**Diplomarbeit**

# The role of noradrenaline in the treatment of painful diabetic neuropathy (PDN)

**Analgesic efficacy of Reboxetine - a selective noradrenaline-reuptake inhibitor**

ein systematischer Review inkl. Planung einer randomisierten, doppelt-verblindeten, aktiv-kontrollierten Pilot Studie

eingereicht von

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*Graz, November 2013* *Antonia Pfretschner*

***Mir brennen die Eingeweide. Die Heftigkeit des bösen Saftes verdreht mir die Glieder, entstellt mich, wirft mich zu Boden. Ich verdurste, ich ersticke, ich kann nicht schreien. Das ist die Hölle, die ewige Qual! Seht, wie das Feuer auflebt! Ich könnte nicht besser brennen. Fort, Dämon!***

*Arthur Rimbaud, 1854-1891*

*Une Saison en Enfer*

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# Zusammenfassung

Die Therapie chronischer Nervenschmerzen gestaltet sich bekanntermaßen schwierig und zum Teil frustran für den Behandler und den betroffenen Patienten. Insbesondere die Subgruppe der Patienten, die unter schmerzhafter diabetischer Polyneuropathie (PDN) leiden, ist vom Problem der unzureichenden Analgesie betroffen. Neben den Einschränkungen, die sich aus der insuffizienten Wirkung bei neuropathischen Schmerzen von nicht steroidaler Antirheumatika (NSAR) und Opioidanalgetika ergeben, wird die etablierte Therapie mit Antidepressiva durch deren Nebenwirkungen und zusätzliche Komorbiditäten der Patienten limitiert.

Seit den 1960er Jahren werden Antidepressiva in der Therapie chronisch neuropathischer Schmerzen eingesetzt. Die Gruppe der trizyklischen Antidepressiva (TCA), allen voran der Wirkstoff Amitryptilin, konnten sich seitdem als first-line Therapeutika etablieren. Als Alternativtherapeutikum konnte sich in den letzten Jahren die Substanz Duloxetin aus der Gruppe der selektiven Serotonin- und Noradrenalin Wiederaufnahmehemmer (SNRI) hervortun und fand als solche in die internationalen Guidelines zur Behandlung chronisch neuropathischer Schmerzen Eingang.

Der genaue analgetische Wirkmechanismus dieser unterschiedlichen antidepressiven Substanzklassen ist weiterhin unklar, jedoch wird der Noradrenalinwiederaufnahmehemmung diverser klinischer Studien zufolge, die den analgetischen Effekt von Antidepressiva untersucht haben, die Schlüsselrolle zugewiesen. Diese Schlussfolgerung ergab sich unter anderem aus einigen Studien, die den Einsatz von selektiven Serotoninwiederaufnahmehemmers (SSRI) erprobten. Sämtliche Ergebnisse konnten kaum oder keine Analgesie nachweisen. Diese Ergebnisse wurden durch Studien, die den Wirkmechanismus der Opioidanalgetika Tapentadol und Tramadol untersuchten, unterstützt. Beide Substanzen verfügen zusätzlich, neben dem opioidtypischen Effekt am μ-Opioidrezeptor, über eine Serotonin- und Noradrenalin-Wiederaufnahmehemmung.

Im Gegensatz zur Analgesie durch die gesteigerte Verfügbarkeit von Noradrenalin im synaptischen Spalt kann erhöhten Levels von Serotonin in diesem Zusammenhang lediglich die typischen Nebenwirkungen wie Müdigkeit, Libidoverlust, gastrointestinale Beschwerden etc. zugeordnet werden.
Reboxetin ist das einzige selektiv noradrenalinwiederaufnahmehemmende Antidepressivum und wird bislang nur mit der Indikation der leichten bis mittelgradigen Depression eingesetzt. Einige klinische Studien an gesunden Probanden und im Tierversuch weisen auf den möglichen Einsatz in der Therapie der chronisch neuropathischen Schmerzen hin, mit ähnlich zufriedenstellendem analgetischen Effekt wie der von Amitryptilin bzw. Duloxetin, jedoch günstigerem Nebenwirkungsprofil.

Zu den standardisierten Screening Tools hat sich in den letzten Jahren die quantitativ sensorische Testung (QST), als bislang objektivste Methode zur Subgrupierung und Verlaufskontrolle, bei der Erfassung chronisch neuropathischer Schmerzen, behaupten können.

Vor dem Hintergrund der aktuellen Forschungsergebnisse, die der Noradrenalinwiederaufnahmehemmung den Schlüsselmechanismus bei der analgetischen Wirkung von Antidepressiva zuweisen und der Studienresultate, die den Einsatz von Reboxetin als alternatives Co-Analgetikum bei derselben Indikation untersucht haben, scheint es uns vielversprechend, dessen Analgesie in einer Pilotstudie an Patienten mit schmerzhafter diabetischer Polyneuropathie zu erproben. Auf Grund des ähnlichen Nebenwirkungsprofils empfiehlt sich Duloxetin als Referenzsubstanz. Für das intiale Screening vor Studienbeginn und zur Erfassung klinischer Veränderungen der PDN während der Studie soll QST zum Einsatz gebracht werden.

# Abstract

Treatment of patients suffering from chronic neuropathic pain is known to be difficult and partial frustrating for patient and therapist. In particular therapy of the subgroup suffering from painful diabetic neuropathy (PDN) tends to be unsatisfying.

Beside the limitations, as a result of insufficient analgesic efficacy of non steroidal anti-inflammatory drugs (NSAIDs) and opioid-analgesics, also the established use of antidepressants is limited by their side effects and co-morbidities the patients bring with.

Since the 1960s antidepressants are applied in treatment of chronic neuropathic pain. International guidelines rank tricyclic antidepressants (TCAs), especially the substance amitryptiline, as first line treatment. During the last years duloxetine, an selective serotonin and noradrenaline reuptake inhibitor (SNRI) with less side effects, was established as alternative to TCAs.

The analgesic mechanism of different antidepressant substance-classes is still unknown in detail. Recent clinical studies, which explored the analgesic effect of antidepressants, found evidence for enhanced levels of noradrenaline in the synaptic cleft, to play the key-role. These suggestions are supported by the results of studies, testing the analgesic effect of selective serotonin reuptake-inhibitors (SSRI). There was hardly any or no effect. Some trials, exploring the mechanism of action of the opioid substances tapentadol and tramadol, had similar results. These two substances show, besides their effect at the opioid μ-receptor, also action as serotonin-reuptake inhibitors.

In contrast to the analgesic effect through enhanced levels of noradrenaline, enhanced levels of serotonin just cause side effects, such as fatigue, dizziness, loss of libido, gastro-intestinal complaints etc.

Reboxetine is the only selective noradrenaline-reuptake-inhibitor, yet only applied as antidepressant for the treatment of mild to moderate depression. Some trials testing its analgesic effect on healthy probands and animal experiment found auspicious results. The analgesic efficacy was similar to amitryptiline and duloxetine but showed less side effects.

In addition to standardised screening tools for neuropathic pain, quantitative sensory testing (QST) is yet the most objective clinical tool to subgroup different types of neuropathic pain and to observe clinical changes.

According to this findings and the background, suggesting enhanced levels of noradrenaline as the key-role in analgesia through antidepressants, we propose a pilot study testing reboxetine as a co-analgesic in PDN. Hence its established use in treatment of neuropathic pain and similar side effects, we suggest duloxetine as reference substance and QST to subgroup and to observe clinical changes in PDN.

# INDEX

# Introduction

Up to 30-41% of adults worldwide are affected by chronic non-cancer related pain. Reduction of life quality and decreased physical functioning is often associated. [1] There are three categories to classify chronic pain: nociceptive pain (caused by tissue disease or damage), neuropathic pain (owed by somatosensory disease or damage) and mixed pain (characterized by the coexistence of nociceptive and neuropathic components) [2].

Neuropathic pain develops as a result of lesions or disease affecting the somatosensory system either in the periphery or centrally [3]. It is characterised by spontaneous ongoing or shooting pain and evoked amplifies pain responses after noxious or non-noxious stimuli, yet the clinical manifestation is similar across the different neuropathic pain syndromes and causes. Typical disorders are postherpetic neuralgia, trigeminal neuralgia, post-stroke pain and painful polyneuropathy, of which the latter appears as part of a syndrome in different diseases. [2] One of the biggest subgroups suffering from chronic neuropathic pain and particular from painful polyneuropathy are patients affected by diabetes [4-6].

In pursuance of estimates of the world health organization, 374 million people were affected by diabetes worldwide in 2013 with an expected rise of up to 438 million people by 2030 and an overall increase in prevalence from 6.4% (2010) to 7.8% (2030) [7]. Furthermore substantial regional variances in particular lifestyles lead to higher share of diabetes within highly industrialized countries such as the United States (8+) or, to a lesser extent, European Union Member States (3-6%, Austria 4%) associated with the acquisition of diabetes mellitus type 2 [7].
Up to 10-20% of diabetics suffer from diabetic peripheral neuropathic pain (DPNP) [4,8].

Management of patients suffering from chronic neuropathic pain is a common known problem in medical care. Interacting factors such as biological, social, psychological and cultural influences as well as comorbidity complicate diagnosis and treatment.

Since the 1960`s antidepressants are used to treat patients suffering from painful diabetic neuropathy (PDN), with a variety of clinical trials to test different substances in the past and still ongoing [9]. Recent clinical guidelines rank tricyclic antidepressants, such as amitryptiline, selective serotonin and norepinephrine reuptake inhibitors (SSNRI), such as venlafaxine and duloxetine together with anticonvulsants (gabapentin, pregabalin) as first line drugs to treat neuropathic pain [10-13]. Between antidepressants TCAs were considered to be gold standard [14] and within that subgroup amitryptiline was established to be first line treatment. These substances intervene in different pathways: the serotonergic and noradrenergic system [11-12,15] and TCA also have effects on muscarinic and histaminic receptors. Due to this unselective mechanism of action they cause many side effects, of which inotropic effects on the heart are the mean, limit their use although they have the most satisfying analgesic efficacy [14].

Recent findings indicate that the analgesic effect of antidepressant is in response of enhanced noradrenaline levels caused by their noradrenaline reuptake inhibition. This implies intervention in the serotonergic pathway is not necessary for analgesia in neuropathic pain [2,9,11-12,14,16-25]. Moreover results of several clinical trials testing selective serotonin reuptake inhibitors on their effect for acute and neuropathic pain relief showed there was hardly or any no analgesia [9,11,14].

Hence the results of recent research in pathomechanisms of chronic neuropathic pain and the high levels of participation of it in patients with diabetes, we propose to test an alternative management in patients suffering from painful chronic polyneuropathy with reboxetine, the only antidepressant substance selectively inhibiting noradrenaline reuptake, expecting satisfying pain relief at more benign side effects, compared to substances already used to treat neuropathic pain.

# Scientific background

Management of patients suffering from chronic pain is a well known problem in medical care. There are somatic/ neuropathic/ visceral entities to classify chronic pain: nociceptive pain (caused by tissue disease or damage), neuropathic pain (owed by somatosensory disease or damage) and mixed pain (characterized by the coexistence of nociceptive and neuropathic components) [8].

# 1. Pain mechanisms

## 1.1. Pain pathways

### 1.1.1. The ascending pain system

Action potentials from nociceptors of Aδ- or C-fibres are transferred by the primary afferent pain neurons via the dorsal root ganglion to the dorsal horn of the spinal cord. Through the excitatory signal due to the noxious stimulus is sent by secondary and third afferent neurons to regions of the brain involved in the sensory components of pain, formed as an important part of the ascending pain transmission pathways by these projection neurons. The affective component of pain is generated in the limbic area on which there are parallel projections. [59]

# 2. Neuropathic pain

The definition of neuropathic pain is „pain arising as a direct consequence of a lesion or disease affecting the somatosensory system“ proposed by the group of neuropathic pain of the international association for the study of pain (NeuPSIG) [2-3, 26-28].

Basic and human research indicates that a lesion of afferent pathways is necessary for development of neuropathic pain. Additionally, data clearly indicate that not a single one but several mechanisms can lead to neuropathic pain. Consequential, many of these mechanisms are not dependent on the cause of the disease: the same mechanism can be found in different diseases. [2]

## 2.1. Pathogenesis

Neuropathic pain can be provoked by various nerve damaging influences in the peripheral or central nervous system. Although the clinical manifestation of different neuropathic syndromes is very similar, they are distinguished from their origins. The typical clinical presentation shows paradoxical sensory perceptions with pain as dominating positive symptom combined with lesion-induced reduced sensations, which are unique and have not been experienced before by the patient. The coexistence of hypersensitivity and hyposensitivity is characteristic for neurological disorders. [2]

Latest research into pathogenesis of neuropathic pain has suggested that a nerve lesion is followed by dramatic changes in the nervous system which makes it different to other types of chronic pain, which have an intact nociceptive system.
An area of sensory deficit in the related innervation territory is the consequence of a lesion to a sensory or mixed peripheral nerve or damage to a central somatosensory pathway.[2]

Mechanoception (A-β) and proprioception (A-β + A-α: Ia +Ib) in the periphery are mediated by large fibres. In contrast, small fibers mediate the sensory qualities of thermoception, nociception and viscероception (Aδ + C) [26]. A deficit in the perception of mechanical vibratory stimuli can be a presentation of damage to large diameter afferent fibres or to the dorsal column tract, whereas a damage of small fibres leads to a loss of thermal and noxious perception. [2]

Patients suffering from neuropathic pain almost always have areas of abnormal sensation or hypersensitivity in the affected area, which can be bordering to or combined with skin areas of sensory deficit. Positive symptoms are paraesthesias (crawling or tingling sensation), spontaneous (without stimulus) ongoing pain and shooting, electric-shock-like sensations. Many patients also have evoked pain (stimulus induced and hypersensitivity). Usually mechanical and thermal hypersensitivity is reported.

Hypersensitivity can be differentiated into two types: allodynia and hyperalgesia. A response to a non-nociceptive stimulus resulting in pain is called allodynia. In case of mechanical allodynia even an everyday innocuous stimulus such as fabric on skin or even a cold breeze can cause painful sensation. Increased pain sensitivity to a nociceptive stimulus is defined as hyperalgesia.

Summation is another evoked feature, which is progressive worsening of pain as a result of slow repetitive stimulation with mildly noxious stimuli. [2]

### 2.1.1. Ectopic nerve activity

Persistent spontaneous pain and paroxysmal shooting pain in the absence of an external stimulus is caused by ectopic impulse generation within the nociceptive pathways. Such spontaneous ectopic activity can be recorded by miconeurography in afferent fibres from a neuroma in patients with stump and phantom pain, as well as in patients with painful diabetic neuropathy. Under physiological conditions, activation of unmyelinated (C-fibre) and thinly myelinated (A $\delta$ -fibre) nociceptive afferent fibres acts as an indicator for potential tissue damage, which is reflected in the high thresholds of nociceptive receptors for mechanical, thermal, and chemical stimuli. These conditions change dramatically in states of neuropathic pain: a peripheral nerve lesion is followed by spontaneous activity which is evident in both injured and neighbouring uninjured nociceptive afferents. Increasing levels of mRNA for voltage-gated sodium channels show correlation with ectopic activity, and increased expression of sodium channels in lesioned and intact fibres.

Similar changes within second-order nociceptive neurons are thought to occur after central lesions, leading to central neuropathic pain.

Furthermore to voltage-gated sodium channels, several other ion channels probably change after a nerve lesion, such as voltage-gated potassium channels, which might also concur to alteration in membrane excitability of nociceptive nerves. Upregulation of various receptor proteins is induced by nerve injury such as the transient receptor potential V1 (TRPV1), which is located on subtypes of peripheral nociceptive endings and is physiologically activated by noxious heat at about 41°C. After a nerve lesion, TRPV1 is downregulated on injured nerve fibres but upregulated on uninjured C-fibres. Clinically, patients with such underlying pain mechanisms can show presence of heat hyperalgesia additionally to ongoing burning pain.

Abnormal responses to cold and topical application of menthol indicate a nerve lesion triggered abnormal function or expression of cold-sensitive receptor of the TRP family. [2]

### 2.1.2. Central sensitisation

Secondary allodynia and hyperalgesia in the area contiguous to the innervation territory of the lesioned nerves implies involvement of the CNS. Central sensitisation might be a consequence of ectopic activity in primary nociceptive afferent fibres and structural damage though the CNS itself might not be necessarily involved. Ongoing discharges of peripheral afferent fibres that release excitatory aminoacids and neuropeptides within the dorsal horn of the spinal cord are followed by postsynaptic alterations of second-order nociceptive neurons, These changes evoke neuronal hyper- excitability that submits low-threshold mechanosensitive A $\beta$ and A $\delta$ afferent fibres to activate second-order nociceptive neurons. Consequently normally innocuous tactile stimuli such as light brushing or pricking the skin become painful. [2]

### 2.1.3. Contributive mechanisms to ectopic nerve activity and central sensitisation

Moreover pathophysiological mechanisms involved in neuropathic pain contribute to ectopic activity and central sensitisation. After a nerve lesion inflammation induces activation and migration of macrophages into the nerve and dorsal root ganglion, which contribute to pain hypersensitivity by releasing proinflammatory cytokines, including tumour necrosis factor $\alpha$. Peripheral and central nerve damage is followed by activation of microglia within the CNS release several immune modulators that also perpetuate neuropathic pain. These inflammatory processes concur to peripheral sensitisation.

Like central sensitisation, peripheral sensitisation can also arise in intact nociceptors without any nerve damage.

A peripheral nerve lesion leads to a loss of inhibitory GABAergic interneurons in the spinal horn.

Furthermore potent inhibitory neurons, such as descending pathways originating in the brainstem, concur to a change of pain processing. Damages that affect these opioidergic and monoaminergic systems also result in pain exacerbation through disinhibition.

In some cases of amputations, postherpetic neuralgia, complex regional pain syndromes, and post-traumatic neuralgias, topical application of norepinephrine and enhancement of physiological sympathetic activity increased spontaneous pain and dynamic mechanical hyperalgesia. [2,32-34]

This finding suggests a pathological adrenergic linkage among sympathetic postganglionic fibres and nociceptive afferent fibres, which might be caused by expression of $\alpha$-receptors on cutaneous afferent fibres or by sprouting of sympathetic fibres within the dorsal root ganglion. [35] Thus, this symptom of sympathetically maintained pain can be treated by use of sympathetic blocks. [2]

#### 2.1.4. Specific sensory profiles

Despite all forms of neuropathic pain involve neuronal lesions, the pattern of sensory abnormalities in the affected skin area can differ between the various disorders or even within individual patients. Some suffer from spontaneous pain, paraesthesias, and electric shocks, whereas in other patients, the affected area is hypersensitive to temperature or touch [2,26-27,29,36-39].

# 3. Diabetes mellitus

Diabetes mellitus is an increasingly prevalent disease with potential health disparity in different populations all over the world.

According to valuations of the world health organization, 374 million people were affected by diabetes worldwide in 2013 with an expected rise of up to 438 million people by 2030 and an overall increase in prevalence from 6.4% (2010) to 7.8% (2030) [7,40]. Diabetes mellitus is showing a range of systemic complications due to severe long-term sequelae as well as complications associated with unmanaged diabetes that includes adult blindness, amputations of extremities, cardiovascular diseases, nephropathies and neuropathies

The treatment of (severe) diabetes usually involves lifelong therapy with anti-diabetic medication and/or the injection of insulin as well as regular monitoring of the patients' health status.

## 3.1. Painful diabetic neuropathy (PDN)

### 3.1.1. Epidemiology

Up to 70 percent of people diagnosed with diabetes have mild to severe forms of nervous system damage, which can be developed at any time, but risk rises with age and longer duration of the disease [5,43-44].

The damage affects nerves throughout the body and leads to different subtypes as autonomic, proximal, focal and peripheral neuropathy (DPN), of which the last one is the most common type [5,43] with 50% [8,52]. 10-20% of them are suffering from diabetic peripheral neuropathic pain (DPNP) [4,8].

Prevalence data for DPN range from 1.6-90% depending on tests used, populations examined, type and stage of disease. [44] Beyond 40 years of age almost 40% of people with diabetes have impaired sensation in the feet. In up to 50% of the population with diabetes DPN is occurring and causes sensory, motor and autonomic dysfunction, whereas in up to 50% it may be asymptomatic, and patients are at risk of insensate injuries.

The prevalence of PDN depends on multiple factors which include: age, glucose control, duration of diabetes, method of diagnosis of DPN, simultaneous conditions or comorbidities, use of certain medications, elevated homocysteine and low cobalamine levels as well as lower socioeconomic status [44].The highest rates occur among people who have had diabetes for at least 25 years, high blood glucose, hyperlipidemia, arterial hypertension and are obesity [5,43].

Using the MNSI score >2 the prevalence of PDN found in Germany was 28% according to the MONICA/KORA Augsburg surveys. Other national trials, using the MNSI score >2, showed similar results: in the Australian Fremantle Diabetes study the prevalence of PDN was 30,9%; a Swedish population-based study found prevalence of PDN in 34% of included diabetics.[46] Another study conducted in Belgium found a prevalence of PDN of 43%, whereby it was higher in patients with type 2 diabetes (50,8%) than in those with type 1 (25,7%). Painful diabetic polyneuropathy (P-DPN) was found in 14% of diabetics. In the United Kingdom a study demonstrated a prevalence of diabetes in 4,5%. P-DPN in this population occurred in 26,4%. Eighty percent of them reported moderate to severe pain.

Suffering from P-DPN has a significant negative effect on quality of life, altering physical and mental components, and increasing DPN is associated with an increasing risk of developing P-DPN. [44]

### 3.1.2. Pathophysiology

At DPN prolonged exposure to high blood glucose causes nerve damage through a combination of factors including metabolic and neurovascular influences, leading to damage the nutritioning vessels of nerves, autoimmune factors that cause nerve inflammation, inherited traits that increase susceptibility to nerve disease as well as lifestyle factors, such as smoking or alcohol [43].

To discuss the matter in detail, causes of DPN are thought to be a multi-factorial metabolic process that increasingly affects tissues. Damage on endothelial tissue due to hyperglycemia, increased sorbitol and protein kinase C, elevated homocysteine, reduced nitric oxide and excessive Reactive Oxygen Species (ROS) is followed by a change of rheological behavior which leads to an increase of vascular resistance and reduction of blood flow to the nerves. [44]

#### *3.1.2.1. Microvascular Hypothesis*

Vascular and neural diseases are closely related. Blood vessels are dependent on normal nerve function, and nerves are dependent on sufficient blood flow. First pathological microvascular change is vasoconstriction. With disease progression neuronal dysfunction impairs, correlating closely with the development of vascular abnormalities, such as thickening of capillary basement membrane and endothelial hyperplasia, which leads to reduced oxygen tension and hypoxia of nerve tissues. Neuronal ischemia is a well-established characteristic of diabetic neuropathy. High homocysteine levels in combination with decrease in vitamin Bl2 and folic acid has been associated with decreased production of nitric oxide and a state of vasoconstriction, as a consequence. [44]

#### *3.1.2.2. Elevated levels of Glycated End (AGE) Products*

Elevated intracellular levels of glucose lead on to AGE products through bonding with proteins that modifies their structure and inhibits their function.

Glycosylated proteins have been implicated in the pathology of DPN and other long term complications of diabetes. Chronic hyperglycemia promotes AGEs generation and consequent accumulation, which could be impaired by nephropathies, because AGE clearance is mainly renal. Glycated myelin is sensitive to phagocytosis by macrophages and can also stimulate macrophages to secrete proteases. This might conduce to nerve demyelization in diabetic neuropathy. High AGE levels have been documented in the peripheral nerves of subjects with diabetes and the AGE pathway is a major pathophysiologic mechanism in the development and progression of diabetic neuropathy. [44]

#### *3.1.2.3. Sorbitol - aldose reductase pathway*

The sorbitol - aldose reductase pathway, also called the polyol pathway, may be involved in diabetic complications resulting in microvascular changes that lead to nervous tissue damage, as well to the retina and kidney. Glucose is a highly reactive compound and needs to be metabolized to avoid reaction with other tissues of the body. In increased levels the polyol pathway activates as an alternate biochemical pathway and causes a decrease in glutathione and an increase in reactive oxygen radicals resulting in direct tissue damage. The pathway depends on the enzyme aldose reductase which is not activated in normoglycemic state. In contrast to most body cells, the cells of the retina, kidney and nervous tissues are insulin-independent. At hyperglycemia, the affinity of aldose reductase for glucose rises, creating much higher levels of sorbitol. Sorbitol has no ability to cross cell membranes, accumulates and produces hyperosmolarity resulting in water drawing into the cell. In summary, excessive activation of the polyol pathway by highly elevated glucose levels leads to increased levels of sorbitol and reactive oxygen species and decreased levels of nitric oxide and glutathione, as well as hyperosmolarity. These factors lead to increased cell damage. Hyperglycemia causes elevated intracellular diacylglycerol, which activates Protein Kinase C (PKC). PKC is supposed to be involved in the pathology of diabetic neuropathy and could result in vascular damage and decreased neuronal blood flow. These changes result in abnormal metabolism in neuronal, axonal and Schwann cell causing impaired function. [44]

#### *3.1.2.4. Homocysteine, Folate and Vitamin B12*

Elevated homocysteine (Hcy) levels increase the risk of developing peripheral vascular disease. There are a growing number of articles documenting an increase in the blood Hcy levels and the risk of developing DPN in patients with DM2 taking metformin. Long duration of intake of metformin is associated with malabsorption of vitamin B12 and elevated fasting Hcy and methylmalonic acid (MMA) levels, which may have harmful effects on peripheral nerves in patients with diabetes. Clinical and electrophysiological measures approved this hypothesis, with cumulative metformin dose strongly correlating with such clinical neuropathic evidences. Adding metformin to insulin therapy in DM2 reduces levels of folic acid and vitamin Bl2, which results in increase in Hcy levels within 12 weeks. [44]

#### *3.1.2.5. MTHFR Polymorphism*

Methylene-tetrahydrofolate reductase (MTHFR) is an enzyme that in humans is encoded by the MTHFR gene [7]. MTHFR catalyzes the conversion of the active form of folate, which acts as a co-substrate for Hcy remethylation to methionine. MTHFR gene is located on chromosome 1, p36.3 in humans. Two of the most investigated single nucleotide polymorphisms (SNPs) in this gene are C677T and Al298C. The 677T allele leads to a valine substitution at amino acid 222 and encodes a thermolabile enzyme with reduced activity. 677TT individuals (homozygous) are said to have MTHFR deficiency, which causes low level of active folate and therefore, increased risk of cardiovascular disease.

Increased risk of DPN with the use of metformin has been reported by several studies. The exact role of MTHFR polymorphisms on the manifestation of such risk is still unknown, its use represents an important potential “geneticiatrogenic” contribution to the development and degree of severity of DPN. As serum folic acid and B12 levels are known to alleviate during intake of metformin and in consequence of that, the Hcy levels might elevate, this effect may be higher in patients being carriers of the MTHFR 677T allele (677TT genotype).

Summarizing, recent reports suggest a potential clinical association between MTHFR genotypes and the metformin-treated patient's risk of worsening DPN. [44,50]

#### *3.1.2.6. Other Genetic Factors*

Early reports expect other genetic polymorphisms to play a role in the development of DPN.

Findings indicate that the ‘low-producer’ IFN gamma +874 A/A genotype and ‘high-producer’ IL 10 -1082 G/G genotype, but not the TNF alpha - 308G/A variant, are significantly associated with cytokine-induced nerve damage in diabetic peripheral neuropathy patients with DM2 [44,51]. Another Italian study found an association between G1181C and T245G polymorphisms in osteoprotegerin with risk of Charcot neuroarthropathy [44,52,53].

Polymorphisms in the eNOS gene may constitute a factor in the genetic tendency to diabetic retinopathy and neuropathy. [44]

Moreover distribution of a VEGF gene polymorphism at promoter region (-7C/T) also was found, that was significantly different between British-Caucasian diabetic subjects with vs. without neuropathy. [44]

A Greek study in patients with neuropathy a significantly reported a higher frequency of the deletion polymorphism in the alpha2B-adrenoceptor gene in contrast to those without neuropathy. Presence of the deletion correlates also with a higher neuropathic score. Findings provide evidence for an association of this polymorphism with both presence and degree of severity of neuropathy in patients with DM2 mellitus. [44,54]

### 3.1.3. Clinical effects

In clinical examination peripheral neuropathy may be asymptomatic. When symptoms are present, they may be negative or positive. Negative clinical symptoms are hyposensitivity, insensitivity, loss of strength and loss of balance and coordination, as a result of affected deep sensitivity. Positive symptoms are presented by abnormal temperature sensation, tingling, burning, prickling sensation, sharp pains or cramps, paraesthesia, hyperalgesia and allodynia [13,43]. These symptoms often emerge during the night and interfere with sleep [6,43,44].

One of the most distressing symptoms that people can suffer from is neuropathic pain and paraesthesia [13]. Chronic painful diabetic peripheral neuropathy (PDN) can cause symptoms that last for years and severely impair quality of life [6].

# 4. Therapy

The treatment of patients from this chronic neuropathic pain subgroup is known to be complicated and unsatisfying [9,13,44]. Various types of drugs, including antidepressants with norepinephrine and serotonin reuptake inhibition, calcium channel $\alpha$2-$\delta$ ligands, anticonvulsants, opioid analgesics, and topical lidocaine, have been shown to have consistent efficacy in randomised controlled clinical trials and meta-analyses. [2]

Since the 1960`s antidepressants are used to treat patients suffering from diabetic painful neuropathy, whereby there was a variety of clinical trials to test different substances in the past and are still going on [9]. Concerning on that the European federation of neurological societies developed an algorithm for the treatment of chronic neuropathic pain (shown in table 1) [79].

**Table 1: European federation of neurological societies (EFNS) guidelines on the pharmacological treatment of PDN – 2010 revision [79]**

a) Amitriptyline, clomipramine, (nortriptyline, desipramine, imipramine)

Abbreviations:

| | |
|---|---|
| SSRIs | selective serotonin reuptake inhibitors |
| TCA | tricyclic antidepressants |
| Venlafaxine ER | Venlafaxine extended release |
| NK1 antagonist | neurokinin 1 receptor antagonist (antidepressant substance) |

| Level A rating for efficacy | Level B rating for efficacy | Level C rating for efficacy | Level A/ B for inefficacy or discrepant results | Recommendations for first line | Recommendations for second or third line |
|---|---|---|---|---|---|
| Duloxetine | Botulinum toxin | Carbama-zepine | Capsaicin Cream | Duloxetine | Opioids |
| Gabapentin-morphine | Dextro-metorphan | Phenytoin | Lacosa-mide | Gabapentin | Tramadol |
| TCAs a) | Gabapentin/ Venlafaxine | | Lamotrigine | Pregabalin | |
| Gabapentin | Levodopa | | Memantine | TCAs a) | |
| Nicotine agonist | | | Mexiletine | Venlafaxine ER | |
| Nitrate derivatives | | | Mianserin | | |
| Oxycodone | | | NK1 antagonist | | |
| Pregabalin | | | Oxcarba-zepine | | |
| TCAs a) | | | SSRIs | | |
| Tramadol alone or with acet-aminophen | | | Topical Clonidine | | |
| Venlafaxine ER | | | Topiramate | | |
| | | | Valproate | | |
| | | | Zonisamide | | |

## 4.1. Antidepressants

Primary the use of antidepressants was proposed by the high incidence of comorbidity with depressive mood alterations in patients affected with diseases resulting in chronic neuropathic pain syndromes. Their analgesic efficacy was secondly approved, thus it is important to distinguish depression that is secondary to the pain, which can be considered as a consequence of a syndrome, where pain is the feature which may increase the chance to develop this type of mood disorder, and primary depression which can be worsened by pain. [14] Literature demonstrates significant relief of pain by using antidepressants as monotherapy for painful diabetic neuropathy [12], and between antidepressants, tricyclics were considered to be gold standard [14].

### 4.1.1. Mechanisms

These substances intervene in two different pathways: the serotonergic and noradrenergic system [11-12,15], whereas both types of neurotransmitters are involved in two descending pathways from the brainstem to the spinal cord, which act on the on the input of afferent painful signals, called analgesic system [14].

Serotonin is the major neurotransmitter in the first pathway, which originates at the level of the midbrain in the periaqueductal grey. In contrast noradrenaline is the major transmitter in the second, which originates at the locus coeruleus in the medulla. These pathways – and drugs, respectively – cause their analgesic and side effects not only in the CNS, but also in the peripheral tissues due to interactions with local receptors [14].

Serotonin or 5-Hydroxytryptamine (5-HT) is synthesized and deposit at the level of enterochromaffin cells in the gastrointestinal system, where it is involved in the regulation of motility. Depending on receptor`s kind binding, gut response to HT-5-secretion may be increased or reduced, whereas enteric ganglion cells and longitudinal as well as circular muscle layers participate to stimulating response. Another target of serotoninergic action are platelets, which take up 5-HT from the blood and store it into their secretory granules. In case of vascular injury due to platelet aggregation causes 5-HT release, which leads to a procoagulative status with clot formation and hemostasis. Enhanced platelet aggregation and smooth muscle cell constriction are results of 5-HT2A receptor activation. Vascular endothelial HT1 receptor binding cases the release of endothelium derived relaxing factor (EDRF).

Furthermore vasoconstriction in splanchnic, renal, pulmonary and cerebral beds as well as bronchoconstriction on bronchial muscle can the stimulated by serotonin.
The effects on the nervous system influence different brain activities such as sleeping, learning, sensorial perception, thermoregulation, appetite, sexual behaviour, hormonal secretion and pain perception [14].

Noradrenaline (NA) binds two adrenergic receptors, α and β, and is the main sympathetic postganglionar transmitter. The function of α-adrenergic receptors is the mediation of arterial vasoconstriction, mydriasis, intestinal smooth muscle relaxation, piloerection, bladder and intestinal sphincter contraction.

There are two different types of β-adrenergic receptors, which mediate different functions in different organs. β1 act selective on cardiac tissue, where they effect inotropic action of the heart, such as increase of contractility and cardiac rate.

β2 in contrast mediate sympathetic action on the pulmonary smooth muscle, effecting bronchodilation , and systemic on arteriola, evoking vasodilatation. They also mediate intestinal, uterine and bladder relaxation and induce thermogenesis and glycogenolysis.[14]

### 4.1.2. Tricyclic Antidepressants

Recent clinical guidelines rank tricyclic antidepressants, such as amitryptiline and venlafaxine, together with anticonvulsants (gabapentine, pregabaline) as first line drugs to treat neuropathic pain [10-13].
Therefor doses used are lower than for antidepressant therapy and the analgesic effect occurs also in absence depressive symptoms and psychiatric comorbidity [12-14]. Between antidepressants TCAs were considered to be gold standard [14] and within that subgroup amitryptiline was established to be first line treatment. Moreover, TCAs have the lowest number-needed-to-treat (NNT) compared to the other substances used in treatment of neuropathic pain [14].

Tricyclic antidepressants intervene in both, the noradrenergic and the serotoninergic pathways, which causes their analgesic efficacy but also their side effects.

Furthermore TCAs act as antagonists on cholinergic muscarinic receptors located on cells, which are activated by parasympathic postganglionar neurons, resulting in anticholinergic side effects. The latter causes problems on patients affected by glaucoma, as it leads to a mydriatic effect but miosis is necessary to stabilise the bulbal pressure. For treating glaucoma, pilocarpine eye drops are used to induce miosis, which are reported to antagonize TCA´s effects. [14]

#### *4.1.2.1. Side effects and limitations*

Before 1980s, TCAs were considered to be the standard therapy to treat different kinds of neuropathic pain, through their action on noradrenergic and serotoninergic pathways. [14] Due to their side effects including 5-HT mediated fatigue, dizziness, weight gain, sexual dysfunction, its antagonism on cholinergic muscarinic receptors leading to constipation, urinary retention, tachycardia, dry mouth and mydriasis/ problems with accommodation and its effect on α1+2 as well as on β1+2 adrenergic receptors mediated by noradrenaline causing symptoms in the intestinal and urinary system (α1+2) and inotropic (β1+2) effects and limit their use

[14]. Even though the latter, resulting in rhythm abnormalities and following cardiac risk, are the main limitations in use of TCA [8,56].

#### 4.1.3. SSRI

Besides of the substances, which interfere in both pathways, selective serotonin reuptake inhibitors (SSRI) were tested on their analgesic effect on acute or chronic neuropathic pain. The results of these trials were disillusioning: enhanced levels of serotonin cause hardly any or no analgesia [9,11,14].

#### 4.1.4. SNRI

Thus its better side effect profile duloxetine was approved as first SNRI as a treatment for painful neuropathy associated with diabetes and established as an alternative to TCAs [1,7,8,56-63,74] but it also has less analgesic effect than amitryptiline. This substance inhibits reuptake of both serotonin and noradrenaline, whereby its noradrenergic actions are supposed to contribute to efficacy for painful physical symptoms [59].

# 5. Reboxetine

## 5.1. Backgrounds

Most recently, Grunenthal introduced an opioid with a novel pharmacological profile. Tapentadol acts on μ opioid receptors and has noradrenaline (NA, = norepinephrine, NE) reuptake inhibiting (NARI) effects. Hence, tapentadol demonstrated analgesic effects in somatic and neuropathic pain. This implies that increased noradrenaline, but not serotonin levels in the synaptic cleft are responsible for the relief of neuropathic pain [9,11-12,14,16-20,23-25].

These results induced a trial of [11] to find out which adrenoceptors were responsible for the analgesic effect of antidepressant drugs. In murine model the antidepressants desipramine [TCA], venlafaxine (NSRI) and reboxetine (SNRI) were tested at their analgesic potential. To find out the responsible receptors for the analgesic effect, the substances yohimibine, as an alpha2-adrenoreceptor-antagonist, and ICI118.551, a specific beta-2-adrenoreceptor antagonist, were used. The results surprisingly showed that there was no analgesia under intake of ICI118.551, which leads to the conclusion that the effect of noradrenaline on beta-2-adrenoreceptors is responsible for pain relief [11]. A murine trial by Rojas-Corrales et al. had findings leading to similar conclusion: Pindolol, a nonselective β1-receptor-blocker, enhances the analgesic effect of tramadol, which is an opioid, known to inhibit reuptake of noradrenaline as well as 5-HT1 [68].

## 5.2. Mechanism

Reboxetine is the only substance, which inhibits selective the reuptake of noradrenaline. It is licensed as an antidepressant and used for mild to moderate depression. Its analgesic effect was tested in few animal and human pilot studies [9,11-12,14,16,18]. The results were motivating: not only analgesic effects on chronic neuropathic pain, but also in acute pain and prevention of allodynia was reported [11,18,21].

Reboxetine blocks very selectively the noradrenaline transporter (NET), which provides the primary mechanism to terminate the action of noradrenaline at noradrenergic synapses, whereas tricyclic antidepressants, such as amitriptyline, imipramine and desipramine, block the same transporter but unselectively. In contrast to TCAs it also shows different binding effects: hardly any or no binding on α1 AR, which causes less and milder side effects on the gastrointestinal and urinary system, but therefore higher binding tendencies on β2 AR, which was supposed to be the “analgesic key mechanism” by Barrot et al. [11]

Results of recent research in pathomechanisms of chronic neuropathic pain, suggest noradrenaline as the responsible neurotransmitter for the analgesic efficacy of antidepressants [2, 11, 14,17, 21, 22, 69]. This conclusion was made in consequence of several trials testing reboxetine in mural model and in healthy probands [9, 11-12, 16, 18,].

# 6. Quantitative Sensory Testing (QST)

Quantitative sensory testing is a widely accepted tool to investigate somatosensory changes in patients suffering from chronic pain. Sensory examination promotes determination of diagnosis and assessment of the function from different submodalities of the somatosensory system (mechanoreception, proprioception, thermoreception, nociception, and viscероception). These different submodalities are transferred through various peripheral and central nervous system pathways. Peripheral, large fibers mediate mechanoreception (A$\beta$) and proprioception (A$\beta$ and A$\alpha$: Ia, Ib) and small fibers mediate thermoreception, nociception, and viscероception (all A$\delta$ and C) [26].

## 6.1. Subgrouping different states of neuropathic pain

Simple QST subgrouping patterns have been described, focusing on the presence or absence of evoked pain types or the preservation or loss of small-fibre functions. Similar to grading systems for tumors, patients were classified according to decreased or increased function of their small and large afferent fibres. 12 different QST profiles are used to subgroup patients with neuropathic pain, although with an arbitrary organisation, 16 different profiles can occur, of which four do not occur in substantial numbers in all aetiologies.

One subgroup predominating presents spontaneous burning pain and prickling, combined with dynamic mechanical allodynia and pain attacks. Numbness is rare, which indicates that innervation of the skin is intact. Obtained and irritable nociceptors might be involved in the pathophysiological mechanism of this group of patients.

Another sensory phenotype is dominated by painful attacks, although other sensory symptoms are rare. In presumption, the patients in this subgroup probably have typical neuropathic shooting pain that occur spontaneously for seconds and is similar to trigeminal neuralgia pain attacks.

One sensory profile a combination of severe painful attacks and pressure-induced pain is unique to patients with painful radiculopathy.

Patients in one subgroup usually mark similar intensity scores for each of the symptoms of the sensory profile in the questionnaire.

Another subgroup shows severe deafferentation of the affected skin, which results in prominent numbness without mechanical or thermal allodynia as the corresponding symptom. This profile is characteristic of diabetic painful neuropathy and painful radiculopathy, and indicates degeneration of the peripheral or central branch of the primary afferent neuron, or both. [36]

## 6.2. Clinical examination in patients with somatosensory abnormalities

Various clinical signs are caused by the damage of different types of nerve fibres and need specific tests to identify the subgroups. Table 2 shows clinical signs with the affected fibres and the appropriate testing options.

**Table 2: Somatosensory submodalities and taxonomy of aberation [26]**

a) Objective test currently not sensitive to gain of function

Abbreviations:

| | |
|---|---|
| NCV | nerve conduction velocity |
| SEPs | somatosensory evoked potentials |
| LEPs | laser evoked potentials |
| EPs | evoked potentials |
| CHEPs | contact heat evoked potentials |
| IENF | intraepidermal nerve fiber |
| n.a. | not available |
| QST | quantitative sensory testing |
| Thermal EPs | thermal evoked potentials |
| Pinprick EPs | pinprick evoked potentials |

| Clinical sign | Fiber classes | Clinical test | Techniques for additional semi-objective tests (QST) | Techniques for additional objective laboratory tests |
|---|---|---|---|---|
| Negative Signs | | | | |
| Mechanical hyposthesia | Aβ | Cotton wool tip<br>Tuning fork | Graded von Frey filaments<br>Tuning fork, vibrameter | NCV, SEP |
| Cold/ warm hypesthesia | Aδ, C | Cold/ warm test tube<br>thermoroller | Thermal test device | Thermal EP, IENF |
| Mechanical hypoalgesia | Aδ | Toothpick<br>Blunt pressure (finger) | Weighted pinprick stimuli<br>Blunt pressure (algometer) | Pinprick EPs, IENF |
| Cold/ heat hypoalgesia | Aδ,C | n.a. | Thermal test device<br>Infrared laser | LEPs, CHEPs, IENF |
| Positive signs | | | | |
| Mechanical hyperalgesia | Aδ,C | Toothpick<br>Blunt pressure (finger) | Weighted pinprick stimuli<br>Blunt pressure (algometer) | a |
| Dynamic mechanical allodynia | Aβ | Cotton wool tip<br>Soft brush | Cotton wool tip<br>Soft brush | a |
| Cold/ heat hyperalgesia | Aδ,C | Isopropanole | Isopropanole | a |

# 7. Methods

## 7.1. Study title

A Randomized, Double-Blind Active Controlled Trial Of Reboxetine In Patients With Chronic Painful Diabetic Neuropathy.

## 7.2. Background

Antidepressants are one of the most powerful coanalgesics for the treatment of chronic neuropathic pain [12, 14]. Substances such as amitryptiline (a tricyclic antidepressant) or Duloxetine (a SNRI) intervene in two different neurochemical pathways: the serotonergic and noradrenergic pathways [9,55]. These pathways – and drugs, respectively – cause their analgesic and side effects not only in the CNS, but also in the peripheral tissues due to interactions with local receptors [14]. Besides of these substances, which interfere in both pathways, selective serotonin reuptake inhibitors (SSRI) were tested on their analgesic effect in chronic neuropathic pain patients. The results of these trials were disillusioning: enhanced levels of serotonin cause hardly any or no analgesia [9,11,14].

Most recently, Grunenthal introduced an opioid with a novel pharmacological profile. Tapentadol acts on μ opioid receptors and has noradrenaline (NA, = norepinephrine, NE) reuptake inhibiting (NARI) effects. Hence, tapentadol demonstrated analgesic effects in somatic and neuropathic pain. This implies that increased noradrenaline, but not serotonin levels in the synaptic cleft are responsible for the relief of neuropathic pain [10-12, 16,-17]. OTHER STUDIES add further evidence to support this observation.

Reboxetine is the only selective NRI and licensed as an antidepressant. Its potential as analgesic was studied in few animal and human pilot studies [9,11-12,14,16,18]. The results are motivating: not only analgesic effects on chronic neuropathic pain, but also in acute pain and prevention of allodynia was reported [11,18,21].

## 7.3. Aim of our study

Summing up, these observations strengthen the hypothesis that the reuptake inhibition with consecutive higher levels of norepinephrine causes analgesia in patients with chronic neuropathic pain.

The analgesic treatment of patients suffering from painful diabetic neuropathy (PDN) often tends to be unsatisfying. Even if antidepressants or anticonvulsants relief pain, the persisting side effects reduce the patients' quality of life, due to gastro-intestinal problems such as nausea, vomiting, constipation, loss of libido, erectile dysfunction. These side effects reduce the otherwise positive results of the coanalgesic therapy.
To increase the analgesic armentarium we aim to test Reboxetine as coanalgesic in patients suffering from PDN in an off label pilot study. We hypothesize that Reboxetine shows equipotent analgesic effects as equivalent doses of Duloxetine, but less side effects due the lack of increased serotonin levels.

## 7.4. Trial design

This study was designed as a randomized, double blind, active controlled pilot study with 10 patients per group, meaning a total of 20 patients. Reporting followed the CONSORT Statement 2010 [71].

## 7.5. Participants

### 7.5.1. Criteria

Patients with painful diabetic neuropathy with the following criteria:

Inclusion Criteria:

- male/ female, any race, >18y
- Diagnosis of painful, distal, symmetrical, sensory-motor polyneuropathy, which is due to diabetes, for at least 1 year
- Patients at screening must have a score ≥ 5 at the numeric rating scale (NRS)
- Blood glucose levels ≤ 200mg/dl

Exclusion Criteria:

- Significant hepatic impairment (>/= 3x)
- Significant renal impairment (GFR </= 50) or st. p. kidney transplantation
- Therapy with β-blockers
- Neurological/ psychiatric disorders unrelated to diabetic neuropathy that may confuse the assessment of neuropathic pain
- Former treatment of the PDN with Duloxetine
- Any pain or other condition that may confound assessment or self-evaluation of the pain due to diabetic neuropathy
- Amputations (except toes)
- Current/ recent diagnoses of major depressive disorder and/ or uncontrolled depression within the last 12 months
- Anamnestic TIA or stroke
- MI or unstable angina pectoris within the last 3 months

## 7.6. Interventions

Interventions followed the study algorithm as it is presented in figure 1:

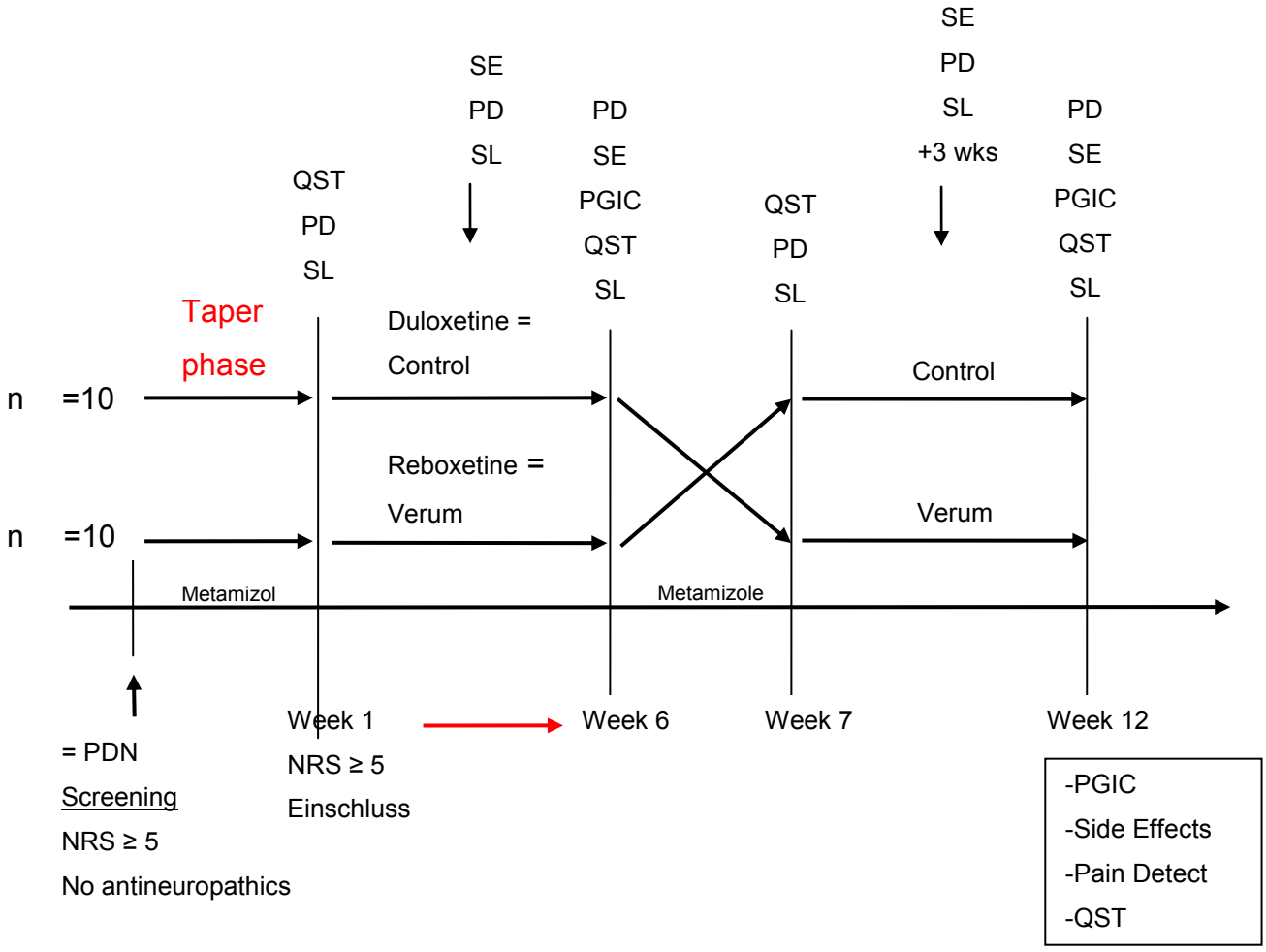


**Fig. 1: Flow Chart of this randomized, double blind, active controlled study comparing the analgesic potencies of reboxetine vs. duloxetine.**

Use of reboxetine (selective NRI) as analgesic treatment in patients suffering from PDN assembled with Duloxetine = sSNRI as an established therapy. We suppose that reboxetine has the same analgesic efficacy level as duloxetine=SNRI, but less/ different side effects. For similar side effects to submit a double blind masking we decided to use duloxetine as an active control.

1. Screening Phase:

Patients will be screened for eligibility and written consent will be obtained.

2. Taper Phase 1 – until the end of week 1:

In case of pain, patients may use metamizole 500mg tablets (Novalgin©) for a maximum of 3000mg per day,

3. Study phase 1 - weeks 2-6:

The patients are assigned to two subgroups – duloxetine 60mg as active control drug (n=10), and reboxetine 4mg as verum.

4. Examination day:

5. Taper Phase 2 – until the end of week 7:

Once again, patients may use metamizole 500mg tablets (Novalgin©) for a maximum of 3000mg per day in case of pain.

6. Study phase 2 (Cross over) - weeks 8-12:

The patients are assigned to the cross over study subgroup, meaning the patients primarily receiving duloxetine will take reboxetine, and vice versa

7. Examination day:

8. End of study

## 7.7. Outcome

### 7.7.1. Primary variable

- Changes in pain intensity (using “Pain Detect“) on screening and on days 1, 21, 35
- QST (days 1, 35) modules
    - Paradoxical heat sensation to cold stimuli → positive in PDN
    - Pallhypaesthesia → negative in PDN

### 7.7.2. Secondary variable

- Pain Detect modules
    - Pain at the moment (0-10)
    - Strongest pain during the last 4 weeks (0-10)
    - Average pain during the last 4 weeks (0-10)
    - Visual scale for pattern of pain fluctuation description (4 grades)
    - Marking the main area of pain
    - Questioning for 7 pain qualities (burning, prickling/tingling, electric-shock-like, allodynia, cold-/ heat-hyperalgesia, numbness, hyperalgesia) divided into 6 grades (never-very strong)
    - Pain behavior pattern (4 grades)
    - Radiating pain
- Sleep (days 1, 21, 35)
- Mood (days 1, 21, 35)
- PGIC (days 1, 21, 35)
- Side effects

Additional Information:

Every drug 5 weeks; taper phase before Cross-Over 1week (HLP= half-life period Duloxetine:8-17h, reboxetine 13h).

In taper the probands may use metamizole 500mg tablets (Novalgin ®) for single dose 500-1000mg and maximum 3000mg per day.

During the whole period of intake – no matter if verum or active control– the probands have to control their blood sugar levels and in case of change from their common ranges the antidiabetic therapy has to be adapted.

Every third week we will control the parameters for renal function (CK, GFR) by taking blood samples.

## 7.8. Statistical information

### 7.8.1. Sample size

Verum- and control-group: each will be n=10 concerning on pilot study characteristics.

### 7.8.2. Randomisation:

Randomisation will be generated by www.randomizer.com

### 7.8.3. Statistical methods

Statistical methods used to compare groups for primary and secondary outcomes
For calculation of descriptive data we use explorative statistics analysis. To compare the dependent and independent variables, depending on the homogene characteristics of the data, parametric variance-analysis or non-parametric Mann-Whitney-U-tests will be conducted.
The analysis of the data-development in between pre- and post-testing will be calculated with paired-samples T-test or with non-parametric Wilcoxon-test. To compare control- and intervention-group, T-test for independent sample or Kruskal-Wallis-H test for the non-parametric properties, will be used.

Methods for additional analyses, such as subgroup analyses and adjusted analyses
The analyses of the interaction of pallhypesthesia or and heat-sensitivity will be carried out with univariat variance-analysis. The side effects are recorded with parametric and non-parametric comparison-tests (see above), as well as with univariat variance-analysis to identify interaction between pallhypesthesia and heat-sensitivity.

### 7.8.4. Recruitment

Patients, who meet inclusion criteria will be asked to participate and enter the study after receiving written consent.

#### 7.8.5. Outcomes and estimation

We expect significant differences between the verum- and active-control-group concerning on side effects, whereas the analgesic efficacy is supposed to be the same or even higher in the intervention group

# 8. Discussion

## 8.1. PDN and neuropathic pain

Diabetic peripheral neuropathy is the most common neuropathic syndrome found in diabetics. According to recent findings it affects up to 50% of patients and is a major cause of morbidity and increased mortality.

Its clinical manifestations include painful neuropathic symptoms and insensitivity, which increases the risk for burns, injuries and foot ulceration.

Painful symptoms such as burning, tingling, shooting or lancing (stabbing) are present in around a third of patients with diabetic peripheral neuropathy and around 20% of all diabetic patients [10, 17]. These symptoms generally worsen at night and disturb sleep [6, 18, 43-44,]. Together with painful symptoms during the day, this often influences individual's ability to perform daily activities and seriously affects life quality in general [6].

Some patients with severe pain symptoms have little sensory deficit, whereas others without painful symptoms suffer from complete numbness of feet, putting them at extremely high risk for foot ulceration.[74] Despite the impact of PDN, management is poor with up to 25% of patients receiving no treatment and many treated with medications having little or no efficacy in managing PDN. [57]

## 8.2. Pathogenesis

A multi-factorial metabolic process leads to PDN that increasingly aggravates tissues. Hyperglycemia, increased sorbitol and protein kinase C, elevated homocysteine, reduced nitric oxide and excessive Reactive Oxygen Species (ROS) impair endothelial tissue and produce a rheological change that increases vascular resistance and reduces blood flow to the nerves. [44]

The mechanism involved in pathogenesis of chronic neuropathic pain is not fully understood in detail, but a growing body of evidence indicates that disinhibition and imbalance of 5-HT and NA could conduce to persistent pain mechanisms [58, 69].
Findings in the study of Morgado et al [75] indicate that PDN can be due to impairments in descending pain modulation, as the study showed that during diabetes, an increase in the numbers of serotoninergic and noradrenergic neurons in key brainstem centers for descending pain is associated with increased levels of serotonin and noradrenaline in the spinal cord.

## 8.3. Diagnosis and examination

The diagnosis of DPN begins with a careful history of sensory and motor symptoms. Quality and severity of neuropathic pain if present should be assessed using a suitable scale. Clinical examination should include inspection of lower extremities and evaluation of neurological reflexes and sensory responses to vibration, light touch, pinprick and the monofilament [74]. Consequently to obtain at most objective results in subgrouping [2, 26-27, 36] patients suffering from PDN and observe changes in PDN it is indicated to use QST, in addition to standardised screening tools, such as the neuropathic pain questionnaire, PainDetect [39], NPSI and PGIC [2, 36]. PDN is a consequence of isolated neuropathy of small fibres (Aδ and C) [2, 29, 36], which are responsible for mediation of thermoreception, nociception and visceroception [26]. In case of PainDetect [39] PDN conforms subgroup 4 [36], whereas the appropriate QST-tools were the thresholds for warm detection (WDT), heat pain (HPT), cold pain

(CPT) and pressure pain (PPT) for affected C-fibres and the thresholds for mechanical pain (MPT), cold detection (CDT) as well as mechanical pain sensitivity (MPS) for Aδ–fibres.

## 8.4. Therapy

Current treatment guidelines for the clinical management of DPN are limited to glucose control and symptomatic pain relief [62]. With proper use of the medications in the guidelines many patients do not achieve more than 30% to 50% pain reduction [44].

The International Consensus Panel on Diabetic Neuropathy recommended TCAs as gold standard [10-14] and together with duloxetine, pregabalin, and gabapentin as first-line agents for the pharmacological treatment of painful PDN, whereas the final drug choice should be tailored to the particular patient based on demographic profile and comorbidities[4, 29, 76].

Different studies showed involvement of noradrenaline in neuropathic pain [15, 30, 55, 64-67, 77-78]. In particular substances which increase the level of noradrenaline act as effective analgesics [2, 9, 11-12,14, 16, 18-25, 36, 69, 77-78]. Known selective noradrenaline and serotonin reuptake inhibitors reboxetine, desipramine, fluoxetine, and paroxetine were evaluated in both in vitro and in vivo assays [77]. The contribution of noradrenaline in mediating antinociception may be greater than that of serotonin[77], as studies testing selective serotonin reuptake inhibitors found hardly any or no analgesic effect [9, 11, 14].

In addition, pain relief medications are often accompanied with adverse effects that include dizziness, drowsiness, gastrointestinal distress, constipation, headaches, dry mouth, and sexual dysfunction [2, 44]. Although it is hoped that improvement in pain will be followed by improvement in functionality, this may not be the case as many of these patients may have other comorbidities [4]. Established pharmacological treatment of painful PDN often is not satisfying because currently available drugs are often ineffective and complicated by adverse events.

Tricyclic compounds (TCAs) are used as first-line agents for many years, but their use is limited by frequent side effects that may be central or anticholinergic, including dry mouth, constipation, sweating, blurred vision, sedation, and orthostatic hypotension[2, 10-13, 56]. Higher doses are been associated with an increased risk of sudden cardiac death, and caution should be taken in any patient with a history of cardiovascular disease [56].

Selective serotonin noradrenaline reuptake inhibitors (SNRI) duloxetine and venlafaxine are used for the management of PDN as an alternative to TCAs. SNRIs lead to pain relief by increasing synaptic availability of serotonin and noradrenaline in the descending pathways that inhibit pain transmission [1, 2, 7, 8, 15, 36, 56-63, 74]. The main side effects include nausea, somnolence, dizziness, constipation, dry mouth, and reduced appetite, although these tend to be mild to moderate and are temporary [56-58].

The initial selection of a particular first-line treatment will be influenced by the assessment of contraindications, evaluation of comorbidities (including sleep disturbance, mood disorders, and other chronic medical/diabetes complications), and cost (29, 76). For example, in diabetic patients with a history of heart disease, elderly patients on other concomitant medications such diuretics and antihypertensives, and patients with comorbid orthostatic hypotension TCAs have relative contraindications.

In patients with liver disease, duloxetine should not be prescribed, and in those with peripheral edema, pregabalin or gabapentin should be avoided. Therefore, treatment has to be individualized to include patients comorbidities in therapeutical decisions and it is advised to start at lower than recommended doses and titrate gradually [4].

# 9. Conclusion

The results of recent research in pathomechanisms of chronic neuropathic pain suggest noradrenaline to be the responsible neurotransmitter for the analgesic efficacy of antidepressants [2, 11, 17, 21, 22, 69].
Trials testing reboxetine in mural model or in healthy probands [9, 11-12, 16, 18] indicate patients who suffer from PDN could benefit of Reboxetine as co-analgesic.

Thus this substance intervenes in noradrenergic pathways only by selective blocking of NET [59, 63], in contrast to TCAs, which block the same transporter but unselectively, and has hardly any or no binding tendencies on α1-adrenoreceptors [11, 59, 63]. In contrast duloxetine intervenes through reuptake inhibition in both, the noradrenergic and serotoninergic system [13, 15, 59, 67], we expect treatment with reboxetine to result in less/ milder side at the same analgesic effects.

Thus we propose to test its analgesic efficacy in patients suffering from painful chronic polyneuropathy in a double-blind clinical pilot trial.

The overall aim of the study is to assess whether reboxetine as an alternative treatment to duloxetine in patients with PDN.

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