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# Therapy-resistant enuresis

*In search of new therapies and prognostic markers*

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ACTA  
UNIVERSITATIS  
UPSALIENSIS  
UPPSALA  
2019

ISSN 1651-6206  
ISBN 978-91-513-0701-5  
urn:nbn:se:uu:diva-389838

Dissertation presented at Uppsala University to be publicly examined in Enghoff-salen, Akademiska Sjukhuset, Ing 50 bv, Uppsala, Friday, 4 October 2019 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Senior professor Sievert Lindström (Institutionen för klinisk och experimentell medicin (IKE) Linköpings Universitet).

### **Abstract**

Lundmark, E. 2019. Therapy-resistant enuresis. In search of new therapies and prognostic markers. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1584. 60 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0701-5.

A large minority of children with enuresis do not respond to treatment with either desmopressin or the enuresis alarm. Anticholinergics have not proven as successful as expected. The fourth evidence-based treatment of enuresis, the tricyclic antidepressant imipramine, is cardiotoxic when overdosed, which has led to diminished use. Since the long-term consequences of enuresis are potentially grave it is important that effective treatments of therapy-resistant enuresis are found.

When investigating the enuretic child a full voiding-chart - in addition to the case history - is the method of choice. However, there is no robust evidence that daytime voiding chart data actually do predict nocturnal detrusor function.

The aim of this thesis was to determine whether there is a role for the noradrenergic antidepressant reboxetine in the treatment of therapy-resistant enuresis, and whether anamnestic data and the voiding chart provides prognostic information regarding response to treatment with anticholinergics and antidepressants respectively in therapy-resistant patients.

In a retrospective evaluation of 61 children who for humanitarian purposes had been treated with reboxetine 32(52%) responded to this treatment, 21 of them after desmopressin had been added. We then proceeded with a randomized placebo-controlled study with 18 patients, in which the reduction of wet nights was much better with either reboxetine in monotherapy or in combination with desmopressin than during the placebo period ( $p=0.002$ ). However, no patient achieved complete dryness. No prognostic markers for therapy-response were found in either of these studies.

In the randomized study we also sought to investigate whether reboxetine had any statistically significant effect on voiding-chart data. No such effect was found, but in respect to this secondary aim the sample size was too small. Nonetheless, this led to the speculation whether reboxetine exerts its antienuretic effect via modulation of arousal mechanisms.

Prognostic markers were sought in a retrospective evaluation of 154 patients treated with anticholinergics or antidepressants, but few and inconsistent differences were found between the groups responding or not responding to the various treatment regimens, and this was true both for anamnestic and voiding chart data.

In conclusion reboxetine seems to be an alternative in the treatment of enuretic children who have not responded to standard treatment, but further trials with higher doses and larger study populations are needed. The internationally recommended assessment of children with therapy-resistant enuresis does not seem to give the prognostic information intended.

*Keywords:* Nocturnal enuresis, Reboxetine, Antidepressants, Desmopressin, Voiding chart

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ISSN 1651-6206

ISBN 978-91-513-0701-5

urn:nbn:se:uu:diva-389838 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-389838>)

*Till minne av min far Sven-Eric Lundmark  
- jag saknar dig så*



# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Lundmark E, Nevéus T. Reboxetine in therapy-resistant enuresis: a retrospective evaluation. *Scand J Urol Nephrol*. 2009;43(5):365-8.
- II Lundmark E, Hägglöf B, Stenberg A, Nevéus T. Reboxetine in therapy-resistant enuresis – a randomized, placebo-controlled study. *J Ped Urol*. 2016 Dec;12(6):397.e1-397.e5.
- III Lundmark E, Stenberg A, Häggöf B, Nevéus T. Effects of antidepressant therapy on voiding chart data and nocturnal urine production in children with enuresis – a randomized controlled study. (manuscript)
- IV Lundmark E, Nevéus T. The prognostic value of voiding chart data in therapy-resistant enuresis. (submitted manuscript)

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# Definitions

Throughout this document the definitions of the International Children's Continenence Society (ICCS) will be strictly adhered to [1].

Enuresis	Urinary incontinence occurring at night while asleep, i.e. bedwetting.
Monosymptomatic enuresis	Bedwetting in a child without any concomittant daytime lower urinary tract symptoms, such as urgency or daytime incontinence.
Non-monosymptomatic enuresis	Bedwetting combined with daytime lower urinary tract symptoms.
Primary enuresis	Enuresis in a child who has never been reliably dry at night.
Secondary enuresis	Enuresis after a previous dry period of at least six months.
Urgency	The sudden, unexpected sensation of an imminent need to void. Usually indicative of detrusor overactivity.
Overactive bladder	Urinary urgency, with or without urinary incontinence, in the absence of urinary tract infection or other obvious pathology.
Detrusor overactivity	involuntary detrusor contractions occurring during the filling phase, as measured by cystometry.
Voiding dysfunction	Contraction of the sphincter during the voiding phase, resulting in staccato flow on uroflowmetry.
Full response	100% reduction of the number of wet nights.
Partial response	50-99% reduction of the number of wet nights.
Nonresponse	<50% reduction of the number of wet nights.



# 1 Introduction

## 1.1 Epidemiology

Nocturnal enuresis is a common condition, affecting between 5 and 10% of 7 year-old children [2], 4% of eleven-year olds [3] and 0.5-1% of teenagers and young adults [4, 5]. The average annual spontaneous cure rate is approximately 15% [6], but the prognosis is worse if the enuresis frequency is severe [7]. Enuresis is, for unclear reasons, more common in boys than girls [8].

The condition can be either monosymptomatic or nonmonosymptomatic according to the presence of daytime lower urinary tract symptoms, such as urgency and/or daytime incontinence [1]. Daytime incontinence has been reported in 15-30 % of enuretic children [9, 10] but since subtle symptoms such as urgency or abnormal daytime voiding frequency are common in enuretic children, the nonmonosymptomatic subgroup is probably larger than the monosymptomatic [11]. This has important pathogenetic implications since concomitant daytime symptoms probably reflect underlying detrusor overactivity, as discussed below.

Secondary enuresis is much less common than primary enuresis [12]. Thus, most enuretic children have never been reliably dry at night. Among children with secondary enuresis there is a small subgroup with enuresis due to underlying disorders such as diabetes or polyuric renal failure.

## 1.2 Consequences

As mentioned above, not all children ever grow out of their condition [5], and it is obvious that the social consequences for these individuals will be considerable. Even in those who do, or are successfully treated, modern research has shown that it negatively affects self-esteem in childhood [13], peer relations and school function in adolescence, [14] and quality of life in general [15].

Recently, it has been suggested that the sleep disturbance underlying enuresis (see below) may have unfavorable consequences regardless of the bed-

wetting *per se*, since it has been demonstrated that enuretic children have cognitive daytime problems linked to disturbed sleep and that these problems disappear when sleep quality improves – regardless of whether the children become dry or not [16].

Thus, enuresis is not a trivial disorder. There are multiple potentially deleterious consequences of the condition, which underscores the importance of successful treatment [13, 17, 14].

### 1.3 Comorbidity

It is not uncommon that children with enuresis, especially of the nonmonosymptomatic kind, also suffer from constipation [18]. Furthermore, psychiatric or psychological problems are more common among enuretic children than in the normal population [19]. This is not due to underlying psychopathology, as was previously thought, but to effects on self esteem and/or sleep disruption, as mentioned above, as well as to the fact that neuropsychiatric disorders such as attention deficit hyperactivity disorder (ADHD) for unclear reasons are overrepresented in the enuretic population [20, 21]. The recognized link between enuresis and these disorders does not, however, imply that treatment of the one condition automatically leads to amelioration of the other; we can, for instance, not expect that successful ADHD therapy with central stimulants makes the child dry at night [22].

Practitioners frequently observe that sleep apnea syndrome seems to be overrepresented in children with enuresis, and it has been confirmed that surgical removal of upper airway obstruction may be curative in enuretic children [23, 24]. Still, among children seeking help for nocturnal enuresis the prevalence of sleep disordered breathing is not high [25], and there is no evidence that enuretic children without sleep apneas or heavy snoring are helped by upper airway therapy.

### 1.4 Etiology

The observation that enuresis tends to run in families was first made as early as in the 19<sup>th</sup> century [26], and has since been corroborated in twin studies and formal genetics [27, 28], as well as in epidemiological studies [8]. Still, the frequently positive family history gives only limited clinically useful information; i.e. it does neither predict the chance of spontaneous remission nor response to therapy [29].

## 1.5 Pathogenesis

Pathogenetically, enuresis has been shown to be a heterogeneous condition. Two major subgroups can be discerned:

(i) the children with nocturnal polyuria who wet their beds because their kidneys produce more urine at night than their bladders can be expected to accommodate [30], and

(ii) those with nocturnal detrusor overactivity who wet their beds because of uninhibited detrusor contractions that occur regardless of bladder filling [31].

Both these groups of children have excessively high arousal thresholds [32, 33] and thus fail to be awakened by the bladder filling or detrusor contractions.

### 1.5.1 Nocturnal polyuria

Urine production is regulated by several hormonal and nonendocrine factors. Vasopressin, an antidiuretic hormone produced in the hypothalamus and secreted by the neurohypophysis is, in normal children, usually released in increased amounts during the night [34]. In pioneer research it was shown that a group of children with enuresis exhibited nocturnal polyuria, as compared with nonenuretic children, and that this polyuria was linked to a lack of the normal nocturnal peak in vasopressin secretion [30]. Since then, this finding has been both corroborated [35] and contradicted [36]. The consensus now is that nocturnal polyuria is relevant in a subgroup of enuretic children [37] and that these children have a large chance of becoming dry if the polyuria is addressed [38].

### 1.5.2 Detrusor overactivity

The notion that some enuretic children suffer from detrusor overactivity has been established in studies involving cystometric evaluations [31], and is indirectly supported by the overlap between enuresis and daytime urge incontinence [2] as well as studies in which voiding chart data show that patients with enuresis, as a group, have smaller voided volumes than their dry peers [39, 40 41]. Furthermore, studies on the "enuresis volume", i.e. the amount of urine voided during the bedwetting episode, have shown that this volume, regardless of urine production, only rarely represents the emptying of a full bladder, indicating that some extent of nocturnal detrusor overactivity may be present in most or all enuretic children [42].

The problem here is that although the role of detrusor overactivity in the pathogenesis of nocturnal enuresis is certain, it is much more difficult to know whether it is present in the individual child, since the evidence for daytime voiding chart data or case history to predict detrusor function is actually not very robust [43]. And the basis for making conclusions regarding specifically nocturnal detrusor overactivity is weaker still [44]. The only reliable way to know if this is present would be to perform ambulatory cystometry, an examination which due to its invasiveness is not defensible in uncomplicated enuresis except in the rare cases when a neurogenic bladder is suspected.

Detrusor overactivity can be either idiopathic or secondary to constipation. The former may, in turn, be subdivided into groups with myogenic, urothelial or central nervous mechanisms [45]. The latter is partly explained by the fact that the distended rectum in a constipated individual exerts pressure on the bladder [46].

### 1.5.3 Sleep and arousal

Individuals with nocturnal polyuria and/or nocturnal detrusor overactivity would be expected to wake up when the bladder is either distended or contracted [47]. The reason why enuretic children do not do this has not been fully determined. That enuretic children are difficult to arouse from sleep is a near universal observation made by their parents [33]. This observation has also been corroborated in a landmark study of objective arousal thresholds [32]. Thus, enuretic sleep is indeed "deep" in the sense that these children have high arousal thresholds. But this does not necessarily mean that they have an abnormal distribution of sleep stages or other polysomnographic differences compared with dry children [48]. There is, in fact, accumulating evidence that sleep in enuretic children is disrupted, and thus, in some sense of the word, actually "superficial" [49, 50], and, furthermore, that this disrupted sleep might affect daytime cognitive function [16].

Another aspect of sleep and arousal is that disrupted sleep is a frequent feature of ADHD [22, 51, 52]. Furthermore, sleep deprivation – regardless of etiology – may cause hyperactivity and difficulties in focusing, and thus simulate ADHD [53, 54, 52]. It can be speculated that disrupted sleep is the common denominator in enuresis and ADHD.

Finally, since repeated sleep disruption is known to result in increased arousal thresholds [56], and the sleep of enuretic children can indeed be assumed to be disturbed by repeated detrusor contractions and/or bladder distension – both recognized as strong arousal stimuli [47, 57] –, it may be

questioned whether the high arousal thresholds represent a cause or a consequence of enuresis.

#### 1.5.4 Underlying brainstem mechanisms

The reticular activating system, a diffuse network of neurons in the brainstem, is the nervous structure directly responsible for arousal from sleep. It has its uppermost center in the locus coeruleus (LC) in the rostral pons [58] (Figure 1). The vasopressin-producing nuclei in the hypothalamus have direct and indirect neural connections to the LC [59], which, in turn, overlaps the pontine micturition center, anatomically as well as functionally [60]. The pontine micturition center is the supraspinal coordinator of detrusor and sphincter control [61]. As mentioned above, nocturnal enuresis usually runs in families [27], but it has been observed that members within the same pedigree often differ in the relative importance of the pathogenetic mechanisms mentioned above, i.e. genotype does not predict phenotype [27]. This observation, combined with the proximity between the structures regulating arousal, micturition and urine production, has led to a hypothesis that nocturnal enuresis may be caused by a disturbance in the LC [62].

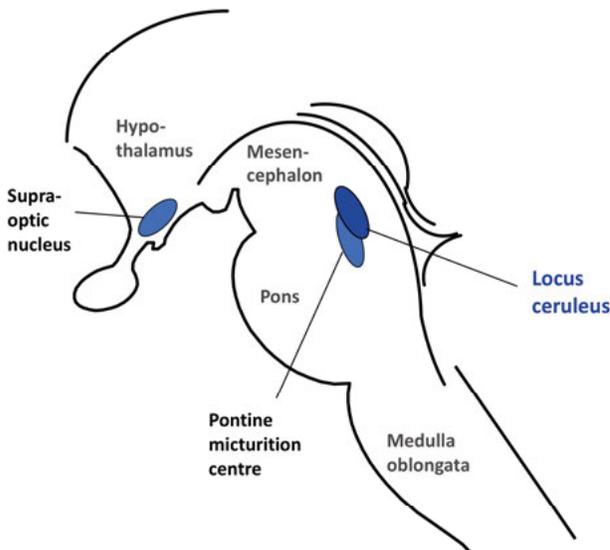


Figure 1. The location of the LC in the rostral pons

This hypothesis is indirectly supported by the importance of the autonomous nervous system in nocturnal dryness. The sympathetic nervous system is active during arousal [63] as well as during the urine storage phase [61], and sympathetic activity in the renal nerve has antidiuretic effects [64]. On the other hand, the parasympathetic nervous system is active during non-REM-sleep [63] and during the voiding phase [61], and parasympathetic suppression may cause antidiuresis via vasopressin release [64]. The LC is the most dense accumulation of noradrenergic neurons in the central nervous system, and the main nucleus of the central branch of the sympathetic nervous system [65]. Studies of autonomic tone during the night, using cardiac frequency analysis, have shown that children with enuresis have higher nocturnal parasympathetic activity compared to dry controls or previously enuretic children [66].

Thus, it may well be the case that what is inherited in a family with enuresis is not nocturnal polyuria, detrusor overactivity or high arousal thresholds *per se*, but a subtle brainstem disturbance manifesting itself in combinations of the three.

## 1.6 Treatment, theoretical considerations

There are only two established and evidence-based first-line treatments of enuresis readily available today: the antidiuretic drug desmopressin and the enuresis alarm [67]. In addition to this, bladder advice, or basic urotherapy, is often recommended as well. Still, a large minority of children with enuresis – probably at least 25% – is resistant to first-line therapy [68]. Recognized second-line alternatives are anticholinergics and imipramine.

### 1.6.1 Desmopressin

Desmopressin is a synthetic analogue of vasopressin [69], and – given at bedtime – decreases urinary output during the night, thus helping the child to stay dry. It rarely has any side-effects, but must be handled correctly [67]. This means that excessive fluid intake at night must be avoided [70]. Desmopressin is not a curative treatment, but the medication can be given for long periods without risk [71]. Efficacy estimates vary between studies but overall one third can be expected to have a full response and one third an intermediate response to desmopressin therapy [72]. The enuresis usually reappears when treatment is discontinued [72].

Voiding chart data are very valuable as prognostic indicators of desmopressin response, since nocturnal polyuria and normal diurnal voided volumes mean that it is very likely that the drug will make the child dry [38, 73].

### 1.6.2 The enuresis alarm

The enuresis alarm is the only known curative treatment of nocturnal enuresis. The alarm consists of a sensor either worn close to the patient's body, or placed on the sheets, which is connected to an alarm unit. The alarm is activated by urine, and the child is wakened from sleep, either by the alarm itself, or – more often – by a parent. The mechanism behind the therapeutic effect is still not fully known, but it can be assumed that the patient either is conditioned to wake before the bladder emptying, or learns to inhibit the bladder contraction without waking up. Approximately two thirds of enuretic children can be expected to respond to alarm therapy, provided the family is well motivated and instructed, and can comply with the hard work that is required [74, 68].

The main positive predictors of enuresis alarm response are that the family is motivated and that the enuresis is frequent [75, 76]. The predictive value of voiding chart data is limited [77, 78].

### 1.6.3 Basic bladder advice

Based on clinical experience [79], and the well-established role of urotherapy as a first-line therapy in daytime incontinence [80], daytime bladder training is often recommended as an initial therapy in enuresis as well. The advice given is, very briefly, that the child should establish regular voiding habits, adopt a sound voiding posture and drink adequate amounts of fluid, evenly distributed during the day [67]. The main problem with this approach is that it is, as yet, not based on prospective randomized, controlled studies [81].

### 1.6.4 Anticholinergics

Since detrusor overactivity is believed to play an important role for some children with enuresis, anticholinergics are often tried if first-line therapy doesn't work. Cystometric studies has indeed indicated that anticholinergics may be useful in enuretic children with underlying detrusor overactivity [82]. Unfortunately, although better than placebo, they have not proven as successful as expected [83] [84] [85], a fact that may be due to pathogenetic heterogeneity within the subgroup of children with detrusor-dependent enuresis – i.e. the detrusor overactivity may be either peripheral or central. The former is elicited at the level of the bladder, the latter in the central nervous system. [86, 87].

The drugs are also burdened by side effects, mainly constipation and – for oxybutynin – mood changes [88]. These circumstances leave a substantial number of patients without effective treatment.

There is scant information regarding prognostic indicators for anticholinergic response. It would be logical to assume that low daytime voided volumes, suggesting detrusor overactivity, would predict a favorable response. This assumption has been given support by some studies [89] [41], but not by others [73].

### 1.6.5 Antidepressants

The fourth evidence-based treatment of enuresis is the tricyclic antidepressant imipramine [90], with noradrenergic, serotonergic and anticholinergic effects. The drug probably exerts its antienuretic effect via central noradrenergic facilitation, although the mechanism is not fully known [91]. Although imipramine has anticholinergic side effects this cannot explain the antienuretic effect, since all imipramine-responding children in a previous study at our center had tried anticholinergic treatment without success [91]. The central noradrenergic effects of imipramine depend on the binding of the active metabolite desipramine to LC [92] [93]. Serotonergic antidepressants have no clear effects on enuresis [94].

However, imipramine – as all tricyclics – is cardiotoxic when overdosed, and lethal reactions have occurred [95] [96], which – in addition to the development of alternative antidepressants with selective serotonergic or noradrenergic action – has led to diminished use. In many countries the substance is no longer available for label prescription, which again leaves many patients without effective treatment.

Prognostic factors indicating therapy success with antidepressants have not been identified [73].

The selective noradrenaline reuptake inhibitor reboxetine has the same noradrenergic action as imipramine, but no clinically relevant serotonergic effect, and no cardiotoxicity [97]. The substance is very similar to atomoxetine, and has been used in pilot studies on children with neuropsychiatric disorders [98]. Selective noradrenaline reuptake inhibitors such as atomoxetine – which is used in the pediatric population – have few serious side-effects [99]. However, reboxetine is not yet registered for pediatric use.

## 2 Management of the enuretic child

What follows below is a simplified outline based on the recommendations issued by the International Children's Continence Society (ICCS) [67]

### 2.1 Initial assessment

The case history is – of course – the primary tool when investigating the enuretic child. With a good history the minority of children who require extended evaluation can easily be detected and treatment can be started for the rest. Much of the history will, of course, be focused on micturition habits; Are there daytime problems as well? How often does the child go to the toilet? Is the enuresis frequent or sporadic? Has there been previous periods of dryness? The presence of urgency and/or daytime incontinence confirms that the enuresis is of the nonmonosymptomatic variety and gives support to the suspicion that it is associated with detrusor overactivity. If the enuresis is secondary, i.e. if it appears after a prolonged period of dryness, extra caution needs to be given to possible underlying extraneous causes. Likewise, weight loss, nausea or excessive thirst mean that conditions such as diabetes need to be excluded without delay, and voiding difficulties indicate that urodynamic investigation with at least uroflowmetry and residual urine measurements are required. Of course, the family's strategies to treat or cope with the problem need to be explored and the child needs to be questioned about how big a problem it is for him/her. Finally, given the psychological impact of enuresis and the psychiatric comorbidity, at least a few exploring questions regarding behavioral issues or problems in the interaction with family and peers should to be asked.

Although pathological findings rarely are found in children with monosymptomatic enuresis, a physical examination is recommended, with special focus on the inspection of the lower back region and tendon reflexes in the lower extremities. If the enuresis is secondary or if there are warning signs in the history this examination is mandatory. If the child presents with any symptoms of constipation a rectal examination should be considered, since the presence of formed feces in the rectum of a child without present urge to go to the toilet is strongly indicative of constipation.

To further determine whether nocturnal polyuria or detrusor overactivity is present, a full voiding chart, as defined by the ICCS [1, 10], is the method of choice. When evaluating the voiding chart data nocturnal polyuria, obviously, is determined directly via the weighing of diapers, whereas a high daytime voiding frequency with low voided volumes are taken as indicators of detrusor overactivity. The lack of firm evidence for daytime voiding chart data to predict nocturnal detrusor function has been discussed above [42, 43].

## 2.2 First-line treatment

After the initial assessment outlined above the healthcare professional can proceed with therapy for the large majority of children who do not need extra evaluation. The child who is old enough to be bothered by his or her enuresis, usually by the age of six or seven, should be offered active treatment with the enuresis alarm or desmopressin. Traditionally, basic bladder advice is also recommended to all children, regardless of age. Given the prognostic indicators mentioned above there are two equally valid ways to choose between the enuresis alarm and desmopressin:

- A) The choice is made based on the prognostic indicators for desmopressin, as determined by a bladder diary. If there is nocturnal polyuria and normal daytime voided volumes desmopressin is given, otherwise the alarm is recommended
- B) The choice is made based on the central prognostic indicator for alarm therapy, i.e. family's motivation. This means that the pros and cons of both therapies are presented to the family and they choose themselves. The parents and child who are highly motivated and whose family situation doesn't preclude the workload that this therapy entails will usually choose the alarm. To the rest desmopressin is offered.

If the alarm treatment is chosen it is crucial that it is thoroughly demonstrated to the child and caregivers and that the healthcare professional maintains contact with the family during treatment. The parents need to know that they will most probably have to help the child to wake when the alarm goes off during the first weeks of therapy. They should be instructed to use the alarm every night without interruption during at least six weeks or until fourteen consecutive dry nights have been achieved, in which case the child is probably cured.

Desmopressin is nowadays most commonly given as a quick-melting oral lyophilisate approximately one hour before the child goes to sleep. The standard full dosage is 240 µg. The child needs to be instructed to limit fluid

intake during the evening and night when desmopressin is given to eliminate the risk for water intoxication. Response or nonresponse to therapy will be immediately apparent. If desmopressin reliably makes the child dry the family may decide either to give the drug every evening or only before "important nights"; if the former is chosen it is important to make regular short "drug holidays" to see whether treatment is still needed.

Regardless of which therapy is tried first the other should be offered should the first choice not work.

### 2.3 Assessment of the therapy-resistant child

If neither the enuresis alarm nor desmopressin helps the child to become dry he/she needs to see a physician, usually a pediatrician or a pediatric urologist, and a full physical examination needs to be done. Additional questions need to be asked, focused on the therapies given, exploring reasons for treatment failure. Signs of constipation need to be looked for with extra care, and laxative treatment considered. Still, the fact that the enuresis has proven to be difficult to treat does not, by itself, mean that invasive examinations such as cystometry or micturating cystourethrography are indicated. But uroflowmetry and residual urine assessment are recommended for all therapy-resistant children. We have, as yet, no way to individualize therapy choice based on prognostic indicators in this patient group.

### 2.4 Second-line treatment

The recommended next step in the treatment of enuretic children is anticholinergics. The choice of drug differs according to availability and concerns regarding side effects. Oxybutynin is the only drug so far available for label use in children, but, for reasons stated above, alternatives such as tolterodine can be defended. Regardless of which anticholinergic drug is chosen constipation and residual urine first need to be excluded or successfully treated. A usual dosage strategy is to give 2-4 mg tolterodine in the evening. Therapy is evaluated after one month and desmopressin may be added to increase the chance of success. During therapy it is important that the child maintains sound, regular voiding habits and good oral hygiene and that the family reacts if there are any symptoms of urinary tract infection. If therapy is successful regular attempts to discontinue should be made approximately every third month.

For children who do not become dry on anticholinergic therapy or who for reasons of contraindications or side effects cannot be given such therapy,

antidepressant treatment can be offered. The crucial contraindication is unstable arrhythmia, i.e. long QT syndrome. Thus, this alternative cannot be given without a prior long-time ECG registration if there is a history of unclear palpitations/syncope or if there is sudden cardiac death in the family. Furthermore, it is absolutely crucial that parents are informed about the risks of drug overdose and that they keep the pills securely locked away. The family should also be informed that a substantial minority of patients will have side effects such as mood swings. Naturally, not all families will be willing to give this kind of drug to their child but if they do and there are no contraindications, it is the recognized treatment of choice after anticholinergic failure. Imipramine is the antidepressant drug with most evidence as an antienuretic therapy; the recommended dosage is 25-50 mg given in the evening, combined with desmopressin if needed. Treatment effect is usually seen within one month. If treatment is effective it is important that the child makes regular substantial "drug holidays" to decrease the risk of tolerance.

Finally, there is a subgroup of enuretic children who snore heavily or who experience sleep apneas due to enlarged tonsils or adenoids. In these cases an otorhinolaryngologist needs to be contacted since tonsillectomy may make the child dry. These children usually need to see an otorhinolaryngologist regardless of their enuresis.

### 3 Aim

The focus in this thesis is children with therapy-resistant enuresis. The overall aims were 1) to evaluate an alternative to imipramine and 2) to explore if there are prognostic indicators that can guide the choice of second-line therapy without having to resort to invasive examinations.

Specific aims were as follows:

**Study I:** To retrospectively evaluate a group of therapy-resistant patients treated with the noradrenergic antidepressant reboxetine for humanitarian reasons.

**Study II:** To further investigate the therapeutic effect of reboxetine in a randomized placebo-controlled study.

**Study III:** To measure reboxetine effects on variables reflecting bladder function and nocturnal urine production in an attempt to investigate the potential therapeutic mechanism of the drug.

**Study IV:** To search for predictors for therapeutic effect of second-line treatment in therapy-resistant enuresis.

## 4 Patients and methods

### 4.1 Study I

This is a retrospective evaluation of children suffering from severe enuresis – i.e. enuresis occurring during most nights – who were treated with reboxetine in the line of clinical practice. The enuresis did not have to be strictly monosymptomatic according to the ICCS guidelines [1], but daytime incontinence had to be successfully treated before the child was eligible for inclusion. All these children had received standard urotherapy, and symptoms of constipation – if present – had been eradicated. All had tried but failed treatment with the enuresis alarm, desmopressin and combined anticholinergic-antidiuretic therapy. Children with urinary tract infections, neurogenic bladder or other relevant concomitant disorders were not included.

All children underwent a thorough physical examination, and a detailed case history focusing on bladder and bowel habits was taken. A urinary dipstick test was done and found normal in all patients, but radiological and invasive examinations – apart from ultrasound, flowmetry and measurement of residual urine – were not performed, since no child had any history or symptoms suggesting malformations or neurogenic bladder disturbance. In a subset of children full frequency-volume charts were acquired, including nocturnal urinary production measurements (i.e. diaper weighing).

Reboxetine was given orally at bedtime with an initial dose of 4 mg daily, which was increased to 8 mg after 1 month in the absence of clinical efficacy. If the effect was still not satisfactory 240 µgram desmopressin (oral quick-melt formulation) was added. Only children who were reported to be completely dry (at most one wet night per month) were considered full responders, whereas those who had an estimated reduction of wet nights of at least 50% were classified as partial responders. Side-effects and tolerance were actively asked for. Prognostic variables were sought in the case history and frequency-volume chart data.

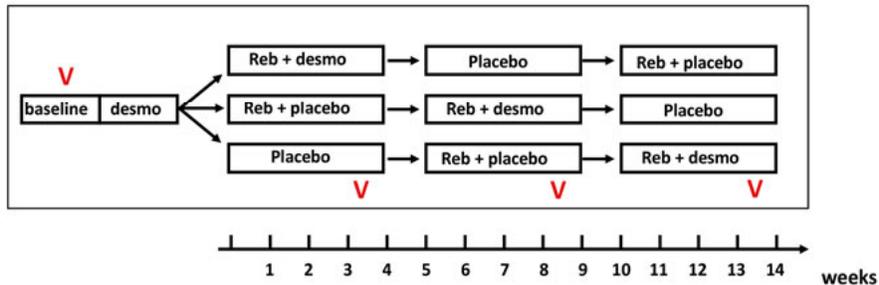
## 4.2 Study II

The study presented here is a randomized, placebo-controlled, trial with a double-blind, cross-over design. The patients included were aged seven or more. They all suffered from enuresis with at least seven wet night out of fourteen, and all had tried but failed treatment with desmopressin. The enuresis alarm had either been tried without effect, or deemed unfeasible due to the family situation. As in the previous study, the enuresis did not need to be strictly monosymptomatic, but constipation, if present, had to be treated, and daytime incontinence eradicated, before the child was eligible for inclusion. The families had to be able and willing to provide a signed informed consent. Although this was not part of the inclusion criteria, all patients had either tried and failed combination therapy with anticholinergics or, due to contraindications, been unable to receive such therapy. Thus, all children were severely therapy-resistant.

Patients with underlying renal, urologic, neurologic, endocrinologic or cardiac conditions were excluded. Depression and other severe psychiatric diseases, with the exception of ADHD, were also criteria for exclusion, as were concomitant medication interfering with kidney or bladder function, sleep or the autonomic nervous system. Before inclusion all patients underwent a thorough physical examination, and a detailed, structured case history, focusing on bladder and bowel habits, was taken. A uroflow examination, with measurement of residual urine, was performed, and a urinary dip-stick test was done. All families were provided with a standard voiding chart to complete at home during two weeks. No treatment for enuresis was given during these two weeks. In this pre-study voiding chart daytime voiding frequency and voided volumes, as well as diurnal and nocturnal urine production, were documented during 48 hours, whereas wet and dry nights were recorded during two weeks. These recordings were used as baseline data. In order to exclude desmopressin responders from participation in the randomized part of the study, the two weeks of baseline observations were followed by two weeks during which the patients were given desmopressin 0.4 mg orally at bedtime. If the number of wet nights during desmopressin treatment was reduced with more than 50% compared to baseline the patient was not included in the randomized study.

After these baseline investigations the patients underwent treatment during three four-week periods: one period with reboxetine 4 mg and desmopressin 0.4 mg treatment, one with reboxetine 4 mg and placebo, and one period with double placebo treatment. The order of these periods was blinded to patients, parents and investigators. The randomization was made by APL Pharma Specials Inc (Stockholm, Sweden), who also prepared the medications so that tablets were put into capsules, all of an identical design. The

randomization was communicated to the investigators in sealed envelopes. Between the treatment periods wash-out periods of at least 48 hours and at most two weeks were interspersed. The families recorded in a log whether the medication was taken or not and were also asked to complete new voiding diaries, similar to the pre-study voiding chart, during all treatment periods. Side effects were actively asked for. After each period the families were seen by the study nurse, and by the treating physician after completing all three treatments. The study-design is illustrated in Figure 2.



**V = recording of:**  
**wet/dry nights during 14 days**  
**voided volumes and nocturnal urine production during 72 hrs**

Figure 2. Study design

Treatment response, i.e. the reduction of wet nights out of fourteen compared with baseline, were compared between the three treatment periods. Prognostic indicators were sought in patient history data and the baseline voiding chart parameters.

### 4.3 Study III

The patients and interventions in the study presented in study III were the same as in study II. As described in more detail above this study had a randomized, double-blind cross-over design, with three treatment periods of four weeks each. The therapies given were 1) placebo, 2) reboxetine 4 mg + placebo and 3) reboxetine 4 mg + desmopressin 0.4 mg, all drugs given orally in the evening. The crucial variables analyzed here were the voiding chart data. We looked at the effects of reboxetine medication – with or without desmopressin added – on the following parameters: number of daytime micturitions, maximum and average daytime voided volumes with and without inclusion of the first morning void, nocturnal urine production during wet nights and enuresis volume. These variables were extracted from voiding charts completed during 48 hours of the last two weeks of each treatment period.

## 4.4 Study IV

The study was a retrospective evaluation of patients with therapy-resistant enuresis, without daytime incontinence, assessed in a tertiary outpatient setting. Their bedwetting was resistant to treatment with desmopressin, and all had either without success tried the alarm, or due to their family situation been unable to undergo such therapy. Since there is no evidence that treatment should be differentiated between enuretic patients with or with urgency, particularly not in the therapy-resistant group, the enuresis did not need to be strictly monosymptomatic. However, patients with current daytime incontinence were not included. Children with concomitant diseases or treatments affecting kidney or urinary tract function were also not included.

Prior to treatment all patients completed a voiding chart similar to the one described above in study II. The management of the patients was done in a semi-structured way, reflecting our everyday clinical practice and the recommended standards of care. Before the bedwetting was addressed constipation was carefully excluded or treated. In accordance with international recommendations [10], the children were first treated with anticholinergics, provided this was not contraindicated. Due to concerns about the central nervous side effects of oxybutynin, and the available evidence indicating efficacy of tolterodine in the relevant patient group [10], the latter drug was the anticholinergic used. If the therapeutic effect was not satisfactory dosage was adjusted (maximum 4 mg in the evening) and desmopressin was added. If sufficient treatment effect was not achieved with combined anticholinergic treatment, or if bothersome side effects appeared, antidepressant therapy was tried next, combined with desmopressin if needed. Due to concerns regarding cardiotoxicity as well as the unavailability of imipramine in our part of the world the antidepressant used was reboxetine (maximum dose 8 mg) in 55 patients, whereas 10 patients were treated with amitriptylin (25-50 mg). One patient was treated with atomoxetine (50 mg) by child psychiatrists because of concomitant ADHD. All therapies were tried for at least one month with adequate dosage.

Since this was a retrospective evaluation of clinical practice, not a randomized trial, treatment success was graded according to family satisfaction, not the actual frequency of wet nights. Thus, only children who reported that they were completely dry were regarded full responders and those who stated that there was a substantial and useful reduction of wet nights were labeled intermediate responders. All other children were classified as non-responders, even though many of them experienced some reduction of the number of wet nights.

## 4.5 Statistics

In *Study I* possible prognostic factors were analyzed using  $\chi^2$  tests or independent samples *t*-tests. In *Study II* the actual reduction in enuresis frequency was used as the main outcome variable, in accordance with the recommendations from the ICCS [1]. It was also determined whether the children were nonresponders, partial responders, or full responders to therapy according to the ICCS definitions (i.e. if the reduction of enuresis frequency was below or above 50% or 90%, respectively) [1]. The reduction of wet nights between baseline and the last two weeks of each treatment period were compared using the Friedman test (since the data were found not to be normally distributed). When looking for prognostic indicators, baseline data for responders and non-responders to therapy were compared using *t*-tests or the Mann-Whitney test for numerical variables, and the  $\chi^2$  test for dichotomized variables. In *Study III* the voiding chart variables were compared between both active treatment periods and the period with placebo treatment. Since the data were found not to be normally distributed, the Friedman test was used. When comparing the voiding chart data of responders and nonresponders to active treatment the Mann Whitney *u* test was used.

The sample size calculation for the randomized controlled trial was made with respect to the primary aim, which was treatment response, i.e. reduction of wet nights, not on effects on voiding chart data. Previous research on comparable groups of enuretic children indicate that their mean number of wet nights out of 14 is approximately  $11 \pm 3.9$  [73]. Given these data a study with 16 children would have a power of 80% to detect a treatment difference of four wet nights or more per two weeks. A smaller difference than this would not be clinically relevant.

When looking for prognostic indicators in *Study IV*, data for responders and non-responders to the treatments given were compared using independent samples *t*-tests for numerical variables, and the  $\chi^2$  test for dichotomized variables. Previous research [73] on comparable groups of children with therapy-resistant enuresis indicate that their daytime micturition frequency is  $6 \pm 1.5$ , and their average voided volume is  $40 \pm 12\%$  of EBC. To detect with a power of 80% a difference in micturition frequency of 2 would require nine patients with each of the six possible treatment outcomes (treatment effect with anticholinergics and antidepressants, respectively, and benefit from addition of desmopressin). To detect with a power of 80% a difference in average voided volumes of 10% would require 25 patients with each treatment outcome. Smaller differences than this would not be clinically relevant. Since most patients are subject to several or all treatment options, a study population of approximately 160 was considered adequate to provide a sufficient number of patients within each outcome.

All voiding chart variables, in studies II, III and IV, except the number of daytime micturitions were expressed as percentages of expected bladder capacity, as calculated from the Koff-Hjälms formula [100]. This way, effects of age were minimized

The statistical significance level was set at 95% ( $p=0.05$ ) in all studies.

## 4.6 Ethical considerations

The randomized placebo-controlled trial (*Study II* and *III*) was approved by the ethical review board at the Medical Faculty of Uppsala University (record nr 2008/203). The study was registered by the Swedish Medical Products Agency (MPA) and the European Medicines Agency (EMA) with EudraCT number 2008-002636-15. The retrospective evaluation in study IV was approved by the Swedish Ethical Review Authority, record nr 2019-938.

As mentioned above, all interventions of *Study I* was part of clinical practice and not a prospective clinical trial. The medication was given as off-label prescription for humanitarian reasons to children whom we otherwise had no therapy to offer, and thus did not at the time (2007-2008) have to undergo an evaluation by the ethical review board.

In studies II (and thus III) informed oral and written consent was acquired from all participants, including the children if they were old enough to be fully involved in the decision. Study I was part of clinical practice, and not a proper clinical trial, and did thus not include a formal informed consent. Still, all families were given full information regarding the assets, drawbacks and potential risks of the therapy offered before consenting to try the medication on their child. The same can be said regarding the therapies offered in the retrospective study IV, in which formal consent to the collecting, processing and publishing of data was not acquired, a strategy which was approved by the Swedish Ethical Review Authority.

All research was conducted in accordance with the Declaration of Helsinki.

# 5 Results

## 5.1 5.1 Study I

The study population consisted of 61 children aged 7- 19 years ( $11.2 \pm 3.0$ ), 12 of whom were girls. (There is a misprint regarding age in the published article.) Of these children 25 (41%) had urgency, 29 (48%) had previously suffered from day-time incontinence, and 13 (21%) had required treatment for constipation. Only 15 patients had managed to fully complete the nocturnal urine output measurements of the frequency-volume charts, the results of which are presented in Table 1.

Table 1. Data obtained from the frequency-volume charts in study I.

Daytime voiding frequency	$5.6 \pm 1.6$
Average voided volume*	$51 \pm 27\%$
Maximum voided volume (first morning void included)*	$104 \pm 83\%$
Maximum voided volume (first morning void excluded)*	$86 \pm 68\%$
Nocturnal urine production (during wet nights)*	$154 \pm 85\%$

\* These variables are expressed as percentages of expected bladder capacity.

All 61 children received reboxetine and 32 (52%) responded to this treatment, 21 of them after desmopressin had been added. Five patients developed tolerance and needed intermittent treatment (usually two weeks without treatment every third month) to maintain dryness. Eighteen children were non-responders, eight discontinued medication due to side-effects and three were lost to follow-up.

No serious adverse events occurred, but side-effects, mainly mood-swings and nightmares, were reported by 24 patients. With the exception of the eight patients who chose to discontinue, these side-effects were minor and transient. In no case did side-effects remain after discontinuation of therapy. No connection was observed between side-effects and the child's age or reboxetine dose.

Beneficial side-effects, although not specifically asked for, were reported by four patients, who all stated that they were calmer and had less trouble focusing when taking the drug.

No anamnestic or voiding chart data was found to be able to predict either anticholinergic or antidepressant effect or need for combination therapy with desmopressin. (Table 2).

Table 2. Differences between the responders and the non-responders to reboxetine treatment in study I.

	Responders	Non-responders	p-value
Age	11.6 ± 2.7	10.4 ± 2.5	0.71
Gender (M/F)	25/7	17/1	0.23
Urgency, presence of	11/18 (61%)	7/10 (70%)	0.70
Previous day-time incontinence, presence of	14/24 (58%)	8/13 (62%)	1.00
Previous constipation, presence of	6/18 (33%)	2/11 (18%)	0.67
Daytime voiding frequency	5.8 ± 1.8	5.2 ± 1.3	0.54
Average voided volume*	44 ± 14%	62 ± 39%	0.31
Maximum voided volume (first morning void included)*	72 ± 30%	152 ± 117%	0.16
Maximum voided volume (first morning void excluded)*	62 ± 28%	121 ± 95%	0.20
Nocturnal urine production (during wet nights)*	127 ± 62%	190 ± 103%	0.18

\* These variables are expressed as percentages of expected bladder capacity.

## 5.2 Study II

In study II and III 18 patients were included, aged 7-21 years (median 10), three of them female. All but one of them completed all parts of the study. Their baseline characteristics are presented in Table 3. No child participated in both study I and II.

Table 3. Baseline characteristics of the study population in study II. Data presented are either proportions or range (median)

Urgency, presence of	5/17 (29%)
Previous daytime incontinence	7/17 (41%)
Previous constipation	5/17 (29%)
Previous fecal incontinence	2/17 (18%)
High arousal thresholds	17/17 (100%)
Daytime voiding frequency	3.0-10.5 (5)
Average voided volume (first morning void included)*	21-53 (36.5)
Average voided volume (first morning void excluded)*	34±11
Maximum voided volume (first morning void included)*	25-83 (67)
Maximum voided volume (first morning void excluded)*	30-74 (60)
Enuresis volume*	15-111 (43)
Nocturnal urine production (during wet nights)*	64-158 (82)

\* These variables are expressed as percentages of expected bladder capacity.

The median number of wet nights were 10 with reboxetine in monotherapy, 11 with combination treatment, and 13 with placebo treatment. The difference in reduction of enuresis frequency between the three treatment periods was highly significant (Figure 3) (Friedman test: chi-square 10.909; df = 2; p = 0.004). When comparing the treatment periods pairwise, using the Wilcoxon signed ranks test, both active treatment periods differed significantly from the placebo period but not from each other: reboxetine monotherapy vs placebo, p= 0.004; reboxetine combination therapy vs placebo, p = 0003; reboxetine monotherapy vs reboxetine combination therapy, p = 0.950.

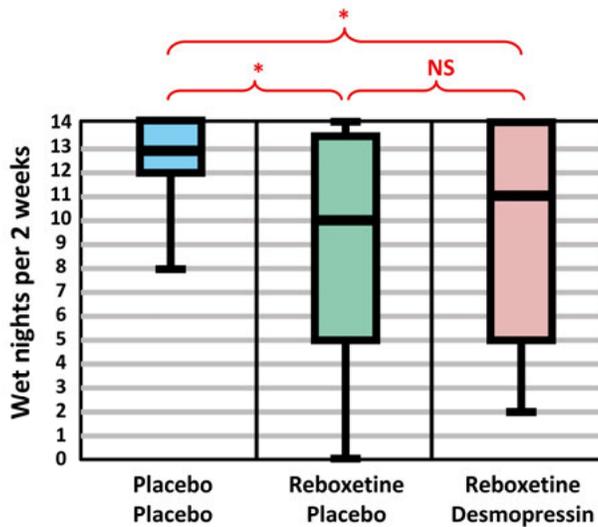


Figure 3. The number of wet nights out of 14 with placebo, reboxetine and combination therapy

With reboxetine in monotherapy one patient achieved full therapy response. Three patients were intermediate responders (i.e. they had at least 50% reduction of enuresis frequency), whereas 13 were non-responders. During treatment with reboxetine combined with desmopressin the number of intermediate responders was five, still leaving 12 children nonresponders. Two intermediate responders in each treatment group were the same individuals. There were no full responders to combination therapy and no participant had any response to double placebo therapy.

Six patients experienced side effects during treatment with reboxetine in monotherapy, whereas three did so when treated with reboxetine combined with desmopressin. These patients were mostly (six out of nine) nonresponders. The side effects mostly consisted of mood swings and – to a lesser degree – sleeping difficulties. All side effects were fully and rapidly reversible, most were both of a mild degree and transient. Only one patient – the study's

only adult - discontinued treatment, and this was due to severe headache, during placebo-treatment as well. One patient reported as a positive side-effect that he became less hyperactive at school. Two patients reported side-effects during placebo treatment, one of them the patient mentioned above.

No significant prognostic factors were found in either the anamnestic data or in the voiding chart variables. (Table 4)

Table 4. Prognostic value of the bladder variables study II. Values presented are - with the exception of one normally distributed variable - range and median, p-values refer to differences between responders and non-responders to each treatment.

	Reboxetine monotherapy			Reboxetin + desmopressin combined		
	Responders	Nonresponders	p	Responders	Nonresponders	p
Daytime micturition frequency	5.5-9.7 (6.3)	3-10.5 ( 4.75)	0.136	3.5-9.7 (5.5)	3-10.5 (5.0)	0.679
Average voided volume, morning void excluded*	23± 4	36± 11	0.069	28± 10	37± 11	0.129
Average voided volume, morning void included*	21-39 (23)	24-53 ( 38)	0.101	23-39 (30)	21-53 (40)	0.206
Maximum voided volume, morning void excluded*	32-73 (45)	30-74 (64)	0.734	30-73 (45)	32-74 (67)	0.310
Maximum voided volume, morning void included*	32-73 (45)	25-83 (69)	0.734	42-83 (54)	25-83 (69)	1.000
Enuresis volume*	43-59 (51)	15-111 (42)	0.769	23-59 (40)	15-111 (46)	0.260
Urine production (wet nights)*	75-82 (78.5)	64-158 (84)	0.308	76-84 (80)	64-158 (90)	0.414

\*these variables are expressed as percentages of expected bladder capacity.

### 5.3 Study III

The patients in this study are the same as the ones reported in study II except the one patient who discontinued treatment due to side effects. Their base-line characteristics are presented in Table 5.

Table 5. Baseline characteristics of the study population. Data presented are either proportions or range (median)

Gender, male	15/17
Age	7- 14 (10) (n=17)
Urgency, presence of	5/17 (29%)
Previous daytime incontinence	7/17 (41%)
Constipation	5/17 (29%)
Fecal incontinence	2/17 (12%)
High arousal threshold	17/17 (100%)
Daytime voiding frequency	3.0- 10.5(5)(n=15)
Average voided volume (first morning void included)*	21-53(36.5)(n=15)
Average voided volume (first morning void excluded)*	15-56 (35) (n=15)
Maximum voided volume (first morning void included)*	25-83 (67) (n=15)
Maximum voided volume (first morning void excluded)*	30-74 (60) (n=15)
Enuresis volume*	15-111 (43)(n=13)
Nocturnal urine production (during wet nights)*	64-158 (82)(n=13)

\*These variables are expressed as percentages of expected bladder capacity.

Since four families did not manage to complete the voiding chart of the last treatment period, only 13 patients provided acceptable daytime data during *all* treatment periods. The nocturnal variables – nocturnal urine production during wet nights and enuresis volumes – were missing in eight patients during one treatment period, and in one patient during two, since both measured nights happened to be without enuresis. Two patients did not provide all measurements necessary to the calculation of nocturnal urine production, one of them during two treatment periods.

Table 6. Voiding chart results during the different treatment periods

	Placebo		Reboxetine monotherapy		Reboxetine + desmopressin	
	mean ± SD	median (range)	mean ± SD	median (range)	mean ± SD	median (range)
Mict/24	5.25 ± 2.07	4.5 (3-10.5)	5.15 ± 1.42	5 (2.5-8.5)	4.53 ± 1.49	4.25 (2.5-8.5)
AVV-m	36.6 ± 15.8	39 (17-78)	33.9 ± 18.6	35 (14-79)	31.7 ± 11.7	30 (14-58)
AVV+m	35.1 ± 10.7	40 (17-49)	35.7 ± 16.5	36 (19-78)	32.3 ± 10.1	30.5 (17-55)
MVV-m	52.9 ± 17.1	51 (30-91)	53.4 ± 25.5	51 (20-115)	50.4 ± 14.7	50 (23-76)
MVV+m	56.5 ± 19.4	56 (30-91)	65.2 ± 23.4	62 (29-115)	54.4 ± 14.4	50 (32-76)
NUP	98.6 ± 23.8	92.5 (62-143)	92.6 ± 42.3	74 (45-157)	80.5 ± 37.8	70.5 (36-148)
enuvol	64.5 ± 27.6	62 (15-126)	47.1 ± 32.1	42.5 (15-116)	44.7 ± 30.3	39 (8-109)

All six volume variables expressed as percentages of expected bladder capacity for age. Mict/24 Number of daytime micturitions AVV+m average voided volumes, first morning void included, AVV-m average voided volumes, first morning void excluded; MVV+m maximum voided volumes first morning void included, MVV-m maximum voided volumes first morning void excluded; NUP nocturnal urine production wet nights, enuol enuresis volume.

Table 7. Differences in voiding chart data between the three treatment periods (Friedman test)

	N	Chi-square	df	p value
Mict/24	13	2.261	2	0.323
AVV-m	13	3.647	2	0.161
AVV+m	13	1.451	2	0.484
MVV-m	13	0.510	2	0.775
MVV+m	13	1.755	2	0.416
NUP	6	1.000	2	0.607
enuvol	6	4.333	2	0.115

all six volume variables expressed as percentages of expected bladder capacity for age. Mict/24 Number of daytime micturitions AVV+m average voided volumes, first morning void included, AVV-m average voided volumes, first morning void excluded; MVV+m maximum voided volumes first morning void included, MVV-m maximum voided volumes first morning void excluded; NUP nocturnal urine production wet nights, enuol enuresis volume.

No clear and consistent reboxetine effects were seen on either voiding parameters or nocturnal urine production (Table 6 and 7). Neither did we find any significant difference in the voiding chart data of responders to active treatment, compared to that of non-responders. This is visualised in Figure 4, in which reboxetine responders are highlighted. No difference in nocturnal urine production was found in the period with desmopressin added to the reboxetine treatment (data not shown).

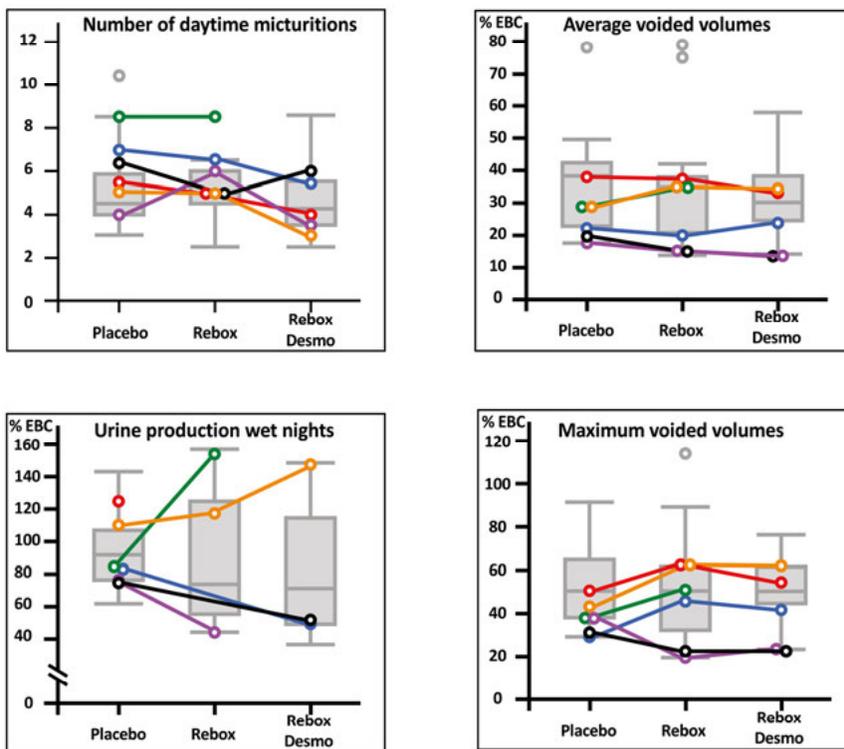


Figure 4. Selected voiding chart data during the three treatment periods

All variables except number of daytime voidings are expressed as percentages of expected bladder capacity according to age. The boxplots represent all children whereas the coloured circles and lines illustrate the values of children who responded to reboxetine therapy (with or without desmopressin).

## 5.4 Study IV

In study IV 154 patients were included, aged 6- 24 (mean  $10.5 \pm 3.0$ ), 127 (82.5%) of whom were boys. The population, and their relevant anamnestic data, are described in Table 7.

Table 7. Baseline characteristics of the study population

Gender (male/female)	127/27 (n=154)
Age	10.5 ± 3.0 (n=154)
Primary enuresis	126/135 (93 %)
Urgency, presence of	74/139 (53%)
Previous daytime incontinence, presence of	71/153 (46%)
Previous constipation, presence of	39/152 (26%)
Previous fecal incontinence, presence of	19/150 (13%)
Daytime voiding frequency	5.7 ± 1.8 (n= 147)
Average voided volume (first morning void included)*	41 ± 15 (n= 146)
Average voided volume (first morning void excluded)*	40 ± 15 (n= 147)
Maximum voided volume (first morning void included)*	73 ± 28 (n= 146)
Maximum voided volume (first morning void excluded)*	66 ± 26 (n= 147)
Enuresis volume*	59 ± 35 (n= 132)
Nocturnal urine production during wet nights*	108 ± 47 (n= 127)

Data presented are either proportions or mean ± 1 SD

\*All volumes and nocturnal urine production are expressed as percentages of expected bladder capacity for age.

Not all children who still experienced wet nights with anticholinergic treatment proceeded to antidepressant therapy, since some families did not feel comfortable with such medication. Some children for whom anticholinergics were contraindicated were given antidepressants as their first second-line treatment. The treatment strategy is shown in Figure 5.

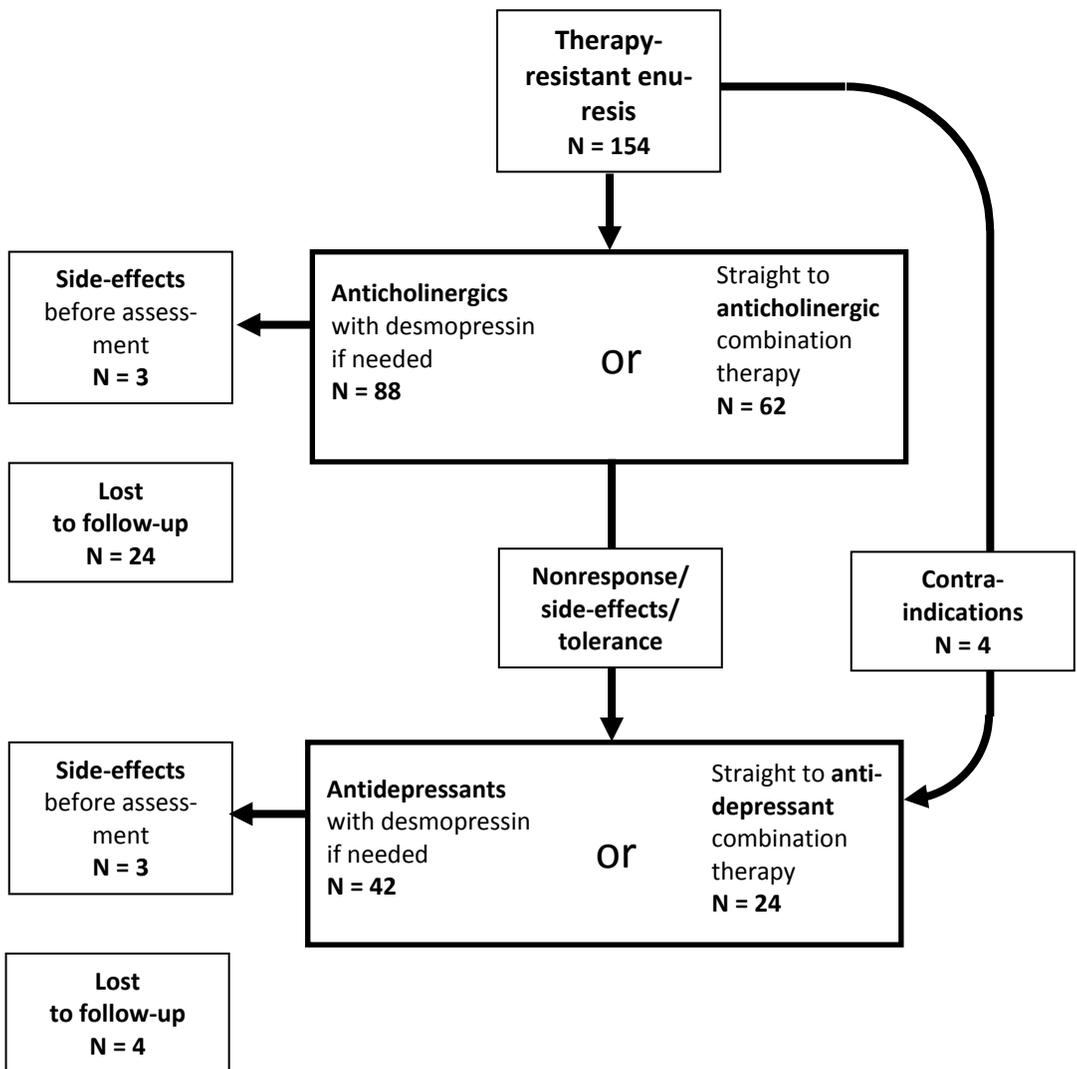


Figure 5. Treatment strategy

Of the 123 patients who could be evaluated with regard to anticholinergic treatment response 47 experienced at least a substantial and useful reduction of wet nights. Of the corresponding 59 patients with antidepressant medication 40 had a similar effect. Three patients with anticholinergics and four with antidepressants discontinued treatment prior to assessment due to side effects. Of the families offered antidepressants 41 declined due to concerns about possible side effects.

For reasons such as previous partial desmopressin response or the patients' desire for speediest possible dryness, 62 patients went straight to desmopressin combination treatment with anticholinergics, and 24 with antidepressant medication. Of these patients, 39 left no further report as to whether desmopressin made any difference in treatment response.

Few and inconsistent differences were found between the groups responding or not responding to the various treatment regimens, and this was true both for anamnestic and voiding chart data (Table 9). The only statistically significant findings were that responders to antidepressant therapy were older than non-responders ( $p=0.013$ ), and patients who benefited from addition of desmopressin had a higher micturition frequency than those who did not ( $p=0.027$ ).

Surprisingly, the children who needed desmopressin as part of combination treatment to become dry did not have significantly higher nocturnal urine production than those who had no such benefit ( $p=0.619$ ). Equally surprisingly, neither the presence of urgency nor a history of previous daytime incontinence was significantly more common in children responding to anticholinergics ( $p = 0.375$  and  $0.072$ , respectively).

Table 9. History data and voiding chart results according to response to therapy

	Anticholinergic response		p	N	Antidepressant response		p	N	Desmopressin benefit		p	N
	yes	no			yes	no			yes	no		
Urgency	24/39	38/72	0.38	111	14/36	10/16	0.12	52	16/26	26/50	0.43	76
Previous daytime incontinence	27/47	31/76	0.07	123	18/40	8/19	0.83	59	15/31	22/55	0.45	86
Micturition frequency	5.9±1.5	5.6±1.7	0.30	119	5.5±1.6	5.5±2.0	0.91	57	6.3±1.55	5.5±1.7	0.03	82
AVV+m*	42±16	38±12	0.12	119	42±13	39±8.7	0.39	56	40±18	40±13	0.85	82
AVV-m*	40±15	37±12	0.19	120	41±14	3±9 10	0.54	57	38±17	39±13	0.06	83
MVV+m*	76±28	68±22	0.06	119	72±24	73±20	0.79	56	71±26	72±25	0.79	82
MVV-m*	66±27	62±23	0.43	120	64±26	68±20	0.51	57	59±23	67±25	0.14	83
Enuvol*	55±38	63±36	0.33	103	56±32	77±56	0.11	46	63±41	63±36	0.97	70
NUP*	108±54	109±48	0.93	101	103±49	121±62	0.29	45	113±65	107±43	0.62	69

Data presented are either proportions or mean ± 1 SD

\* all six volume variables expressed as percentages of expected bladder capacity for age. AVV+m average voided volumes, first morning void included, AVV-m average voided volumes, first morning void excluded; MVV+m maximum voided volumes first morning void included, MVV-m maximum voided volumes first morning void excluded; Enuvol enuresis volume; NUP nocturnal urine production

## 6 Discussion

The focus of this thesis has been therapy-resistant enuresis. We have, in one open and one randomized, controlled study, evaluated the non-cardiotoxic antidepressant reboxetine as a potential new second-line therapy, and looked at the value of anamnestic data and the voiding chart as prognostic indicators. We found that reboxetine is indeed at least moderately useful in this patient group but that neither anamnestic data nor the voiding chart gives much prognostic information in enuretic children needing second-line therapy.

### 6.1 Reboxetine antienuretic effect

Of the 61 children presented in study I who had received treatment with reboxetine at our center 52% responded to this treatment.

Since this study is a retrospective evaluation of patients assessed and treated in the line of clinical practice, it has obvious limitations. Although all children had recorded day-time micturition habits, voided volumes and nocturnal urine production measurements could be obtained from only a minority of them. As a consequence, all patients could not be assessed properly regarding pathogenetic factors such as detrusor function and nocturnal urine production. Evaluation of the third relevant pathogenetic factor – arousal mechanisms – demands complicated polysomnographic examinations.

This study was neither blinded nor placebo-controlled, which of course means that bias and placebo effects cannot be ruled out as possible explanations to the therapeutic effect. However, since the patients and their families reported whether or not the child became dry the risk of reporting bias should be somewhat less than with a more subjective outcome variable. The fact that the families were not asked to record the exact number of dry and wet nights before and after treatment is another limitation. The results of the study would have been easier to compare with those of other studies, had this been done. Since therapy response was obtained after approximately one month of treatment, placebo effects were deemed unlikely, but could of course not be ruled out. And the lack of an untreated control group makes it difficult to know how many children would have become dry without treatment.

Still, the results of this study indicated that reboxetine might be a treatment option for some patients with therapy-resistant enuresis, and encouraged us to proceed with the randomized placebo-controlled trial, i.e. study II.

In this trial we found that patients treated with reboxetine – with or without added desmopressin – had significantly fewer wet nights than those treated with placebo, although only one child became completely dry. Three children had an intermediate treatment response.

As this study was randomized, placebo-controlled and double-blinded it provided much stronger evidence.

Since recruitment of children for this study was expected to be ongoing for several years it was decided that if a family, after completion of all three treatment periods, wanted to continue with reboxetine therapy, the blinding for this patient could be removed even though the trial had not been completed for all patients. This strategy, although slightly unorthodox, was approved by the Ethics Review Board as well as the Medical Products Agency. It may of course be argued that this might lead to investigator bias when subsequent children were recruited. It turned out, however, that although thirteen children were given open-label reboxetine after the randomized study, only three families insisted on having the blinding removed. And in no case, of course, was the randomization order revealed to the researchers before all three treatment periods had been completed. Thus, we believe that the possible bias was unimportant.

Treatment with reboxetine was not as effective as our pilot studies [101] (study I) gave us reason to hope. Only one child became completely dry with the dosage given during the randomized study. We decided to use the low dosage of 4 mg in order to avoid side effects to the largest extent possible. However, the correlation between dosage and side effects is not proven, but only assumed on the basis of general clinical experience. After completion of the study the patients who had responded to at least one treatment period were offered reboxetine 8 mg, with desmopressin added if needed. With this treatment four more children became completely dry, and another two had just three or four wet nights per month. This indicates that the drug may be more effective when the higher dose is given.

Another possible explanation for the better treatment effect in our previous retrospective studies may be that partial responders to desmopressin may have been included in those trials, but not in the present one.

Side effects did occur in a minority of patients, but they were not serious, and we already know from other studies that the drug is not cardiotoxic [99].

Still, mood swings – the most frequent side effect experienced by the study patients as well as by those with reboxetine prescribed off-label – are often bothersome and limit the usefulness of the drug. In our clinical practice we have observed that development of tolerance is common after a few months of treatment, but that the therapeutic effect can be maintained with regular drug-free intervals.

The observation – although not based on randomized comparisons – that reboxetine seems less effective than imipramine leads to the question whether imipramine’s serotonergic action contributes to the substance’s effect, as both drugs share the same noradrenergic characteristics.

## 6.2 Effects of antidepressant therapy on voiding chart data

In the randomized placebo-controlled study (study II) we also sought to investigate whether reboxetine had any effect on variables reflecting bladder function and nocturnal urine production (study III). We found this not to be the case.

The obvious drawback of this study is that the lack of differences found may very well be due to small sample size. The power calculations were made based on treatment response, not on effects on voiding chart data. When it comes to the question at hand in the present analyses, the sample size is too small to exclude true differences between the treatment periods.

Still, the lack of reboxetine effects on voiding chart data, if true may, have several different explanations, one of which is that the anti-enuretic effect of reboxetine is explained by the drug’s noradrenergic effects on arousal mechanisms in the LC.

We can also speculate as to whether a higher dosage would have given a different result. In this study we decided to use the lower dosage of 4 mg. As mentioned above, in our clinical practice we have experienced that response rate is improved when the dose is increased to 8 mg. The question is whether this would be reflected in a significant effect of reboxetine treatment on bladder function or nocturnal urine production.

There is only slight evidence of the accuracy of the voiding chart in reflecting daytime bladder function in patients with neither daytime incontinence nor severe urgency [41]. In study II (and thus III), several parents of the patients commented that their children voided less frequently than usual, in

order to avoid the procedure of measuring the voided volumes. Furthermore, there is even more scanty evidence that the registration of daytime voiding habits can be used to detect nocturnal detrusor overactivity. [42] It is thus not ascertained that the lack of reboxetine influence on the bladder variables actually precludes that the drug can indeed affect detrusor function.

The lack of difference in nocturnal urine production between treatment periods with and without desmopressin may indicate that nocturnal polyuria is of minor importance in therapy-resistant enuresis.

### 6.3 Prognostic indicators

Our first two studies gave us no way to predict response to treatment with reboxetine, in monotherapy or combined with desmopressin. In study IV we looked at a substantially larger sample of children receiving either antidepressant and/or anticholinergic therapy. Still, no clear and consistent differences in either anamnestic factors or voiding chart data were found between the patients responding or not responding to the various treatment regimens. Small voided volumes and/or increased voiding frequency, as an indicator of underlying detrusor overactivity, could have been expected to predict anticholinergic response, but this was not the case. Not even urgency could predict anticholinergic efficacy.

This study was not a randomized trial but a retrospective evaluation of clinical reality. We wanted to look at ordinary therapy-resistant enuretic children, evaluated and treated according to modern guidelines, and see if their background data gave any information regarding treatment success. The obvious drawback of this strategy is that the patient group is heterogeneous and not meticulously evaluated – only as much as medically required – and some data regarding drug dosage or desmopressin addition is missing. On the other hand, the asset is that the material does reflect ordinary families, not just the ones who are willing and able to take part in elaborate clinical trials.

Another possible limitation may be the choice of drugs, particularly the use of reboxetine in the group who received antidepressant therapy. The reason for our choice of antidepressant was threefold: 1) we wanted to avoid the cardiotoxicity of imipramine, 2) imipramine is no longer available in Sweden, and 3) we have, as described above, found that reboxetine is better than placebo in the relevant patient group (study II). Furthermore, we see no reason that the predictive value of voiding chart data or anamnestic factors would differ between the two alternatives. In earlier research on imipramine no such prognostic indicators were found [73].

Likewise, it may be questioned whether our choice of anticholinergic – tolterodine – may have influenced the results in any direction. Perhaps more useful prognostic indicators would have been gained if oxybutynin had been chosen? We doubt this. First, tolterodine is the only anticholinergic with proven efficacy in the situation at hand, i.e. therapy-resistant enuresis [83]. Second, we have no reason to suspect that the potential antienuretic mechanisms of the two alternatives differ.

It should be noted that the sample size of non-responders to antidepressant therapy in study IV was too small to confidently conclude that the absence of differences observed was true.

The fact that the antidepressant responders were older than nonresponders may reflect that the older children were on their way towards spontaneous remission and the drug just hastened this process. This effect would be easier to discern in the children given antidepressants, since this therapy is usually given after anticholinergics have been tried. In addition, any treatment given to older children with enuresis is more likely to coincide in time with spontaneous remission.

The voiding chart is a much used tool in the assessment of children with nocturnal enuresis. It has been conclusively shown that nocturnal polyuria combined with normal daytime voided volumes predict a high chance of success with desmopressin treatment in treatment-naïve patients [102]. But apart from that the prognostic value of the voiding chart is much less well established. Most crucially, we do not know if low voided volumes and/or high micturition frequency predicts response to anticholinergics. To our knowledge, no previous investigation of the prognostic value of the voiding chart in patients with therapy-resistant enuresis has been performed.

The higher daytime micturition frequency found in children who benefited from desmopressin combination therapy is hard to explain, but since it was not accompanied by decreased voided volumes we do not take the finding as indicative of detrusor overactivity. More likely this is either a chance finding or reflect that they for some reason drink more than the other enuretic children.

Somewhat surprisingly, no association between nocturnal polyuria and desmopressin benefit was found. The children who needed desmopressin in order to achieve a favorable response to anticholinergics or antidepressants did not have higher nocturnal urine production than those who had no such benefit. This is yet another finding regarding desmopressin benefit that is difficult to explain. Either the desmopressin-induced decrease of urine production, and thus decreased bladder distension, indirectly diminished the

detrusor's tendency to contract or desmopressin had effects on sleep and arousal.

The main value of the voiding chart appears to be what is already known, i.e. that nocturnal polyuria can predict whether desmopressin will be an effective first-line treatment [102]. In our clinical practice we have also found (although not scientifically proven) that the chart may constitute a valuable tool to assess the families' ability to comply with labor-intensive instructions: the family who finds it difficult to complete a full voiding chart will also find it difficult to use the enuresis alarm accurately or comply with standard urotherapy. Apart from this, the predictive value of the voiding chart in enuresis may not be that great. There is no evidence that daytime voiding chart data give any information regarding night-time bladder function. In a recent study from our center, we observed that the actual voided volume at the time of enuresis correlated very poorly with daytime voided volumes [42]. The results of the present study should be confirmed in a larger and prospective study, but perhaps daytime voiding chart data reflect behavior more than bladder function.

## 7 Conclusions and clinical implications

We have, in first an open pilot investigation and then a proper randomized, placebo-controlled trial, found the noradrenergic antidepressant reboxetine to be a safe and potentially useful treatment alternative for children with therapy-resistant enuresis, in mono-therapy or combined with desmopressin. The main importance of this finding is that it provides a noncardiotoxic alternative to imipramine. Still, most children did not become completely dry by the treatment.

We also investigated the role of history and the voiding chart – the recognized cornerstones in the evaluation of enuretic children – in predicting response to second-line therapy. We found the usefulness of these data to be frustratingly limited, regarding both anticholinergic and antidepressant therapy as well as these drugs combined with desmopressin. Thus, with the standard tools of investigation we have, as yet, no way of individualizing management by predicting which child with therapy-resistant enuresis will benefit from which treatment. This means that we should continue to start with the least toxic alternative, i.e. anticholinergics.

Our results also provided grounds for speculations about enuresis pathogenesis, antienuretic therapy mechanisms and investigative procedures that may, if proven true, have clinical implications. Serotonergic as well as noradrenergic pathways may both, as well as urine production, be important in enuresis pathogenesis, and greater treatment success may possibly be reached when all these are modified. And the voiding chart may not be an adequate way to evaluate nocturnal bladder function.

## 8 Further research

Considering the short- and longterm consequences of untreated enuresis, there is still a need to provide therapy-resistant patients with efficient treatment. As mentioned above, the noradrenergic antidepressant reboxetine may become such a treatment, but further trials with higher doses and larger study populations are needed.

To shed further light on the mechanism explaining the therapeutic effect of reboxetin and other antidepressants a study including ambulatory polysomnography during treatment would be useful. We also need to intensify the search for prognostic indicators that can make it possible to tailor treatment to the individual and thus decrease the number of frustrating failures for the families. Since the voiding chart may not live up to expectations, we need clinically useful indicators for detrusor overactivity. There is some evidence to suspect that the ultrasonographical measurement of bladder wall thickness [103, 104] or the biomarker urinary growth factor [105, 106] may fill such a role. Inclusion of these measurements in prospective, randomized studies of anticholinergic treatment in therapy-resistant enuresis could prove fruitful.

## 9 Populärvetenskaplig sammanfattning på svenska

Nattväta, dvs att efter sex års ålder kissa på sig nattetid, är ett vanligt tillstånd som bland sju-åringar förekommer hos 5-10%. Av tonåringar har upp till 1% kvar problemet. Det är av oklar anledning betydligt vanligare bland pojkar än bland flickor. De allra flesta av de drabbade blir spontant torra före 18 års ålder, men trots det riskerar obehandlad nattväta få svåra och långsiktiga konsekvenser, bla för självkänslan.

Nattväta behandlas i första hand antingen med läkemedlet desmopressin, som minskar den urinmängd som bildas under natten, eller med ett larm som utlöses när det blir blött i säng eller underkläder. Det är dock upp mot 25% av barnen och ungdomarna som inte har effekt av någon av dessa behandlingar. Man provar då ett läkemedel som har en lugnande effekt på blåsan, men det är vanligt att dessa läkemedel också är ineffektiva. I sista hand brukar man – enligt internationella rekommendationer – prova vissa typer av äldre antidepressiva läkemedel. Varför dessa hos många patienter har effekt även mot nattväta är inte klarlagt, men det beror inte på att nattväta har psykiatrisk orsak. Dessa äldre läkemedel är dock mycket farliga att ge i för hög dos, och i delar av världen, bla i Sverige, finns de inte längre att tillgå.

I det här projektet har vi därför försökt finna ett modernare och säkrare läkemedel för de barn och ungdomar som inte har effekt av någon av de rekommenderade behandlingarna. Vi har undersökt om läkemedlet reboxetine, som även det är antidepressivt men utan de behandlingsrisker som följt med de äldre preparaten, kan vara ett behandlingsalternativ för dessa patienter. I en första utvärdering av de barn och ungdomar som dittills behandlats på Akademiska barnsjukhuset visade sig ca hälften ha effekt, antingen av reboxetine ensamt, eller i kombination med ovan nämnda desmopressin. Vi gick då vidare med en sk randomiserad, dubbel-blind studie som ger betydligt mer tillförlitliga resultat. I den kunde vi statistiskt säkerställa att reboxetine minskade antalet våta nätter betydligt bättre än placebo (sk sockerpiller), men däremot var det mycket få som blev helt torra. Vidare studier behövs därför för att kunna ta reda på om det berodde på t ex för låg dos, eller om inte heller detta läkemedel har tillräcklig effekt för de allra mest svårbehandlade.

Vi har i projektet också undersökt om det finns något bra sätt att förutspå vilka barn som har bäst effekt av respektive läkemedel. Det internationellt rekommenderade är att familjerna fyller i en sk kiss-lista, vilket innebär att de under två dygn dokumenterar hur ofta och hur mycket barnet kissar på dagen. De ombeds också väga våta liggunderlägg. Från dessa uppgifter kan sedan behandlande läkare bedöma hur barnets urinblåsa verkar fungera dagtid, och beräkna mängden urin som bildas nattetid. Tanken är sedan att man utifrån det ska kunna avgöra vilken behandling som blir bäst för det enskilda barnet. Problemet är dock att det inte finns några bevis för de samband man bygger bedömningen på, och alltså inte för att metoden fungerar.

I en studie med 154 barn och ungdomar som behandlats för svårbehandlad nattväta på Akademiska Barnsjukhuset, utvärderade vi om det fanns något statistiskt samband mellan de olika fynden från patienternas kiss-listor och den eventuella effekt de sedan hade av den behandling de provade. Tyvärr fann vi i denna studie inget sådant samband. Vidare forskning behövs för att bekräfta detta, men den samlade forskning som finns idag talar för att man för de flesta barn i första hand bör prova det ofarligaste läkemedelsalternativet först, om det inte finns något specifikt som talar emot detta hos det enskilda barnet. Det behövs också vidare forskning kring andra metoder att förutspå behandlings-effekt.

## 10 Acknowledgements

Många är de som på olika sätt bidragit till att jag – till sist – ändå fick min avhandling klar. Jag vill särskilt tacka

**Alla barn och föräldrar** som deltagit i våra studier, inte minst behandlingsstudien. Utan er ingen forskning!

**Tryggve Nevéus**, min huvudhandledare, för allt ditt driv, din gedigna ämneskunskap och entusiasm. Och FÖRSTÅS (här finns det inga originalitetspoäng att hämta...): dina alltid lika snabba svar på mina mail-frågor! And for a shared love for the English language!

**Arne Stenberg**, min bihandledare och medförfattare, för dina så kloka synpunkter i alla lägen, och den trygghet du utgjort. Förlåt att disputationen blev så tätt inpå hummer-premiären!

**Bruno Hägglöf**, min medförfattare, för din kunskap och input kring allt från enuresens konsekvenser till antidepressiva läkemedel. Och för allt ditt stöd för vår forskning!

**Birgitta Karanikas**, forsknings-sjuksköterska, tidigare arbetskamrat på barnnjur-mottagningen och vän, för ditt noggranna handhavande av våra studiepatienter, och så mycket stöd, pepp och goda råd. Jag glömmer inte ”Dagen D” i Boston! Mycket skoj har vi haft, både i samband med forskningen och helt vid sidan av den.....

**Lena Hellström-Westas** och **Kjell Alving**, tidigare och nuvarande studie-rektorer för forskarutbildningen, för goda råd i kniviga situationer.

**Martin Selinus**, **Hanna Taylor**, **Hans Lindgren** och **Kerstin Ståhlberg**, för tålmodig hjälp med administrativa spørsmål, datorer och finanser.

Alla statistiker på **Uppsala Clinical Research Center**, för att ni finns.....

Betygsnämnden vid min halvtids-kontroll för ovärderliga synpunkter: **Anna Bjerre**, **Åsa Neumann** (som dessutom ställde upp med väldigt kort varsel)

och **Mia Herthelius**, vars insats som ordförande - inkl M&M's ! – varit så värdefull i det fortsatta arbetet.

**Tina Dahlgren** och inte minst **Monica Mäki Karlstrand**, min tidigare och nuvarande chef på Sektionen för Barns Vård och Hälsa, har - i en mycket pressad klinisk verksamhet – sett värdet i att vi producerar vetenskap också, och varit så generösa med att bevilja ledig tid för forskning, även i tider då dess finansiering varit god.

**Alla medarbetare** på Uppsala Barnspecialistmottagning och Sektionen för Barns Vård och Hälsa, för allt samarbete och ”vardags-samvaro”, OCH för att ni ställt upp vid - och stått ut med - min frånvaro för forskningen. TACK!

**Carola Engström**, medicinsk sekreterare på Sektionen för Barns Vård och Hälsa, för att du förebyggt mycken oro genom att på ett så ypperligt sätt hålla reda på min väntelista under tider med sparsam klinisk tjänstgöring. Och för så gott samarbete genom många år nu.....

Inspirerad av min Barnsjukhus-kollega Åsa Myreliid önskade jag mig länge en ”forsknings-storasyster”. **Charlotte Heijkenskjöld Rentzhog** blev mot slutet av forskar-utbildningen en värdefull sådan.

**Lillemor Berntson**, en gång min ST-handledare och mentor i Falun, nu kollega på Barnsjukhuset, är fortfarande den jag gärna vänder mig till när jag behöver råd i de allra svåraste kliniska situationerna, de som ställer stora krav på både omdöme och klokhet. Och som på senare år funnits där till ovärderlig tröst och stöd när livets allra största frågor ställts på sin spets.

**Gunnar Bergman**, kollega på Barnsjukhuset och en gång på Barnkliniken i Sundsvall, lotsade mig fram under mina första steg på den barn-nefrologiska banan. Tack för alla goda råd i stort och smått, och för att du öppnade ert hem för mig när min själ var lite stukad efter en trafikolycka.

Jag har genom år och arbetsplatsbyten byggt upp ett professionellt kvinnligt nätverk, där flera också blivit kära vänner. Dit hör förutom ovan nämnda **Charlotte** och **Lillemor**: **Susanna** (även nämnd nedan), **Eva H**, **Kerstin S** och **Ruzica**. Och så dök min gamla arbetskamrat från Falun **Malin B** plötsligt upp som doktorand-kollega i forskargruppen!

Alla **grannar och andra Blidö-bor** - ingen nämnd, ingen glömd – ger mig ett sammanhang, att landa, finna kraft och glädje i.

**Marianne** och **Jan-Erik**. Ibland blir grannar de allra käraste vänner.

Min farmor **Edla Karlsson** gick bort för många, många år sedan, men är fortfarande central för min ”inre kompass”, så viktig i såväl forskning som livet i stort.

Jag är så tacksam och glad att jag har resten av **släkten Karlsson** med sina allt fler förgreningar, som också ger ett så viktigt sammanhang.

**Min mor Birgitta.** Tack för allt ditt stöd, såväl med råd från din egen forskarbana inom annan disciplin, som praktiskt när livet plötsligt kärvade till sig rejält. Utan dig hade det faktiskt inte gått!

**Pappa,** för allt du gav, och allt du var, för mig. Jag önskar så att vi hade fått dela den här resans målgång också!

Flera riktigt nära vänner har jag tappat bort längs vägen, men desto tacksammare är jag över de som hängt kvar, och som finns där när det verkligen gäller: **Susanna, Peter** och **Lena.**

The studies in this thesis were kindly supported by the Gillbergiska foundation, the Jerring foundation, the Kronprinsessan Lovisa foundation and the Samariten foundation.

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