Research Project

The Efficacy of L-cysteine in Prevention of Cluster Headache.

Randomized intervention trial with a medical device (Acetium® Capsules)*

Executed by:

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SYNOPSIS

Background: Cluster headache belongs to a group of idiopathic headache entities, the trigeminal autonomic cephalalgias (TACs), all of which involve short-lasting, unilateral, severe headache attacks and typical accompanying autonomic symptoms. The life-time prevalence of cluster headache for adults of all ages is around 125/100,000, or approximately 0.1%, with clear male dominance (4:1). Prophylactic treatment is an important part of the total management of cluster headache patients, having twofold goals: i) to reduce the frequency, painfulness, and/or duration of headache attacks, and ii) to increase the effectiveness of abortive therapy. During the past several decades, a large number of optional modalities have been tested as preventive measures of cluster headache. Not unexpectedly, the effects of any such preventive therapies are highly variable, and in many patients, the attack frequency is not under satisfactory control. Many of these drugs also have untoward side effects that offset their potential benefits.

Recently, spontaneous case testimonials were received by Biohit Oyj from migraine patients (and one with cluster headache) reporting that our new medical device, Acetium® capsule (containing 100mg L-cysteine, developed for inactivation of acetaldehyde in the stomach contents after alcohol intake), proved to be highly effective against their headache attacks. Their attacks disappeared almost immediately after onset of Acetium® capsule administration, all of them remaining in complete remission for several months up to several years.

These patient testimonials prompted us to formulate a novel study hypothesis that could possibly explain this dramatic effect of Acetium® capsules in prevention of headache attacks, to be tested in this RCT. This novel hypothesis is starting from the fact that, in cluster headache (a vascular-type of headache), swelling and dilatation of the blood vessels is necessary to provoke the attack.

It is known that Nitric Oxide (NO) is the final trigger of vascular headache attack, operating through phosphorylated protein kinase G (PKG) and Ca2+ channels, slowing the influx of calcium into the cell, which leads to smooth muscle relaxation and vasodilation. Histamine is a potent inducer of NO Synthase, making NO available locally on the vasculature, acting through endothelial H1-receptors. Histamine is synthesized from histidine in tissue mast cells, which are ubiquitous cells and their activation e.g. in the meninges has long been suspected to be involved in generating migraine headaches. Finally, one of the potent liberators of histamine from the mast cells is acetaldehyde, which, in turn, is effectively inactivated by L-cysteine (Acetium® capsule). This led us to rational that by eliminating acetaldehyde in the stomach, L-cysteine could block (or reduce below the threshold levels) histamine liberation from the tissue mast cells and ECL cells in the stomach, thus arresting its multitude of functions, of which vasodilatation is critically involved in the cluster headache attack.

Objective: To validate the novel hypothesis that daily use of Acetium® capsules (2 capsules three times a day) is an effective means to decrease the frequency of (or completely abort) the headache attacks in patients suffering from cluster headache.

Study design: A double-blind, randomized placebo-controlled multi-centre trial comparing Acetium® capsules (2 capsules 3 times a day) and placebo in prevention of headache attacks during a 1-month trial period. In brief, a cohort of 60 (up to100) voluntary subjects (women and men, with periodic or chronic cluster headache) are invited to participate. To be eligible for the study, the subjects should: i) have the frequency of typical attacks up to 5 per day; ii) age between 18 and 65 years; and iii) have a minimum of co-morbidity. There are no restrictions on duration since the

diagnosis, or the age at the onset of cluster headache. They should discontinue other prophylactic medication prior to study entry. Before enrolment, all subjects are requested to sign a written consent. The study protocol will be subjected for approval by the Committee on Medical Research Ethics.

Methods: The study setting is triple-blinded (participant-blind, investigator-blind, sponsor-blind). Placebo preparation with design and package identical to the test preparation will be used. Parallel group design instead of cross-over design is used. Randomization will be performed using a random number generator, with block size of 4, and stratified by gender, cluster headache type and duration of the on-going cluster episode.

The treatment period in both study arms will be 1 month. The participants should use (but accurately report) their usual symptomatic or acute treatment, because not anticipated to interfere with the study medication. During the 1-month treatment period, participants will be evaluated at weekly intervals by the study coordinator.

In addition to the baseline assessment of attack frequency, each subject will be requested to fill in a structured Questionnaire recoding their detailed headache history and other pertinent data on potential triggers. The headache diary is the main research tool used to monitor the efficacy of the test preparations, recording all predefined assessment measures (efficacy, tolerability and safety), and submitted to the study monitor on each FU visit.

In statistical analysis, both conventional techniques (e.g. non-parametric paired-samples and non-paired samples t-test), and more sophisticated methods will be used. The latter include i) life-table methods like Kaplan-Meier and Cox proportional hazards regression, as well as ii) generalized linear models (GEE and panel Poisson) and as a new technique in cluster headache RCTs, a competing risks regression to model the natural outcomes of the headache during the intervention. This study (n=60; the most modest scenario) is adequately powered (Type II error 0.80, type I error 0.05) to detect a true difference in attack frequency between 15 attacks/week in the placebo and 13.1 attacks/week in the Acetium® arm. Given that the study subjects are enrolled among patients with typically 1-3 attacks/day, i.e., 7-21 (up to 35) attacks per week, and assuming a 10% reduction by placebo, these figures seem reasonable estimates for the power calculations.

Specific aims: The null hypothesis of the study implicates that Acetium® capsules are no better than placebo in prophylaxis of cluster headache during the 1-month intervention period. Rejection or not of the null hypothesis is based on comparison of the two arms for the primary study endpoint and (to lesser extent) for a series of secondary endpoints. The **primary study endpoint** (efficacy measure) is the number of attacks per week. Potentially useful secondary endpoints include: i) intensity of headache (4-tier nominal scale); ii) drug consumption for symptomatic or acute treatment; and iii) patients' preferences and satisfaction.

Study execution and time-table: Meanwhile the final protocol is under evaluation for ethical approval by HUS, preparatory measures have been taken by informing the FMA about the planned study and asking their co-operation in encouraging the interested migraine patients to contact the study coordinator. Given the preliminary interest shown in the study, the required cohort of volunteers should be enrolled within a reasonably short time. However, because the subjects with episodic cluster headache must be enrolled during their on-going cluster episodes that typically

follow a seasonal pattern, the enrolment of the total cohort (even n=60) inevitably needs to be completed over several months (up to one year).

Impact of the study: Given that L-cysteine is a natural (semi-essential) amino acid, converted to inert substance (MTCA) in the alimentary tract, it would comprise an ideal means to conduct prophylaxis of cluster headaches for years, without concern about the side effects that are inherent to many of the current treatment modalities. If the efficacy is proven in this formal RCT, the concept of using Acetium® capsules in prophylactic treatment of cluster headache would represent a major step forward in a better clinical control of these frequently excruciating headaches.

1.BACKGROUND

Headache disorders are among the most prevalent, burdensome, and costly diseases in the world, and there is an urgent need for acceptance, education, and scientific interest.[1-6] Indeed, the magnitude of the burden associated with headache has not been fully acknowledged until now. Globally, the percentage of the adult population with an active headache disorder is 47% for headache in general, 10% for migraine, 38% for tension-type headache, and 3% for chronic headache that lasts for more than 15 days per month.[1-6] Most of the severely affected patients also have profound comorbid disorders, which complicate their overall management and outcome. Thus, the burden of headache on the patient, their families, and on society is considerable.

The large costs of headache to society, which are mostly indirect through loss of work time, have been reported. On the individual level, headaches cause disability, suffering, and loss of quality of life that is on a par with other chronic disorders. Most of the burden of headache is carried by a minority who have substantial and complicating comorbidities.[1,2] Renewed recognition of the burden of headache and increased scientific interest have led to a better understanding of the risk factors and greater insight into the pathogenic mechanisms, which might lead to improved prevention strategies and the early identification of patients who are at risk.

Limited knowledge of the underlying pathophysiology combined with a lack of academic interest has previously resulted in the use of non-specific treatments, although management of migraine has improved and interest has increased during the past decade.[7,8] Headache-related disability can be markedly reduced by increasing general awareness of headache, better education among health-care professionals, and the identification of trigger factors combined with pharmacological management, although we still lack specific preventive modalities.[9-11] Most importantly, early intervention, the identification of risk factors, and lifestyle associations might lead to effective strategies to prevent headaches turn chronic, which will have considerable benefits for the patient and the society.

2.CLUSTER HEADACHE

Migraines were first comprehensively classified in 1988 by Olesen et al.[12] The International Headache Society most recently updated their classification of headaches in 2004.[13] According to this

classification, migraines are considered as the primary headaches along with the tension-type headaches and **cluster headaches**.[13,14]

Cluster headache is a disease that involves as its most prominent feature, an immense degree of pain that is almost always on only one side of the head. The first complete description of cluster headache was given by a London neurologist Wilfred Harris in 1926, who named the disease as **Migrainous neuralgia**.[15] In subsequent literature, cluster headaches have been called by several other names, including Erythroprosopalgia of Bing, Ciliary neuralgia, Erythromelalgia of the head, **Horton's headache**, **Histaminic cephalalgia**, Petrosal neuralgia, sphenopalatine neuralgia, Vidian neuralgia, Sluder's neuralgia, and Hemicrania angioparalyticia.[15]

Cluster headache belongs to a group of idiopathic headache entities, the trigeminal autonomic cephalalgias (TACs), all of which involve short-lasting, unilateral, severe headache attacks and typical accompanying autonomic symptoms.[16,17] Cluster headache is the most prominent of these entities. The concept of the TACs is useful for clinicians seeking a pathophysiologic understanding of the primary neurovascular headaches and a rational therapeutic approach to treating or preventing these headaches. There are many who describe the pain resulting from cluster headaches as **the most intense pain that a human can tolerate,** being worse than giving birth, having burns or broken bones. Cluster headaches often occur periodically, i.e., spontaneous remissions interrupt active periods of pain, although there are some 20% of sufferers in whom the cluster headaches never remit. Some people affected with cluster headache have committed suicide, leading to the nickname "suicide headaches."[15]

2.1.Epidemiology

The prevalence of cluster headache is <1 percent and mostly affects men.[17-20] In a recent meta-analysis of 16 population-based epidemiologic studies,[20] the following key observations emerged: 1) The life-time prevalence of cluster headache for adults of all ages was 124 per 100,000 (95% CI 101-154), or approximately 0.1 percent; 2) The 1-year prevalence of cluster headache was 53 per 100,000 (95% CI 26-95); 3) he overall male to female ratio was 4.3:1.[20]

However, there is some evidence that the male preponderance in cluster headache is decreasing, particularly in patients with headache onset after 1960 or 1970.[21-23] A likely explanation could be an

improved understanding of the pathophysiology of this syndrome and consequently a higher acceptance and awareness leading to more frequent diagnosis.[23,24] However, this finding has not been confirmed in all studies.[18,20]

2.2.Signs and symptoms

Cluster headaches are agonizing unilateral headaches of extreme intensity.[24] Cluster headache is characterized by attacks of severe orbital, supraorbital, or temporal pain, accompanied by autonomic phenomena.[13,25] The stereotypical attacks may strike up to eight times a day and are relatively short-lived. The duration of the common attack ranges from as short as 15 minutes to three hours or more. Cluster headache is strictly unilateral, and the symptoms remain on the same side of the head during a single cluster attack. However, the symptoms can switch to the other side during a different cluster attack (so-called side shift) in approximately 15 percent of cases, and even rarer, simultaneously (within the same cluster period)to appear as bilateral headache.[26,27]

The onset of an attack is rapid and most often **without the preliminary signs** that are characteristic of a migraine. Similarly, in contrast to migraine, the patients with cluster are restless and prefer to pace about or sit and rock back and forth. However, some patients report preliminary sensations of pain in the general area of attack, often referred to as "shadows", that may warn them of an imminent attack.[27] Although trigeminal neuralgia can cause headaches with similar qualities, the pain is mostly located around the facial area, and is described as being like stabbing, electric shocks, burning, pressing, crushing, exploding or shooting pain that becomes intractable. The attacks of cluster headache can be so vicious that patients may commit suicide if the disease is not diagnosed or treated.[28]

2.2.1.Pain

The pain of cluster headaches is remarkably more severe than in other headache conditions, the most severe migraine attacks included, and many experts have suggested that it may be the most painful condition known to medical science. Female patients have reported it as being more severe than childbirth.[29] The pain is lancinating or boring/drilling in quality, and is located behind the eye (periorbital) or in the temple, sometimes radiating to the neck or shoulder. The condition was originally named **Horton's Cephalalgia** after B.T Horton, who postulated the first theory as to their

pathogenesis.[30] Already his original paper describes the severity of the headaches as being able to take normal men and force them to attempt or complete suicide.[28,30]

2.2.2.Autonomic symptoms

The principal symptoms of the cluster headache attack are the severe or very severe unilateral orbital, supraorbital and/or temporal pain lasting 15–180 minutes, if untreated, and the attack frequency of one to 16 attacks in 48 hours. The headache is accompanied by at least one of the following autonomic symptoms: i) ptosis (drooping eyelid), ii) miosis (pupil constriction) iii) conjunctival injection (redness of the conjunctiva), iv) lacrimation (tearing), v) rhinorrhea (runny nose), and, less commonly, vi) facial blushing, swelling, or sweating, all appearing on the same side of the head as the pain.[31]

These unilateral autonomic symptoms occur only during the pain attack and are ipsilateral to the pain. These symptoms are indicative of both parasympathetic hyperactivity and sympathetic impairment. In some patients, the signs of sympathetic paralysis (miosis and ptosis) persist indefinitely,[32] but intensify during attacks. Sweating and cutaneous blood flow also increase on the painful side, particularly in areas of sympathetic deficit.[33,34] However, some 3 percent of all patients lack these autonomic symptoms.[35] In rare cases, sympathetic disturbances persist on the previously-affected side of the face in patients whose cluster headache has switched sides.[25]

In contrast to migraine, nausea rarely accompanies a cluster headache, though it has been reported. Less frequently, the victim can have an aversion to bright lights and loud noise during the attack The neck is often stiff or tender after the headache, with jaw or tooth pain sometimes present. Some sufferers report feeling as though their nose is stopped up and that they are unable to breathe out of one of their nostrils.[25,27] Secondary effects are inability to organize thoughts and plans, exhaustion and depression. Patients tend to dread facing another headache, and may adjust their physical activities or ask for help to accomplish normal tasks, and may hesitate to schedule plans in reaction to the clock-like regularity of the pain schedule leading to social isolation.[31]

2.2.3.Circadian periodicity

Another clinical landmark of the cluster headache syndrome is the circadian rhythmicity of the relatively short-lived (15 to 180 minutes) painful attacks.[25,28,29,31] Because of this, cluster headaches

are occasionally referred to as "alarm clock headaches" because of their ability to wake a person from sleep and because of the regularity of their timing. Both the individual attacks and the clusters themselves can have **a metronomic regularity**; attacks striking at a precise time of a day each morning or night is typical, even precisely at the same time a week later. The clusters tend to follow daylight saving time changes and happen more often around **the spring and autumn equinox**. This has prompted researchers to speculate an involvement of the brain's "biological clock" or circadian rhythm.[25,28,29,31]

Cluster headaches occurring in two or more cluster periods lasting from 7 to 365 days with a pain-free remission of one month or longer between the clusters are considered **episodic.** In the episodic form, attacks occur daily for some weeks followed by a period of remission. On average, a cluster period lasts 6 to 12 weeks while remissions can last up to 12 months or longer. The episodic form is the most common, affecting 80 to 90 percent of patients with cluster headache. It is characterized by periods of attacks (clusters or bouts) and periods of remission. In a bout, patients may experience one to eight attacks per day, and bouts may last from seven days to 12 months.[13] When not in a bout, patients are usually asymptomatic.

If the attacks occur for more than a year without a pain-free remission of at least one month, the condition is considered **chronic**.[13,31] In approximately 10–15% of the cluster headache victims, the condition is chronic, and they can experience multiple headaches **every day for years**. Chronic clusters run continuously without any "remission" periods between cycles. Chronic sufferers may, however, have "high" and "low" cycles, meaning the frequency and intensity of attacks may change for a period of time, although the amount of change during these cycles varies between individuals and is not the same as the complete remission episodic sufferers experience. The condition may change from chronic to episodic and from episodic to chronic. Remission periods lasting for decades before the resumption of clusters have been known to occur.[13,31]

2.3.Causes of cluster headache

Cluster headaches have been classified as vascular headaches. The intense pain is caused by the **dilation of blood vessels** which creates pressure on the trigeminal nerve.[13,25,31] While this process is the immediate cause of the pain, the etiology (underlying cause or causes) is not fully understood.

2.3.1.Role of hypothalamus

Among the most widely accepted theories is that cluster headaches are due to an abnormality in the hypothalamus. This can explain why cluster headaches frequently strike around the same time each day, and during a particular season, since one of the functions of the hypothalamus is regulation of **the biological clock**. In addition to being responsive to light, i.e., day length and photo period, the hypothalamus responds to i) olfactory stimuli, including sex steroids (some researchers have linked low testosterone to cluster headaches)[36] and corticosteroids; ii) neurally transmitted information arising in particular from the heart, the stomach, and the reproductive system; iii) autonomic inputs; blood-borne stimuli, including leptin, ghrelin, angiotensin, insulin, pituitary hormones, cytokines, blood plasma concentrations of glucose and osmolarity, etc.; as well as iv) to stress. It may well be that these particular sensitivities could underlay the causes, triggers, and methods of treatment of cluster headache.[31,36] (for more details, see Section 2.6.)

2.3.2.Genetics

Before 1990, cluster headache was not thought of as an inherited disorder.[37] Subsequently, however, the importance of considering genetic factors in the etiology has been highlighted by several important observations. First, in a report of monozygotic twins, both had cluster headache.[38] Second, epidemiologic studies have reported a family history of cluster headache in 5 to 20 percent of patients with cluster headache.[18,39-41] Compared with the general population, the risk of cluster headache for first-degree relatives was increased by 14 to 39-fold, and for second-degree relatives by 2-to 8-fold.[39-41]

This convincing evidence of increased familial risk clearly implicates the hypothesis that cluster headache has a genetic component, at least in some families. Results of a complex segregation analysis in one study suggested that an autosomal dominant gene may play a role in cluster headache inheritance in some families,[42] although there is also evidence for autosomal recessive or multifactorial inheritance in others.[17] It must be taken into account that cluster headache can start between the age of 7 and 83, and that the distinction between affected and unaffected individuals is clearly provisional.[43,44]

2.3.3.Smoking

Tobacco smoking may trigger cluster headaches, particularly among those with a heavy addiction to

cigarette smoking. There is evidence that up to 85 percent of patients with cluster headache are also chronic cigarette smokers.[45,46] Quitting smoking has no effect on the disease. However, **smoking may be a risk factor for the development of cluster headache, possibly on the basis of a genetic predisposition.**[47] Interestingly, there was a marked decline in the incidence of cluster headaches between 1979-81 and 1990-91 in Olmsted County, Minnesota, during a time when the incidence of smoking declined in the population.[48]

2.3.4.Severe brain injuries

Anecdotal evidence points to a correlation between a severe blow to the head, leading to unconsciousness, and the onset of cluster headaches in genetically predisposed subjects. The correlation seems to be specific to a 3-year period between the head injury and the onset of the first cluster attack.[31] Similarly, some studies have noted an apparently high incidence of history of head trauma among patients with cluster headache, up to 15 percent.[45] However, the average time between head injury and cluster headache onset (10 years) seems too long to support a causative role.[13,31]

2.4.Diagnosis

Cluster headaches often go undiagnosed for many years, being confused with migraine or other causes of headache.[31] However, cluster headache, in its typical form, is unmistakable. The diagnosis is exclusively a clinical task based upon a compatible history and diagnostic criteria from the second edition of the International Classification of Headache Disorders (ICHD-2).[13] No single instrumental examination is able to define, ensure, or differentiate idiopathic headache syndromes.[3] Nevertheless, neuroimaging is suggested to exclude a cranial lesion in patients with suspected cluster headache.

To confirm the diagnosis of cluster headache, the ICHD-2[13] requires at least five headache attacks fulfilling the following criteria:

1) Severe or very severe unilateral orbital, supraorbital, and/or temporal headache attacks, which last untreated for 15 to 180 minutes. During part (but less than half) of the time course of the cluster headache, attacks may be less severe, less frequent, or of shorter or longer duration.
2) The headache is accompanied by at least one of the following symptoms:

- Ipsilateral conjunctival injection or lacrimation

- Ipsilateral nasal congestion and/or rhinorrhea
- Ipsilateral eyelid edema
- Ipsilateral forehead and facial sweating
- Ipsilateral miosis and/or ptosis
- A sense of restlessness and agitation

3) The attacks have a frequency from one every other day to eight per day.
4) The history and physical and neurologic examinations do not suggest any other disorder, and/or such a disorder is ruled out by appropriate investigations, or such disorder is present but attacks do not occur for the first time in close temporal relation to the disorder.

Attacks fulfilling all but one of the criteria for cluster headache are diagnosed as **probable cluster headache.**[13]

The ICHD-2 diagnostic criteria for episodic and chronic cluster headache are as follows:
1) Episodic cluster headache is diagnosed when at least two cluster periods lasting seven days to one year are separated by pain free periods lasting one month or longer.
2) Chronic cluster headache is diagnosed when attacks occur for more than one year without remission or with remission for less than one month.

2.5.Prevention of cluster headaches

Pharmacotherapy of cluster headache is divided into two broad categories: acute treatment and preventive treatment.[49] Acute treatment is given during the attack to decrease the pain duration and intensity of an individual attack. Preventive treatment is taken on a daily basis, mainly to decrease the frequency of the attacks. In this study, we are interested in the preventive treatment only, and for this reason, the state-of-art acute therapy falls outside the scope of this text.

Preventive therapy should be started as soon as possible at the onset of a cluster episode. The goal is to suppress attacks over the expected duration of the cluster period. An effective preventive regimen is of utmost importance because patients typically have one to eight cluster headaches a day, and repeated use of abortive medications may result in toxicity and/or rebound.[50]

A wide variety of prophylactic medicines are in use, and not unexpectedly, the response of the patients to these measures is highly variable.[49,50] Current European guidelines suggest the use of the calcium channel blocker **verapamil** at a dose of at least 240 mg daily.[49] Steroids, such as prednisolone/prednisone, are also effective, with a high dose given for the first five days or longer (in some cases up to 6 months) before tapering down. Methysergide, lithium and the anticonvulsant topiramate are recommended as alternative treatments.[49,50] Melatonin has also been demonstrated to bring significant improvement in approximately half of episodic patients, and psilocybin, dimethyltryptamine, LSD, and various other tryptamines have shown similar results. Detailed discussion of the treatment regimens, dosages and efficacy results in prevention therapy of cluster headache is found in the recent uptade.[50]

2.6.Pathogenesis of the pain attack in cluster headache

The potential causes and triggers of cluster headache are discussed in sections 2.3.1.-2.3.4. There seems to be a reasonable agreement that the intense pain is caused by the **dilation of blood vessels** which creates pressure on the trigeminal nerve.[13,25,31] While this process is the immediate cause of the pain, the underlying cause or causes still remain incompletely understood. Although a unifying pathophysiological explanation of cluster headache is not yet available, any attempt to understand this syndrome must take into account the three cardinal features of the disorder. As discussed above, these principal characteristics include i) pain, ii) autonomic symptoms, and iii) stereotyped periodicity. Seminal observations on the neurobiology of cluster headache have been made recently, which cast some further light on the understanding of the basic mechanisms behind the pathogenesis of the pain attacks in cluster headache.[51]

First, cephalic pain is relayed to the central nervous system via nociceptive ophthalmic branches of the trigeminal nerve, which innervates pain-sensitive intracranial structures such as the dura mater and **dural blood vessels**. Substance P and calcitonin gene-related peptide (CGRP) are trigemino-vascular neuropeptides that are released when the trigeminal fibers or ganglion are activated. CGRP, the most potent **vasodilator** in the human body and in animal models, when released, leads to the production of neurogenic inflammation and **dilation of dural blood vessels**. Activation of the trigemino-vascular system in cluster headache has been corroborated by recent evidence demonstrating markedly elevated blood levels of CGRP in the external jugular vein (EJV) of patients during a cluster attack.[51,52]

Second, the autonomic features of cluster headache indicate activation of the cranial parasympathetic fibers. These fibers originate from the first-order neurons within the superior salivatory nucleus, which has a functional brainstem connection to the trigeminal nucleus caudalis. These fibers travel with the seventh cranial nerve and synapse in the pterygo-palatine ganglia. Post-ganglionic fibers provide vasomotor and secretomotor innervation to the cerebral **blood vessels** and the lacrimal and nasal mucosal glands, respectively. Activation of this pathway has similarly been supported by the finding of dramatically elevated blood levels of vasoactive intestinal polypeptide (VIP) in the EJV of the patients during an acute attack.[51]

Third, the typical presence of a post-ganglionic Horner's syndrome during the attacks of cluster headache clearly indicates an involvement of the carotid sympathetic plexus.[51,52] The cavernous carotid artery is the likely location since it is at this level where the parasympathetic, sympathetic, and trigeminal fibers converge. Indeed, evidence from a variety of imaging sources has demonstrated the presence of **arterial dilatation** and venous outflow obstruction in the region of the cavernous sinus. This evidence collectively provides an explanation for the pain and autonomic features underlying a cluster attack, but importantly, does **not account for the periodicity** of the syndrome.[51,52] This is neatly explained by the important involvement of hypothalamus, as described in section 2.3.1.

2.6.1.Histamine and vascular headaches

Different as classical migraine and cluster headache are in their clinical manifestations, both are vascular headaches, in which substances inducing swelling and dilatation of the blood vessels among susceptible individuals can provoke an attack of headache.[51,52] Nitroglycerin or **histamine**, smoking or minimal amounts of alcohol can precipitate or increase the severity of the attacks as the sufferer's blood vessels seem to change and become susceptible to the action of these substances. The blood vessels are not sensitive to these substances during headache-free periods. Hormonal influences in women do not appear to be a factor in cluster headaches.[13,28]

During the past years, also the possible mechanisms responsible for the actions of histamine as a vasodilator have been elaborated.[52,53] Indeed, there is firm experimental and clinical evidence implicating that **histamine** induces the enzyme Nitric Oxide (**NO**) Synthase, making NO available to act locally on the vasculature as a vasodilator. In primates, histamine is known to activate cerebral

endothelial H1-receptors leading to formation of NO. This was nicely shown by Lassen et al. (1995) who examined 20 migraine patients with pretreatment by placebo or the histamine-H1-receptor antagonist, mepyramine, in a randomized, double blind fashion, followed in both groups by i.v. histamine.[54] In patients receiving placebo, histamine administration caused immediate headache during the infusion followed by a delayed migraine attack fulfilling IHS criteria for migraine without aura.[13] Mepyramine pretreatment abolished both immediate headache and delayed migraine attacks, suggesting that a migraine attack can be caused by NO formation in the endothelium of cerebral arteries.[54]

These results clearly confirm the earlier data reported by the same group.[55] Starting from the fact that nitroglycerin, which may be regarded as a prodrug for NO, induces a mild to moderate headache in healthy subjects, the authors tested, whether migraine patients are more sensitive to NO than non-migrainous subjects, using four different doses of i.v. nitroglycerin in a double-blind setting with age and sex matched healthy controls and subjects with tension-type headache.[55] The nitroglycerin-induced headache was significantly more severe in migraine sufferers, lasted longer and fulfilled diagnostic criteria for migraine more often. This implicates that migraine patients are supersensitive to i) exogenous NO from nitroglycerin as well as to ii) endothelially produced NO,[54] providing additional support to the concept that NO may be partially or completely responsible for the pain attacks in vascular headache.

The next logical step was to assess, how histamine and its antagonists exert their effects on cerebral (dural) arteries, as elegantly studied by Ackerman et al. (2002),[53] using intra-vital microscopy to directly assess the diameter of dural arteries in rats. Histamine infusion caused immediate and reproducible dilation of **meningeal** blood vessels that could be blocked by H1- (mepyramine) and H2-(famotidine)-receptor antagonists as well as a NO-synthase inhibitor (N(G)-nitro-L-arginine methylester. Neurogenic **dural** vasodilation was not inhibited by H2-receptor antagonists, but was significantly inhibited by a H1-receptor antagonist. These data lend further support to the concept that **histamine** is likely to activate NO synthase and promote NO production.[52-55] At molecular level, NO binds to guanylyl cyclase in vascular smooth muscle cells, which leads to production of cyclic GMP, resulting in phosphorylated protein kinase G (PKG). PKG phosphorylates Ca2+ channels, slowing the influx of calcium into the cell, which leads to smooth muscle relaxation and **vasodilation**, resulting in characteristic migraine attack.[52]

2.6.2.Liberators of histamine: acetaldehyde

Most histamine in human body is generated in granules of the **mast cells** (in tissues) or in white blood cells called basophils. Mast cells are especially numerous at sites of potential injury, including the nose, mouth, **gastrointestinal tract**, skin, and **blood vessels**. Non-mast cell histamine is found in several tissues, including the brain, where it functions as a neurotransmitter.[56] Another important site of histamine storage and release is the **enterochromaffin-like (ECL) cells** of the gastric mucosa.

Mast cells play a crucial role in **hypersensitivity**, allergic, and inflammatory reactions by secreting chemical mediators, e.g. histamine, proteases, and cytokines as a response to various immunologic and non-immunologic stimuli. Given the fact that also alcohol intake causes, especially in Oriental individuals (with hereditarily deficient acetaldehyde metabolism), **hypersensitivity** symptoms that can be blocked by antihistamines, as well as urticaria and anaphylactoid reactions in Caucasian individuals as well, led Koivisto et al. (1996) to speculate the possibility that **acetaldehyde** (the first metabolite of alcohol) might enhance these reactions by directly affecting the tissue **mast cell** functions.[57] Indeed, these authors were the first to demonstrate that this is the case. The authors used purified rat peritoneal mast cells incubated with different concentrations of acetaldehyde demonstrating that acetaldehyde, already at relatively low concentrations (50μM), directly induces histamine release from the mast cells.

The authors reasoned that such acetaldehyde-induced histamine release from the mast cells may contribute to different hypersensitivity reactions caused by alcohol intake, including some alcohol-associated gastrointestinal disorders.[57] Subsequently, the validity of this concept has been confirmed also in other clinical conditions, including human bronchial mast cells.[58] In their elegant experiments, Kawano et al. (2004) demonstrated that acetaldehyde (>3 x 10^{-4} M) increased airway muscle tone, which was associated with a significant increase in the release of histamine. A histamine (H1-receptor) antagonist completely inhibited acetaldehyde-induced bronchial smooth muscle contraction. Acetaldehyde also induced a significant histamine release from human **lung mast cells** and their degranulation. These results strongly implicate that acetaldehyde stimulates human airway mast cells to release histamine, which seems to be involved in bronchial smooth muscle contraction following alcohol consumption.[58]

2.6.3.Acetaldehyde: Group 1 carcinogen (IARC)

Tobacco smoke contains several classes of carcinogens that include among others polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines. Tobacco smoke contains also high concentrations of toxic aldehydes,[59] of which the most abundant is acetaldehyde, its concentrations in tobacco smoke being >1,000 times greater than those of polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines.[60] Acetaldehyde is also the first metabolite of ethanol oxidation. It binds to DNA, forming stable DNA adducts that are observed in alcohol consumers. Numerous epidemiological studies in alcohol drinkers with alcohol dehydrogenase (ADH2) deficiency or low aldehyde dehydrogenase (ALDH1B) activity provide the most compelling evidence for the carcinogenicity of acetaldehyde.[61] This deficiency results in accumulation of acetaldehyde locally into the saliva during ethanol metabolism and also in markedly increased risk for many upper gastrointestinal tract cancers.

Similarly, it was recently shown that acetaldehyde from the tobacco smoke is easily dissolved into the saliva during smoking.[62] Thus, toxic aldehydes could mediate the carcinogenic effect of tobacco smoke through saliva to oral cavity and further to the larynx, esophagus, and stomach. Based on firm epidemiological and toxicological documentation, IARC proclaimed (in 2009) acetaldehyde as Group I carcinogen, equivalent to asbestos, formaldehyde and others.[63]

2.6.4. Acetium® eliminates acetaldehyde in the stomach and in saliva

Cysteine is a non-essential amino acid, which was shown (almost 40 years ago) to be capable of eliminating the toxicity of acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA).[64] MTCA is an inert and non-toxic compound that is eliminated from the body through feces and urine, without being absorbed into the blood circulation. The recent innovation of Biohit **Acetium® capsule** (containing 100mg L-cysteine) is based on this simple principle, reported in 1975.[64]

In the proof-of-concept study, oral administration of Acetium® was confirmed to effectively bind acetaldehyde originated from ethanol metabolism in achlorhydric stomach.[65] In that setting, the mean acetaldehyde level of gastric juice was 2.6 times higher with placebo than with Acetium® (13 vs. 4.7 μM, $p<0.05$), implicating that Acetium® can be used to decrease acetaldehyde concentration in achlorhydric stomach during alcohol exposure.

This led the authors to examine the concept, whether it would be possible to eliminate alcohol-derived acetaldehyde also from the saliva, using L-cysteine slowly released from a special buccal (Acetium®) tablet.[66] Indeed, this was shown to be the case in tested volunteers, in whom, up to two-thirds of acetaldehyde (after alcohol intake) could be removed from the saliva with a slow-releasing buccal L-cysteine formulation (Acetium®). This might have important implications e.g. in prevention of upper GI-tract cancers among individuals with high acetaldehyde exposure (heavy drinkers, smokers).[66]

As the logical next step, Biohit Oyj developed an Acetium® sucking tablet (lozenge) that releases L-cysteine into the oral cavity during smoking, and tested this formulation as a potential chemopreventive agent against toxicity of tobacco smoke, i.e., in harm reduction.[67] Seven volunteers smoked five cigarettes, and during every smoking period, sucked a blinded tablet containing 0, 1.25, 2.5, 5, or 10 mg of L-cysteine. Acetium® reduced highly significantly the salivary acetaldehyde, and carcinogenic acetaldehyde could be totally inactivated in the saliva during smoking by the sucking tablet containing 5 mg of L-cysteine.[67]

3.STUDY HYPOTHESIS

The striking novel hypothesis of this study is simply the following: L-cysteine administered by daily intake of **Acetium® capsules** is an effective prophylactic remedy preventing the attacks of cluster headache or at least significantly decreasing the attack frequency.

As described above, Acetium® capsules and lozenges were originally developed for inactivation of acetaldehyde in the stomach and in the saliva after alcohol intake and smoking, respectively. This novel concept on the potential prophylactic effect of Acetium® capsules against migraines (based on acetaldehyde inactivation and the consequent block of acetaldehyde-induced histamine liberation from tissue mast cells), emerged purely by chance. Importantly, the trigger to formulating this hypothesis is derived **from the patients** suffering from or intractable cluster headache (or migraines), who described to us that daily intake of Acetium® capsules (for their original indications) completely eliminated their headache attacks.[68]

3.1.Case histories of patients with cluster headache (and migraines)

Because of their pivotal role in stimulating us to formulate the novel study hypothesis, these case

histories submitted to us until to date are referred to in brief here.

Case 1: A 36-year-old woman (an employee of Biohit Oyj), whose migraine deteriorated in 2006 when the attacks started to be accompanied by aura as well as other symptoms e.g. weakness in the upper extremities. She was thoroughly examined, and the condition was diagnosed as hormonal migraine. She received a specific medication (Maxalt, Migard), but with some side effects and little effect on the attacks. Attacks continued appearing in 3-4 days a week, not infrequently severe enough to prevent daily work and other activities. Different physical therapies were of no help at all, and attacks continued with the same frequency. This lead her to seek help from a wide variety of drugs, including Panadol and Panacod, with prolonged administration for protracted headache of over one week's duration. Her situation gradually escalated to the stage when continuous use of analgesics was necessary to keep the headache at least in some control to enable daily work and other normal activities.

She reporting starting the intake of Acetium® capsules on December 22, 2012, with 2 capsules in the morning and 2 in the evening. After a few months, she could reduce the dose to one capsule in the morning and one in the evening. To her major relief and surprise, all headache attacks have remained practically absent. During this period, she has had a few episodes, but all associated with a specific trigger (unrelated to her migraine). She does need no painkillers any longer, and if occasionally needed, they give a good relief. Interestingly, she once forgot taking Acetium® capsules for one week (due to non-availability), and the headache attacks returned immediately. She learned not to repeat that mistake, and at the time of this testimony (June 2013), she has remained attack-free for over 4 months.

Case 2: A 12-year-old girl, daughter of Case #1, who started suffering from severe headaches when 7 years old (in 2008). The attacks were characteristic to migraine, and in 2010, migraine with aura was diagnosed. A combination therapy with Ketorin, Panadol and Burana was instituted, but with little help on the frequency of the attacks. The condition was aggravated in late 2012, with attack rate increasing up to 2-3 times a week. The migraine (possibly aggravated due to hormonal changes) diagnosis was confirmed by pediatric neurologist, who administered beta-blockers for attack prophylaxis. The patient and her mother, however, decided to test the efficacy of Acetium® also for the daughter, with one capsule twice a day.

Given the striking effect in her mother, the same effect was noticed also in the daughter, who has remained without a single migraine attack since the onset of Acetium® intake (April 2013). She feels herself completely healthy, fully capable of attending the school with no periods of absence ever since. Both the mother and her daughter have a feeling that with the use of Acetium® capsules, they have obtained a completely new life, with no concern of migraine on daily basis.

Case 3: A 41-year-old woman (from Norway), with a long-term history of severe migraine attacks who was introduced with Acetium® capsules by one of the Biohit partners in Sweden. She has also a diagnosed celiac disease and follows a strict gluten-free diet. Otherwise generally healthy, with no regular medication, except occasional anti-depressants. She started the daily use of Acetium® capsules as soon as they entered into the market, and after a few weeks, her migraine attacks disappeared. She has been attack-free now for a number of years, and continues taking one Acetium® capsule every day for maintenance prophylaxis.

Case 4: A 48-year-old male, with cluster headache (Horton's syndrome). He provided a very detailed description of his headaches, which fulfills the criteria of a classical cluster headache, diagnosed with the name Horton's syndrome by his physician some 16 years ago. The attacks start at distinct time of the day (at 1-2 o'clock at night in his case), are unilateral and have a maximum duration of two hours.

Some 6-7 years ago, the condition was aggravated and the attack rate increased, extending to other seasonal periods that during the first few years. The cluster periods lasted up to 8 weeks, having a definite negative impact on the quality of his daily life. He also listed the most important triggers of the clusters, including stress, associated with short sleeping hours and use of even small a mounts of alcohol. During the years, he has tested

several different modalities for cluster prophylaxis. The best combination, with some reduction in cluster frequency but not total disappearance, proved to be magnesium food supplement, melatonin (5mg), abundant drinking of water before going to sleep, refrain from alcohol intake, and highly regular sleeping. As said, the cluster frequency diminished, but the attacks did not disappear.

He started taking Acetium® capsules in December 2009, following a very intense cluster. Since then, he has continued taking one capsule in the morning. Occasionally, while feeling prodromal symptoms, he has raised the dosage to 2-4 capsules per day. Some six months since initiation of Acetium®, he was able to abandon his daily routines (described above), including the use of magnesium supplement, melatonin drinking abundant water before going to sleep. Interestingly, also the previous triggers of his clusters (stress, sleeplessness, alcohol), have not evoked new attacks since the onset of Acetium® prophylaxis. According to his written testimonial (July 2013), this patient has experienced not a single attack of cluster headache since December 2009, when he started the daily intake of Acetium® capsules, one capsule per day.

The above spontaneous testimonials[68] given in writing by these four persons who all have suffered from either severe migraines (3 subjects) or intractable cluster headache (Case #4) for years, prompted us to consider, whether a plausible mechanism could be discovered to explain these dramatic effects experienced by all these subjects soon after onset of Acetium® intake on daily basis. Given that L-cysteine is a natural (semi-essential) amino acid, converted to inert substance (MTCA) in the alimentary tract, it would comprise an ideal means to conduct prophylaxis of cluster headache for years, without concern about the side effects that are inherent to many of the current treatment modalities.[49,50] If the efficacy is proved in a formal randomized controlled trial (RCT), the concept of using Acetium® capsules in prophylactic treatment of cluster headache, would represent a major step forward in a better clinical control of these frequently intractable syndromes.[13]

3.2.Components of the study hypothesis

The current study hypothesis is built up of several elements, all based on solid experimental and/or clinical evidence. These elements include both i) the known pathways involved in the provocation of a characteristic attack of cluster headaches, as well as ii) established and postulated mechanisms, how Acetium® capsule administration could interfere with this sequence of events.

From up-stream to down-stream, the sequence of events leading to vasodilatation and pain attack in cluster headache is as follows.

3.2.1.Nitric oxide (NO) is the final trigger of headache attack

NO binds to guanylyl cyclase in vascular smooth muscle cells, which leads to the production of cyclic GMP, which in turn forms phosphorylated protein kinase G (PKG). PKG phosphorylates Ca2+

channels, slowing the influx of calcium into the cell, which leads to smooth muscle relaxation and vasodilation, resulting in characteristic headache attack.[52]

3.2.2.Histamine induces Nitric Oxide (NO) Synthase

There is firm experimental and clinical evidence implicating that histamine induces the enzyme Nitric Oxide (NO) Synthase, making NO available to act locally on the vasculature as a vasodilator. Histamine is known to activate cerebral endothelial H1-receptors, leading to formation of NO.[52-55]

3.2.3.Histamine is synthesized in tissue mast cells and basophils

Histamine is synthesized in tissue mast cells and basophils by histidine decarboxylase converting histidine to histamine. Another important source of histamine are **enterochromaffin-like (ECL) cell**s that are abundant in **gastric (corpus) mucosa**. Histaminic cephalalgia is the old name for cluster headaches, implicating that histamine has been linked with the development of vascular headaches since their description. Mast cells are ubiquitous, and their activation (e.g. in the meninges) by migraine triggers is now believed to contribute to genesis of migraine headaches.[56]

3.2.4.Histamine is liberated from mast cells by acetaldehyde

Mast cells play a crucial role in hypersensitivity, allergic, and inflammatory reactions by secreting chemical mediators, e.g. histamine, proteases, and cytokines as a response to various immunologic and non-immunologic stimuli. One of the potent liberators of histamine from the mast cells is acetaldehyde (a Group 1 carcinogen[63]), both in the human and in experimental animals.[57,58] This could neatly explain several hypersensitivity-like reactions associated e.g. with alcohol intake and smoking, both being abundant sources of acetaldehyde.

3.2.5.Acetaldehyde in the saliva and stomach is inactivated by Acetium® capsules

The phenomenon that L-cysteine eliminates free acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (MTCA),[64] was exploited by Biohit Oyj while developing their **Acetium® capsule** containing 100mg L-cysteine. Both the capsule and the recently introduced Acetium® lozenge (3 mg L-cysteine) effectively eliminated acetaldehyde derived from alcohol and/or cigarette smoking, both in the stomach and in saliva.[65-67]

3.2.6.The efficacy of Acetium® capsules in prophylaxis of cluster headache?

We are convinced that the dramatic disappearance of cluster headaches (and migraines) immediately after regular intake of Acetium® capsules as described in the case histories[68] cannot be by change. Instead, we believe that the mechanism must be based on the capacity of Acetium® capsules to interfere with the above decoded sequence of events, whereby elimination of acetaldehyde in the stomach could block (or reduce below the threshold levels) histamine liberation from the tissue mast cells and ECL cells in the stomach, thus arresting its multitude of functions, of which vasodilatation is critically involved in inducing the pain attack in cluster headache.[51-56]

In the nervous system, histamine acts mainly on H1- and H3-receptors.[69] While H1-receptors mediate inflammation, H3-receptors are much more sensitive to histamine and serve as negative feedback to inhibit further excessive release of histamine by C-fibers (afferent fibers of the somatic sensory system).[70] It is tempting to speculate, whether **reduction of total histamine burden** by eliminating acetaldehyde would be equivalent to so called "modulation of histamine" that has been successfully employed in the treatment of cluster headaches (and migraine) for years by now.[52] Indeed, for cluster headache, H3-receptor mediated negative feedback loop has been exploited by intermittent very low doses of sc. histamine. In theory, low histamine concentrations in the body would lead to preferential stimulation of the more sensitive H3-receptors, while leaving the H1-receptors relatively untouched (see 2.6.1.), thus activating the negative feedback loop and reducing the potential of the C-fibers to become activated.[52]

The present study is designed to validate this novel hypothesis that daily use of Acetium® capsules (2 capsules 3 times a day) is an effective means to decrease the frequency of (or completely abort) the attacks of cluster headache, as tentatively suggested by a case testimonial from such a patient, reporting complete remission almost immediately after onset of Acetium® capsules administration.[68]

4.STUDY DESIGN

This **multi-centre**, double-blind, placebo-controlled randomized trial (RCT) is designed to test the efficacy of intervention by Acetium® capsules (used twice a day) in reducing the frequency of (or completely aborting) the headache attacks among patients with cluster headache. The study design follows the IHS **Guidelines for Controlled Trials of Drugs in Cluster Headache**, recently elaborated

by the International Headache Society Committee on Clinical Trials in Cluster Headache.[71] A cohort of 100 subjects with clinically diagnosed cluster headache will be enrolled utilising the membership registry of the National Migraine Association (NMA) and/or by public invitation, and randomly allocated to two groups (n=50 in each), receiving either Acetium® capsules or placebo. All subjects will be requested to fill in a structured questionnaire recoding their detailed headache history and other clinical data pertinent to this study. The subjects will be administered a headache diary on daily basis, submitted to the study monitor on monthly basis for recording the compliance of each subject with the medication. The total treatment period will be 1 month in both study arms.[71]

4.1.Aims of the study

The single most important goal of this study is to establish whether Acetium® capsule is an effective measure in decreasing the frequency (or totally aborting) the headache episodes among patients suffering from cluster headache, as implicated by the spontaneous testimonials of individual patients.[68] The null hypothesis of the study implicates that Acetium® capsules are no better than placebo in prophylaxis of these headache attacks, during the intervention period of 1 month. Rejection or not of the null hypothesis is based on comparison of the two strata (Acetium® and placebo) against the **primary study endpoint** (efficacy measure): **The frequency of attacks per week.** The frequency of headache attacks per week, either during the entire treatment period or during the last treatment interval is compared with the baseline frequencies, to disclose differences between the two study arms.

In addition to these primary efficacy endpoints, the IHS Guidelines recommend using a number of potential **secondary efficacy endpoints** in analysing the results.[71] Such potentially useful secondary endpoints include: i) Intensity of headache (4-tier nominal scale; ii) Drug consumption for symptomatic or acute treatment (e.g. number of attacks that required treatment per week and the number of drug administrations for acute therapy); and iii) Patients' preferences and satisfaction (a secondary global outcome).[71]

In addition to the conventional statistical approaches used to analyse the efficacy of cluster headache prophylaxis trials,[71] our intention is to estimate the role of Acetium® capsules as an independent covariate of cluster prophylaxis in multivariate (Cox) proportional hazards (HR) regression model, controlled for potential confounders (age, gender, treatment, baseline attack rate, alcohol, smoking,

heredity, etc.). Another aim is to assess whether these longitudinal data on Acetium® intervention in cluster headache prophylaxis can be modelled using the newly described statistical technique, **competing risks regression**.[72,73] (more details in Methods section).

4.2.Patient selection (=criteria of being eligible)

This intervention trial is designed and conducted in conformity with the current guidelines of the International Headache Society,[71] jointly by **specialist neurologists** (experts in vascular headaches) in Hospital X (City Y), and by the Clinical Research Department of Biohit Oyj (Helsinki). These guidelines stipulate detailed recommendations for patient selection, trial design, as well as evaluation of the results.

4.2.1.Definition of cluster headache

All subjects to be enrolled have a clinically confirmed diagnosis of cluster headache, based on the ICHD-II classification.[13] The diagnostic criteria of the ICHD-II are highly reproducible and universally endorsed, which ensures homogeneity of the cohort and better interpretation of data.[71] Furthermore, strict adherence to the ICHD-II criteria for cluster headache only precludes a relatively small patient group. In this study, both the patients with **episodic** and **chronic** cluster headache are eligible, but will be analysed as subgroups, if indicated (see: Stratification).

4.2.2.Patients with interval headaches

Following the recommendations of the Guidelines,[71] this proof-of-concept intervention trial shall **not necessarily** exclude the participants with so called interval headaches.[13] The exclusion of these "non-target headaches" is dependent whether or not the patients are able to differentiate these interval headaches from typical cluster headache. According to the Guidelines, this can usually be dome using an appropriately designed headache diary.[71]

4.2.3.Frequency of attacks

To be eligible for the study, the subjects should report cluster headache attacks with the frequency from **one every second day up to five per day**. A typical cluster headache patient reports attack frequency of one to three per day.[71] The individual attacks should last **from 15 minutes to 3h**, with the **maximum** of 8 attacks per day, and 15 min duration each. The reasoning behind this selection criteria is that with these extreme limits, it will be difficult to make distinction between cluster

headache and paroxysmal hemicrania. In general, patients with headache attacks of less than 30 min duration and occurring more than 5 times per day, will be not eligible.

4.2.4.Duration of disease

Only subjects in whom cluster headache has been clinically confirmed are eligible for the study. In contrast to the 1-year requirement since diagnosis for migraine, there is no absolute timing since the diagnosis other than the patients should not be enrolled while on their first episode of cluster headache. In addition, the **expected duration of the episode** after randomization should be at least **one month** after initiation of the trial. In case of episodic cluster headache, spontaneous remission must always be kept in mind, but if randomization is properly done, the rate of remission should be equal in both study arms.

4.2.5.Age at onset of cluster headache

Because of the fact that cluster headache can be diagnosed at any age,[43,44] there will **no age limitations** for diagnosis of cluster headache to make the subject eligible for the study.

4.2.6.Age at study entry

Following the general guidelines for adult migraine RCTs, our cohort will accept only participants **between 18 and 65 years of age.** This is simply because the intervention studies on paediatric patients are different, and after 65 years of age, there is an increased risk of cerebrovascular and cardiovascular illnesses that might increase the co-morbidity.[71]

4.2.7.Gender

Both **male** and **female** participants will be eligible in the present cohort. Cluster headache is up to five times more common among men than in women, easily resulting in gender selection bias in all RCTs for cluster headache prophylaxis. In this study, every effort is done to minimize such a gender selection bias by encouraging particularly the women to participate, except those who are pregnant, who will be excluded.

4.2.8.Concomitant medication

The subjects enrolled, will be allowed to **continue** their current **acute therapy** for individual attacks, because abortive therapy for agonizing pain cannot be denied. Because Acetium® capsules have no

known interactions with other drugs, the other drugs not taken for cluster headache are not contraindicated. However, other **prophylactic medication** for cluster headache should be **discontinued** prior to the study entry.

Excluded are the following subjects: patients who meet the ICHD-II criteria for medication overuse;[13] patients who have taken anti-psychotics or anti-depressant medications during the previous month; patients who abuse alcohol or other drugs; and potentially fertile and sexually active women who do not practise contraception.

4.2.9.Co-morbidity

The intention is to enrol a cohort of subjects with minimum co-morbidity. Specific co-morbid medical conditions that **exclude participation** in this trial follow the general recommendations for cluster headache and migraine RCTs,[71] and include the following categories of patients: other acute or chronic pain disorders, severe psychiatric disease, infections, malignancy, short life expectancy, cardiovascular disease, cerebrovascular disease, uncontrolled hypertension, degenerative central nervous system diseases, as well as pregnant and lactating women.

4.3.Trial design

Also the design of this multi-centre RCT follows the guidelines of the International Headache Society,[71] with only a few deviations from the protocol.

4.3.1.Pre-trial observation period

In contrast to RCTs for prophylaxis of migraines, where a reliable assessment of the baseline attach frequency necessitates a 3-month retrospective history and 1-month prospective baseline (run-in) period, **no such pre-trail observation period** is needed for enrolment of cluster headache patients. Self-obviously, the subjects must be enrolled **during their cluster episodes**, which makes the difference between the subjects with chronic cluster headache and those with the episodic form. In the former category, enrolment can take place any time of a year, whereas for the latter, the seasonal (circadian) fluctuation in the natural history of the episodes must be taken into account. In addition, the expected duration of the on-going episode after randomization should be at least one month after initiation of the trial, to allow enough time for the eventual efficacy measures to become detectable (without confounding by spontaneous resolution). This complexity of the natural history

of cluster headache makes it inevitable that the **enrolment** of the whole cohort can only be done during a prolonged period of time, i.e., **over several months**.

4.3.2.Blinding

Following the most stringent recommendations of the guidelines,[71] this company-sponsored trial with Acetium® capsules will be conducted in **triple-blind fashion**; i.e., 1) participant-blind, 2) investigator-blind, and 3) sponsor-blind (=statistician evaluating the study results), to exclude the possibility of undue bias caused by analysis of the results.

4.3.3.Placebo control

Placebo preparation with design and package identical to the test preparation (Acetium® capsule, 100mg) will be used in this trial, received by one half of the randomly allocated study subjects.

4.3.4.Parallel-group design

Only the **parallel study design** is recommended for RCTs testing prophylactic therapy for cluster headache,[71] and this recommendation will be followed in the current trial, which will be conducted using a **parallel group** design.

4.3.5.Randomization

Because patients in this trial will be recruited over an extended period, it is strongly suggested to randomize the subjects in relatively small blocks, to avoid the potential bias due to varying the selection criteria over time.[71] In this trial, randomization will be performed using the random number generator (https://www.sealedenvelope.com/simple-randomiser/v1/lists) with block size of 4, and creating unique randomization codes for each study subject. The latter will be used as the identifier of each subject in all datasets. Printed list (CSV Excel) is sealed in an envelope and stored in the company safety box, until opened at the completion of the study and data analysis.

4.3.6.Stratification

Randomization alone may not ensure full comparability between participants in the two treatment arms, and stratified randomization is needed to remedy this potential imbalance between the two arms. Of the baseline characteristics of cluster headache that potentially affect the efficacy outcomes in the trials, the most obvious include the following: **i) gender**; and **ii) cluster type** (episodic vs.

chronic). Furthermore, the subjects with episodic cluster headache should be stratified by iii) the **duration of the present cluster period**. The rational is straightforward. Stratification is intended to create two groups that are matched by the key characteristics that might influence on the efficacy measures. Cluster headache among women is rare, justifying the use of gender as one of the stratification variables. Similarly, the natural history of episodic and chronic cluster headache is distinct enough to expect different efficacy outcomes, which, unless stratified, can lead to biased estimates on the drug efficacy. Finally, stratification by the duration of the present cluster period (e.g. by using 2-week cut-off) is intended to result in study groups with a similar expectancy of spontaneous remission rates. These three covariates impacting the natural history of cluster headache in this trial are most likely too powerful to be remedied only by statistical treatment without stratification. As recommended in the Guidelines,[71] these baseline stratification variables will be entered as covariates in the final multivariate models to control for their potential confounding of the efficacy endpoints (see Statistical Methods).

4.3.7.Duration of the treatment period

Cluster headaches are chronic conditions with protracted and fluctuating course, posing challenges in the statistical modelling of its natural history. In this cluster headache intervention trial, the primary efficacy endpoint is the attack frequency per week. In contrast to the RCTs of migraine prophylaxis, in which the IHS Guidelines recommend a 3-month treatment period, only **2 weeks** treatment period is recommended for RCTs of cluster headache.[71] The reasoning is that in migraine, keeping the treatment periods relatively long will help providing more stable estimates of the attack frequency (calculated per 1 month). In cluster headache, however, the primary endpoint is attack frequency per one week, and albeit that also in this disease, only the **sustained effects** are **clinically relevant**, the spontaneous remissions make prolonged observation periods problematic.

In this trial, we decided to use the **treatment period of 1 month**, instead of the 2 weeks recommended in the IHS guidelines.[71] The rational is that this longer treatment period, once completed, will enable us to calculate the estimates of efficacy outcomes also for the recommended 2-week period, if the confounding by the spontaneous remissions start to be disturbing for the 1-month period. This also makes possible to estimate the stability of the efficacy outcomes, while having the possibility to compare the effect size (attack frequency per week) at different study endpoints.

4.3.8.Symptomatic (acute) treatment

The optimal treatment for acute attacks in cluster headache prevention RCTs is both ethically and clinically indicated. In our trial, the participants should use and accurately report their usual symptomatic or acute treatment, because it is not anticipated to interfere with the study medication.

4.3.9.Follow-up visits

Each study subject will be examined by the neurologist twice during the study: i) at baseline visit, and ii) after the treatment period. During the 1-month treatment period, participants will be evaluated **at weekly intervals** by the study coordinator (**a certified GCP monitor**), to review the diaries, monitor adverse events, ensure compliance and promote continued participation in the study. The follow-up is concluded by the last visit at the end of the observation period.

4.3.10.Compliance

Evidence of poor compliance in prophylactic RCTs of cluster headache is not unusual, and can be falsely interpreted as drug failure.[71] Therefore, it is crucial to monitor patients' compliance with the test preparations during the entire study period. One such approach is the drug or pill count at every follow-up visit, and repeated emphasis on the values of adherence to the protocol requirements. The same applies to proper completion of headache diaries (see below).

5.METHODS

5.1.Baseline data

Before enrolment in the cohort, all subjects are requested to sign **a written consent**, after having been explained the details of the study and the commitment requested from each subject for the successful completion of their study protocol.

Before enrolled in the study, each subject will be requested to fill in a structured **Questionnaire** recoding their detailed headache history, including the on-going medications (**ANNEX 1).** This Questionnaire also records the headache attack frequency during the on-going cluster period, to be used as the baseline data for the primary study endpoint (attack rate per week).

5.2.Headache diary

The headache diary (**ANNEX 2**) will be the main research tool used to monitor the efficacy of the

test preparations on the natural history of cluster headache on daily basis throughout the entire study period. In the IHS Guidelines, there are no specific recommendations about the design of such a headache diary, other than it should be an **easy-to-use**, either a paper-and-pencil report form or an electronic diary that captures all predefined assessment measures (**efficacy, tolerability and safety**).[71]

These diaries are submitted to the study monitor on weekly basis, so as to confirm the compliance of each subject with the study protocol as well as to record the efficacy endpoints, tolerability and safety of the test preparations.

5.3.Study endpoints

The single most important goal of this study is to establish whether Acetium® capsule is an effective measure in decreasing the frequency (or totally aborting) the headache episodes among patients suffering from cluster headache, as implicated by the testimonial of an individual patient.[68] The null hypothesis of the study implicates that Acetium® capsules are no better than placebo in prophylaxis of cluster headache, during the intervention period of 1 month.

5.3.1.Primary endpoints

Rejection or not of the null hypothesis is based on comparison of the two strata (Acetium® and placebo) against the **primary study endpoint** (efficacy measure): **The frequency of attacks per week.** The frequency of headache attacks per week, either during the entire treatment period or during the last treatment interval is compared with the baseline frequencies, to disclose differences between the two study arms.

5.3.2.Secondary endpoints

In addition to these primary efficacy endpoints, the IHS Guidelines recommend using a number of potential secondary efficacy endpoints in analysing the results.[71] Such potentially useful secondary endpoints include: i) **Intensity of headache** (4-tier nominal scale; ii) **Drug consumption** for symptomatic or acute treatment (e.g. number of attacks that required treatment per week and the number of drug administrations for acute therapy); and iii) **Patients'** preferences and **satisfaction** (a secondary global outcome).

5.4.Statistical analysis

All statistical analyses will be performed using the SPSS 25.0.0.1 for Windows (IBM, NY, USA) and STATA/SE 15.1 software (STATA Corp., Texas, USA). The descriptive statistics will be conducted according to routine procedures. Frequency tables will be analyzed using the χ2-test, with the likelihood ratio (LR) or Fisher's exact test for categorical variables. Differences in the means of continuous variables (e.g. attack frequency) are analyzed using non-parametric (Mann-Whitney or Kruskal-Wallis) test for two- and multiple independent samples, respectively.

5.4.1.Conventional techniques

Standard statistics are used to compare the efficacy of the two study arms on cluster headache attack frequencies. The effects of test preparation and placebo can be analysed separately in non-parametric paired samples t-test (Wilcoxon signed ranks test) to compare the pairs of the baseline- and post-trial attack frequencies. Another way is to calculate the effect size in both arms (reduction of attack frequency by treatment as compared with baseline) and compare these effects between the two arms. Similarly, the statistics used to compare the two arms with regard to the secondary endpoints are straightforward. For categorical outcomes, conventional regression models can be used, where the results are expressed as crude OR (odds ratio), and their 95% confidence intervals (95% CI).

5.4.2.Life-table techniques

In addition, several more sophisticated approaches can be used to analyse these longitudinal data, as determined by the obtained results. If, e.g. complete and sustained abortion of the attacks are recorded, different life-table techniques can be used to compare the two study arms, using univariate survival (Kaplan-Meier) analysis, where the attack disappearance dates represent the event, and the stratum-specific (test vs. placebo) estimates are calculated using the log-rank (Mantel-Cox) statistics. The same approach can be used to calculate the difference in the duration of attack abortion (remission time, RT) between the two study arms. The independent effect of Acetium® (adjusted for potential confounders) can be analysed using the Cox proportional hazards regression model (in multivariate), where all recorded baseline characteristics of cluster headache (Questionnaire) can be entered as covariates, including the 3 stratification variables (gender, type of cluster headache, duration of the "baseline" attack period).

5.4.3.Generalised linear models (GEE, panel Poison)

In addition, using the permanent attack disappearance (abortion) as the event, the effect of Acetium® versus placebo can be modelled also using the regression techniques based on count variables, i.e., Poisson regression. In that case, attack disappearances are expressed as events per person time (weeks) at risk, and the two arms can be compared using the incidence rate ratio (IRR) statistics. When applied to panel type of data (Panel Poisson), the covariates subject to intra-subject variation (at FU visits) can be adequately controlled in this longitudinal setting. A similar type of approach based on panel data, i.e., generalized estimating equation (GEE) modelling, can be used to estimate the effect of Acetium®/Placebo on persistence (=sustainability) of such an attack remission (AR), using the AR (yes/no) recorded at each follow-up visit as the dependent variable, and adjusted for potential confounders in multivariate GEE. A wide variety of such potential confounders should be examined, including e.g. age, gender, disease type, disease onset, severity, attack frequency, triggers like alcohol and cigarette smoking, previous interventions, current medication, etc.

5.4.4.Modelling of outcomes by competing-risks regression

This type of prevention trial is likely to be more complex than merely showing a reduction in attack frequency vs. no such reduction, as dichotomous (yes/no) outcome. Indeed, it can be anticipated that there are several possible outcomes to be observed during the Acetium® intervention, which can be treated as **competing events**, including at least the following: **i) no effect** (weekly attack frequency remain unchanged as compared with the baseline), **ii) abortion** of attacks (=disappearance of all headache episodes since the onset of intervention)**, iii) relapse** (=disappearance of attacks for a period of time but subsequent reappearance of the attacks), and **iv) reduction** of attacks (attack frequency is reduced at study endpoint, but attacks are not completely aborted).

Thus, it was decided to apply another method for modeling these complex data, by taking into account the fact that i) the longitudinal data be utilized in full, ii) dependence of the repeated measurements at follow-up visits be taken into account, and iii) the multiple-outcome dependent variable (no change, quit, relapse, reduction) be treated in a single statistical model. All these prerequisites are met by **the competing-risks regression**,[84,85] which will be used to model the impact of Acetium® intervention (and other covariates) on the competing risks outcomes of this trial.

5.5.Power analysis

Due to the fact that several optional tools are available for statistical analysis of these data, also the power of the study can (and needs to) be analysed differently, following the algorithms specified for each of these statistical techniques. In the simplest approach (for attack frequency or the effect on it, i.e., post-trial reduction compared with baseline), the power can be calculated using the two-sample mean test, comparing mean attack rates (or reduction) in the Acetium® and placebo arms. The study (n=30 per study arm; the most modest scenario) is adequately powered (Type II error 0.80, type I error 0.05) to detect a true difference in attack frequency between **15 attacks/week** in the placebo and **13.1 attacks/week** in the Acetium® arm, i.e., the difference in effect size of **1.9 attacks/week**. Within this range, the study power is sensitive to any discount in this effect size and also critically dependent on the SD in both arms. Given that the study subjects are selected among patients with typically 1-3 attacks/day (i.e., 7-21/week)(up to 5 daily attacks; 35 per week), and assuming a 10% reduction by placebo, there figures seem reasonable estimates for the basis of these power calculations.

6.STUDY EXECUTION AND TIME-TABLE

For execution of the study, the company decided to build up a multi-centre consortium with specialist neurologists working in clinical centres, supervised by internationally recognized experts in the field of vascular headaches (prof. X.Y & Z.X.). The execution of the study is **monitored** following the principles of **good clinical practice** (GCP) by a certified GCP monitor. For this purpose, the company recently hired a project coordinator, who (in addition to study monitoring) will also assist the investigators in all practical steps during the study execution.

Given that each study subject shall complete the 1-month trial period, the trail itself would not take too long. **However, because of the fact that the subjects with episodic cluster headache must be enrolled during their cluster episodes that typically follow a seasonal pattern, the enrolment of the total cohort (n=60 or 100) inevitably needs to be completed over several months (up to one year).**

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ANNEX 1. **RECORD OF CLUSTER HEADACHE HISTORY**

Date of Interview:	Day:	Month:	Year:	
Name:				
Date of Birth:	Day:	Month:	Year:	AGE:
Gender:	Female:		Male:	
Profession:				
Chronic co-morbidities	Yes:		No:	
If yes, list the most important ones:				
Cluster headache diagnosis made:	Year:		Age at onset:	
The exact diagnosis (ICD-10 code):	G44.0: Cluster headache syndrome		Chronic paroxysmal hemicrania	
	Chronic cluster headache		Episodic cluster headache	
Symptoms of typical attack:	Prodromal symptoms (Y/N):		Autonomic symptoms (Y/N):	
Pain:	Unilateral:	Bilateral:	Severity (Mi/Mo/S):	
	Duration (h):	Aggravated by:	Ass. symptom:	
Autonomic symptoms:	Conjunctival:	Nasal:	Eyelid:	
	Forehead:	Facial:	Pupil:	
	Lacrimal:	Agitation:	Restlessness:	
Frequency of attacks (typical):	No./Day:	No./Week:	No./Month:	More rarely:
Attack rate since the diagnosis	Remained Constant:	Increased:	Decreased:	
Duration of typical cluster period:	No. days:	No. weeks:	No. months:	Constant:
Duration of remission:	Over one month:		Less than one month:	
Asymptomatic during remission:	YES:		NO:	
Condition changed since diagnosis:	From episodic to chronic:		From chronic to episodic:	
Causes and Triggers:	Genetic background (Y/N, details):			
	Smoking (Y/N):			
	Head injury in history (Y/N):			
	Other known or suspected trigger:			
Seasonal period :	Spring:	Summer:	Autumn:	Winter:
Circadian period:	Y/N:	Attack time of the day:		
	Frequency of attacks during the CURRENT period (IMPORTANT)			
	No./Day:	No./Week:	No./Month:	No attacks:
Preventive measures ever tested:	YES:		NO:	
	Describe:			
Preventive medical treatment:	YES:		NO:	
	List your current preventive medicines:			
	1	2	3	
Current treatment of acute attacks:	List the medicines:			
	1	2	3	
	4	5	6	
Your self-estimation of your headache:	Under good control:		Not in satisfactory control:	
	Improved during the years:		Aggravated during the years:	
	Debilitates my daily life:		I can live with it:	
SOME OF YOUR LIFE-STYLE PRACTICES				
Alcohol consumption:				
Regularity and type	No:	Social:	Daily:	Excessive:
Type of alcohol typically used	Beer:	Wine:	Liquors:	Spirits:	Other:
Weekly use	Estimated dosages per week:			
Alcohol intake triggers your headache?	NO:		YES:	
Did you stop drinking due to headache?	NO:		YES:	
Smoking history:				
	Never:	Past:	Current:	
Age when started smoking				
Regular smoker ever since	Yes:		No:	

If not, describe			
Cigarettes per day (currently)			
The same number, for how long (yrs)?			
Trend in the daily numbers of cigarettes	Kept constant:	Increasing:	Decreasing:
Other forms of tobacco	Cigars:	Pipe:	Smokeless:
If any of the above, list the amounts			
Smoking triggers your headache?	NO:	YES:	
Did you stop smoking due to headache?	NO:	YES:	
SPACE FOR FREE COMMENTS:			

ANNEX 2. **HEADACHE DIARY FOR CLUSTER HEADACHE PATIENTS**

MONTH:	**SUBJECT CODE:**

Day	**Attacks Y/N**	**No. of Typical Cluster Attacks**	**No. of Interval Attacks**	**Intensity* of headache** (1-4)	**Acute Medicine (=AM) (Y/N)**	**How many pills of AM?**	**Your satisfaction on the test drug#** (1-5)	**Test drug taken; how many?**	**Side effects ascribed to the test drug:** (see footnote for coding)		
									ES (1-3)	**ESR** (1,0)	**D** (h)
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
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31											

RESEARCH PERSONNEL FILLS OUT:

OVERALL EVALUATION OF THE ATTACKS OF THE WEEK

Days	No. attack days	Total No Typical	Total No Interval	Average Intensity	AM Used (Y/N)	No. AM Used	Average Score	No. Test Drugs	No. of Side Effects	Total D

*__Typical attacks, before taking symptomatic medicine__; (1=mild; 2=moderate, 3=severe, 4=excruciating); **#Satisfaction**: (1=very dissatisfied; 2=somewhat dissatisfied; 3=neither satisfied nor dissatisfied; 4=somewhat satisfied; 5=very satisfied); **SIDE EFFECTS**: 1. Event severity **(=ES)**: (1=mild, 2=moderate, 3=severe); 2. Event seriousness (=**ESR**): (1=serious, 0=non-serious); 3. Duration **(=D)**: (hours).