1-inhibitor deficiency

2.1.1 Evaluation of the effects of long-term danazol therapy on hematological parameters in hereditary angioedema

Taking into account the possible occurrence of erythrocytosis and/or polyglobulia as an adverse effect of danazol, the objective of our follow-up study was to appraise the effects of long-term (1-, 3-, and 5-year long) danazol therapy on the hematological parameters of our patients with C1-INH-HAE.

2.1.2 The effect of danazol on the growth of pediatric patients with angioedema caused by C1-inhibitor deficiency

Premature closure of the epiphyseal growth plates is described in the literature as an adverse effect of long-term danazol therapy. Therefore, we investigated the effects of the latter in our pediatric C1-INH-HAE patients.

2.1.3 Appraisal of thromboembolic risk in HAE patients treated with plasma-derived C1-inhibitor concentrate

Considering that thromboembolism has been reported from a study as an adverse effect of therapy with plasma-derived C1-inhibitor concentrate, we appraised the prevalence of thromboembolic events in our patient population.

2.2 A new option for prophylaxis in hereditary angioedema caused by C1-inhibitor deficiency

2.2.1 Experience with a new prophylactic method in hereditary angioedema caused by C1-inhibitor deficiency by administering pdC1-INH concentrate upon the occurrence of erythema marginatum

Plasma-derived C1-inhibitor concentrate was administered as a means for attack prophylaxis to patients experiencing erythema marginatum as a prodromal symptom of impending acute episode of C1-INH-HAE.

2.2.2 Short-term prophylaxis with recombinant human C1-INH concentrate in acquired C1-INH deficiency

In a patient with acquired C1-INH deficiency, recombinant, human C1-inhibitor concentrate (rhC1-INH – Ruconest®, Pharming Group NV, Leiden The Netherlands) was administered as short-term preoperative prophylaxis.

3. METHODS

3.1 Evaluation of the effect of long-term danazol therapy on hematological parameters in hereditary angioedema – subjects and study design

In our prospective study conducted in the National Angioedema Center, we investigated – by analyzing laboratory findings recorded between 1993 and 2015 – the occurrence of erythrocytosis and polyglobulia among 145 C1-INH-HAE patients, diagnosed and followed up in compliance with the international criteria. In the age- and sex-matched group of healthy controls, the hematological parameters were analyzed in a single blood sample drawn for the purposes of a health status screening.

The hematological parameters – white and red blood cell counts, hemoglobin level, hematocrit value, and platelet count – were measured with a digital analyzer.

In the first stage of the study, we compared the incidence of erythrocytosis and of polyglobulia in C1-INH-HAE patients who have never been treated with danazol, and in healthy controls. (See I/1 and I/2.)

In the second stage of the study, we compared the hematological parameters measured before danazol therapy with those recorded after 1, 3, and 5 years of danazol treatment in 39 out of 145 C1-INH-HAE patients. (See II.)

In the third stage, we investigated the occurrence of erythrocytosis and polyglobulia in in C1-INH-HAE patients who had received danazol (50 to 200 mg/day) longer than 5 years. (See III.)

3.2 The effect of danazol on the growth of pediatric patients with hereditary angioedema caused by C1-inhibitor deficiency – patients & methods

We conducted a retrospective study on 45 out of the 145 C1-INH-HAE patients diagnosed and followed up – in compliance with the international criteria – between 1986 and 2014 at the National Angioedema Reference Center.

In the first part of our study, we included patients over 21 years of age, who had been diagnosed with C1-INH-HAE before they turned 21 years old. The study population comprised 42 patients (20 boys and 22 girls). Twelve of the 42 patients (8 boys and 4 girls) received danazol on a regular basis, 4 patients (2 boys and 2 girls) took danazol as required, and 26 patients (9 boys and 17 girls) have never received danazol before the age of 21 years. (See I.)

In the second stage of our study, the inclusion criteria for stage I were modified. Out of the 12 patients receiving danazol on a regular basis, 7 (6 boys and 1 girl) were included into the second study part – these patients received danazol treatment before the age of 16 years. (See II.)

Periodic follow-up evaluation of the study subjects included, among others, the measurement of body height. The body height of the parents was measured at the annual follow-up visits on one hand. When this was not feasible, it was recorded as quoted by the patient, or taken from medical

records. Next, we calculated the expected deviation from mid-parental target body height (ΔSD). Then we compared the ΔSD values of the patients taking *vs*. not taking danazol, as well as of male *vs*. female patients. In patients taking danazol on a regular basis, we calculated the cumulative dose of danazol and the total duration of treatment up to 21 years of age. We grouped these patients into subsets below or above the median of cumulative danazol dose (median: 150,438 mg, which corresponded to a daily dose of 84 mg [min. 33 mg, max. 300 mg]), and by the duration of danazol treatment (median: 4.9 years). We calculated these indices also in patients treated with danazol before 16 years of age, as well as we checked for a correlation between these values and ΔSD.

3.3 Appraisal of the risk of thromoembolism in patients with hereditary angioedmea treated with plasma-derived C1-inhibitor concentrate – patients & methods

We conducted a retrospective study on the data accumulated between 1986 and 2015 from 144 C1-INH-HAE patients (79 females and 65 males), followed up at the National Angioedema Reference Center. After diagnosis, every patient was supplied with pdC1-INH concentrate. The dosage of this medicinal product during this study was as follows: The patients received 500 to 1000 IU pdC1-INH as acute treatment, as well as for short-term, and for intermittent prophylaxis. Starting from 2011, the recommended dose of C1-INH changed to 20 IU/body weight.

During our study, we observed the following arterial and venous thromboembolic complications: deep vein thrombosis (DVT), pulmonary embolism (PE), stroke, and acute myocardial infarction. All patients were queried for the possible occurrence of these thromboemolic events at every annual follow-up visit.

We investigated the occurrence of thromboembolic diseases and their predisposing factors both in patients treated with pdC1-INH, and in those not treated with this medicinal product. The following risk factors were identified:

Risk factors predisposing for cardiovascular disease: Hypercholesterolemia, hypertriglyceridemia, smoking, hypertension, diabetes mellitus, obesity. The survey of these parameters was based on the data gathered at the annual follow-up visit of 2015.

Risk factors predisposing for pulmonary embolism: Age (in the year 2015), major orthopedic or oncologic surgery, oral contraceptive use, pregnancy, history of venous thromboembolism.

Risk factors related to hereditary or acquired angioedema, or to hematological parameters – the presence of the following: Lupus anticoagulant (LA), dilute Russel's viper venom time (dRVVT), lupus anticoagulant-sensitive partial. thromboplastin time (PTT LA), anticardiolipin antibodies (ACA), anti-β_2-glycoprotein-I antibodies (anti-beta2-GP-I AT), Leiden mutation, prothrombin G20210A mutation.

The laboratory tests were conducted on samples obtained from the patients in 2014. Finally we checked whether the patients received long-term prophylaxis with an antifibrinolytic (tranexamic acid), or with an attenuated androgen (danazol).

Laboratory methods

Coagulation studies (with the Siemens BCS-XP automate – Siemens Healthcare Diagnostics Product GmbH, Marburg, Germany), and molecular genetic testing (Genosign kit, real-time polymerase chain reaction LightCycler® – Microtrade, Budapest, Hungary) were performed in the blood samples drawn from every patient at the annual follow-up visits. When these tests indicated an abnormality, we supplemented the assessment of thrombotic risk by measuring antithrombin activity (Innovance® chromogenic assay; anti-Xa), as well as with the determination of protein C and protein S levels.

3.4 A novel prophylaxis with plasma-derived C1-INH concentrate during erythema marginatum in hereditary angioedema caused by C1-inhibitor deficiency – Patients & methods

For the purposes of this study, we selected two C1-INH-HAE patients with frequent, acute episodes of HAE, which are often preceded by the occurrence of erythema marginatum (EM). They were instructed to administer pdC1-INH as soon as possible after the onset of EM and then, to record relevant information on this treatment (time of administration, drug dose), and on the details of the possible subsequent HAE episode in their patient diary.

With regard to all of our investigations, the study protocol was approved by the institutional review board of Semmelweis University, Budapest, and informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

3.5 Short-term prophylaxis with recombinant, human C1-INH concentrate in acquired C1-inhibitor deficiency – Patients & methods

Recombinant, human C1-inhibitor (rhC1-INH), derived from the breast milk of transgenic rabbits, was administered as short-term preoperative prophylaxis to a 66-year old patient with acquired C1-INH deficiency.

4. RESULTS

4.1 Evaluation of the effect of long-term danazol therapy on the hematological parameters in hereditary angioedema

I/1. We did not find any difference between C1-INH-HAE patients never treated with danazol (n=76; 31 males and 45 females) and healthy controls (n=141; 57 males and 84 females), with regard to the incidence of erythrocytosis and of polyglobulia ($p>0.05$).

I/2. Comparing the laboratory parameters obtained in the year 2012 from C1-INH-HAE patients who had never before received danazol (n=51/145; 21 males and 30 females) with those of age- and sex-matched healthy controls (n=210; 89 males and 121 females) did not reveal any difference either in the incidence of erythrocytosis and/or polyglobulia ($p>0.05$).

II. Compared with baseline, there were no significant changes in the hematological parameters of male patients after 1, 3, or 5 years of danazol therapy. In female patients, the same applies to red blood cell count, hemoglobin level, and hematocrit values; however, significantly lower white blood cell counts ($p=0.0067$) and platelet counts ($p=0.0203$) were found compared to baseline values after 5 years of danazol treatment. RBC and hematocrit value were beyond the reference range in just one female patient. In this patient, erythrocytosis developed after one year of danazol treatment, and persisted during the 3rd and the 5th years of follow-up. Considering that all other underlying causes could be excluded, erythrocytosis in this patient might have been related to danazol therapy. Hematocrit value was also elevated in this same female patient with erythocytosis. Moreover, we observed also polyglobulia in this patient after 1 and 3 years of danazol therapy. However, polyglobulia was no longer detectable after 5 years of treatment and hence, its occurrence is possibly unrelated to danazol therapy.

III. Fifty patients altogether (25 males and 25 females) received danazol for more than 5 years. Neither erythrocytosis, nor polyglobulia occurred among the male patients in this subset, whereas erythrocytosis was observed in 3 females.

4.2 The effect of danazol therapy on growth in pediatric patients with hereditary angioedema caused by C1-inhibitor deficiency

I. We did not find any significant difference between the ΔSD values of patients taking *vs*. not taking danazol (n=12 *vs*. n=30), of males *vs*. females (n=8 *vs*. n=4), of patients taking lower *vs*. higher cumulative doses (n=6 vs. n=6) of danazol, or of those taking danazol for a shorter *vs*. for a longer period (n=6 vs. n=6).

II. In patients treated with danazol before the age of 16 years, there was no significant correlation between ΔSD values and the duration of danazol therapy, or the cumulative dose of danazol administered.

None of the subjects had an abnormal body height (ΔSD min.: -1,04, max.: 1,29).

4.3 Appraisal of the risk of thromboembolism in patients with hereditary angioedema treated with plasma-derived C1-inhibitor

During the observation period, 104 of the 144 patients (62 females and 42 males) received pdC1-INH, whereas 40 patients (17 females and 23 males) have never received pdC1-INH during their lifetime. The mean cumulative dose per patient was 573.59 IU. The administered dose was 500 IU on 3155 occasions, 1000 IU in 434, 1500 IU in 38, and 2000 IU in 7 instances – one patient received 2500 IU pdC1-INH on a single occasion. Median exposure to pdC1-INH was 10.5 years, and the number of cumulative years was 1031 in the group treated with pdC1-INH. In a single case, the maximum cumulative dose over a year was 136,500 IU, administered to a C1-INH-HAE patient during pregnancy. The number of cumulative years of observation was 365 in patients not receiving pdC1-INH. None of the patients administered pdC1-INH through an indwelling central catheter.

In both groups, approx. half of the patients were older than 45 years, and half of them had hypercholesterolemia. The groups treated/not treated with pdC1-INH were similar also in respect of other risk factors – with several exceptions. In particular, 4 women in the group receiving pdC1-INH took a progesterone-containing oral contraceptive *vs*. none in the group not treated with pdC1-INH. Obesity was more common in the group receiving pdC1-INH. The number of patients on long-term prophylaxis was 3 to 4 times higher in the group taking vs. that not taking pdC1-INH. We identified risk factors much more often in patients receiving long-term danazol therapy, than in those on long-term treatment with tranexamic acid. Additional, common risk factors in the group taking danazol included age over 45 years, pregnancy, oral contraceptive use, and a history of a thromboembolic event.

Abnormalities detected in the thrombophila panel were the most important risk factors for thromboembolic events. Fifteen out of the 144 patients tested positive for lupus anticoagulant and prothrombin G20210A mutations. Two of these patients belonged to the subset with poor risk, whereas nine to that with a moderate risk of thromboembolism. Except for a single patient with a heterozygous Factor II polymorphism, all these eleven patients were treated with pdC1-INH.

We observed thromboembolic events in five patients; two of them received pdC1-INH. Patient N° 1 sustained cerebral infarction 9 months after the administration of pdC1-INH concentrate. During the 14 years before the occurrence of cerebral infarction, this patient received a cumulative dose of 2,500 IU pdC1-INH. Treatment with pdC1-INH was no longer necessary after the thromboembolic event, because HAE episodes have not recurred since then. Patient N° 2 sustained venous thromboembolism, which involved the left external iliac vein, the common femoral artery, and the great saphenous, as well as the superficial femoral veins 7 months after the administration of the last 500-IU dose of pdC1-INH. This patient also had arterial cerebral

infarction 3 years later – that is, 5 months after the administration of the last 500-IU dose of pd-C1-INH. Furthermore, this patient received further 500 IU of pdC1-INH after the occurrence of cerebral infarction without any subsequent thromboemboilc events. Both patients with thromboembolism received pdC1-INH in fixed (500-IU) doses.

4.4 A novel prophylaxis with plasma-derived C1-INH concentrate during erythema marginatum in hereditary angioedema caused by C1-inhibitor deficiency

Two young female patients were enrolled into this study.

Patient N° 1: The 21-year-old woman was diagnosed with Type 1 C1-INH-HAE at the age of three, by complement testing. Between the ages of 12 and 18 years, she had three times (exactly 3.1×) more HAE attacks preceded by erythema marginatum, than those without this skin manifestation. As the correlation between EM and HAE attacks had become conspicuous after the age of 19 years, we instructed the patient to administer 500 IU pdC1-INH as soon as possible after the onset of EM, in order to prevent the HAE attack from occurring. Accordingly, the patient injected pdC1-INH during EM on 16 occasions, and this intervention prevented the HAE attack in all instances. On 26 occasions, when the patient was unable to administer pdC1-INH while EM was present (i.e. at school or at the workplace), angioedema always developed. In these cases, the patient administered 500 to 1000 IU pdC1-INH as an acute treatment. At the age of 20, pdC1-INH was administered during EM on 9 occasions, and this treatment always prevented edema formation. During this period, she did not administer pdC1-INH during EM on 19 occasions – and in these, EM was always followed by edema (18 subcutaneous and 1 abdominal attacks). The patient had significantly fewer HAE attacks when pdC1-INH was administered during EM ($p<0.0001$).

Patient N° 2: In this 28-year old female patient, Type II C1-INH-HAE was diagnosed based on her clinical symptoms, on the results of the complement screen, and of genetic testing. As the correlation between EM and HAE attacks had become conspicuous after the age of 26 years, and unplanned pregnancy occurred, which might have aggravated the frequency and severity of HAE symptoms, we instructed the patient to administer 500 IU pdC1-INH as soon as possible after the onset of EM, in order to prevent the HAE attack from occurring. Accordingly, at the age of 27, the patient injected pdC1-INH during EM on 19 occasions, and this measure prevented the HAE attack in all instances. On 39 occasions, when the patient was unable to administer pdC1-INH in the presence of EM (i.e. because it would have been socially inconvenient, or she had to care for her baby), a HAE attack always developed. At the age of 28, she was administered pdC1-INH during EM on 34 occasions, and this measure prevented the HAE attack in all cases. Unfortunately, during this period, she unable to have pdC1-INH administered on 45 occasions – HAE attack always developed in these instances. This patient

had significantly fewer HAE attacks when pdC1-INH was administered than not being given during EM ($p<0.0001$).

4.5 Short-term prophylaxis with recombinant human C1-INH concentrate in acquired C1-INH deficiency

This is the first case report on short-term prophylaxis in C1-INH-AAE, with the administration of recombinant human C1-inhibitor (rhC1-INH). The 66-year-old female patient had hypertension known for 15 years and treated with enalapril. C1-INH-AAE was diagnosed, based on the clinical symptoms and on the results of complement testing; the family history was negative. As the known trigger factors of bradykinin-mediated angioedema attacks include treatment with ACEIs, we suggested replacing enalapril with bisoprolol when the patient first presented at our outpatient clinic. During follow-up pancytopenia was diagnosed and bone marrow biopsy performed, which confirmed the presence of the non-Hodgkin lymphoma of the splenic marginal zone type. Splenectomy was performed after pre-treatment with erythropoietin – the latter was necessary because the patient rejected blood transfusions for religious reasons. Surgery was performed with pdC1-INH at hand; however, the operation and the postoperative were uneventful with regard to angioedema. Edematous attacks have not recurred during the last two years and therefore, neither acute, nor maintenance therapy was necessary. The patient's hematological and complement parameters have been checked regularly since then. During the past 2.5 years, she had surgery on three occasions. Although she was symptom-free, short-term prophylaxis was administered before these interventions, because her complement status remained unchanged and C1-INH deficiency persisted. On two occasions, she received 1000 IU pdC1-INH concentrate before oral surgery (multiple dental extractions and suture). On one occasion, we introduced short-term prophylaxis before cataract surgery; the patient received 2,100 IU rhC1-INH. Both drugs were administered by the intravenous route, one hour before the intervention. We switched the patient from the plasma-derived to the recombinant preparation, because rhC1-INH concentrate had become available in the meantime also in Hungary. Furthermore, the patient preferred the latter to the plasma-derived product on religious grounds. All interventions were uneventful with regard to the occurrence of HA episodes; drug adverse reactions did not occur.

5. CONCLUSIONS

The main findings of the study are as follows:

1. We did not find any difference with regard to the occurrence of erythrocytosis and polyglobulia between C1-INH-HAE patients older than 18 years of age and never treated with danazol and controls. Comparing the laboratory parameters of these two populations, there were no differences in the number of cases with erythrocytosis and/or polyglobulia. Monitoring the changes of the hematological parameters of 39 patients receiving long-term danazol therapy from baseline to the end of 1, 3, and 5 years of treatment, we did not ascertain any significant changes compared to baseline values in male patients. In females, the same applies to red blood cell count, hemoglobin level, and hematocrit values. However, compared to baseline values, we observed significantly lower white blood cell counts and platelet counts in women after 5 years of danazol therapy. Red blood cell counts and hematorcrit values beyond the reference range were detected in just a single female patient. Considering that all other underlying causes could be excluded, erythrocytosis and polyglobulia in this patient might have been related to danazol therapy.

2. With regard to the deviation from expected mid-parental target body height, we did not find any statistically significant differences between C1-INH-HAE patients taking *vs*. not taking danazol before 21 years of age, between males vs. females, or patients taking lower vs. higher cumulative doses of danazol, or those taking danazol for a shorter or a longer period. Therefore, our findings suggest that the warnings regarding the premature closure of epiphyseal growth plates occurring as a theoretical adverse effect of danazol and causing growth retardation in children may be ignored.

3. We observed thromboembolic complications in five patients – two of them received pdC1-INH therapy; however, the occurrence of these events could not be linked to pdC1-INH administration even in these cases. Based on our findings, therefore, we can conclude that – when administered according to the approved therapeutic indications, in the recommended doses, and into a peripheral vein – plasma-derived C1-INH concentrate may be used safely even in the presence of preexisting risk factors, and without causing thromboembolism.

4. In two female patients, we introduced a novel prophylactic treatment during the occurrence of erythema marginatum, which is the sole objective prodromal symptom of an impending acute episode of C1-INH-HAE. In both patients, treatment with plasma-derived C1-INH concentrate was 100-per-cent effective in preventing the occurrence of an acute edematous episode of HAE.

5. In a patient with C1-INH-AAE, we administered recombinant C1-INH concentrate as short preoperative prophylaxis before cataract surgery. The procedure was uneventful with regard to

the occurrence of an acute edematous episode of HAE, and drug adverse effects did not occur either.

We have conducted all these studies with a view to improving the quality of life of C1-INH-HAE patients, by appraising the adverse effects of the medicinal products administered, and by creating new means for prophylaxis. When developing novel treatment protocols, we focused on administering medicinal products in the lowest effective doses, avoiding unnecessary therapy, and taking into account also cost-effectiveness.

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Cumulative impact factor of the publications unrelated to the thesis: 36,618.

Total impact factor of the indicated publications: 54,508.