REDUCING ANTICHOLINERGIC BURDEN IN A FRAIL ELDERLY POPULATION

Translating the inappropriateness of anticholinergic drugs into patient-related outcomes

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(Hege Kersten 2012, inspirert av Olav O Hauge/Valkyrien Allstars og Arthur Arntzen)

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PREFACE AND ACKNOWLEDGEMENTS

My source of inspiration for this thesis has been the intention to translate reduced drug risks into patient-related improvements. The research process began with a pilot project in 2007, went through a long phase of planning, applications, and preparation, before the first patient was included in September 2008. Running a randomized controlled trial in 21 centers, including very elderly frail participants, has been challenging and would not have been possible without the trustful assistance of our engaged research nurse, Inga Kristin Tolo. We both learned a lot from all the unforeseen events that had to be solved during the conduction. Analyzing and reporting this research project has been a stimulating, but also a demanding, educational process. My utmost gratitude goes to my three deeply respected supervisors who have guided me and extended their valuable expertise and assistance from the design of the study to the completion of this dissertation.

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ABBREVIATIONS

AA	Anticholinergic Activity		IQCODE	Informant Questionannaire on Cognitive Decline in the Elderly
ACB	Anticholinergic Cognitive Burden		MDRD	Modification of Diet in Renal Disease
ADE	Adverse Drug Events			
ADL	Activity of Daily Living		PCR	Polymerase Chain Reaction
ADR	Adverse Drug Reaction		PM	Poor Metabolizers
ADS	Anticholinergic Drug Score		EM	Extensive metabolizers
ANCOVA	Analysis of Covariance		MMSE	Mini Mental State Examination
ARS	Anticholinergic risk scale		NPI-NH	Neuropsychiatric Inventory-Nursing Home version
BI	Barthels Index			
CAM	Confusion Assessment Method		PgP	Permeability glycoprotein
CDR	Clinical Dementia Rate		RCT	Randomized Controlled Trial
CERAD	Consortium to Establish a Registry of Alzheimer's Disease		SAA	Serum Anticholinergic Activity
			SIB	Severe Impairment Battery
CCI	Charlson Comorbidity Index		^{3}H-QNB	tritiated quinuclidinyl benzilate
CNS	Central Nerve System		AB	Anticholinergic Burden
DBI	Drug Burden Index		USD	Urinary Spasmolytic Drugs
DRP	Drug Related Problem		PRADA	Pharmacist-initiated Reduction of Anticholinergic Drug Activity
GFR	Glomerular Filtration Rate			
IDP	Inappropriate Drug Prescription			

CONTENTS

SUMMARY

Background: Evidence-based knowledge of drug safety in geriatric patients with polypharmacy is limited, but the focus on risk assessment tools is increasing. The risks related to pharmacological treatment are often assessed by the number of inappropriate drug prescriptions. Anticholinergic drugs display a high risk of adverse effects and are denoted as inappropriate in elderly people. The overall objective of this thesis was to achieve better clinical understanding of the risks related to anticholinergic drugs in frail elderly patients.

Anticholinergic drugs are prescribed to treat a variety of medical conditions such as psychotic disorders, depression, sleeping disorders, and urinary incontinence. Despite that the overall use of anticholinergic drugs has decreased over the past decades, drugs used to treat urinary incontinence will become increasingly prevalent as the elderly population increases. Anticholinergic drugs acts towards muscarinic receptors in the central and peripheral nervous system and inhibit acetylcholine mediated responses through competitive receptor binding. Anticholinergic adverse effects are caused by the unselective muscarinic antagonism and give symptoms that are easily misinterpreted as age-related complaints, e.g. dry mouth, constipation and reduced memory. Many drugs have shown competitive binding activity towards muscarinic rat brain receptors *in vitro* and have potential anticholinergic activity (AA) *in vivo*. The AA of drugs is previously rated with an ordinal 4-point scale, and the sum of each drugs' AA score expresses the overall anticholinergic burden of a patient. A high anticholinergic drug scale (ADS) score has been associated with increased risk of cognitive and functional impairments in many observational studies. However, little is known about the pharmacodynamic interactions between potentially anticholinergic drugs used concurrently. There is also a lack of evidence-based knowledge of the clinical effects of a reduced anticholinergic burden. Four studies are included in this thesis: one in vitro study, two clinical studies with cross-sectional design, and one randomized control trial (ClinicalTrials.gov; NCT00854438). All studies were targeted to gain knowledge of anticholinergic drug safety.

Methods: We developed and validated a high-throughput version of the published radio receptor bioassay used for determination of the AA of individual drugs. The method is described in the in vitro study, but was also used to measure AA in the patients' serum in the three clinical studies. In the in vitro study, we used the bioassay to compare the potential risk of central anticholinergic adverse effects between five urinary spasmolytic drugs (USDs) used to treat the symptoms of overactive bladder.

We conducted a single-blinded randomized controlled trial in order to investigate the clinical effects of a reduced anticholinergic burden in nursing home residents with an ADS score ≥ 3 and no severe dementia. The participants were recruited from 22 nursing homes and randomly allocated (1:1) to intervention or control. The intervention was a pharmacist-initiated reduction of ADS score after multidisciplinary drug reviews. Primary end-point was the CERAD-10 wordlist test for immediate recall. Secondary end-points were MMSE, delayed recall and recognition of words (CERAD), saliva flow and serum anticholinergic activity. The participants were re-tested after 4 and 8 weeks, and the study groups were compared after adjusting for baseline differences. At baseline we sampled blood for pharmacogenetic analyses of hepatic enzymes involved in the metabolism of anticholinergic drugs. The baseline data were used to investigate whether patients' serum AA were related to their genotype-determined activity in the drug-metabolising CYP2D6/2C19 enzymes. We also used the baseline data to evaluate the additive properties of the ADS score model by investigating whether increasing ADS scores above 3 were associated with a gradual decline in cognitive function.

Findings: In our *in vitro*-based risk assessment of the five USDs marketed in Norway, we found that darifenacine had the lowest potential for central anticholinergic side-effects, while the active metabolite of the prodrug fesoterodine, 5-HMT, and tolterodine were shown to have the highest detectable AA.

The cross-sectional evaluation of the ADS score model did not support a progressive decline in cognitive function with ADS score above 3. However, ADS scores above 5 were associated with reduced saliva flow and elevated AA in serum.

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The interventional study showed that the pharmacist-initiated drug changes significantly reduced the patients' ADS scores with two units, whereas the score remained unchanged in the control group. This reduction did not translate into significant improvements of cognitive function or peripheral anticholinergic symptoms compared to the control group.

The preliminary findings from the genotyping suggested that elderly poor metabolizers of CYP2D6/2C19 are at higher risk of elevated serum AA than extensive metabolizers. Whether poor metabolizers are more prone to experience adverse anticholinergic effects, needs to be further studied in larger patient samples.

Conclusion: There are many determinants of the central anticholinergic drug response and the anticholinergic burden in the brain is difficult to predict in elderly patients using multiple drugs. The clinical utility of the anticholinergic risk assessment tools might be limited by the simplification of complex pharmacological mechanisms into compulsive categories without considering the high intra-individual differences in drug response in elderly people.

Our findings need to be confirmed in larger study samples, and there is a general need for more interventional studies in pharmacological geriatric research.

LIST OF PAPERS

I. Stine Mjåvatn Jakobsen, Hege Kersten, Espen Molden.
    Evaluation of brain anticholinergic activities of urinary spasmolytic drugs
    using a high-throughput radio receptor bioassay
    J Am Geriatr Soc. 2011. 59(3); 501–505.

II. Hege Kersten, Espen Molden, Tiril Willumsen, Knut Engedal,
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    Higher anticholinergic drug scale (ADS) scores are associated with peripheral
    but not cognitive markers of cholinergic blockade. Cross sectional data from
    21 Norwegian nursing homes.
    Br J Clin Pharmacol. 2012 Aug 26. doi: 10.1111/j.1365-2125.2012.04411.x

III. Hege Kersten, Espen Molden, Inga Kristin Tolo, Eva Skovlund, Knut Engedal,
     Torgeir Bruun Wyller.
     Cognitive effects of reducing anticholinergic drug burden in a frail elderly
     population: a randomized controlled trial.
     J Gerontol A Biol Sci Med Sci. 2012 Sep 14. doi: 10.1093/gerona/gls176

IV. Hege Kersten, Torgeir Bruun Wyller, Espen Molden,
    Poor-metabolizing phenotype of CYP2D6/2C19 is associated with elevated serum
    anticholinergic activity in elderly patients
    Submitted

INTRODUCTION

A DRUG CRESCENDO

Never before have physicians had such an abundance of prescribing options, and elderly people are taking more medications than ever. The use of medications among the elderly is disproportionate in relation to their population share. In Norway, people above 65 years comprise only 15% of the population, but use approximately 50% of all prescribed drugs (1). The population share of elderly (≥ 65 years) is growing faster than any other age segments, and the number of people above 80 years increases most rapidly. 4.5% (221 000) of the Norwegian population are 80 years or older and the number is expected to double within the next 30 years (2). Old age is accompanied by an increased likelihood of illness and several chronic diseases indicate prescription of multiple medications. Furthermore, the availability and use of drugs to prevent, treat, or relieve pathological or age-related complaints continue to rise. A Finnish cohort study of medicine use among the elderly reported that the prevalence of older people using more than 5 medicines had increased to 67% from 1998 to 2003 and the proportion using 10 or more medications had increased to 28% (3). Accordingly, a rapid increment in the prevalence of older people receiving multiple concomitant drugs is seen in Norway and other countries (2,4,5). As the population is aging and the number of drugs taken by aged people still increases, a crescendo in the drug market can be expected over the next decades.

POLYPHARMACY

Various terms are used to define polypharmacy in the literature. Commonly, polypharmacy is defined either in terms of inappropriate medication intake e.g. "the administration of more medicines than clinically indicated, representing unnecessary drug use" (6), or according to the number of concomitant medication used, e.g. the use of five or more medications concurrently (7). An increasing number of drugs taken daily is associated with a

number of negative health outcomes, but on the other hand prescribing of multiple medications may be required to achieve appropriate treatment of many chronic medical conditions. To ensure that under-treatment is not neglected in the elderly, it is important to look beyond a simple medication count when evaluating the drug therapy. A commonly used quality indicator of appropriate polypharmacy is drug related problems (DRP)s (8). A DRP is defined by the Pharmaceutical Care Network Europe, as «an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes» (9). The definition of DRPs encompasses potentially and manifested problems caused by inappropriate prescriptions, unnecessary drug-use and under-treatment. It has been shown that the number of DRPs such as adverse drug reactions (ADR), unfavorable drug-drug interactions and poor adherence to the drug regimes, increases approximately linearly with the number of drugs used (10,11). Interestingly, it has also been shown that in geriatric patients receiving more than five drugs concurrently, the probability of under-prescription increased significantly with the number of drugs prescribed (12). For the purpose of this thesis, polypharmacy is discussed according to the definition of concomitant use of five or more medications.

POLYPHARMACY IN THE ELDERLY

The etiology of polypharmacy in the elderly is multifactorial. Application of disease-specific prescription guidelines to older patients diagnosed with several chronic medical conditions might result in excessive polypharmacy. Additionally, older people are the major users of complementary and alternative medicines (13,14). Polypharmacy is of special concern in the elderly because older people are at higher risk for adverse drug events (ADEs) which are often more severe than in younger individuals. ADE is defined as an injury caused by the drug therapy – and may result from adverse drug reactions (ADR) or medication errors. Between 5% and 10% of hospital admissions among older people are related to ADEs (15). Polypharmacy is associated with a significant decline in activity of daily living (ADL), increased risk of hospitalization, and mortality (16-19). It is also reported that polypharmacy represents a markedly increment in medical costs (20). Hence, reducing unnecessary polypharmacy is an essential medication safety issue in the elderly and interventions

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designed to improve appropriateness of multiple drug regimes in older people is of major importance (8)

POLYPHARMACY IN NURSING HOMES

Older people in long-term care are of increased risk of receiving multiple drug regimes and the prevalence of polypharmacy among nursing home residents is significantly higher compared with non-institutionalized elderly people (13). Norwegian nursing home residents are characterized by a mean age of 85 years and poor ADL function. Moreover, 80 % of the residents have dementia (21). The mean regular daily intake of drugs among Norwegian nursing home residents is 6-8 medications (22,23). This is in line with prevalence studies of polypharmacy among nursing home residents in other European countries (13). Polypharmacy in nursing homes is consistently associated with an increased number of potentially inappropriate drugs, negative health outcomes and increased nursing time needed for medication administration (24).

INAPPROPRIATE DRUG PRESCRIPTION

The phrase "Inappropriate drug prescription (IDP)" is another term used to express the quality of drug regimens and encompass all prescriptions that are assumed to pose more risk than benefit, particularly when safer alternatives exist (25). IDP compass misprescribing such as suboptimal dose and/or duration of drug exposure, under-prescribing of beneficial drug treatments, and unnecessary drug use (26). Judging of appropriateness includes more than pharmacological rationalism; the patients' preferences, the expectations of the relatives, the traditions for pharmacological treatment among the caregivers, and the social aspect which in its widest context embrace the attitude and tolerance for good health and disease. "Inappropriate drug prescription" is an elusive term, but as for other term indicators, it could be assessed with explicit (criterion-based) outcome measures or implicit (judgment-based) outcome measures (27). Explicit measures are usually drug-oriented and/or disease-oriented while implicit measures are usually based on information from the patient (26). Hence, the

explicit indicators of IDPs represent the evidence based perspective of the health care professionals while the implicit approach represents the individual view of disease and treatment. However, since evidence-based knowledge of efficacy and safety of drugs in older people is limited, the explicit criteria used to measure IDPs are commonly based on expert consensus. There are many lists of IDPs to elderly, but the most widely cited explicit tool is Beers' criteria while Medication Appropriateness Index is the predominantly used implicit measure (25). According to consensus-based lists, the prevalence of IDPs among the elderly is reported to be high. In Norwegian nursing homes it has been reported that 76% of the patients had potentially drug-related problems due to IDPs (28). Two more recent studies in Norway have used the Norwegian General Practice (NORGEP) criteria, which is a prescription tool based on Beer's criteria to assess IDPs in people aged 70 years or older (29). These studies reported that among elderly people acutely admitted to hospital, the number of IDPs increased from 24% to 35% during the hospital stay (30), and the number of IDPs among home-dwelling elderly was 35%, of which 65% was psychoactive medications (31). However, to be clinically relevant, the use of inappropriate prescription tools must translate into clinical outcomes relevant for geriatric patients. Relevant outcomes could be reduced rates of ADRs and falls, increased cognitive and physical function, and enhanced self-care capacity (32). Despite that consensus-based lists of IDPs in the elderly have been linked to a number of negative health outcomes in the literature, further studies in real-life settings are needed to test the clinical utility of such lists (26).

DRUG SAFETY IN THE ELDERLY

The safety and effectiveness of medications are thoroughly investigated in premarketing studies during the drug development process. The determination of drug dosage and the prediction of the risks and benefits of every new drug launched are based on the evidence from the premarketing trials. Post marketing randomized controlled trials (RCTs) are essential to evaluate the safety and efficacy of drugs in the target population. The very elderly population (≥ 80 years old) is mainly excluded from both the premarketing and post marketing RCTs and there is a discrepancy between the prevalence of elderly among the investigated population and the population actually receiving the medications (33). It is a

paradox that the underrepresentation of older patients in clinical trials occurs for the same reasons that cause the risk:benefit ratio of the drug treatment to differ greatly from that in adults as a whole. Frequently used exclusion criteria are existing disease characteristics, the use of concomitant medications, frailty and advanced age per se (34,35). In parallel, reduced homeostatic control, multiple comorbidities, and functional and cognitive status are all determinants of the overall risk:benefit ratio of multiple drug therapies and provide uncertainty to the extrapolation of results from younger and/or healthier adults to older adults. Hence, there is a gap in evidence based information regarding the overall effects of multiple medication exposure to older people with multiple illnesses. To fill the gap between evidence based knowledge and prescribing practice in the elderly, the regulatory authorities have encouraged the inclusion of older people in RCTs for the last 15 years, but data from such studies remain very scarce (36).

AGE-RELATED DIFFERENCES IN DRUG RESPONSE

The progress of aging is a heterogeneous and an individual multifactorial process depending on exposure to intrinsic and extrinsic factors over time. Accumulation of deficits in multiple organ systems and regulatory processes affect pharmacological features and homeostatic control at different rates and results in a greater variability in drug response in aged versus younger adults. Furthermore, patophysiological changes that occur in diseases that are common in advanced age provide additionally diversity in older people's drug response. The debility in compensation mechanisms to regain homeostasis after drug exposure and the general decline in physiological reserves in geriatric patients result in increased sensitivity to certain drugs and poorer recovery from ADEs.

PHARMACOKINETIC CHANGES

The aging process affects pharmacokinetic processes that alter the serum concentration of different drugs. Reduction in active transport mechanisms and first-pass metabolism might change the bioavailability of some drugs. Major changes in body composition with a relative increase in body fat, enlarges the distribution volume of lipid soluble drug, whereas the reduction in plasma protein concentration increases the free fraction of active drugs which

might be of clinical relevance for drugs with a high degree of protein binding. Yet, the most important factor for dose adjustments in elderly people is the age-related reduction in drug clearance that leads to prolonged elimination half-life of several drugs (32). This is mainly due to reduced renal excretion caused by lowered glomerular filtration rate (GFR), but age-related alterations in hepatic metabolism, foremost reduced liver size and hepatic blood flow might also prolong the drug elimination half life (37). The implication of renal and hepatic aging on drug clearance depends on the pharmacokinetic profile of the drugs (38).

PHARMACODYNAMIC CHANGES

Age-related pharmacodynamic changes are related to effectors' system function and may be explained by changes in physiological factors, receptor abundance, receptor affinity and post receptor responses, or intracellular signaling pathways. Pharmacodynamic changes influence the end-organ responses, tend to be drug-specific and for the most part the mechanisms of the age-related differences are not well defined (35). The aging process may result in both decreased and increased post receptor responses to drug exposure which in turn influence the sensitivity to drug dosage. For example, older people have shown decreased sensitivity for beta adrenergic receptor agonists and antagonists while the sensitivity for drug effects such as sedation with benzodiazepines and cognitive impairments with anticholinergics are increased (39,40).

ADVERSE DRUG REACTIONS (ADR)

The World Health Organization has defined ADRs as harmful, unintended reactions to medicines that occur at doses normally used for treatment. The risk of ADRs is a very important measure of medication exposure in the elderly because ADRs often have more serious outcomes in older than in younger adults (41). A meta-analysis has shown that the odds for elderly people to be hospitalized of ADR is 4 times higher than for younger ones, that is 16.6 % versus 4.1% (41). It has also been shown that most of these hospital admissions are avoidable, meaning that an intervention by a caregiver could have prevented the admission (42). ADRs are among the leading causes of death in many countries (43). In Norway, 18 % of the deaths in a medical department of a major hospital have been related

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to one or more medications, and the fatal ADRs were significantly associated to age, multiple diseases and polypharmacy (44).

APPROACHES TO IMPROVE DRUG SAFETY

MULTIDISCIPLINARY DRUG REVIEWS

Prescription of medicines is a predominant part of geriatric health care and should not be viewed as a solitary activity undertaken by physicians (26). A collaborative approach to prescription, involving a clinical pharmacist in structured medication reviews within the context of geriatric evaluation and management units, have shown to improve the appropriate use of medicines (45). A medication review is defined by the National Prescribing Center in UK as a "*structured critical examination of the patient medicines with the objective of reaching agreement with the patient about the continued appropriateness of the treatment, optimizing the impact of medicines, minimizing the number of medication-related problems and reducing waste*" (46). The objective of medication reviews includes both pharmacological rationalism and the patients' preferences and is an advocated method to optimize drug therapy (47).

Many studies report that clinical pharmacists identify a large amount of drug related problems (DRPs) and contribute to prevent DRPs like adverse drug reactions, drug-drug interactions and unnecessary drug use (48,49). It has also been reported that clinical pharmacists' interventions improve compliance, reduce polypharmacy, and increase prescription quality (50-53). An RCT in patients 80 years or older in Sweden reported a significant reduction in hospital re-admissions and net cost per patient in the intervention group that received pharmaceutical care at ward level throughout their hospital stay (54). Reviews of pharmacist interventions have concluded that pharmaceutical care appear to improve appropriate polypharmacy in older people in terms of reducing IPs and DRPs, but multicenter studies with larger sample sizes and reproducible interventions are needed to measure the effects on patient-related health outcomes (8). However, the health authorities

in different countries have recently emphasized clinical pharmacists as essential resources to improve medication safety in elderly people and reduce unnecessary health costs (49,50).

CLINICAL PHARMACY IN NURSING HOMES

Nursing homes encompass approximately 40 000 beds and are the largest health institutional level in Norway (22,55). Nursing home residents are characterized by multiple chronic diseases and functional and cognitive impairments for which multiple medications are prescribed. About 70 % of the residents are women and the mean age is 86 years (56). It is reported that almost 80 % of residents in nursing home care have dementia and 90 % have one or more neuropsychiatric symptoms which is a group of psychological symptoms such as depression, hallucinations, apathy and anxiety (56,57). In order to provide good medical care and services to this complex patient group, multifaceted approaches like involvement of nurses and care givers in treatment strategies, educational training in geriatrics, continuance in the physician staffing and interaction with clinical pharmacists in the prescribing and evaluation of medication treatment have been suggested (23,26). Although interaction by clinical pharmacists at the time of prescribing is not always feasible in Norwegian nursing homes, studies have reported that the involvement of clinical pharmacists in regularly multidisciplinary medication reviews improved quality of drug prescribing in nursing homes (28,58). Still, clinical pharmacists are almost absent in nursing homes and in the community health services in Norway.

REDUCED POLYPHARMACY

Due to the increased risk of DRPs associated with polypharmacy, it is likely to expect that elderly people would benefit from drug withdrawal in general. Furthermore, reduced polypharmacy increases patient adherence to drug regimes which is essential to achieve maximum benefit and minimized ADE (11). However, withdrawal of medications might be difficult to implement and is not always beneficial in elderly patients. Discontinuing a drug is a multidimensional action that involves many parts in addition to the prescribing physician. That is, the patient, the relatives and the care-givers. Drug withdrawal is an active

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pharmacological intervention that needs the same attention and monitoring as prescription of a new drug and certain drugs need to be tapered off over time to avoid withdrawal symptoms and rebound syndromes. This is of particularly importance in frail elderly people with diminished regulatory responses and reduced capacity to regain homeostatic control after drug changes. However, there have been reported that reduced polypharmacy have increased patient satisfaction without any clinical consequences (59-61). Furthermore, there are several trials reporting beneficial effects in older people after reduced exposure to certain classes of medicines, i.e. withdrawal of psychotropic medications are shown to reduce incidence of falls (62). On the opposite, a recent RCT that discontinued antidepressants in Norwegian nursing home residents with dementia and neuropsychiatric symptoms, reported that the drug withdrawal was unfavorable leading to increasing symptoms in a subgroup of the discontinued patients after 25 weeks (63). The result contradicted with previous cross-sectional efficacy studies and highlights the needs for more RCT studies conducted in real-life setting to increase evidence-based guidance to what drugs should be continued and what could safely be withdrawn in nursing home patients.

GENOTYPING

The aging process affects organs and compensatory mechanisms with great interindividual differences and this amplifies the differences in drug response between older individuals. Other determinants of interindividual drug responses are inherited and are thought to be stable throughout the lifetime. Sequence variations in the genes encoding for hepatic drug metabolizing enzymes are of major importance for the variations in drug response (64), but inherited differences in hepatic drug metabolism phenotype might be a consequence of other factors than changes in the DNA sequence (epigenetic).The possible impact of epigenetics on drug clearance is an upcoming research area of great interest and is likely to be of special importance for the treatment outcome in aged people who are exposed to environmental factors over many years (38). The influence of aging and genetic variability on hepatic drug clearance and secondarily on the serum concentration of drugs, adverse drug effects, and therapeutic outcomes, depends on many factors including the pharmacokinetic profile of the drugs. Many lipophilic drugs, including anticholinergics, are

metabolized by the hepatic cytochrome P450 2D6 and 2C19 enzymes before renal excretion. These enzymes are highly polymorphic and carriers of variant alleles encoding deficient enzyme activity in CYP2D6 or CYP2C19 is phenotypically classified as poor metabolizers (PMs). The prevalence of CYP2D6 PMs are 5-10 % in Caucasians, while 2-3 % of Caucasians are CYP2C19 PMs. Compared to patients carrying functional alleles, i.e. extensive metabolizers (EMs), PMs are at risk of concentration dependent adverse drug reactions and drug-drug interactions (65). An age-related increase in pharmacodynamic sensitivity and the narrow therapeutic range of some anticholinergic drugs such as tricyclic antidepressants, emphasize the need for specific anticholinergic dose recommendation for elderly people. Nevertheless, genotyping have the potential to identify patients with increased risk of anticholinergic toxicity and might guide the physician to prescribe a non-anticholinergic drug alternative or reduce the anticholinergic dosage and thereby increase drug safety in elderly.

ANTICHOLINERGIC DRUGS

HISTORY AND USE

Anticholinergic agents occur naturally as alkaloids in Atropa belladonna and other plants in the Solanaceae family, native to Europe, North Africa and Western Asia. Belladonna alkaloids and their synthetic analogs have been used for their cosmetic, therapeutic, and toxic effects throughout centuries. Extract from Atropa belladonna was used as poison-tipped arrows thousands of years ago and the ancient Romans used it to murder contemporaries. The term belladonna refers to the beautiful Italian women who desired dilated pupils to appear more seductive. In modern times, synthesized anticholinergic drugs have been used for a variety of medical conditions such as parkinsonism, depression, urinary incontinence, allergy, travel sickness, obstructive lung diseases, sleep disorders, peptic ulcer disease, cardiac arrhythmias and psychoses among others. As late as in the 1950s, atropine induced coma was used as a treatment for psychosis (66). In 1991 there were as many as 600 drugs with anticholinergic activity on the United States market (67). Currently, many of these drugs are withdrawn, but a high number of drugs with potential anticholinergic properties are still present on the

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market (68). As a consequence of increased knowledge about the high risk of anticholinergic side-effects and the development of non-anticholinergic drug alternatives, the use of anticholinergic medications have declined over the past decades. Anticholinergic drugs are now considered as potential inappropriate drugs in the prescription appropriateness tools for elderly people (29,69,70). Still, such drugs are frequently prescribed for several indications and are the most common inappropriate drugs used in elderly people. Epidemiological studies have reported that 20-50 % of the elderly population use a medication with possible anticholinergic properties (71,72).

RISK FACTORS ASSOCIATED WITH ANTICHOLINERGIC DRUG USE

Risk factors for anticholinergic drug use are frequently present in the elderly and include polypharmacy, institutionalization, and the presence of certain medical conditions, e.g. urinary incontinence and dementia (24,73,74). Despite the anticholinergic hypersensitivity present in patients with dementia, studies have shown that elderly patients with dementia are more frequently exposed to definite anticholinergic drugs than those without dementia (73,75). It has also been shown, in four different epidemiological studies, that people using cholinesterase inhibitors have increased risk of receiving an anticholinergic drug with the potential to antagonize the effects of the cholinesterase inhibitor (75-78). This might be due to misinterpretation of the adverse effects of the cholinesterase inhibitor (77). Elderly people in nursing home care use significant more anticholinergic drugs than home-dwelling elderly (71,79). One study reported that 60 % of nursing home residents used anticholinergics, while the prevalence was 23% among the home-dwelling elderly, and among nursing home residents 10 % used several anticholinergic drugs concurrently (71). Furthermore, it has been reported that among hospitalized people above 65 years the prevalence of anticholinergic prescription increased significantly during their hospital stay (80). Finally it has been shown that within palliative care, the use of anticholinergic drugs increase as death approaches (81).

ANTICHOLINERGIC EFFECTS

Acetylcholine was identified in 1914 and was the first neurotransmitter to be chemically isolated and characterized (82). Acetylcholine is synthesized in certain neurons by the enzyme choline acetyltransferase and mediates parasympathetic nerve impulses in the central and peripheral autonomic nervous systems. The enzyme acetylcholinesterase rapidly hydrolyses acetylcholine into its inactive form in the synaptic cleft. Acetylcholine acts on two different cholinergic receptors in the parasympathetic nerve system, that is nicotinic receptors which are ligand-gated ion channels located on autonomic ganglia and skeletal muscles, and transmembrane muscarinic G-protein coupled receptors located in the brain and in the heart and in smooth muscles innervated by autonomic effector cells. The peripheral and central anticholinergic effects of drugs are caused by the activity towards muscarinic receptors.. There is no nicotine receptor antagonists marketed in Norway, and drug-induced anticholinergic antagonism refers to inhibition of muscarinic effects. Hence, "antimuscarinic drugs" would be a more pharmacologically precise term, but in accordance with the terminology used in the literature, we use "anticholinergic" throughout this thesis. There are five subtypes of muscarinic receptors, M1, M2, M3, M4 and M5, that have been characterized by molecular cloning (83). The expression of the muscarinic subtypes in the cholinergic nerve system and the response of acetylcholine-mediated activation are overviewed in table 1. Muscarinic receptors are widely distributed throughout the human body and mediate distinct physiological responses according to location and receptor subtype (84). However, there is a potential interplay between the receptor subtypes responsible for the acetylcholine-mediated functional response. The M1 receptor, predominantly distributed in the brain, outnumbers the other receptor subtypes posing 40-50 % of the total amount of muscarinic receptors (84). The M3 receptor is the predominant receptor in the peripheral nerve system; distributed in smooth muscles and exocrine glands and activation mainly cause contraction and secretion respectively (84).

Table 1: Distribution of muscarinic receptor subtypes and acetylcholine-mediated responses

Organ	Functionally predominant receptor(s)	Acetylcholine-mediated response
Brain	M1 (M2, M4 and M5)	Promote learning and memory processes, controls dopamine release
Eyes	M3	Contractility responses, tear production
Mouth	M1 and M3	Salivation
Heart	M2	Modulation of contraction and atrio-ventricular conduction, slows heart beat
Lungs	M3	Constricts bronchi
Gastrointestinal tract	M2 and M3	Promote acid secretion contractility and mobility
Liver	M3	Stimulates release of bile-acids
Blood vessels	M3	Vasodilation
Sweat glands	M3	Sweat secretion
Bladder	M2 and M3	Detrusor contraction

ANTICHOLINERGIC ADVERSE EFFECTS

Anticholinergic adverse effects are caused by the drugs' unselective antagonism of muscarinic receptor subtypes and the symptoms are related to the inhibition of acetylcholine-mediated responses. The symptoms may be dramatic, but are more often subtle and easily discounted as natural consequences of aging, leading to prescription of symptom-relieving drugs rather than adjustment of the responsible drug(s) (85).

PERIPHERAL ANTICHOLINERGIC ADVERSE EFFECTS

Peripheral anticholinergic adverse effects are related to the inhibition of acetylcholin-mediated muscle contraction and secretion. Symptoms of peripheral adverse effects range from mild reduction in salivary flow to fatal heatstroke caused by the inhibition of sweating and peripheral vasodilatation (86). But even the most common peripheral anticholinergic complaint, dry mouth, may affect important aspects of life such as speaking, the enjoyment and ingestion of food (and thus an adequate nutrition), and wearing of dental prostheses (87). A reduction in saliva flow increases the susceptibility to oral infections and caries, and many elderly people describe great discomfort related to xerostomia. There are many antimuscarinic side-effects in the eyes i.e. dilation of the pupils, increased intraocular pressure, dry eyes and blurred vision. Visual disturbances might lead to serious outcomes in elderly people such as falls and decreased function. Anticholinergic drugs can also induce tachycardia which is reported to have serious consequences in older people (82). Other common symptoms of peripheral anticholinergic adverse effects are urinary retention and constipation (87).

CENTRAL ANTICHOLINERGIC ADVERSE EFFECTS

The risk of central anticholinergic adverse effects is determined by the drugs' distribution to the brain and their competitive binding affinity to muscarinic brain receptors. The concentration of the drugs within the brain is regulated by the balance between passive influx and active efflux across the blood-brain-barrier (BBB) (84). Factors that influence on the permeability across the BBB are contributing factors in the risk assessment of adverse central effects (84). Drug characteristics such as small molecular size, no polarity, and great lipophilicity enable passive influx through BBB, while the efflux is facilitated by the drugs' specificity for the active transporter molecule permeability-glycoprotein (PgP). Moreover, the PgP-mediated efflux might be affected by genetic polymorphism and/or drug-induced inhibition of the transporter protein (84). As an example, anticholinergic drugs used to treat urinary incontinence show different propensity to cause central anticholinergic effects due to different molecular characteristics that affect the BBB penetration (84). Symptoms of

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anticholinergic effects in the brain are linked to the inhibition of acetylcholine transmission in certain brain areas including the forebrain, cerebral cortex, hippocampus, and corpus striatum. Acetylcholine transmission is especially involved in memory processes, in particular short-term memory and attention (88), but cholinergic blockade in the brain has been related to a widespread of undesired side-effects such as behavioral disturbances, reduced cognitive function, altered emotions, and declined motor function (89-92).

ANTICHOLINERGIC HYPERSENSITIVITY

Normal aging is accompanied by an increased pharmacodynamic sensitivity to blockade of muscarinic receptors in the central nerve system (CNS). Different changes in the cholinergic nerve system lead to decreased cholinergic reserves in the aging brain. A structural change in the muscarinic binding sites that led to lower binding affinity to acetylcholine, has been identified in rat models (93). Furthermore, a reduction in the activity of the presynaptic enzyme choline acetyltranseferase reduces the amount of acetylcholine in the CNS followed by a significant down-regulation of the number of muscarinic receptors in the brain (40,94). Moreover, the age-related pharmacokinetic changes contribute to a further increase in the susceptibility to central adverse effects, in particular the increased permeability of the BBB. An auxiliary leakage through the BBB is reported in certain conditions that are common in the elderly such as neurodegenerative diseases and diabetes (84). Mechanisms involved in increased BBB permeability include epithelial shrinkage, opening of tight junctions and dilation of the blood vessels resulting in increased blood flow and a leakage of larger molecules, as shown in a rat model (84). The vulnerability to central muscarinic antagonism is further increased in patients with Alzheimer's disease. These patients have severely impaired cholinergic neurotransmission, secondary to the degeneration of neurons, and it has been shown that older people with dementia are hypersensitive to central anticholinergic side-effects compared with age matched controls (95).

ANTICHOLINERGIC BURDEN(AB)

The term of drug "burden" might evoke linguistic pedantry as physicians prescribe medication based on the anticipated benefits rather than the burden, but as many elderly people use several anticholinergic drugs concurrently, the cumulative anticholinergic drug exposure might be denoted as a burden. There are in vitro methods and several anticholinergic scales that attempt to measure drug-induced AB, but the in vitro methods are not standardized and there is no consensus on how to define the drug exposure in the scales (96-99). Most of the scales rank the anticholinergic activity (AA) of drugs into four levels ranging from 0; that is no anticholinergic activity to 3; that is definite anticholinergic activity (96,97,99). Only one scale is adjusted for dose, but this drug burden index (DBI) includes both anticholinergic and sedative medications (98). The anticholinergic score of drugs is based on the drugs' in vitro AA and/or clinician-rated risk of anticholinergic side-effects. The total AB of a subject is determined by summation of each drugs' anticholinergic score. Hence, AB is an expert-based measure based on the assumption that anticholinergic pharmacodynamic activity is additive in a linear pattern.

SERUM ANTICHOLINERGIC ACTIVITY (SAA)

The potential anticholinergic properties of many drugs are determined by in vitro detection of their competitive binding inhibition of a specific radio-labeled ligand, tritiated quinuclidinyl benzilate (^{3}H-QNB), to muscarinic rat forebrain receptors (100). Many drugs not generally considered as anticholinergic, are identified with the ability to displace ^{3}H-QNB from muscarinic receptors with this assay technique (68). In 2008 Chew et al. assessed the dose-AA relationship of 107 medications at typically doses administrated to older adults (68). This method might be useful in quantifying and comparing the AA of different drugs. For instance, the dose-AA relationship of atypical antipsychotic medications showed that clozapine, olanzapine, and quetiapine had an increase in AA within their therapeutic range, while aripiprazole, risperidone and ziprasidone did not (101). Clinicians might use these results when choosing between equally efficacious medications, but the interpretation into risks of anticholinergic side-effects needs to be considered within the overall clinical status

of each patient. Prediction of anticholinergic toxicity depends on functional level, disease state, individual pharmacokinetic functions, and pharmacodynamic sensitivity.

The bioassay was originally developed to quantify the total AA in the serum of individuals caused by medications and/or endogenous anticholinergic substances. High SAA is previously associated with clinical outcomes such as delirium and impaired cognitive functions (102,103). However, SAA only indicates that a patient's serum contains compounds that affects binding to one or more of the five muscarinic receptor subtypes, but whether elevated SAA is caused by medication or endogenous compounds is unclear (104).

ANTICHOLINERGIC DRUG SCALE (ADS) SCORE

The anticholinergic drug rating scale (ADS) was the first anticholinergic assessment tool developed to quantify anticholinergic burden. The ADS score model is developed from a 3-level anticholinergic classification system of 62 medications that was published in 1978 (105). In 2001, Han et al. rated the AA of 340 medication into 0 (none) to 3 (high), based on the list from 1978, their clinical experiences, and the available ratings of the drugs' in-vitro AA (106). In 2006, Carnahan et al. validated and renamed this clinician-rated anticholinergic scale to anticholinergic drug scale (ADS) (96). The validation analyses (n = 201) showed that ADS was significantly associated with SAA (linear regression), although ADS only explained 9.5 % of the variation in SAA (96). The amount of variance explained by ADS did not improve with a dose-adjusted ADS score model. Moreover, the 1:2:3 weighting strategy appeared to be reasonable although 1:2:5 weighting was thought to be a more precise three-point ratio of the relative AA of different drugs (96). Anyhow, the simple 4-point model of anticholinergic ratings makes it user friendly in clinical practice. High ADS scores is associated with clinical outcomes such as poor memory and executive function (81,90). However, the ability of ADS to guide drug interventions and improve outcomes related to AA needs to be further studied.

Previous cross-sectional studies have related anticholinergic drug use in elderly to increased risk of delirium, cognitive impairments, neuropsychiatric symptoms, and reduced physical function (107-110). Furthermore, in longitudinal studies, cumulative anticholinergic drug exposure is related to deficits in cognitive functioning, but not to elevated risk of dementia, and the relation to mortality is uncertain (72,90,111,112). In contrast, one longitudinal study did not find any long-term cognitive impact of chronic anticholinergic drug use (113), but the overall conclusion is that anticholinergic drugs should be avoided in elderly patients due to their high risk of central adverse effects. This conclusion is mainly based on the comparison between users and non-users of definite anticholinergic drugs. Still, the presumed additive AA of different drugs that have given rise to the assessment of an overall AB is not thoroughly validated. AB express the overall risk of anticholinergic toxicity caused by multiple drugs. Some of these drugs might interact with each other, some drugs have markedly anticholinergic side-effects, while other drugs have potentially AA. Review articles have consistently associated high AB with negative cognitive and psychomotor outcomes in elderly patients, but there are gaps in the literature (89,114). Randomized prospective clinical trials that assess the presumed cognitive improvement of reduced anticholinergic burden are lacking and strongly encouraged in various populations (114). Furthermore, there are discrepancies in the quantification of anticholinergic burden between the studies and no consensus on a complete anticholinergic drug list exists. **Table 2** gives an overview of several commonly used methods to determine anticholinergic burden in people ≥ 65 years and the relation to anticholinergic measures evidenced by observational studies over the last 25 years. Different methods makes it difficult to compare the studies, but it is also difficult to compare the studies that have used the same method, SAA, because they use different cut-off values. Table 2 shows that the relation between the anticholinergic cognitive burden (ACB) scale and cognitive measures is weak. Moreover, the finding of a positive relation between ACB and mortality have been criticized because the covariates correlated with the outcome measure (115). The association between the drug burden index (DBI) and physical function is evidenced in different elderly populations and in the one study that disentangled between anticholinergic DBI and sedative DBI, the anticholinergic DBI had a stronger negative association with physical function than sedative DBI (92). ADS is the only scale that

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have been significantly related to SAA and high ADS score has also been associated with reduced cognitive and executive functions, and the presence of behavioral and psychiatric symptoms.

Table 2: Association between methods used to determine anticholinergic burden (AB) in the elderly and clinical outcomes. Findings from observational studies over the last 25 years.

Method	Estimation of AB	Study population	Clinical Outcomes	
Serum Anticholinergic Activity (SAA); measures drugs' displacement of [3]HQNB from muscarinic receptors expressed in atropine equivalents (100).	An objective measure of the ∑ *in vitro* AA of serum compounds	N = 61 > 65 years, hospitalized patients	**Delirium** Confusion Assessment Method (CAM)(102)	+
		N=61 > 80 years, hospitalized patients with and without dementia	CAM(116)	-
		N = 67 ≥ 75 years, medically ill inpatients	Delirium Symptom Interview(117)	+
		N = 90 ≥ 65 years, cognitively intact, community-dwelling	**Psychomotoric function** (gait speed, simple response time)(118)	+
		N = 201 ≥ 65 years, cognitively intact, community-dwelling	**Cognitive impairment** Mini mental state examination (MMSE)(103)	+
		N=26, geriatric patients with dementia and behavioral disturbance.	MMSE Severe Impairment Battery (SIB) (119)	+ -
		N=61 > 80 years, hospitalized patients with and without dementia	Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE)(116)	-
		N=152 ≥ 65 years, cognitive intact, community-dwelling	Working memory (120)	-
		N=22 demented nursing home patients	**Self-care capacity** Psychogeriatric Dependency Rating Scale (91)	+
Anticholinergic drug scale (ADS); 4 ranked scale of AA of drugs: 0 = no known AA 1= potential AA according to *in vitro* studies 2 = AA observed in high doses 3 = marked anticholinergic effects (96).	Expert-based ∑ AA of drugs based on drug characteristics, clinical experience and SAA.	N = 278 ≥ 65 years, medical inpatients with and without dementia	**Delirium** Evaluation of the severity of delirium symptoms based on CAM (106)	+
		N = 364 >65 years, hip fracture inpatients	CAM (121)	-
		N= 297 elderly in long-term care	**SAA** Significant correlation (96)	+
		N = 544 men ≥65, community-dwelling*	**Cognitive function** Verbal recall test(90)	+
		N =461 elderly patients in palliative care	**Quality of life** McGill life quality scale(81)	+
		N =461 elderly patients in palliative care	**Functional status** Australian Karnofsky Performance scale (81)	+
		N = 544 men ≥65, community-dwelling*	(ADL)(90)	+

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Anticholinergic cognitive burden (ACB); 4 ranked scale of drugs' AA 0 = no AA 1 = possible *in vitro* AA 2 = definite anticholinergic evidenced by association with clinically significant cognitive anticholinergic side-effects 3 = drugs with BBB permeability and association with delirium (122)	Expert-based ∑ AA of drugs based on drugs' BBB-permeability, risk assessments of drugs' cognitive side-effects and SAA.	N = 147 ≥ 65 years cognitively impaired inpatients N = 13 004 ≥ 65 years, community-dwelling with and without cognitive impairments N= 224 elderly patients with Alzheimer's dementia(123)	**Cognitive function** CAM(124) Only those using definite anticholinergic drugs had lower MMSE (72) MMSE Severity of cognitive decline	- + / - - -
		N = 13004 ≥ 65 years, community-dwelling with and without cognitive impairments	**Mortality** After two years (Covariate correlated with outcome measures)(72)	+
		N = 87 ≥ 65 years nursing home patients with dementia	**Quality of life** Multiple engagement observations(125)	-
Anticholinergic risk scale (ARS); 4 ranked scale of drugs' AA : 0 = limited or no risk 1 = moderate risk 2 = strong risk 3 = very strong risk (97)	Expert-based ∑ AA based risk assessments of drugs' peripheral and central adverse effects	N = 132 ≥ 65 years in geriatric evaluation management and N = 117 males ≥ 65 years in primary care clinics	**Central effects** (falls, dizziness, and confusion) **Peripheral effects** (dry mouth, dry eyes, and constipation)(97)	+ +
		N = 1004 elderly in long-term care	**Mortality(126)**	-
Drug burden index; Anticholinergic and sedative effects of drugs ranked 0-1 with a hyperbolic dose-response function (98)	Expert-based ∑ AA based on risk for adverse drug events.	N= 1705 males ≥ 70 years, community-dwelling with no, mild, or severe dementia N = 3075 ≥ 70 years, well functioning community-dwelling N = 932 women ≥ 65 community-dwelling	**Cognitive function** Addenbrooke's cognitive examination and trail making test(127) Digit Symbol Substitution test (98) MMSE(92)	- + +
		N = 3075 ≥ 70 years, well functioning community-dwelling N = 1705 males ≥ 70 years, community-dwelling with no, mild, or severe dementia N = 602 ≥ 70 community-dwelling N = 932 women ≥ 65 community-dwelling**	**Physical function** Health ABC performance score (98) Physical function ADL(128) Falls(129) Physical function ADL (92)	+ + - + + +

*ADS adjusted for drug exposure time
**Anticholinergic drug burden Index

THE PRESENT STUDY

OBJECTIVES

The powerhouse for this thesis was to contribute to improve clinical knowledge about appropriate polypharmacy and drug safety in elderly patients with multiple diseases. Pharmacotherapy is an important and complex field of geriatric and nursing home medicine, and the motivation was also to highlight the need for a multidisciplinary approach to optimize drug treatment in elderly patients.

The overall objective was to achieve better clinical understanding of the inappropriateness of anticholinergic drug use in elderly patients. This objective was chosen because high anticholinergic drug burden has previously been associated with an elevated risk of adverse effects in observational studies, but the association is not evidenced in prospective randomized controlled trials (RCTs). Therefore, we conducted an RCT with the intention to translate reduced AB into patient-related improvements in outcomes associated with anticholinergic side-effects in elderly people.

The specific aims of the four studies included in this thesis were:

i) To compare the potential risk of central anticholinergic adverse effects among five drugs used to treat urinary incontinence.

ii) To evaluate the additive properties of the ADS score model by investigating whether increasing ADS scores above 3 was associated with a gradually decline in cognitive function in aged people using multiple anticholinergic medications.

iii) To investigate the clinical effects of reduced anticholinergic drug burden after multidisciplinary medication reviews in frail older patients with a high risk of anticholinergic side-effects.

iv) To evaluate the contribution of a clinical pharmacist in the multidisciplinary geriatric health care team with patient-related outcomes.

v)   To evaluate whether genotyping can be used as a clinical tool to identify elderly patients at high risk of anticholinergic toxicity by relating anticholinergic measures to genotype determined activity in relevant hepatic enzymes involved in the metabolism of anticholinergic drugs.

DESIGN

Paper I)   An in vitro study of the anticholinergic activity of five drugs used to treat urinary incontinence.

Papers II) and IV)   Prospective cross-sectional studies of nursing home residents with high anticholinergic activity and no severe dementia

Paper III)   A multicenter, randomized controlled single-blinded trial, with repeated measure design, conducted in nursing home residents with high ADS scores and no severe dementia. The effect of the intervention was measured four and eight weeks following baseline, except for mouth dryness that was only re-measured after four weeks.

MATERIALS

Paper I)   Reference compounds of urinary spasmolytic drugs (USDs) were prepared in pooled drug-free plasma obtained from healthy individuals and analyzed in their steady state concentration ranges.

Papers II), III) and IV)   Nursing home residents in long-term care were recruited from 22 nursing homes in Oslo and Akershus from September 2008 to August 2009.

METHODS

STUDY PARTICIPANTS AND DATA COLLECTION

The inclusion criteria in the RCT study entitled "Pharmacist-initiated Reduction of Anticholinergic Drug Activity" (the PRADA- study), were being a nursing home resident in long-term care and having a total ADS score ≥ 3. Prior to inclusion, a local caregiver evaluated the patients' physical and mental eligibility to conduct the tests included in the protocol. Patients considered to have a shorter life expectancy than two month, and patients with blindness, deafness, aphasia, delirium, or severe dementia were excluded.

ADS SCORE

A trained research nurse or the principal investigator determined the ADS sum scores from the patients' drug schedules. In the original ADS, 537 drugs were classified into level 0, 1, 2 or 3 according to their in vitro AA and clinician-rated AA (96). We modified the AA ranking of some drugs in the original ADS according to a more recent comprehensive in vitro study of the AA of 107 drugs at therapeutic doses (10). This study detected AA of some drugs not present in the original ADS and these drugs were added to the modified ADS (68). We considered drugs with less AA than 0.5 pmol/mL atropine equivalents as level 0-drugs. In addition we added five new drugs to the modified ADS as we considered these drugs to have AA according to the previously published ratings of similar drugs and the specific drug characteristics. **Table 3**, shows the ADS score of drugs with AA used by the study participants.

Table 3: ADS score of anticholinergic drugs used by the study participants (n = 87)									
ADS = 3			**ADS = 2**			**ADS = 1**		
DRUG	**ATC code**	**n**	**DRUG**	**ATC code**	**n**	**DRUG**	**ATC code**	**n**
Hydroxycine	N05BB01	13	Olanzapine	N05AH01	5	Furosemide	C013CA01	27
Clorprotixen	N05AF03	12	Nortriptyline	N06AA10	3	Prednisolon	H02AB06	14
Alimemazine	R06AD01	12	Paroxetine	N06AB05	1	Ecitalopram	N06AB10	14
Tolterodine	G04BD07	8	Oxacarbacepine	N03AF02	1	Digitoxin	C01AA05	13
Amitriptyline	N06AA09	5				Ipatropium-bromide	R03BB01	12
Dexchlor-pheninamine	R06AB02	5				Citalopram	N06AB04	9
Solifenacine	G04BD08	4				Mirtazapine	N06AX11	9
Levome-promazine	N05AA02	3				Fentanyl	N02AB03	8
Trimipramine	N06AA06	2				Oxycodone	N02AA05	6
Promethazine	R06AD02	1				Quetiapine	N05AH04	3
						Diazepam	N05BA01	3
						Ranitidine	A02BA02	2
						Captopril	C09AA01	2
						Clonazepam	N03AE01	2
						Theophylline	R03DA04	2
						Clindamycine	J01FF01	1
						Zuclopenthixol	N05AF05	1
						Fluoxetine	N06AB03	1

CLINICAL DEMENTIA RATING (CDR) SCALE

A local caregiver determined the patients' cognitive function using the clinical dementia rating (CDR) scale. The CDR scale is a global assessment tool that ranks six domains of cognitive and functional performances into four categories: 0 = no cognitive impairments, 1 = mild dementia, 2 = moderate dementia and 3 = severe dementia. The six domains are: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. An overall CDR score is calculated by using an algorithm giving precedence to the memory domain (130). The CDR is an effective and reliable informant-based inventory that is validated for use in Norwegian nursing home residents and in multicenter studies (131,132). Patients with CDR > 2 were excluded from the PRADA study as they were considered to be incapable of executing the test battery.

CHARACTERISTICS OF THE PARTICIPANTS

We recorded sociodemographic characteristics, i.e. age, gender, weight, educational level, smoking habits, and wearing of dental prostheses. We also recorded medical information about diagnosis, medical complaints and laboratory measures from the patients' written medical journals. The total number of drugs used, and all available information about each drug, (doses, administration route, strength and formulation), were recorded from the drug regimens. The patients' morbidity was assessed from the recorded diagnosis and drug therapy using Charlson's Comorbidity Index (CCI). CCI is a estimate of the prognostic morbidity based upon the number and severity of comorbid diseases and is a widely used morbidity index in longitudinal studies (133,134). Although, it has been shown that CCI might not provide optimal risk adjustment for 1-year mortality in geriatric patients, the CCI was the most appropriate and sufficient morbidity assessment tool to be used in this study (133). Renal function was measured by glomerular filtration rate (GFR) calculated according to the Modification of Diet in Renal Disease (MDRD) study equation (135). Normal renal function is defined as GFR > 90 ml/min, while most people above 70 have decreased GFR despite normal serum creatinine (136).

NEUROPSYCHOLOGICAL INVENTORY NURSING HOME VERSION (NPI-NH)

Psychiatric symptoms and behavioural disturbance were assessed by the Norwegian version of the Neuropsychiatric Inventory Nursing Home Version (NPI-NH). This was originally a 10-item informant-based scale developed for determination of behavioural and psychological symptoms in patients with dementia (137). A nursing home version (NPI-NH) that comprises 12 items has been developed and the Norwegian version of NPI-NH has shown good reliability and validity (138,139). The items included in NPI-NH are delusions, hallucinations, agitation/aggression, depression, anxiety, euphoria, apathy, disinhibition, irritability, abnormal motor behaviour, night-time behaviour and eating behaviour, and the questionnaire rates frequency (four categories), and intensity (three categories) of each item. Frequency is multiplied with intensity (F x I) to generate a item score ranging from 0 to 12 and adding the item scores gives a sum NPI-NH score between 0 and 144. In addition, a care-giver rates his/her own distress related to each item in a five-point scale from 0-no distress to 5-very distressful. According to previous literature we defined symptoms with (F x I) ≥ 4 as clinically significant (56). The NPI-NH was included in the protocol as an outcome measure, but as the questionnaire is based on information from the nursing home staff, and the inter-rater reliability was poor, we decided to use some of the information from the baseline NPI-NH solely as descriptive information about patients.

RANDOMIZATION AND BASELINE DATA

Among the 230 nursing home residents with ASD ≥ 3, 101 were enrolled in the PRADA-study and randomly allocated 1:1 to intervention group and control group. The randomization was done by a research assistant with no other affiliation to the study. The randomization was stratified for 21 nursing homes, as there were no residents recruited from one of the nursing homes. The size of the strata varied from 2 to 15, with a median of 4 residents. Of the 101 residents allocated for the PRADA-study, 87 conducted the baseline assessment. All the 87 participants were assigned for pharmacogenetic analyses, but 5 were missed due to difficulties in blood sampling. Blood and data from the baseline assessment were withdrawn for the cross-sectional analyses presented in paper II) and IV).

INTERVENTION-DRUG REVIEWS

The intervention in the PRADA-study was based on a multidisciplinary drug review within 3 days after the baseline assessment. Prior to the multidisciplinary drug reviews that took place at each nursing home, a clinical pharmacist (the principal investigator), evaluated all the participants' drug regimens guided by the intention to reduce the patients' overall ADS score. In the multidisciplinary drug reviews, possible drug changes were discussed between the respective nursing home physician, a nurse with good knowledge of the patients, and the clinical pharmacist. Agreements were made whether to discontinue or replace anticholinergic drugs with drug alternatives with less or no anticholinergic activity. Drug withdrawals were performed stepwise to avoid anticholinergic rebound syndrome. When drug alternatives were unavailable, reduction in dosage was attempted in order to reduce the anticholinergic burden, but dose reductions did not affect the patients' overall ADS score. The drug treatments of all participants were discussed in similar drug reviews, but drug changes were only carried out immediately in the patients allocated to the intervention group. In the control group the drug changes were conducted by the nursing home physician after the last follow up.

ASSESSMENT TOOLS

The research nurse who worked in this project performed all the clinical assessments of all the participants. The whole test battery at each assessment took about one hour to administer.

COGNITIVE TESTS

RECALL OF WORDS

Three subtests from the neuropsychological test battery developed for the American consortium to establish a registry for Alzheimer's disease (CERAD) were used in this study. CERAD's neuropsychological inventory was established to discriminate between normal age-related alterations in memory skills and abnormal memory decline that occurs in mild

Alzheimer's disease (AD) (140). The neuropsychological assessment has later been used to identify episodic memory deficit associated with mild cognitive impairments (MCI) (141,142). The assessment includes neuropsychological measurements of acquisition and learning skills which are often the first domains to be impaired in very early AD and MCI. The subtests included are verbal fluency, naming, MMSE, 4 wordlist learning tests, 3 constructional praxis and recall tests, and one clock drawing test. CERAD's neuropsychological test battery has been found to have high re-test reliability, inter-rater agreement and longitudinal validity (140,142). Based on several reports of the association between anticholinergic drug use and poor performances on verbal memory tests, we included the CERAD's wordlist tests for immediate recall, delayed recall and recognition in the present study. Free recall of words relies heavily on semantic organization which is shown to be impeded by anticholinergic drugs (143,144). It has also been shown that wordlist learning by free immediate recall is a sensitive measure to detect mild scopolamine-induced increments in SAA in elderly presurgical patients (145). The Norwegian version of the CERAD wordlist test for immediate recall was therefore chosen as primary end-point in the PRADA-study (140,146). Prior to the administration of the verbal memory tasks, the study participants were asked to memorize the words in the wordlist. The wordlist consisted of ten unrelated nouns, each written on a card and shown one by one to the participants who were asked to read them out loud. The patients immediately recalled as many words as possible in a free order. The task was repeated twice and the cards were reorganized between the repetitions. Maximum score from the three wordlist learning trials was 30 words, and people between 65-95 years recall in average 19-21 words, while less than 18 words is unusual and less than 16 words is considered to be abnormal (140). Another task was performed before the participants were scored for the correct words recalled after five minutes. In the delayed recall test, 65% of the patients with AD do not to remember any words, while cognitively intact people above 80 years usually remember more than 4 words (140,141). Finally, the ten nouns were mixed with ten new nouns and the patients were asked to recognize the words previously read. Normally people aged 65-90 years recognize 9-10 words and we therefore decided that the recognition task should be the last test in the test battery hoping for the participants to have a good feeling of coping after each assessment.

Mini Mental Sate Examination (MMSE), revised for use in Norwegian nursing homes, was included in the test battery as a measure of the overall cognitive function of the participants (147). The MMSE is a 30-point questionnaire test that includes 20 items within the cognitive functions orientation, memory, abstraction, language use and visuospatial ability (148). A total score above 27 is considered as normal, while people scoring 25-27 points require additional assessments, and below 24 points indicate cognitive impairments. The degree of dementia based on the MMSE sum score is given as: 21-24 points defines mild dementia, 10-20 points defines moderate dementia and below 10 points defines severe dementia (148). The MMSE is the most widely used screening tool for global cognitive function, but MMSE is an inadequate screening test for specific alterations in memory skills (142). We recorded the participants' general impressions of their own memory skills and their attention towards the MMSE during the test situation.

PERIPHERAL ANTICHOLINERGIC MEASURES

Measures of peripheral anticholinergic activity were included in the test battery to investigate the relation between ADS score, SAA and symptoms of adverse drug effects not affected by the drugs' distribution to the brain.

MOUTH DRYNESS

Saliva flow and secretion are primarily stimulated via muscarinic receptors and mouth dryness is a multi-glandular condition. Hence, whole mouth resting saliva flow is a relevant measure of drug-induced mouth dryness (149). The saliva flow was only re-measured at four weeks follow-up because the peripheral antimuscarinic response in saliva flow was thought to be completed within four weeks after the drug changes. We chose a swab technique to measure whole mouth resting saliva flow because of its simplicity and feasibility in patients with dementia. The method is validated and commonly used, although intra-individual variability due to oral anatomical differences have been reported with this method (150). We first placed two pre-weighted dental cotton rolls in the patients` mandibula for three

minutes and then in the upper vestibules at the opening of the parotid gland ducts for three minutes. The cotton rolls were placed in locked tubes after the saliva collection and before weighed. The weight difference of the cotton rolls was used to determine the salivary flow rate (g/min). Typically, mean resting whole-saliva flow rate in adults is 0.3 mL/min (1 mL is equal to 1 gram), but the flow rate is affected by age, gender, and time of measurements (149,151,152). In the present study we recorded the time of the measurement, time since food, drink, and medicine intake, smoking habits, and dental prosthesis as possible confounders of the salivary flow. We strived to measure unstimulated salivary flow rate at approximately the same time of the day at each assessment.

RADIO RECEPTOR ANTICHOLINERGIC ACTIVITY BIOASSAY

The bioassay was first described in 1980 and has been used in research to quantify AA of single compounds and peoples' overall anticholinergic burden for more than 30 years (68,100). We used the bioassay for both purposes: paper I describes the quantification of AA of five different USDs dissolved in drug-free plasma in their estimated therapeutic concentration ranges, while papers II, III and IV include the quantification of the AA present in the serum samples withdrawn from the patients participating in the PRADA-study. The therapeutic concentration ranges of the USDs were based on references in the literature and reported pharmacokinetic data. Blood sampled for measurement of SAA were drawn on containers without anticoagulants and kept 1-2 hours in room temperature to facilitate coagulation before the serum was isolated by centrifugation (1500 G for 10 minutes). The serum was dispensed in two Nunc tubes and frozen at -70 degrees before measurement of serum AA. By a mistake, blood samples for measurement of SAA were put directly on ice from some of the initially included patients, prohibiting isolation of the plasma matrix. During the proceeding patient inclusion, we therefore sampled both full blood and plasma to see if radioactive measurements of bound/undisplaced radioligand correlated in paired samples of the respective matrixes. A linear correlation analysis showed that AA in paired full blood and serum samples was significantly associated P<0.0001. Thus, the full blood radioactive values (CCPM) from the initial patients were transformed to 'plasma values' by application of the formula describing the significant linear correlation between radioactive

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measurements in full blood and plasma [CCPM$_{plasma}$ = (CCPM$_{blood}$ * 0.9543) + 50.96]. The assay technique measures the AA of compounds by the degree of displacement of ^{3}H-QNB from the muscarinic receptors isolated from rodent brain. We conducted and validated the bioassay at an academic research laboratory at The School of Pharmacy based on previous literature (68,100). To enable more effective analyses, we modified the original bioassay by using 96-well plates instead of single containers and reduced the incubation volume to 240 µL compared to 2 mL in the original method. The bioassay was performed directly on the plates by mixing the samples in the wells, incubate for 1 hour in room temperature before separating the ^{3}H-QNB-muscarin complex from unbound ^{3}H-QNB by vacuum filtration and washing. In each well the precipitated ^{3}H-QNB-receptor complex was dissolved in 30 µL scintillation fluid, gently shook for 30 minutes and the undisplaced radioligand was counted using a MicroBeta Plus liquid scintillator detector. High AA of serum compounds is proportional with a high degree of displacement and low radioactive counts per minute (CCPM). All measurements were performed in duplicates on the same day and in addition the USDs were analyzed on three separate days (6 parallels). The mean AA was calculated and used in further statistical analyses. For each plate, a standard curve with atropine was used as the reference for AA. Prior to determination of the atropine concentrations to be used in the standard curve, concentrations of atropine ranging from 0.001nM to 1000nM were analyzed to detect which concentrations that captured maximum and minimum displacement from the ligand in a one-site competitive binding model. The standard curves were fitted to a one-site competitive binding model using GraphPad Prism version 5.01 (GraphPad Software Inc, CA), and the concentration interval for atropine was set to 0.05-100 nM. Ten concentrations within 0.05-100nM, were analyzed in four parallels on five separate days (20 parallels). In the validation procedure the regression coefficient (R^2) and the variation coefficient of the estimated values of inhibitory concentration 50% (IC$_{50}$), maximum binding (B$_{max)}$ and minimum binding (B$_{min}$) were calculated. The B$_{max}$ is the binding of ^{3}H-QNB to muscarinic receptors when no competitive AA compounds are present. When the system is saturated with AA competitors, less than 20% of ^{3}H-QNB is displaced = B$_{min}$, and further displacement of ^{3}H-QNB is caused by unspecific binding. The values of Y, B$_{max}$ and B$_{min}$ are measured in radioactive counts per minutes (CCPM), while log IC$_{50}$ is determined from the curve that is fitted by nonlinear regression. The value of X is expressed

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in pmol/mL atropine equivalents and calculated from log[x] plotted on the x-axis using the equation for competitive binding. The equilibrium below describes the competition of ^{3}H-QNB and AA competitors (C) for receptor (R) binding.

$$^3\text{H-QNB} + \text{C} + \text{R} \rightleftharpoons (^3\text{H-QNB-R}) + (\text{C-R})$$

Competitive binding model:

$$Y = \text{Bottom} + \frac{(\text{Top-Bottom})}{1+10^{(\log[x]-\log(IC50))}}$$

Total binding of ^{3}H-QNB

log (IC_{50})
log [unlabeled ligand(s)]

Non specific binding
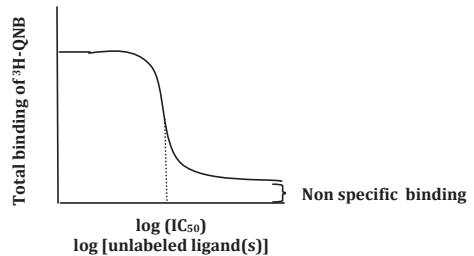
SELF-CARE CAPACITY

A nurse or a nurse assistant in the nursing homes assessed the patients' self-care capacity using Barthel's Index (BI). BI is a questionnaire including 10 items such as urinary and fecal continence, ingestion of food, mobility, and personal care and each item is staged in terms of self-care ability; completely self-dependency gives the highest score. BI is a validated and widely used inventory to assess basic ADL functions (153). Unfortunately, the questionnaire was filled in by different care-givers at each assessment in the PRADA-study and the inter-rater agreement was poor. We thereby decided not to use BI as a repeated outcome measure, but BI was included in the cross-sectional analyzes in paper II.

GENOTYPING

Participants were genotyped for the polymorphic cytochrome P450 2D6 (CYP2D6) or CYP2C19 hepatic enzymes that are involved in the drug-metabolism of many anticholinergic drugs (154). The blood sampled for genotyping was drawn on containers with anticoagulant (EDTA tubes) and frozen at -20 degrees before mutation analyses. The genotyping was performed at Center for Psychopharmacology, Diakonhjemmet Hospital, using validated and certified assays developed for routine analysis (155). Genomic DNA was extracted from

blood samples using E.Z.N.A Blood DNA Kit (VWR International, Oslo, Norway). The routine genotyping procedure included detection of the single nucleotide polymorphisms *CYP2D6 2549A>del* (*2D6*3*), *1846 G>A* (*2D6*4*), *1707T>del (2D6*6)* and *100C>T (2D6*10)* and *CYP2C19 430C>T (2C19*2)* and *1075A>C (2C19*3)* by TaqMan-based real-time polymerase chain reaction (PCR) assays, while *CYP2D6* gene deletion *(2D6*5)* and gene multiplication was detected by copy number analysis. The TaqMan real-time PCR assay specifically amplified genomic CYP2D6 and CYP2C19 by using a specific set of amplification primers and probes which effectively prevent amplification of pseudogenes. Absence of the listed mutations was interpreted as presence of the wild-type allele *1-allele encoding functional enzyme activity (CYP2C19*1 or CYP2D6*1) (154,155). Phenotypes of CYP2D6 and CYP2C19 of the study participants were interpreted from genotype analysis. Patients who carried three or more functional CYP2D6 alleles (ultrarapid metabolizers), were excluded and the rest of the study population were divided into two phenotype subgroups. Homozygous carriers of the detected variant alleles encoding deficient CYP2D6 or CYP2C19 enzyme activity were classified as poor metabolizers (PMs), while patients with one or two functional genes were classified as extensive metabolizers (EMs).

STATISTICAL CONSIDERATIONS

The data in this study was analyzed with SPSS (Statistical Program for Social Science) version 16.0-19.0.

INDEPENDENT VARIABLES

The independent variables were both continuous and categorical. Two-tailed Spearman's rank correlation matrix was inspected to identify the correlation between all the independent variables and to identify possible confounders with significance level $p \leq 0.1$. Distribution of the independent variables across the study groups were compared by Kruskal-Wallis test in paper II, and by t-tests or Mann Whitney test (continuous variables) and Fisher's exact test (categorical variables) in paper III and IV.

DEPENDENT VARIABLES

All the dependent variables were continuous, but the cognitive measures were discrete attaining values on at least 10-point scales. The frequency distributions were inspected by histograms to screen the data for skewness, kurtosis and outliers. The discrete variables appeared to be normally distributed within the study groups and were therefore analyzed as continuous variables in linear models. However in paper III, we used Poisson regression in a secondary model of the interventional effect as count data. The two peripheral measures salivary flow and SAA, were log 10 transformed to fit the linear models. The assumptions of normality and homoscedasticity of the residuals of the outcome measures were tested with Q-Q plot and Levene's test respectively. The reasons for outliers were checked in the raw data and solely the incorrectly entered cases were removed, otherwise the outlying cases were considered as consequences of natural variance within the study population. The influence of the outliers on the linear models was checked by Cook's distance.

MISSING VALUES

One of the reasons why very elderly subjects are excluded from clinical trial is the presumed high rate of dropouts and poor adherence to the study protocol. To account for the expected high rate of missing data, we targeted a sample size of 40% more participants than required to reach a statistical power of 95%, n = 60. The reasons for dropping out were recorded and did not seem to be related to the intervention as described in paper III. Furthermore, there was no association between missing values and group allocation in a regression model and the distribution of the missing values were well balanced between the intervention group and the control group in the PRADA-study. We therefore considered the missing data to be missing at random and analyzed the interventional effect primary without imputing data. However, in the secondary analyses we imputed the missing data by last observation carried forward which is a conservative approach, increasing the risk for type II errors.

In addition to the drop outs, some of the participants got tired during the assessment and lost motivation to complete the test battery. This is reflected by the fact that the final test

(the recognition test), had most missing values. In addition there was a low adherence to the salivary flow rate test, presumably because this test could be unpleasant for some of the participants, especially those with most complaints of mouth dryness. This might have caused a selection bias of the observational effect in paper II, probably underestimating the relation between ADS score and salivary flow.

EFFECT SIZE IN THE PRADA STUDY

The required effect size of 30 % improvement in immediate recall was based on efficacy studies of cholinesterase inhibitors. We justified that a clinical meaningful cognitive improvement of reduced anticholinergic load should be similar to the previously required effect size of cholinesterase inhibitors (156). The required cognitive change is also in accordance with a recently published study of discontinuation of drugs in nursing homes (63). In the a prior power analyses we assumed that the mean score in CERADs immediate recall at baseline would be 15-17 ± 5 words. This assumption was based on a previous Finnish study and knowledge about the high prevalence of MCI and dementia in Norwegian nursing homes, (approximately 80 %) (57,141). However, the observed mean score in our study population was less than expected (mean recall of words was 11-12), and hence it might have been too optimistic to require a 5 words (30%) improvement of the memory performance in patients with probably more severe brain pathology. Although our definition of clinically meaningful difference in the CERAD test might have been to strict, it is interesting to notice that large observational studies have reported e.g. a 0.32 point decline in an immediate recall test of maximum 36 words as statistically significant (90). It is relevant to question whether a difference of this magnitude is clinically important in nursing home patients with a high prevalence of dementia.

RANDOMIZATION

The intervention was dependent on the collaboration within the multidisciplinary team and the local pharmacotherapeutic interest and culture at each nursing home. To stratify for this impact, we block-randomized the patients by nursing homes, each nursing home representing one stratum. It might be argued that cluster randomization would have been more appropriate to avoid leaking from the interventional group to the control group. However, this would have required a much larger sample size. The possible leaking of the intervention to the control patients is further discussed in paper III.

TYPE I ERROR

Some people might argue that the level of the statistical analyses should have been adjusted for multiplicity to avoid type I errors. However, we decided that since the p-values was far from 5% and no significant difference was demonstrated in the PRADA study, adjusting for the risk of type I error was superfluous. Furthermore, when our study groups were rather equally sized, the F-statistics in the linear models used, is relatively robust in terms of violations of normality and homogeneity of variance.

TYPE II ERROR

The main concern about the choice of statistical models used in this research is the risk for type II error. To be sure not to mask a possible effect we have checked all the assumptions for linear models in paper II and paper III. Additionally, we have performed secondary non-parametric analyses of the data. The risk for type II error is reflected in more details in the discussion chapter.

ETHICAL CONSIDERATIONS

Information about the study was given in meetings with the affiliated nursing home staff and the participation of each nursing home was contracted. Prior to enrollment, we met all the participants assessed as eligible for inclusion, and informed them orally about the study. Before randomization, written informed consent was obtained from the all the participants. For patients with reduced capacity to assent, their closest relative provided assumed written consent. Because people with Alzheimer's disease have increased sensitivity for anticholinergic drugs, it was important to include these patients, despite their lack of ability to consent. All the medical records that were used in this project were anonymized.

We strived to choose methods regarded as feasible and time effective to minimize the test burden in this frail study population. This is reflected by our decision to measure mouth dryness solely four weeks following intervention. In light of this approach, it might as well be argued that one follow up, eight weeks after baseline, would have been sufficient to measure the interventional effect.   The control group was offered the same drug review as the interventional group, but no changes in prescription were effectuated before after the last follow up. However, if serious DRPs had been identified during the reviews, these would have been solved immediately. The objective of the intervention was to reach agreement with the patients about the drug withdrawal and in those cases where the patients disagreed to discontinue the anticholinergic medication, this was respected. Finally, those patients with mutations in the hepatic enzymes involved in drug metabolism were informed of their genotype.

The results from our study could easily be transformed to clinical practice and thereby benefit the patients in terms of better understanding of appropriate drug prescriptions in the elderly.

The study was approved by the Regional Committee for Medical Research Ethics, the Norwegian Directorate of Health and the Data Protection Officer at Oslo University Hospital.

RESULTS

The results of the present study are presented in four papers that are briefly summarized in this chapter. The summaries include scientific rationale, main results and conclusion of each paper while more details are provided in the original papers, attachment I-IV.

PAPER I

This study was conducted to assess the relative in-vitro AA for the five urinary spasmolytic drugs (USDs) used to treat urinary incontinence. In particular, the AA of the newer USDs were of special interest as these drugs are claimed to be more specific to M3 receptors. The in-vitro AA reflects the pharmacodynamic potency to induce cognitive side-effects and the relative in-vitro AA may assist appropriate selection among commonly used USDs in order to reduce the risk of central anticholinergic side-effects. However, the risk of central anticholinergic side-effects is also determined by pharmacokinetic factors; in particular the degree of brain distribution and the most appropriate USD would be that with the lowest AA and the lowest brain distribution. The secondary purpose of this paper was to publish the modified radio receptor bioassay (RRA) developed and validated as part of this research project. The rationale for modifying the RRA was to enable more effective analyses of AA which might enhance the utility of the bioassay.

In summary this in vitro evaluation suggests that fesoterodine and tolterodine have the highest pharmacodynamic potential of central anticholinergic side-effects while darifenacin displayed the lowest AA. Moreover, darifenacin is ranked with the lowest brain anticholinergic activity. In conclusion darifenacin probably display the lowest risk of inducing central anticholinergic side-effects of the five USDs evaluated in this study.

PAPER II

This prospective cross-sectional study describes the prevalence of multiple anticholinergic drug use in 87 Norwegian nursing home residents with and without dementia. The paper

explores a gap in the literature regarding anticholinergic polypharmacy and the proposed additive properties of the ADS score model in relation to cognitive impairments, activity of daily living and peripheral anticholinergic side-effects. The ability of the ADS score model to predict central anticholinergic side-effects was evaluated by investigating whether the cognitive function gradually declined when the ADS scores increased above 3 in a frail elderly study cohort.

The study showed that about one fifth of the nursing home residents had an ADS score of 3 or higher and about 10 % used at least two anticholinergic drugs concurrently. Nursing home residents with dementia had significantly lower ADS scores than those without dementia. After adjustment for clinical dementia rate, there was no evidence of a progressive decline in cognitive function or ADL when ADS scores increased above 3 (p> 0.10), but *in vivo* (mouth dryness) and *in vitro* (SAA) measures of peripheral anticholinergic activity were significantly higher in patient with ADS score ≥ 6 (p< 0.01). The findings might indicate that the ADS score model has limited potential to predict the risk of central anticholinergic side effects in frail elderly patients receiving multiple anticholinergic drugs.

PAPER III

This paper presents the results from the main study entitled Pharmacist-initiated Reduction of Anticholinergic Drug Activity (the PRADA-study). The study is an RCT conducted to supply the previous pharmacoepidemiological research with clinical relevancy of high anticholinergic drug burden. We aimed to translate reduced ADS score into improvements in relevant geriatric health outcomes in a real-life setting. Secondary, we intended to evaluate the contribution of a clinical pharmacist in nursing home treatment care teams with patient-related outcomes.

Pharmacist-initiated drug changes significantly reduced the ADS score by 2 units (p< 0.0001) in the intervention group, while it remained unchanged in the control group. After eight

weeks the adjusted mean difference in immediate recall (primary end-point) was 0.54 words between the intervention and control group (95% confidence interval: -0.97, 2.05; p = 0.48). The study groups did not differ significantly in any of the other cognitive end-points, saliva flow or SAA at either follow-ups (p > 0.19). The significant reduction in ADS score did not translate into improved health outcomes which might indicate limited applicability of the ADS score to prevent prescription risks in an aged population with high degree of cognitive impairments, comorbidities, and polypharmacy.

PAPER IV

This paper presents the relation between genetic variants of CYP 2D6/2C19 enzymes and anticholinergic measures. Genetic heterogeneity in drug clearance has not previously been related to AA. We aimed to investigate whether genotyping might be a clinical tool to identify patients at increased risk of anticholinergic toxicity. Based on pharmacogenetic analyses of mutations encoding absent CYP2D6 or CYP2C19 metabolism, the study cohort was divided into subgroups of poor (n=8) and extensive (n=72) metabolizers and anticholinergic measures between the two subgroups were compared.

 Poor metabolizers (PMs) had significantly higher SAA than extensive metabolizers (EMs) (p= 0.012) and the difference persisted after adjusting for ADS. No significant differences in mouth dryness and cognitive function were observed between the subgroups (p>0.3). These preliminary findings suggest that older individuals being PMs via CYP2D6 or CYP2C19 are at increased risk of higher serum anticholinergic activity than EMs. Whether PMs are also more prone to experience anticholinergic side effects needs to be further studied in larger patient samples.

ADDITIONAL RESULTS

CHARACTERISTICS OF THE STUDY COHORT

In paper II, the study population was divided into four subgroups based on ADS-scores, in paper III, and IV, the study population was divided into two subgroups based on randomization and genotyping respectively. **Table 4** was included in this chapter to give an overview of the baseline characteristics of the whole study cohort. The study population was represented by 69 women and 18 men, all above 73 years old and less than 10 % had higher education. The study cohort was characterized by high comorbidity score, slightly reduced renal function (the reduction in GFR was related to increasing age), reduced salivary flow, and substantial polypharmacy (58,136,151,157). Almost 70 % of the study population had mild-moderate dementia and two patients had MMSE sum score below 10 indicating severe dementia (148). Psychiatric symptoms and behavioral disturbances were highly prevalent in the study cohort with almost 80% of the patients having at least one symptom and above 40% had 1-5 clinically significant symptoms (F x I ≥ 4) (138). Night-time behavior was described in 11 patients being the most frequent clinically significant symptom while depression, anxiety and irritability was the three most common psychiatric symptoms. The patients' self-care capacity ranged from minimum to maximum points when assessed by Barthel's Index (BI), and approximately 20% was dependent of considerable help in daily activities having a BI ≤ 20 (153).

Table 4	Characteristics of the study population at baseline (n= 87)	
Characteristics	**N**	**Median (IQR) and range**
Age	87	85 (82-90), 73-99
Female gender, n (%)	87	69 (79%)
Education ≤ 12 years, n (%)	82	75 (91%)
Daily smoking, n (%)	72	13 (16%)
Dental prostheses	72	
0 prostheses, n (%)		38 (53%)
1 prostheses, n (%)		16 (22%)
2 prostheses, n (%)		18 (25%)
CDR	87	
0 = no dementia, n (%)		27 (31%)
1 = mild dementia, n (%)		36 (41%)
2 = moderate dementia, n(%)		24 (28%)
Scheduled drugs	87	9 (7-12), 1-18
Estimated glomerular filtration rate (mL/min)	80	70 (48.3-85.8), 18-207
Comorbidity score Charlson's Index	87	4 (3-5), 1-9
Anticholinergic Drug Score (ADS)	87	4 (3-5), 3-10
Serum Anticholinergic Activity (SAA)	81	4.53 (2.45-8.51), 0.18-53.4
(pmol/mL Atropine equivalents)		
Mini Mental State Examination score (MMSE)	81	20 (16-30), 8-30
Verbal immediate recall (CERAD)	86	12 (8-15), 0-23
Verbal delayed recall (CERAD)	85	2 (0-4.5), 0-10
Verbal recognition (CERAD)	69	6 (4-8), 0-10
Neuropsychiatric symptoms (NPI-NH)	83	69 (79%)
(FXI) ≥ 4, n (%)		36 (43%)
Whole mouth resting salivary flow (g/min)	69	0.22 (0.12-0.43), 0.03-1.18

GENDER DIFFERENCES

There were some differences between women and men in the study population. The women were older (median age = 87) than the men (median age = 81) and the men produced significantly more resting saliva than women (p< 0.010). Interestingly the men's impression of their own memory was better than the women's, despite no correlation between gender and the performance on any of the memory tasks, and the median score on MMS was actually the same across the gender.

DIFFERENCES BETWEEN CDR SUBGROUPS

The study population was divided into three subgroups based on their clinical dementia ratings (CDR). The baseline characteristics of the subgroups were compared with Kruskal-Wallis test for the continuous variables and Fisher's exact test for categorical variables.

Table 5 summarize the median (IQR) of the observed values and the difference between the subgroups. The performance of the cognitive test decreased significantly with increasing CDR and all the cognitive tests were significantly correlated. Moreover, the number of scheduled drugs and ADS decreased with increasing CDR. As pointed out in paper II, no residents with moderate dementia had an ADS score above 5.

Table 5: Baseline characteristics of the study participants with different dementia ratings				
	CDR			**p-value**
Clinical characteristics	**0**	**1**	**2**	**difference between subgroups**
Age	84 (81-90)	86.5 (82-91.5)	86 (82-89)	0.54
Female gender (n)	23	30	16	0.20
Scheduled drugs	11 (8-14)	9 (7-11)	8 (5-9)	0.01*
Weight (kg)	61 (51-91)	58.5 (51-73)	65 (54-76)	0.63
Estimated glomerular filtration rate (mL/min)	73.5 (47-94)	61 (42.5-82.5)	70 (59-84)	0.22
Charlson's comobidity	3 (3-49)	4 (83-59)	3 (2-5)	0.08
Whole mouth resting salivary flow (g/min)	.17 (.06-0.33)	0.32 (0.13-0.6)	0.21 (0.17-0.4)	0.19
MMSE	23 (19.5-26.5)	20 (16-23)	17 (14.5-20)	0.00*
CERAD Immediate recall	15 (11-19)	12 (9-14)	8.5 (4.5-12)	0.00*
CERAD delayed recall	4 (2-6)	2 (0-5)	0 (0-2)	0.00*
CERAD recognition	7 (5-9)	7 (5-9)	6 (4-6)	0.05*
NPI sum score	1.5 (0-7)	5 (1-18)	6.5 (2.8-12)	0.07
ADL-BI	65 (37.5-90)	50 (30-75)	47.5 (28.8-76.3)	0.10
Serum AA (pmol/mL atropine equivalents)	5.9 (3-10.7)	4.5 (2.8-7.4)	3.8 (1.9-6.2)	0.86
ADS	5 (3-6)	4 (3-5)	4 (3-4)	0.03*

*Significant difference p≤ 0.05.

The change in ADS score on CERAD gives insightful additional information of the evaluation of ADS score and we therefore performed an ANCOVA with change in ADS as fixed variable. The change in ADS score, ranging from +2 to -6, did not affect the CERAD tests significantly ($p > 0.35$) Neither the comparison of those having their ADS score reduced with ≥ 2 with the remaining patients showed significant differences in immediate recall eight weeks after the intervention. We have included a scatter plot that shows the relationship between ADS score and CERAD 10 word test for immediate recall eight weeks after intervention, **figure 2**. The plot might indicate that there is a pivotal point between ADS = 0 and ADS =1 associated with improved immediate recall. As discussed in paper III, this interpretation is limited by the low number of patients with ADS = 0 in our study.

Figure 2: The relation between ADS score and CERADs immediate recall eight weeks after intervention
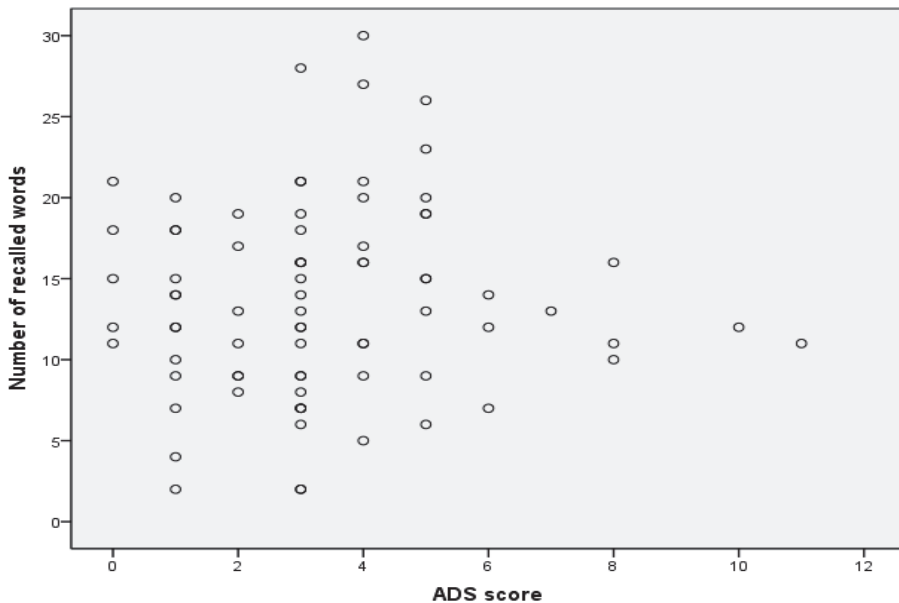
DISCUSSION

THE PRESENT FINDINGS RELATED TO PREVIOUS FINDINGS

Darifenacine had the lowest potential for central anticholinergic side-effects among five drugs used to treat urinary incontinence

Overactive bladder (OAB) frequently causes urinary incontinence in elderly people and will become an increasingly prevalent problem as the elderly population share increases. Management of the urinary symptoms is important for dignity and life quality, but also to reduce the risk of falls and fractures related to urge incontinence (158). Drug-induced blocking of M3 receptors in the bladder is an effective symptom reliving pharmacological treatment, but the urinary spasmolytic drugs (USDs) display a high risk of cognitive impairment due to poor muscarinic receptor-selectivity. The potential to cause cognitive impairment depends on the drugs' activity towards muscarinic brain receptors (predominantly M1), and the ability to cross the BBB (158,159). Knowledge of the USDs relative muscarinic receptor-selectivity and brain distribution might guide the physicians to prescribe the drug with lowest risk of cognitive impairments. In premarketing RCTs, darifenacine is found to be an effective, well tolerated M3-selective USD with a low degree of brain distribution (160). In our post marketing in-vitro based safety assessment of the five USDs marketed in Norway, we confirmed the premarketing findings of darifencine. Our results suggested that fesoterodine and tolterodine have the highest pharmacodynamic potential of central anticholinergic side-effects which also is in accordance with previous literature. Overall we found that the relative risk for central side effects was in line with the previous findings and the information given by the manufactures of the USDs (158,161-166).

The prevalence of Norwegian nursing home residents with a high anticholinergic drug burden was approximately 20%, while 10% used more than one anticholinergic drug concurrently. The anticholinergic drug burden decreased with increasing severity of dementia.

We reported that each study participant used on average 9 scheduled drugs daily which is slightly higher than recently reported in Norwegian nursing homes (58). The use of anticholinergic drugs is known to increase with the number of drugs used, and it is therefore likely that our inclusion criteria of ADS ≥ 3, biased the selection of nursing home residents. We also reported that 21.5 % of the 1101 nursing home residents screened had an ADS score above 2 and that 10 % used multiple anticholinergic drugs concurrently. In general, the prevalence of anticholinergic drug use has decreased over the past decades (71), but the use of anticholinergics among Norwegian nursing home residents was still relatively high compared to recent data from community-dwelling elderly (167). Hence, our data might support, as previously reported by others, that institutionalization is a risk factor for receiving anticholinergic prescriptions (74,80). The cross-sectional analyses in paper II revealed that the patients with moderate dementia used significantly less anticholinergic drugs than those without dementia. However, the difference in ADS score was no longer significant after adjusting for the number of drugs taken. In existing literature it is reported that dementia is a significant risk factor for receiving anticholinergic drugs and that approximately 30% of those using cholinesterase inhibitors receive an anticholinergic drug concurrently (73,75,77). Contrary, our results might indicate that the Norwegian nursing home physicians are aware of the anticholinergic hypersensitivity related to the pathophysiological process of Alzheimer's dementia and hence avoid anticholinergic drug prescriptions to patients with more severe dementia. However, as the ADS score difference not persisted significant after controlling for the number of drugs taken, it is more likely that the observed difference reflects that patients with dementia generally receive less drugs than cognitively intact patients and are as well at risk of under-prescription because of communication problems (74).

ADS had limited clinical utility as a prescription tool to assess risks of central anticholinergic side-effects

Based on previous literature of anticholinergic burden, our clinical findings was contra-intuitive (114). Our cross-sectional baseline-results did not support a progressive decline in cognitive function when the ADS score increased above 3. Neither did a significant reduction in the patients' ADS sum scores translate into cognitive improvements or reduced peripheral anticholinergic activity in our interventional RCT. Our results were consistent and the overall conclusion is therefore that we do not support implementation of the ADS score model as a prescription tool to reduce the risk of central anticholinergic adverse effects in a frail elderly population. However, It is important to notice that our findings do not counteract with previous observations of a significant increased risk of cognitive impairments in older adults with high AB compared with non-users of anticholinergic drugs (72,103,109,168). Accordingly, we might have achieved another result if the ADS score had been reduced to zero in the interventional group. It might seem quite obvious that the risk of side-effects is significantly increased in users compared to non-users of anticholinergic drugs, but the cross-sectional studies have thoroughly increased our knowledge about what harm these drugs might cause in elderly people. Longitudinal observational studies have further enhanced our knowledge of the cognitive risks associated with cumulative anticholinergic drug exposure and that discontinuation of anticholinergic treatment reversed the elevated risk of cognitive decline (90,112,169). The one interventional study conducted prior to the PRADA-study, is unfortunately limited by the risk of type I error (67) while another RCT have solely investigated the effects of biperiden withdrawal (170). Overall, the risks associated with anticholinergic drugs used in the elderly is thoroughly investigated, but as shown in table 2, the results from the observational studies of AB is not as conclusive as previously reported (114). Even so, our lack of interventional effect was unexpected and should be confirmed in larger elderly populations.

The pharmacist-initiated reduction of the patients' ADS score was significant, but the reduction did not translate into improvements in patient-related outcomes.

The RCT was partly motivated by the intention to show that clinical pharmacists contribute to improve health outcomes in geriatric patients with complex drug regimes. We found that

the contribution of a clinical pharmacist in the multi-disciplinary drug reviews did result in a significant reduction in the ADS score, but this reduction did not translate into improvements in patient-related outcomes. Previous trials have shown that the inclusion of clinical pharmacists in geriatric evaluation and management units, and in nursing homes, improved the appropriate use of medicines and reduced potentially harmful drug-related problems (45,58). However, studies that translate these potentially positive effects into patient-related health outcomes are limited and inconclusive (49,50). We did not succeed in our intention, but the drug changes performed in the drug reviews were all guided by the ADS score model. Therefore, our intervention should not be considered as a general evaluation of complete multi-disciplinary drug reviews. Furthermore, the patient-related outcomes were solely related to anticholinergic drug effects. It is important to underline that the PRADA-study was not designed to evaluate the effect of pharmaceutical care in nursing homes. Pharmaceutical care includes more than one drug review guided by the intention to reduce the patients' ADS scores. However, despite no effects on the patient-related outcomes with our approach, the result reflects the possibilities of reducing a potential risk and hence increase drug safety by including a clinical pharmacist in geriatric health care teams.

SAA was significantly higher in poor versus extensive CYP P450 2D6/2C19 metabolizers in an elderly population with high ADS scores.

To our knowledge the relation between genetic heterogeneity in drug clearance and anticholinergic measures has not previously been investigated. We found that high levels of serum anticholinergic activity (SAA), was significantly associated with mutations in cytochrome P450 2D6 and 2C19. Those with no activity in these drug metabolizing hepatic enzymes (PMs) had significantly higher SAA compared to those with enzyme activity (EMs). We did not find any significant differences in clinical outcomes between PMs and EMs, but the clinical importance of our preliminary findings needs to be further investigated. However, other clinical studies have reported that the serum concentration of many anticholinergic drugs is substantially increased in CYP2D6 or CYP2C19 PMs versus EMs and it is therefore likely that the present elevated SAA is related to reduced anticholinergic drug clearance (65,171).

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METHODOLOGICAL WEAKNESSES

LIMITATIONS OF THE IN-VITRO DETERMINED SAA

We developed a more effective method for determination of *in vitro* AA which was used in all the papers included in this thesis, but the bioassay has certain drawbacks. SAA is a measure of peripheral circulating anticholinergic compounds, and does not consider the concentration of the anticholinergic drugs in the brain. One study has even shown that SAA did not correlate with the cerebral cholinergic function as measured by EEG (116). Still SAA has been related to several central anticholinergic side-effects (91,102,103). However, the prediction of central side-effects need to be adjusted for the degree of brain distribution of the drug. In example, we reported that the in vitro AA of oxybutynin was relatively low compared to other urinary spasmolytic drugs, but clinical studies have shown that oxybutynin can cause cognitive impairments and this might be explained by relatively higher brain distribution (172,173). Other limitations of the binding assay is that it does not disentangle between antagonistic and agonistic activity towards the muscarinic receptors nor the specific binding to each muscarinic subtype. It has also been criticized that the levels of SAA vary considerable between different reports (104). This might partly be explained by intra-laboratory heterogeneity in the assay methodology. For instance different curve models might have been used to calculate the atropine standard curves used as a reference for AA (96,174). Furthermore, the bioassay is not standardized and several different units of the AA exist in the literature (104). Finally, the level of SAA is determined by medications, drug metabolites, and endogenous anticholinergic substances and there has been shown that the level of SAA increased during acute illness, without any relations to drug changes. The SAA increment was reversed following illness recovery and it was suggested that SAA might be increased due to a nonspecific stress response to illness in older persons (175,176). Accordingly, naturally occurring substances such as cortisol have shown binding affinity to muscarinic receptors in vitro (104). Moreover, plasma proteins have inhibited the binding of the ^{3}H-QNB in the bioassay and interfered with the resulting SAA levels (177).

LIMITATIONS OF ADS

ADS is an expert-based score model to determine the overall AA of a subject based on the in vitro detected AA of different drugs. Hence, the limitations of SAA and ADS are overlapping, but ADS has some additional drawbacks related to the 3-propotional categorization and the summation of drugs' AA. Firstly, the ADS is developed to estimate an overall drug response, but the dose-response relationship of each drug is not considered in the model. This means, for instance, that a small dose of digitoxin, furosemide and ecitalopram, all having an ADS score of 1, are considered to cause the same anticholinergic burden (ADS sum score = 3), as one high dosage of hydroxycine. Secondly, the relative AA of different drugs is probably not proportional to a 1:2:3 ratio. Thirdly, the overall anticholinergic drug response is influenced by differential aging, drug-drug interactions, and multiple diseases, in particular the stage of dementia, but the inter-individual heterogeneity is not considered in the ADS-score model. This is a general and important drawback of all inappropriate drug lists used as geriatric prescription tools. Finally, there is a lack of consensus on how to define the anticholinergic drug exposure in the different methods used to determine anticholinergic burden and a gold standard is required to achieve internal as well as external validity (33). In conclusion, all the ADS score models simplifies complex pharmacological mechanisms into categories and it is reasonable to question whether ADS sum score possible can predict an overall brain effect of multiple anticholinergic agents in older people with multiple diseases.

LIMITATIONS OF GENOTYPING

We suggest that genotyping could be a clinical tool to identify patients at increased risk of anticholinergic drug toxicity, but the clinical importance of determining genetic variants might be questioned by the effect size of the clinical outcome (178). Furthermore, the relationship between genetic variants and anticholinergic drug responses is still unclear. However, clinical implication of pharmacogenetic testing is strengthened by the unpredictable drug response in the elderly, and in the cases of long-term treatment of anticholinergic drugs with a small therapeutic range.

We divided the study population into two phenotype subgroups according to CYP2D6 and CYP2C19 genotypes. There are limitations in interpreting the genotype-phenotype relation into poor and extensive metabolizers based on the most relevant alleles. Despite the limitations, the genotype determination of CYP 2D6/2C19 deficiency is consistent and of major importance in this relatively small study. We don't think a more accurate prediction of the catalytic activity within the EMs would interfere with our findings.

OTHER LIMITATIONS

The interpretation of the results in paper I and IV is limited by the uncertainty of the clinical relevance of the in vitro AA. Although high levels of SAA have previously been related to cognitive impairment (103), we could not confirm this relationship. Hence, the results in paper I must be understood as a relative pharmacodynamic potential of USDs to cause central anticholinergic effects. The results in paper IV was regarded as preliminary and is limited by the few PMs.

CONCERNS OF TYPE II ERROR

We unexpectedly retained the null-hypothesis in this study and this generated a general concern of type II error. Test sensitivity is an important determinant of the interventional effect and we can not rule out that other measures such as executive functions and physical performance would have been more sensitive to possible improvements of the drug changes. The time schedule is another determinant of the effect, and longer observation time might have been required to regain the dynamics in cholinergic brain transmission after drug changes. However, the consistency between our cross-sectional and interventional results , and the reliability between the primary and secondary statistical analyses of the effects, strengthened our conviction in the null-hypothesis.

INTERNAL VALIDITY

Internal validity is a general problem with studies conducted in very elderly populations because of selection bias. To ensure that our study population consisted of a representative sample, our inclusion strategy was broad. Nevertheless, we experienced a low inclusion

rate, which might reflect that very elderly people and their proxies generally are more reluctant to participate in research trials (36). The great heterogeneity of very elderly subjects represents a further challenge to the internal validity, but even so, age itself should no longer be considered as a reason for exclusion from RCTs (36). A high drop out rate is also a difficulty that needs to be adjusted for because very elderly study participants with multiple diseases often have lower adherence to the protocol than younger healthier ones (33). Despite the challenges that our study population represents, the overall impression was that the participants were positive to conduct the tests. We even observed a significant within group improvement in cognitive performances in both study groups which might reflect their willingness to cope with the cognitive tasks given them.

EXTERNAL VALIDITY

Achieving external validity is challenging when conducting a study under real-life conditions as the characteristics of the study populations might affect the results (33). Despite lowered generalizing strength of the results, it is very important to evaluate drug effects and combinations of drugs in the patients that actually use the drugs, under those circumstances they use them. The external validity of our results might be reduced by our modifications of the original ADS score. The lack of consensus of how to determine anticholinergic burden reduces the external validity of all the studies using anticholinergic assessment tools and it makes it difficult to compare the findings between the studies. The external validity of the in vitro study might be limited by the distribution of muscarinic receptor subtypes in the sonicated rat cerebrum that we used. As biological material always differ, this is a general limitation in all studies of biological nature.

CONSEQUENSES OF THE RESEARCH

This study underlines the need for more RCTs in real-life settings to validate the clinical utility of anticholinergic risk assessment tools. Generally, there is an increasing amount of pharmacoepidemiological studies reporting high risk:benefit ratio related to the pharmacological treatments of elderly people. The risk:benefit ratio is based on the amount

of expert-rated inappropriate medicines used (30,31). These studies are often presented in an overwrought press which might generate a general scare and poor adherence to drug prescriptions in the elderly. Anticholinergic drugs are defined as inappropriate in older people. To be in line with the observational studies we might interpret the lack of improvements related to reduced anticholinergic burden, as a consequence of the study limitations. On the other hand, the lack of effects might also be interpreted as a consequence of the complexity within geriatric clinical pharmacology. We might, in our attempt to predict risks by compulsive classification of central drug effects, have manufactured an unnecessary worry of cognitive side-effects related to certain drugs. There is a need for further investigations, and our results needs to be confirmed in larger samples, but anyhow, the practical consequences of our research might be summed up in one sentence written by James Malone-Lee in a letter in British Medical Journal in 2011: "Nature is inimicial to categories-biological variations is continuously dispersed."

FUTURE DIRECTIONS

Postmarketing pharmacoepidemiologic safety studies are important to identify risks of adverse drug effects in elderly people (33). However, withdrawal of high-risk medicines has to be translated into relevant health improvements before changes in geriatric prescription guidelines should be made. Generally, there is a need for more RCTs in different elderly populations to confirm or reject the findings from the observational safety studies (33).

Working with this thesis has inspired to conduct another RCTs with the objective to evaluate the possible improvement from discontinuation of drugs with high risk of adverse CNS-effects in cognitively intact and cognitively impaired elderly patients. Based on recent observational studies, the clinical outcomes of such drug changes could be for instant grip strength, falls, and ADL in addition to global cognitive status (MMS) (128,179). These are all relevant outcomes of drug safety in geriatric patients.

It is well established that definite anticholinergic drugs (ADS = 3) are inappropriate in elderly patients due to their high risk of cognitive side-effects. However, the anticholinergic scales categorize many drugs, not normally considered as anticholinergic, as potentially

anticholinergic based on their previously shown in vitro AA (ADS =1) (96,122). To our knowledge, the anticholinergic potential of the 71 drugs with ADS =1, is not confirmed in observational studies. It would have been interesting to further evaluate the *in vivo* AA and possible cognitive impact of these drugs in larger population samples. Maybe the drugs with ADS = 1 do not represent any clinically important risk of anticholinergic side-effects? Furthermore, it would have been valuable to assess whether our relative in vitro detection of potentially brain AA of the five USDs was confirmed in vivo studies. In particular, the potentially favorable safety profile of darifenacine need to be investigated in elderly study populations.

Despite that 70 % of the present nursing home population had mild-moderate dementia, only four patients were treated with cholinesterase inhibitors. Several pharmacoepidemiological studies have reported that 30% of those using cholinesterase inhibitors use anticholinergic drugs concurrently (75,76). Some previous preliminary results have suggested that chronic concomitant exposure to anticholinergics are associated with significant deleterious effects on cholinesterase therapy (180). The clinical importance of this drug-drug interaction would have been interesting to study further. There are also diverging observational findings of the association between chronic anticholinergic drug exposure and the development and progression of dementia, and this should be clarified in further longitudinal studies (112,169).

One study reported that anticholinergic drugs had more severe negative impact on physical function than sedative drugs in elderly patients (92). This would be interesting to analyze further because there might be an impression of less harm related to anticholinergic sedatives compared to traditional hypnotics used to treat sleeping disorders in the elderly.

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I

II

TITLE PAGE

Title

Higher anticholinergic drug scale (ADS) scores are associated with peripheral but not cognitive markers of cholinergic blockade. Cross sectional data from 21 Norwegian nursing homes.

Running head

Association between high ADS scores and anticholinergic symptoms in elderly.

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Keywords

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Drugs with anticholinergic properties are frequently used in older people despite their high potential of precipitating central and peripheral adverse effects

- Many institutionalized older persons use several drugs with potential anticholinergic effects concurrently

- Observational studies have reported that patients with a high anticholinergic burden, i.e. a score of 3 or higher on the anticholinergic drug scale (ADS), have increased risk of cognitive impairment compared to non-users of anticholinergics (ADS score '0').

WHAT THIS PAPER ADDS

- About one fifth of nursing home residents had an ADS score of 3 or higher

- Residents with dementia had lower ADS scores than those without dementia.

- After adjustment for clinical dementia rate, there was no evidence of a progressive decline in cognitive function when ADS scores increased above 3 in frail nursing home residents.

Summary

AIM

This study evaluated a presumed gradual decline in cognitive function in nursing home residents when the anticholinergic drug scale (ADS) score increased above 3.

METHODS

The study population was recruited from 21 nursing homes in Norway. Criteria for inclusion were ADS score ≥ 3 and no severe dementia, defined as Clinical Dementia Rating (CDR) score < 3. Primary cognitive end-points were CERAD 10-word lists for recall and Mini Mental State Examination (MMSE). Secondary end-points were activity of daily living (ADL), mouth dryness and serum anticholinergic activity (SAA). The patients were stratified into subgroups according to ADS score, i.e. a reference group with score 3, and test groups with scores 4, 5 or ≥ 6. End-points were compared by analyses of covariance (ANCOVA).

RESULTS

Overall, 230 of the 1101 screened nursing home residents (21%) had an ADS score ≥ 3. After exclusion 101 residents were recruited and among these, 87 managed to participate in the study. No significant differences were detected in cognitive function or ADL when ADS increased above 3 (p> 0.10), but *in vivo* (mouth dryness) and *in vitro* (SAA) measures of peripheral anticholinergic activity were significantly higher in patient with ADS score ≥ 6 (p< 0.01).

CONCLUSION

The present study does not support a progressive decline in cognitive function with ADS score above 3. This might indicate that the ADS score model has limited potential to predict the clinical risk of central anticholinergic side effects in frail elderly patients receiving multiple anticholinergic drugs.

2

Introduction

Many commonly prescribed drugs have shown affinity to muscarinic receptors *in vitro* and may cause central and peripheral antimuscarinic symptoms *in vivo* [1]. Aged people, especially those who are cognitively impaired, have increased sensitivity to central antimuscarinic adverse effects due to reduction in cholinergic neurotransmission, and use of antimuscarinic drugs (in the following referred to as 'anticholinergic') has been associated with reduced cognitive and physical function in the elderly [2-4]. Still, anticholinergic drugs are frequently prescribed to elderly people, and previous studies have shown that nursing home patients often use several anticholinergic drugs simultaneously [5;6].

It is believed that drug-induced anticholinergic activity (AA) is additive and that the overall anticholinergic burden determines the risk of adverse effects [4;7-9]. In 2006, Carnahan and colleagues published the Anticholinergic Drug Scale (ADS) score model for estimation of the overall anticholinergic burden. The ADS has four score levels for each included drug, ranging from level '0' ('no known AA') to level '3' ('markedly AA') [8]. Summation of each medication's ADS score reflects the total anticholinergic burden of a subject.

Large observational studies using the ADS score model have previously reported that high anticholinergic burden increases the risk for peripheral and central side effects when comparing patients with ADS score '0' (non-users of anticholinergics) with those using anticholinergics [4;9-11]. However, the proposed additive properties of the ADS inventory have not previously been evaluated. Thus, it is unknown whether cognitive function gradually decline in aged people who are exposed to an increasing number of anticholinergic medications. The aim of the present study was therefore to investigate the cognitive function when the ADS scores increased above 3 in frail nursing home residents.

3

Methods

Study population

The participants were long-term nursing home residents recruited from 21 institutions in two different Norwegian counties during 2008-2009. Anonymized medical records were screened for anticholinergic drugs by a clinical pharmacist (HK) and a trained study nurse. Anticholinergic drug score was assessed using the ADS score model published by Carnahan et al. in 2006, with some modifications based on a more recent, comprehensive in vitro screening of anticholinergic activities at therapeutic serum concentrations published by Chew et al. in 2008 [1;8]. Patients with overall ADS score ≥ 3 were considered for inclusion by a local caregiver who evaluated their physical and mental eligibility to participate. Patients with blindness, deafness, aphasia, delirium, or severe dementia, i.e. score 3 on the Clinical Dementia Rating scale (CDR) were excluded [12;13].

Outcome measures

Cognitive function

One study nurse, blinded for the patients' ADS scores, performed the cognitive tests on all the participants. The cognitive test battery included the Norwegian translated version of the 10 words tests of immediate recall, delayed recall, and recognition, from the Consortium to Establish a Registry for Alzheimer Disease (CERAD) neuropsychological test battery [14] and the Mini Mental State Examiation (MMSE), revised for use in Norwegian nursing homes [15]. The CERAD subtests were chosen as they can sensitively differentiate between cognitive impairments of different severity [16].

4

Self-care capacity

The patients` self-care capacity was assessed using the Barthel`s Index of Activity of Daily Living (ADL) [17]. The ADL scores of the patients were determined by a nurse or auxiliary nurse at each nursing home included.

Mouth dryness

A swab technique was used to measure the resting whole mouth saliva flow rate. The test was performed by first placing two pre-weighted dental cotton rolls in the patients' lower jowl for three minutes and then in the upper vestibules at the opening of the parotid gland ducts for three minutes. The weight difference of the cotton rolls was used to determine the salivary flow. This test has been shown to be reliable and practicable in cognitive impaired elderly adults [18].

Serum anticholinergic activity

Blood was sampled from the patients for measurement of serum AA (SAA) using a modified version of the radio receptor assay published by Tune and Coyle in 1980 [19]. In the modified assay, samples of 20 µl were applied in 96 well plates for high throughput analyses of SAA [20]. A standard curve with atropine (0.05 to 100 nM) was used as reference for anticholinergic activity. Standard curves were fitted to a one-site competitive binding model using GraphPad Prism version 5.01 (GraphPad Software Inc, CA).

Covariates

Information about age, gender, educational level, smoking habits, time since last meal and medication intake, and use of dental prostheses, was collected from the patients' nursing home records. Further, information about other possible confounders, such as diagnoses,

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neuropsychiatric symptoms, serum creatinine, the use of cholinesterase inhibitors and the total number of drugs used was recorded. Co-morbidity was assessed by the Charlson Co-morbidity Index [21]. The frequency (F) and intensity (I) of neuropsychiatric symptoms were rated by use of the Norwegian version of the neuropsychiatric inventory for nursing homes (NPI). Each symptom with item score ≥ 4 (F x I) was assessed to be of clinical relevance [22]. Glomerular filtration rate (GFR) was calculated according to the Modification of Diet in Renal Disease (MDRD) study equation [23].

Statistical Analyses

Prior to the statistical analyses, the patients were stratified into four subgroups according to overall ADS scores; i.e. a reference group with score 3 and three test groups with scores 4, 5, and 6-10, respectively. Distribution of the covariates across the four strata was compared by Kruskal-Wallis test. All the outcome measures were explored by distribution plots and descriptive analyses. Log 10 transformations of SAA and saliva production were conducted to attain normal distribution. Two-tailed Spearman's rank correlations between the covariates and the outcome measures were inspected to identify possible confounders and collinearity between the covariates. Analyses of covariance were performed to compare the mean difference in each outcome measures between the test groups (ADS = 4, 5 and $\geq$ 6) and the reference group (ADS = 3). We adjusted for the possible effects of the imbalanced covariates with significance level ≤ 0.1. In addition, analyses of cognitively intact patients and patients with mild-moderate dementia were performed separately in each ADS-subgroup. We included the 45 patients with severe dementia in a Mann-Whitney test performed to compare the ADS scores in patients with dementia (CDR 1, 2 and 3) versus patients without dementia (CDR = 0). All statistical analyses were performed using PASW Statistics for Windows version 19 (SPSS Inc., Chicago, USA).

6

Ethics

We obtained written informed consent from all participants. For participants with substantial cognitive impairment, written informed consent was collected from the closest relative, in accordance with Norwegian legal regulation. The study was approved by the Regional Committee for Medical Research Ethics, the Norwegian Directorate of Health and the Data Protection Officer at Oslo University Hospital.

RESULTS

Of the 1101 screened residents, 230 (21 %) had an ADS sum score $\geq$ 3. After exclusion based on predefined criteria, 101 nursing home residents were recruited. Among these 87 managed to participate and comprised the finally included study population. **Figure 1** shows the sample selection and the reasons for exclusion. The nursing home staff considered 99 of the patients with ADS $\geq$ 3 to be incapable of executing the tests; 45 due to severe dementia (CDR = 3) and 44 because of physical impairments such as aphasia, loss of hearing or sight. The 87 patients finally included were allocated to the ADS subgroups (score 3, n=35; score 4, n=22; score 5, n=16; and score $\geq$ 6, n=13).

Clinical characteristics of the study participants stratified by their ADS score are presented in **table 1**. The study population (n= 87) comprised 69 women and 18 men, all aged above 73 years, women being older (median age 87 years, interquartile range (IQR) 84-92) than men (median age 81 years, IQR 79-85). Their median Charlson co-morbidity score was 4 (IQR 3-5), almost 70 % of the included patients had mild to moderate dementia (CDR 1-2), and four patients were recorded with clinical significant symptoms of depression (NPI item score $\geq$ 4). The median number of drugs used on a regular basis was 9 (IQR 7-12), and four patients were treated with cholinesterase inhibitors. The distribution of number of scheduled medications and degree of dementia (CDR) were significantly different across the categories of ADS score, (p = 0.004 and p = 0.007 respectively, in Kruskal-Wallis test). Patients with ADS score $\geq$ 6 had less dementia and used more drugs on a regular basis than the other groups and none of the patients with moderate dementia (CDR = 2) had an ADS score $\geq$ 6. The distribution of patient with dementia (CDR = 1, 2 or 3) versus patients without dementia (CDR = 0), was significantly asymmetrical across the ADS categories (p = 0.011), and the patients with dementia had significantly lower ADS scores than the patients without dementia (p = 0.024).

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The 31 different anticholinergic drugs used by the participants are listed in **table 2**. Fifty-nine patients used one drug with an ADS score of 3 (hydroxyzine, chlorprothixene, and alimemazine most frequent, being used by 15 %), while three patients used two drugs in this category. The most frequently used anticholinergic drug regardless of score was furosemide (ADS = 1). Psychotropic drugs with anticholinergic properties were common in the nursing home patients. Overall, 50 % used an antidepressant and 27 % used an antipsychotic listed in the ADS score model. Two patients used five different anticholinergic drugs and five patients had a total ADS score ≥7. Altogether, the 87 patients used 204 prescribed anticholinergic medications on a regular basis.

The median values of immediate recall, ADL, salivary flow and SAA in each ADS subgroup are illustrated in **Figure 2. Table 3** shows unadjusted and adjusted mean differences in end-points between the ADS subgroups with reference to ADS = 3. CDR and number of scheduled drugs were significantly imbalanced between the ADS subgroups, but only CDR was identified as a covariate with sign level $p \leq 0.1$ and thus adjusted for in the multivariate model. In the unadjusted and adjusted models, no significant differences in cognitive end-points were detected between patients with increasing ADS scores ($p > 0.11$). Separate analyses of cognitively intact patients (n = 27) and patients with dementia (n = 60) did not show any significant cognitive decline with increasing ADS scores in any of the subgroups ($p > 0.45$ and $p > 0.65$, respectively).

The self-care capacity increased significantly with increasing ADS scores ($p = 0.05$), and the participants with ADS ≥ 6 had approximately 23 % better self-care capacity than the

participants in with ADS = 3, p = 0.014 **(table 3)**. After adjusting for the imbalance in CDR the difference in ADL did not persist statistically significant (p = 0.13).

The measure of peripheral circulating anticholinergic activity (SAA), and the peripheral clinical measure mouth dryness, were both significantly increased when participants with ADS score $\geq$ 6 were compared to participants with ADS score = 3 (p < 0.01) **(table 3)**. No differences in peripheral activity measures were observed between test groups with ADS score of 4 or 5 compared to score 3 (p > 0.15). The significant increase in SAA and mouth dryness in patients with ADS score $\geq$ 6 persisted after controlling for differences in CDR (p < 0.02).

Discussion

We found that one fifth of nursing home residents had an ADS score ≥ 3, while around 10 % had ADS scores 4-10. This implies that a substantial proportion of the patients used at least two anticholinergic drugs concurrently. Large observational studies have reported that a high ADS score is a significant predictor of cognitive impairments, but the 'dose-response' relationship in ADS ≥ 3 has not previously been investigated [2;4;8-11]. As no significant differences in cognitive outcomes were observed between the patient subgroups, our results do not support a gradual decrease in cognitive function when ADS score increases above 3. However, the number of nursing homes patients eligible for inclusion was limited, and larger studies would be desirable to confirm the present findings.

The lack of association between ADS score and cognitive function in the current study could be explained by several factors:

Firstly, the ADS score model has a rather simple concept which does not take into account systemic drug exposure, distribution to the brain or pharmacodynamic interactions. The cognitive decline is dependent of the AA in the brain which is previously reported to be dose-dependent, especially in people with dementia. [24] Moreover, the pharmacodynamic brain effects of multiple anticholinergic drugs are probably not additive in a linear pattern that can be predicted by the ADS score model.

Secondly, since aging and Alzheimer Dementia (AD) have previously been associated with hypersensitivity to anticholinergic drugs due to loss of cholinergic neurotransmission, it is possible that a saturation of the receptors might be reached by excessive anticholinergic

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activity (ADS ≥ 3). [25;26] As a consequence, a further anticholinergic increase cannot displace more acetylcholine from the muscarinic receptors.

Finally, the present variability in anticholinergic drug sensitivity related to advanced age, multi morbidity, different degree of dementia and the multiple comedications might affect the results. Thus, we adjusted for the differences in dementia between the ADS subgroups, but the adjusted models did not show significant decline in any of the cognitive test scores when the ADS score increased above 3. In addition, we controlled for the influence of comorbid depression and concurrent use of cholinesterase inhibitors due to the potential importance of these covariates on cognitive test scores, but this did not alter the results. However, the NPI recordings might have underestimated the prevalence of depression (< 5 %) which is supported by the fact that 50% of the residents were treated with antidepressants.

Furthermore, the high prevalence of cognitive impairment in our study population might have reduced the sensitivity of the anticholinergic drug burden on the cognitive test scores. This is consistent with two previous studies reporting no cognitive impact of high anticholinergic burden in old patients with dementia [6;27] However, the separate analyses of cognitively intact participants (CDR = 0; n = 27) in the present study did not show a greater decline in cognitive test performances than observed in the patients with dementia (CDR = 1-2; n = 60). Unfortunately, the small number of participants in each ADS subgroup limits the interpretation of these data. Nevertheless, among nursing home residents with no, mild or moderate dementia, the ADS score model appears to have a limited potential to predict the clinical risk of central anticholinergic side- effects.

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In addition to the cognitive tests included, we decided to measure activity of daily living (ADL). The inclusion of ADL was based on a small previous study reporting greater impairment in ADL in demented patients with high versus low anticholinergic burden [28]. Unfortunately, the validity of the current ADL registration is considered to be reduced because the registration forms for Barthel's Index were filled in by 21 different caregivers with variable knowledge about the patients. However, we observed a significant increase in ADL with increasing ADS score above 3, but the increase in ADL did not persist statistically significant after adjusting for the imbalance in CDR which indicates that the observed increase in ADL is explained by the absence of patients with moderate dementia in the group with the highest ADS scores.

In the present material, nursing home residents with dementia had significantly lower ADS scores than those without dementia. This observation might indicate that patients with dementia are prescribed less anticholinergic drugs than others, which is appropriate from a pharmacological point of view. However, whether the lower ADS score in patients with dementia was due to rational medical decisions, or simply reflected a generally restrictive prescription policy in this patient subgroup, is unclear. Nevertheless, as the pathophysiological changes in cholinergic brain transmission in Alzheimer's dementia increase the sensitivity to temporary cholinergic blockade [24], it would be favorable to avoid or limit use of anticholinergic drugs in people with dementia.

Interestingly, we observed that the overall ADS score was significantly associated to peripheral anticholinergic end-points in terms of a 1.2-fold higher serum anticholinergic activity and 0.7-fold lower saliva production in subjects with ADS ≥ 6 compared to those with ADS score 3. The significant increase in SAA and in peripheral, but not central adverse

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effects demonstrated for subjects with ADS ≥ 6, might be understood in terms of how the ADS score model was developed. The potential anticholinergic effects of many drugs included in the model were characterized by *in vitro* activity to muscarinic receptors measured by the same bio-assay as used for determination of SAA. The drugs were further graded as mild (ADS = 1), moderate (ADS = 2), or markedly anticholinergic (ADS = 3) based upon a consensus of clinical experience, previously reported adverse effects and knowledge of the drugs' properties. As symptoms of central anticholinergic side-effects may be subtle in patients with cognitive disorders (e.g. mild alterations in verbal short-time memory and attention), it's possible that the model was primarily based upon symptoms of peripheral anticholinergic activity.

The interpretation of the present results is restricted by the cross-sectional design and the relatively low and imbalanced number of patients in each ADS category. A randomized, controlled study investigating the potential improvement in cognitive function after an interventional reduction in ADS score would be more conclusive to clarify the clinical utility of the risk score model for evaluation of adverse drug effects. In similar with the validity restrictions of all prescription risk tools in the elderly, the external validity of the present results is limited by the great variability in drug response associated with advanced age, multi morbidity and polypharmacy. On the contrary, the prospective design and the consistent findings within the cognitive and peripheral end-points strengthen the validity. The results are further strengthened by the fact that all cognitive measurements were performed by one study nurse who was blinded to the participants' ADS scores.

In conclusion, the current study does not support a progressive decline in cognitive function with ADS scores above 3. Despite the relatively low number of participants included and

restrictions in the external validity, the findings might indicate that the ADS score model has limited potential to predict the clinical risk of central anticholinergic side-effects in frail elderly patients receiving multiple anticholinergic drugs.

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Table 1 Characteristics of the study cohort represented by the four strata with different anticholinergic drug scale (ADS) score.

Characteristics	ADS = 3 n = 36	ADS = 4 n = 22	ADS = 5 n = 16	ADS ≥ 6 n = 13
Age	87 (84-93) 73-99	85 (83-87) 77-93	84 (81-91) 74-96	83 (81-90) 77-93
Female gender	29 (81%)	16 (73%)	13 (81%)	11(85%)
Education > 12 years	4 (11%)	2 (9%)	1 (6%)	0
Daily smoking	5 (14%)	3 (14%)	1 (6%)	4 (31%)
No dental prostheses	16 (44%)	11 (50%)	7 (44%)	4 (31%)
CDR*				
0 = no dementia, n (%)	8 (22%)	5 (23%)	5 (31%)	9 (69%)
1 = mild dementia, n (%)	17 (47%)	8 (36%)	7 (44%)	4 (31%)
2 = moderate dementia, n (%)	11 (31%)	9 (41%)	4 (25%)	0
Scheduled drugs*	8 (6-10) 1-18	9 (7-10) 4-16	10.5 (8-13) 5-14	12 (9.5-5.5) 7-17
[a)]Glomerular Filtration Rate	69 (54-84.5) 33-207	72 (46-87) 18-125	72 (82.5-87) 38-147	55 (41-77) 24-129
Charlson Co-morbidity score	3.5 (3-4) 1-6	4 (2-6) 1-9	3 (2.5-5) 1-9	3 (3-5) 2-7
No. of participants using Cholinesterase inhibitors	1	1	1	1
Prevalence of clinical significant symptoms of depression (Fx I) ≥4 in neuropsychiatric inventory (NPI)	1		2	1

Data represents median, interquartile range (IQR) and range or frequency and percentages within the stratum; n gives the valid data for each characteristics; [a)]Calculated by the Modification of Diet in Renal Disease (MDRD) formula; CDR = Clinical Dementia Rate.*Significant different distribution across the ADS subgroups, $p < 0.05$, Kruskal-Wallis test.

Table 2 Drugs with anticholinergic properties ranked by modified anticholinergic drug scale (ADS) within the study population, n = 87.

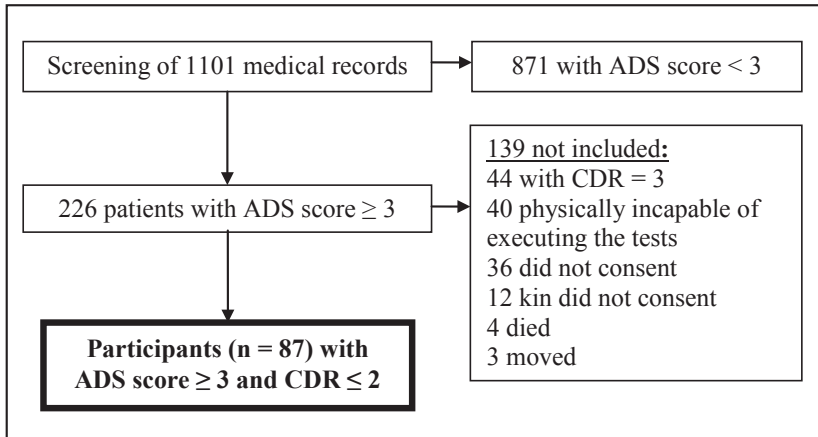| Therapeutic drug group	Frequency (% of n)	Drug name		
		ADS = 3 62 (71.3%)*	ADS =2 10 (10.3%)*	ADS = 1 67 (77.0%)*
Antidepressants	44 (50.6)	amitriptyline trimipramine	noritriptyline paroxetine	escitalopram citalopram mirtazapine fluoxetine
Antipsychotics	24 (276)	chlorprothixene levomepromazine	olanzapine	quetiapine zuclopenthixol
High-ceiling diuretics	27 (31.0)			furosemide
Antihistamines for systemic use	31 (35.6)	hydroxyzine alimemazine dexchlorpheninamine promethazine		
Opioids	14 (16.1)			fentanyl oxycodone
Glucocorticoids	14 (16.1)			prednisolone
Drugs for obstructive airway disease, inhalant and systemic use	14 (16.1)			theophylline ipatropiumbromide
Cardiac glycosides	13 (14.9)			digitoxin
Urinary spasmolytics	12 (13.8)	tolterodine solifenacine		
Anxiolytics	3 (3.4)			diazepam
Antiepileptics	3 (3.4)		oxcarbazepine	
H2-receptor antagonists	2 (2.3)			ranitidine
ACE inhibitors	2 (2.3)			captopril
Lincosamides	1 (1.1)			clindamycin
Total drug prevalence	**204**	**65**	**10**	**129**

Frequency represent the number of participants exposed to drugs in present therapeutic group;*number of participants using at least one drug in present ADS category. (Several patients used more than one drug in each category)

Table 3 Analysis of covariance to compare the mean differences in anticholinergic end-points between the different subgroups of ADS score with reference to ADS score = 3.

End-points	ADS = 3 Reference		ADS = 4			ADS = 5			ADS ≥ 6		
	Mean (SD)	n	B (95% CI) Unadjusted	Adjusted	n	B (95% CI) Unadjusted	Adjusted	n	B (95% CI) Unadjusted	Adjusted	n
CERAD verbal immediate recall, no. of words	10.9 (4.9)	35	1.0 (-1.7, 3.8)	1.3 (-1.1, 3.8)	22	1.5 (-1.6, 4.5)	1.0 (-1.8, 3.7)	16	2.7 (-0.69, 6.0)	0.2 (-2.9, 3.4)	13
CERAD verbal delayed recall, no. of words	2.5 (2.5)	34	0.5 (-1.4, 1.5)	0.2 (-1.1, 1.7)	22	0.3 (-1.5, 1.8)	0.1 (-1.4, 1.6)	16	1.0 (-0.9, 2.7)	-0.1 (-1.9, 1.6)	13
CERAD verbal recognition, no. of words	6.4 (3.2)	26	-1.4 (-3.2, 0.5)	-1.2 (-3.1, 0.7)	17	-0.6 (-2.6, 1.5)	-0.6 (-2.7, 1.4)	13	0.6 (-1.5, 2.6)	0.1 (-2.0, 2.3)	13
Mini mental state examination (MMSE)	19.5 (5.1)	31	0.6 (-2.2, 3.4)	1.1 (-2.9, 2.8)	22	0.5 (-3.1, 3.2)	0.1 (-2.9, 2.8)	15	2.1 (-1.2, 5.5)	-0.1 (-3.4, 3.1)	13
Whole mouth resting salivary flow (g/min)[a]	0.3 (0.1, 0.7)[b]	26	0.0 (-0.2, 0.3)	0.0 (-0.3, 0.3)	17	-0.1 (-0.3, 0.2)	-0.1 (-0.3, 0.2)	14	-0.5** (-0.7, -0.2)	-0.4** (-0.7, 0.1)	12
Serum anticholinergic activity (SAA) (pmol/mL Atropine equivalents)[a]	3.6 (2.0, 6.5)[b]	34	0.1 (-0.2, 0.3)	0.1 (-0.2, 0.3)	22	0.2 (-0.1, 0.4)	0.2 (-0.1, 0.4)	15	0.4** (0.1, 0.6)	0.3* (0.1, 0.6)	12
Barthel's Index of activity of daily living (ADL)	46.8 (35.9, 57.7)	33	3.6 (-11.42, 18.7)	4.5 (-10.5, 19.6)	22	15.8 (-1.2, 32.9)	15.67 (-1.3, 32.6)	15	23.18* (4.7, 41.6)	19.11 (-0.2, 30.4)	12

Controlled for CDR in the adjusted models; B, estimates of mean difference with reference to ADS score = 3; [a] analyze of covariance performed with log-transformed data; [b] data given as median and interquartile range; CI confidence interval; SD, standard deviation; *<0.02; **<0.01; only significant P-values shown; ADS, anticholinergic drug score; n, number of subjects in the subgroup completing each test; g, gram; min, minute; pmol, picomolar; mL, milliliter.

Figure 1 Selection of study population

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│  ┌──────────────────────────────────┐        ┌─────────────────────────┐  │
│  │ Screening of 1101 medical records│───────▶│ 871 with ADS score < 3  │  │
│  └──────────────────────────────────┘        └─────────────────────────┘  │
│                    │                                                       │
│                    │                          ┌─────────────────────────┐  │
│                    ▼                           │ 139 not included:       │  │
│  ┌──────────────────────────────────┐         │ 44 with CDR = 3         │  │
│  │ 226 patients with ADS score ≥ 3  │────────▶│ 40 physically incapable │  │
│  └──────────────────────────────────┘         │ of executing the tests  │  │
│                    │                           │ 36 did not consent      │  │
│                    │                           │ 12 kin did not consent  │  │
│                    ▼                           │ 4 died                  │  │
│  ┌──────────────────────────────────┐         │ 3 moved                 │  │
│  │   Participants (n = 87) with     │         └─────────────────────────┘  │
│  │   ADS score ≥ 3 and CDR ≤ 2      │                                      │
│  └──────────────────────────────────┘                                      │
│                                                                           │
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ADS = Anticholinergic Drug Scale

CDR = Clinical Dementia Rating Scale

Figure 2 Median, IQR and range (10-90 percentiles) for CERADs wordlist for immediate recall (a), whole mouth resting saliva flow (b), serum anticholinergic activity (c), and Activity of daily living (d) in nursing home patients with anticholinergic drug scale (ADS) score of 3 (control group), 4, 5 and 6-10 (test groups). Lines indicate median values.

a) CERADs worldlist test for immediate recall

b) Whole mouth resting saliva flow

c) Serum anticholinergic activity

d) Activity of daily living
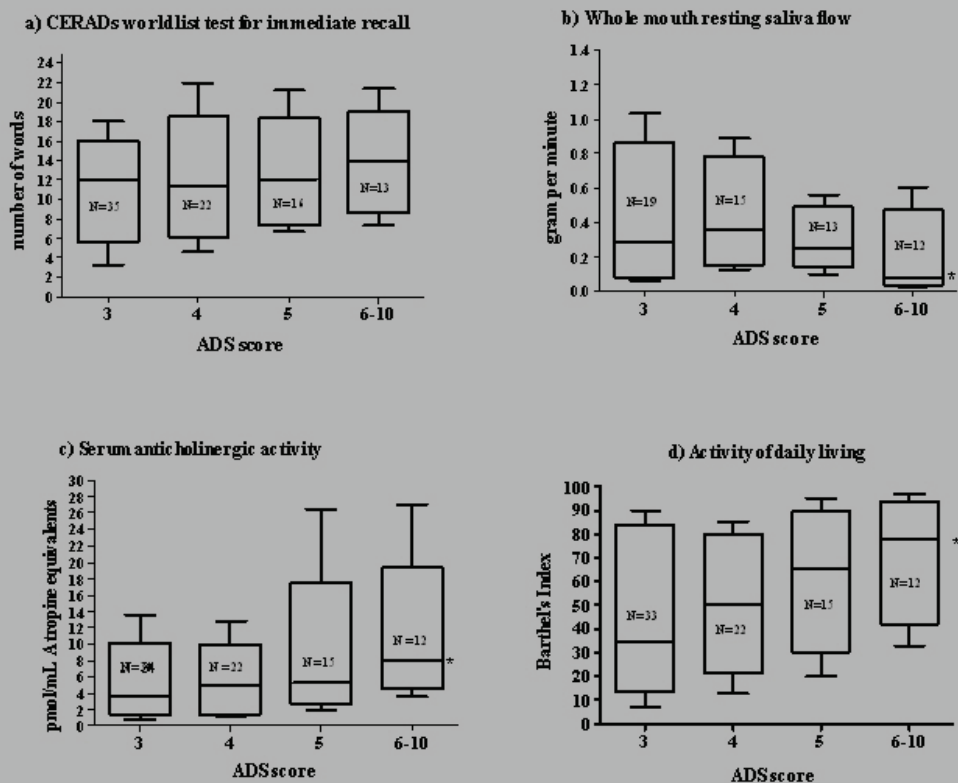
* Significantly different distribution across the four ADS subgroups, p<0.05, Kruskal-Wallis test.

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