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Amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia
A systematic review and meta-analysis

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Dedication

I dedicate this dissertation to all my Medicine Professors who shaped the future doctor I will be.

Acknowledgements

To my adviser Nuno Silva, to my parents Manuel Santos and Rosa Santos, to my brother Manuel Santos, to my partner Sandro Alves, and last but not the least, to my best friend Miguel Fernandes, a thank you of the size of the World because without you all I could not stand where I stand today. I hope one day you all will be as proud of me like I am of you for being part of my life.

Resumo

Introdução: A esquizofrenia é o paradigma da doença mental grave e o seu tratamento tem sido um verdadeiro desafio desde a sua descoberta. Cerca de ⅓ a ½ dos doentes não responde ou apresenta apenas uma melhoria parcial com os antipsicóticos, sendo recomendada a utilização de clozapina, eficaz em 30-50% dos doentes com esquizofrenia resistente e carecendo de monitorização regular devido ao risco de agranulocitose, entre outros. Assim, uma minoria significativa destes doentes mantém sintomas importantes mesmo apesar do tratamento. Para estes, não existem intervenções eficazes, tendo sido publicados estudos com várias combinações de fármacos e terapia electroconvulsiva. A amissulprida, como adjuvante à clozapina, tem sido equacionada como potencial alternativa terapêutica, tendo em conta o seu perfil farmacológico complementar e a mitigação dos efeitos adversos induzidos pela clozapina.

Métodos: Uma pesquisa de literatura foi realizada no PubMed (MEDLINE), SCOPUS e Cochrane Library, com as palavras “clozapina”, “amissulprida” e “esquizofrenia” ou “psicose”, tendo resultado em 856 artigos. Após averiguação, 13 estudos foram adequados para a revisão sistemática, dos quais 7 são do tipo de intervenção (tais como ensaios clínicos e pré-pós), 1 estudo é observacional (retrospectivo) e 5 estudos são casos clínicos descritivos. Apenas 2 estudos foram pertinentes para a meta-análise.

Resultados: A utilização da amissulprida como adjuvante da clozapina aparentou ser eficaz nos estudos descritivos e observacionais e também em alguns estudos de intervenção. Contudo, não mostrou superioridade em alguns estudos de intervenção e de melhor qualidade. A aparente redução de alguns efeitos secundários associados à clozapina (principalmente a sialorreia) foi observada em alguns destes estudos.

Discussão: A amissulprida como adjuvante da clozapina revelou aparentemente uma ligeira melhoria clínica em doentes com esquizofrenia resistente, comparativamente ao placebo. Contudo, a escassez de estudos que explorem a eficácia da adjuvância de

amissulprida, com a qualidade dos estudos variando entre moderada a muito baixa, impede conclusões definitivas. Futura investigação é necessária para que esta terapia possa ser incluída nos algoritmos de tratamentos padrão. Devido à possibilidade de um largo espectro de benefícios observados, é aconselhado que alguns distúrbios concomitantes, como a dependência alcoólica ou a violência, sejam também avaliados.

Palavras-chave

Esquizofrenia;psicose;clozapina;amissulprida

Resumo Alargado

A esquizofrenia é o paradigma da doença mental grave e o seu tratamento tem sido um verdadeiro desafio desde a sua descoberta. A introdução dos antipsicóticos veio trazer uma luz de esperança, permitindo um controlo dos sintomas positivos da esquizofrenia, o que contribuiu para que muitos doentes pudessem ter uma vida integrada na comunidade. No entanto, cerca de ⅓ a ½ dos doentes não responde ou apresenta apenas uma melhoria parcial com os antipsicóticos (típicos e atípicos), sendo nestes casos recomendado a utilização de clozapina. A clozapina é eficaz em 30-50% dos doentes com esquizofrenia resistente, carecendo de monitorização regular devido ao risco de agranulocitose, sendo também frequentes vários outros efeitos adversos. Assim, uma minoria significativa de doentes com esquizofrenia mantém sintomas importantes mesmo apesar do tratamento com clozapina, acarretando múltiplas hospitalizações, diminuição significativa da qualidade de vida do doente e um marcado impacto familiar e social. Para esta minoria relevante de doentes não existem intervenções eficazes, tendo sido publicados estudos com várias combinações de fármacos e terapia electroconvulsiva. A amissulprida, como adjuvante à clozapina, tem sido equacionada como potencial alternativa terapêutica, tendo em conta o seu perfil farmacológico complementar à clozapina e pelo eventual papel no sentido de mitigar efeitos adversos induzidos pela clozapina. Tendo por base este facto, uma pesquisa de literatura foi realizada no PubMed (MEDLINE), SCOPUS e Cochrane Library, com as palavras de busca “clozapina”, “amissulprida” e “esquizofrenia ou psicose”, até ao dia 20 de Agosto de 2020, sem restrição de linguagem ou data, tendo resultado em 856 artigos. Após averiguação, 13 estudos foram adequados para a revisão sistemática, dos quais 7 são do tipo de intervenção (tais como ensaios clínicos e pré-pós), 1 estudo é observacional e 5 estudos são casos clínicos descritivos. Dos 4 estudos inicialmente elegíveis, apenas 2 estudos foram pertinentes para a meta-análise. Segundo 11 dos 13 estudos incluídos, a utilização da amissulprida como adjuvante da clozapina aparentou ser mais eficaz que a adjuvância de quetiapina ou que o placebo, evidenciando uma relativa melhoria clínica nos doentes, constatada pela redução na pontuação das médias de todas as escalas de eficácia, demonstrada pela PANSS nos estudos de Barnes, Kreinin, Koen, Porcelli, Chiu e Munro; pela BPRS nos estudos de Genç, Assion, Ziegenbein, Bogorni e Munro; pela SANS nos estudos de Genç e Munro; e pela SAPS no estudo de Genç. Não obstante, no estudo de Barnes e de Assion, estatisticamente verificou-se que a amissulprida não era mais eficaz que o placebo. A aparente redução de alguns efeitos secundários associados à clozapina (principalmente a sialorreia)

também foi observada em alguns dos estudos incluídos. Relativamente aos efeitos adversos provocados pela combinação da clozapina com a amissulprida, os mais frequentes foram a hiperprolactinemia e os sintomas cardíacos. No entanto, uma vez que a qualidade dos estudos varia de moderada a muito baixa, torna-se difícil e incerta a tomada de conclusões definitivas. Futura investigação é necessária para que esta terapia possa ser incluída nos algoritmos de tratamentos padrão. É aconselhado que alguns distúrbios concomitantes, tais como a dependência alcoólica ou a violência, sejam também avaliados.

Abstract

Introduction: Schizophrenia is the paradigm of severe mental illness, being its treatment a great challenge since its recognition. About ⅓ to ½ of patients do not respond or only show a partial response to antipsychotic treatment, for whom clozapine is recommended, which is effective in 30-50% of patients with treatment-resistant schizophrenia but requires regular monitoring due to agranulocytosis risk and several other common adverse effects. In addition, a significant minority of these patients maintain important symptoms, despite treatment with clozapine. For those, there are no approved interventions, with studies hypothesizing benefits with drug combinations and electroconvulsive therapy. Amisulpride, as an adjunctive to clozapine, has been considered a potential therapeutic alternative for its complementary pharmacological profile and the possibility of counteracting adverse effects induced by clozapine.

Methods: A literature search was performed in PubMed (MEDLINE), Scopus and Cochrane Library, with “clozapine”, “amisulpride” and “schizophrenia” or “psychosis”, yielding 856 articles. Thirteen were suitable for this comprehensive systematic review, of which 7 were interventional studies (randomized controlled trials and pre-post), 1 observational study (retrospective) and 5 descriptive studies (case series and case reports). Only two studies were suitable for meta-analysis.

Results: Amisulpride as an adjunctive to clozapine appeared to be effective in descriptive and observational studies and in a few interventional studies. However, it did not show superiority in some interventional and higher quality studies. A reduction in some of clozapine’s side effects (sialorrhea mostly) was reported in some studies.

Discussion: Amisulpride when added to clozapine therapy apparently revealed a slight clinical improvement in patients with treatment-resistant schizophrenia, compared to placebo. However, the scarcity of studies exploring the efficacy of amisulpride as an add-on, with studies quality ranging from moderate to very low, preclude definite conclusions. Further research is needed so this add-on therapy can be included in standard treatment algorithms. Due to the possibility of a wide range of benefits to be

observed with amisulpride, it is advised that some concomitant disturbances, such as substance use, violence, and clozapine's side effects, should be also assessed.

Keywords

Schizophrenia, psychosis, clozapine, amisulpride.

Index

List of Figures

List of Tables

List of Acronyms

$5\text{-}HT_2$	5-Hidroxitriptamina 2
AIMS	Abnormal Involuntary Movement Scale
ANNSERS-E	Antipsychotic Non-neurological Side Effects Rating Scale
BARS	Barnes Akathisia Rating Scale
BMC-ISRCTN	Biomedcentral – International Standard Randomised Controlled Trial Number
BPRS	Brief Psychiatric Rating Scale
CGI	Clinical Global Impression
CI	Confidence Interval
CIH	Clozapine-induced Hypersalivation
CR	Case Report
CS	Case Series
ESRS	Extrapyramidal Symptom Rating Scale
EPSE	Extrapyramidal Side-Effects Rating Scale
GAF	Global Assessment of Functioning
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
MADRS	Montgomery-Asberg Depression Rating Scale
NHRS	Nocturnal Hypersalivation Rating Scale
NIH-ClinicalTrials.gov	National Institutes of Health – Clinical Trials
NS	Non-significant
OR	Odds Ratio
PANSS	Positive and Negative Syndrome Scale
PP	Pre-Post
PPCT	Pre-Post Controlled Trial
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
REES	Registry of Efficacy and Effectiveness Studies
RPP	Retrospective Pre-post
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
SAS	Simpson Angus Scale
SD	Standard Deviation
SMD	Standardized Mean Difference
UKU	Udvalg for Kliniske Undersogelser
WHO-ICTRP	World Health Organization - International Clinical Trials Registry Platform

Chapter 1

Introduction

Schizophrenia was first coined in 1908 by Eugen Bleuler. It is the paradigm of severe mental illness, being its treatment a great challenge since its recognition. Many treatments were tried, ranging from fever therapy to lobotomies and trepanations. The first antipsychotic used was chlorpromazine in 1950, which provided an improvement in positive symptoms but numerous adverse effects. Around 1970, clozapine was introduced, the first atypical antipsychotic, however, due to several consecutive deaths provoked by agranulocytosis, it was discontinued. Following 20 years (in 1990), clozapine reemerged as an atypical antipsychotic, having a lower affinity for dopamine D2 receptors but higher for D4 receptors, a selectively higher antagonism for 5-HT_2 receptors and an antagonism for adrenergic α1 and muscarinic receptors. Despite having a wide range of possible adverse effects, it does not induce and it may in fact ameliorate negative symptoms. About ⅓ to ½ of patients do not respond or only show a partial response to antipsychotic treatment(1,2). Treatment-resistant schizophrenia is the persistence of positive symptoms despite treatment with two or more trials of adequate dose and duration of antipsychotic medication with a documented adherence(3,4). For these patients, the clozapine's use is recommended, which is only effective in 30-50% of them (5). For patients with clozapine-resistant schizophrenia, there are no approved interventions, with studies hypothesizing benefits with drug combinations and electroconvulsive therapy. Amisulpride, as an adjunctive to clozapine, has been considered a potential therapeutic alternative, for its complementary pharmacological profile(2), where it selectively only blocks the dopamine D2 and D3 receptors(5) in limbic brain structures, rather than striatal structures, as well as the possibility of counteracting adverse effects induced by clozapine.

Chapter 2

Methods and Materials

The PRISMA 2020(6,7), provided guidance to ascertain the amisulpride efficacy as an adjunctive to clozapine in treatment-resistant schizophrenia. In order to investigate this matter, the following research question was constructed based on the Population, Intervention, Comparison and Outcome (PICO) strategy:

Is amisulpride add-on effective in reducing symptoms and adverse effects in patients with schizophrenia under treatment with clozapine?

Section 2.1 Literature Search Strategy

A literature search was performed by the authors, up to the 20th of August of 2020, using three databases: PubMed (MEDLINE), Scopus and Cochrane Library and "clozapine", "amisulpride" and "schizophrenia" or "psychosis" were searched within index or mesh terms, keywords, titles, and abstracts. One general search key was created (Table 1) as well as one "only title" key to confirm if none of the relevant studies were missing, always regarding the syntax rules of each database (Table 2).

This search strategy was performed without language or date restriction and resulted in 856 articles, namely 83 in PubMed (15 articles were obtained with the "only title" key, which were all repeated), 41 in Cochrane Library (16 articles were obtained with the "only title" key, of which 15 were repeated) and 732 in SCOPUS (16 articles were obtained with the "only title" key, of which 4 were repeated). Only one article was added to this list by searching through the selected articles references.

Section 2.2 Eligibility Criteria

The authors included studies of patients with schizophrenia under treatment with clozapine, who were initiated with amisulpride as an add-on, to target schizophrenia symptoms or clozapine's side effects. A broad strategy was used, regarding the types of studies, such as interventional, observational, and descriptive ones (case series/ case reports).

We excluded duplicated or repeated samples between databases, reviews and meta-analysis, studies with an unreliable outcome or not being an add-on study and registered studies without published results.

The articles were selected based on these eligibility criteria and then screened by title and abstract reading, following full-text reading and evaluation. The ones that did not meet the inclusion criteria or had exclusion characteristics were removed (Figure 1).

Section 2.3 Study Selection

The literature search yielded 856 articles, where 799 were excluded after title and abstract assessment for presenting other than the relevant research topics for this study, resulting in 57 selected articles within the three databases. Of these, 18 were duplicated, 13 did not meet the inclusion criteria and 11 were review articles, summing up a total of 16 eligible studies for full-text assessment. After full-text reading, 4 studies were excluded: one had an unreliable outcome and it was not an add-on study and the others were registered studies without published results. One study was included by hand-searching the selected studies references, yielding 13 studies to include in this systematic review. Regarding the meta-analysis, we assessed for eligibility 4 of the 13 selected articles, alluding only to randomized controlled trials testing the efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia. As some measures were missing, it was not possible to assemble the data, with only 2 studies suitable for meta-analysis (Figure 2).

Section 2.4 Data Extraction

The data was extracted and organized in a spreadsheet by author, year of publication, country, study design, population, follow-up, comparator, assessments, quality, and main findings.

Section 2.5 Quality Assessment and Certainty of Evidence

To rate the quality of the included studies and their outcomes, the authors used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system software(8) and Cochrane Handbook(9) Chapter 8, regarding how to assess risk of bias. Four quality traits exist: very low, low, moderate, or high. Randomized trials start with high evidence and its quality decreases if serious (-1) or very serious concerns (-2) related to risk of bias, inconsistency, indirectness, imprecision, and publication bias exists.

The authors identified 4 moderate quality studies and 10 very low quality studies (Table 3). Moderate quality studies were naturalistic pilot studies, where no control group was included or an appropriate analysis was used to estimate the effect of assignment to intervention, once there were patients' dropouts. Concerning the very low quality studies, they had methodological issues such as inadequate control of confoundings, small sample size or no threshold of important benefit value was defined (see explanations in Table 3).

Chapter 3

Results

Section 3.1 Characteristics of Included Studies

The characteristics are summarized in Figure 3. The articles were published between 2004 and 2018, in 10 countries from all over the world. All studies assess the efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia. 7 of the 13 included articles were interventional studies, from which 4 were randomized controlled trials and 3 were pre-post controlled trials; 1 observational study (retrospective pre-post); and 5 descriptive studies (1 case series and 4 case reports). The sample sizes ranged from 1 to 68 inpatients and outpatients with treatment-resistant schizophrenia and their follow-up assessments ranged from 1 week to 3 years and 9.5 months. Regarding efficacy scales, BPRS, PANSS, SANS and SAPS were used.

Section 3.2 Outcomes from Interventional Studies

This study included 3 RCT, 1 RCT with crossover and 3 PPCT, totaling 242 patients. They all assessed the efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia.

Barnes et al. (2018)(5), in a RCT, treated 68 inpatients with schizophrenia with amisulpride 400 mg/day or placebo, with only 52 patients completing the study. Between both groups, a significant difference in PANSS score and other secondary outcome scales such as CGI and CDSS were not achieved. At 6th week assessment, the amisulpride group attained a lower PANSS total score (mean 80 ± 15) compared to placebo group (mean 85 ± 23). Relatively to the 12th week assessment, 44% of patients in the amisulpride group had at least a 20% reduction in PANSS total score (OR= 1.17, 95%CI [0.40; 3.42]) compared to 40% in the placebo group. The study analysis discloses an association between the 20% or more reduction in the PANSS score and time, including the randomized condition and control for baseline PANSS score, from the 6th to the 12th week (OR is 4.19 times greater, 95%CI [1.20; 14.56]) and it is shown a lower value in PANSS negative subscale score (–1.32; 95%CI [–2.20; –0.44]).

Assion et al. (2008)(10), in a RCT, pursued a trial with 30 patients with chronic schizophrenia unresponsive or partially responsive to clozapine monotherapy, with only 16 patients completing the study. Of those, 7 patients were in the clozapine and

amisulpride 400 mg/day group, 6 patients in the clozapine and amisulpride 600 mg/day group and 3 patients in the clozapine and placebo group. A clinical improvement in both amisulpride groups was not achieved, once BPRS total score (primary outcome) failed to decrease significantly. The BPRS subscore activity had potential to improve on the amisulpride 600mg/day group compared to the placebo group, at endpoint (P = 0.073). Other BPRS subscores and the BPRS total score were statistically similar between all groups. Regarding the CGI, MADRS and GAF scores (secondary outcomes), a clinical improvement was achieved, with the CGI score remaining with higher differences after the correction of Bonferroni [F(2.32)= 7.277 and p= 0.024]. The extrapyramidal side effects did not significantly vary in ESRS score between all groups and no severe adverse effects were reported.

Genç et al. (2007)(2), in a RCT, included 56 university hospital inpatients and outpatients, who were partially responsive to clozapine treatment, with 50 patients completing the study, of which 23 patients were assigned to 595 ± 125 mg quetiapine group and 27 patients were assigned to 437 ± 104 mg amisulpride group. At week 8, an improvement was observed in both groups, with a higher improvement seen in the amisulpride group. Controlling for baseline scores, the clozapine and amisulpride group presented a decrease in BPRS (t=9.84; df=26; P=0.0001), SAPS (t=7.694; df=26; P=0.0001) and SANS (t=7.214; df=26; P=0.0001) scores and an increase in CGI (t=9.603; df=26; P=0.0001) score. On SAPS score, no difference was noticed (t=0.335; df=22; P=0.741). Both drugs were well tolerated, as measured by the UKU Side Effects Rating Scale and by the SAS.

Kreinin et al. (2006)(11), in a RCT, studied 20 inpatients in Tirat Carmel Mental Health Center, with CIH and clozapine treated schizophrenia, 9 patients being assigned to an amisulpride 400 mg/day add-on group and 11 patients assigned to placebo. From baseline to endpoint, amisulpride group had a significantly lower average NHRS index, compared to placebo [1.79 ± 1.25 versus 2.63 ± 1.33; F(1.38) = 5.36, P < 0.05]. In PANSS negative symptom subscale there was a greater improvement with amisulpride [F(3.57) = 3.76, P < 0.05]. No significant differences were noted in other PANSS subscales [F(3.57) = 0.94, NS], in general subscale of PANSS [F(3.57) = 1.43, NS], in the CGI severity [F(3.57) = 1.70, NS] and in the CGI change score [F(2.38) = 0.69, NS], comparing to placebo.

Ziegenbein et al. (2006)(12), in a PPCT, included 15 patients with schizophrenia, unresponsive or partially responsive to adequate clozapine monotherapy. At the 3rd month assessment, there was a response (>20% decrease in BPRS total score) in 10

patients (67%). At the 6th and 12th month assessments, a response was observed in 11 patients (73%). From baseline to endpoint, it was observed only a reduction in the BPRS positive symptom subscale (mean 5.7 ± 4.1, range 0 to 13), but not in the negative subscale. Of note, clozapine and amisulpride association allowed a 13% decrease in clozapine's daily dose over time. No increase in adverse effects was noted. Regarding non responders, most were women and the daily amisulpride and clozapine doses were lower than in treatment responders. Despite reported differences no statistical tests were performed.

Munro et al. (2004)(13), in a PPCT, studied 33 patients with schizophrenia, of which only 28 patients completed the study. Of these, 20 patients (71%) showed a response (>20% reduction in BPRS score) to amisulpride add-on up to 800mg/day. More specifically, at 6 months, there was a significant improvement in the PANSS, BPRS, GAS and SANS scores, including positive and negative symptoms subscales. There were no relevant changes in depression or anxiety measures.

Koen et al. (2006)(14), in a PPCT with 20 inpatients (16 with treatment-resistant schizophrenia and 4 with schizoaffective disorder) recruited from Stikland Hospital in Cape Town. From baseline to endpoint, there was a significant improvement in PANSS total score ($t = 3.49$, $df = 18$, $p = 0.003$); PANSS negative subscale score ($t = 3.22$, $df = 18$, $p = 0.005$); and PANSS depression factor score ($t = 3.89$, $df = 19$, $p = 0.001$).

Section 3.3 Outcomes from Observational Studies

One observational study was included, totaling 14 patients.

Kampf et al. (2003)(15), using a retrospective pre-post design, studied 14 patients (8 with schizophrenia and 6 with schizoaffective disorder) who started a mean dose of amisulpride of 514 ± 235 mg/day as an add-on to clozapine. There was a significant improvement in illness severity, reflected by a reduction in the mean CGI score from 5.6 ± 0.5 to 3.9 ± 0.99 (Wilcoxon test, $p=0.0015$), more specifically: 3 patients (21%) were "very much improved"; 8 patients (57%) were "much improved"; 2 patients (14%) were "minimally improved"; and, 1 patient (7%) was "not improved". No significant correlation was found between drug dosage and CGI score.

Section 3.4 Outcomes from Descriptive Studies

Five descriptive studies were included, 1 case series (n=8) and 4 case reports, totaling 12 patients.

Hotham et al. (2013)(16) reported 8 inpatients with schizophrenia with a history of violent behavior, who started amisulpride with a mean dose of 667 mg/day (ranging from 400 mg/day to 1000 mg/day) as an add-on to clozapine. Six patients completed the study. After treatment with clozapine and amisulpride, there was a general improvement in illness severity, reflected by a reduction in the mean CGI score, more specifically, 3 patients “very much improved”; 1 patient was “much improved"; and 2 patients were "minimally improved". There was also an apparent decrease in violent behavior towards others.

Bogorni et al. (2015)(17) reported a case with an add-on up to 50 mg/day amisulpride in an inpatient with refractory schizophrenia. At the 2nd assessment (4 weeks after amisulpride treatment initiation), BPRS score had improved. At the 3rd assessment (10 weeks after hospital release), the patient’s positive symptoms remained in remission. The effects in hypersalivation were inconsistent across assessments.

Porcelli et al. (2014)(18) studied a patient with treatment-resistant schizophrenia where, as a first approach, was assigned to an add-on up to 6 mg/day haloperidol, with worse treatment tolerability. As no improvement was seen after 3 months, the patient was gradually switched up to 1000 mg/day amisulpride. At 2 months assessment, the patient demonstrated improvement in his clinical condition, exhibited by a reduction in PANSS total, general, positive, and negative subscales, with a reduction of 28, 13, 4 and 11 points, respectively. Treatment tolerability also improved.

Chiu et al. (2011)(19) reported a case of an inpatient with refractory schizophrenia to whom 600 mg/day amisulpride was given. The patient’s clinical assessment demonstrated an important improvement on the PANSS score, as well as in thought disorganization, delusions, and auditory hallucinations. Hypersalivation also improved throughout time.

Dervaux et al. (2007)(20) studied a patient with paranoid treatment-resistant schizophrenia. The patient started an add-on of 600 mg/day amisulpride, revealing an important clinical improvement, reflected by an amelioration in thought disorganization, delusions, and auditory hallucinations, as well as remitted violent and alcohol addictive behaviors.

Chapter 4

Statistical Analysis (Quantitative Synthesis)

The next step was to summarize data from clinical trials using meta-analysis. Four Randomized Controlled Trials were assessed for eligibility. However, due to lack of data, only 2 studies were suitable for inclusion. Regarding the missing data of the other 2 studies, the authors were asked to provide it, although with no success. The authors collected sample sizes, means and SD into RevMan 5 software, obtaining a random effect model, with SMD and 95% CI of efficacy measures (PANSS total score) at post-intervention or mid-intervention. To determine the variability of the intervention, statistical heterogeneity was addressed with Cochrane's Chi2 Test and to quantify for inconsistency I2 Statistic was used. A p-value <0.05 was set to determine statistical significance. The results show a non-significant reduction in schizophrenia symptom severity with amisulpride add-on. (SMD -0.27, 95% CI -0.69 to 0.15, p=0.20) (Figure 1).

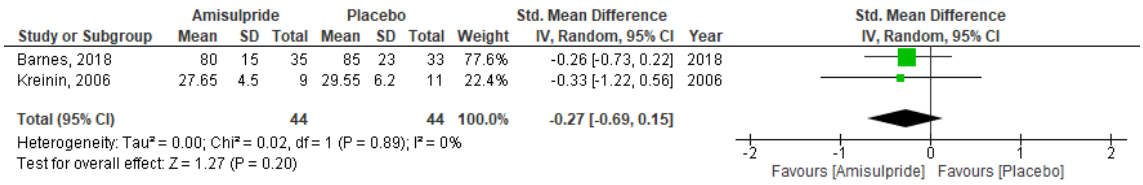

Figure 1 Forest plot of the meta-analysis

Chapter 5

Side Effects of Clozapine and Amisulpride Intervention

Side effects reported by patients or assessed by clinicians were screened in all included studies, through text and tables, and calculated on a percentage basis. The effect of assignment, using the intention-to-treat population, was considered.

The most common adverse effects associated with amisulpride augmentation are outlined in Table 4. The most frequent one was hyperprolactinemia (26%), followed by cardiac symptoms (11%).

Chapter 6

Conclusion

To the best of authors knowledge, this is the first systematic review assessing the efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia.

Clozapine, as an atypical antipsychotic, has a low affinity for dopamine D2 receptors but a high affinity for D4 receptors, a selectively higher antagonism for 5-HT_2 receptors and an antagonism for adrenergic α1 and muscarinic receptors, ameliorating some of the schizophrenia symptoms. Amisulpride selectively binds to dopamine D2 and D3 receptors, appearing to ameliorate some of the schizophrenia negative and positive symptoms and, therefore, demonstrates a complementary pharmacological profile to clozapine. There is also the possibility that amisulpride counteracts some adverse effects induced by clozapine, whether by its complementary pharmacological profile to clozapine or by allowing the reduction of clozapine dosage.

To assess for publication bias, the authors searched in several study registers databases, namely WHO-ICTR, NIH-ClinicalTrials.gov, BMC-ISRCTN, Research Registry, REES, and Clinical Trials Register. Two search keys were used: “schizophrenia AND clozapine AND amisulpride” (1st key) and “schizophrenia” (2nd key). The register research yielded 30 results with the 1st key and 8712 results with the 2nd one. Of these, only 4 results were relevant for the present study: the AMICUS study(21); the ClozAmi study by Krivoy, Amir (withdrawn in 2015) and the “Amisulpride Augmentation Therapy for Clozapine resistant Schizophrenic Patients: A 14-week Randomized, Double-blind and Placebo-controlled Trial” by Wang, Sheng-Chang (not included in this systematic review as its results were not published and not provided after being requested by email).

The results from our systematic review show that amisulpride as an add-on to clozapine in patients with treatment-resistant schizophrenia might be a valuable option for such a difficult to treat condition for which therapeutic options are very scarce. However, results are inconsistent across studies, with some show benefit and others showing no improvement. Also, the quality of included studies is very low in general, with a few studies having moderate quality, which precludes strong conclusions to be taken. Of note, the study with the largest sample and higher quality methodology, the AMICUS

study, by Barnes et al. (2018)(5), did not show a benefit with amisulpride add-on. The benefits of amisulpride seemed more consistent and of a higher magnitude regarding negative symptoms, assessed by the PANSS (Barnes(5), Kreinin(11), Koen(14), Porcelli(18), Chiu(19) and Munro(13) studies); SANS (Genç(2) and Munro(13) studies) and BPRS (Genç(2), Assion(10), Ziegenbein(12), Bogorni(17) and Munro(13) studies). Also, there was an improvement on positive symptoms in some studies, stated by PANSS Positive (Kreinin(11), Porcelli(18) and Munro(13) studies), SAPS (Genç(2) study) and BPRS (Ziegenbein(12) and Bogorni(17) study). In general, this treatment combination was well tolerated and relatively safe with only few adverse effects being reported (the most frequent ones being hyperprolactinemia and cardiac symptoms), assessed by prolactin level monitorization, ANNSERS-E, BARS, AIMS, EPSE, UKU, ESRS and patient's subjective report. As it is shown in literature, clozapine has several adverse effects, with agranulocytosis being one of the most feared and sialorrhea one of the most common(2). Amisulpride may counteract hypersalivation or allow a decrease in clozapine dosage, as reported by is supported by Kreinin(11), Ziegenbein(12) and Chiu(19) studies.

Main limitation is the absence of high quality studies exploring the efficacy of amisulpride as an add-on. Most of the trials have small sample sizes. We chose to adopt a broad criteria in terms of types of studies, including interventional, observational and descriptive studies, to allow a comprehensive review of this topic.

Finally, due to the possibility of a wide range of benefits to be observed with amisulpride add-on, namely an improvement in other domains besides positive symptoms, it is advised that a comprehensive assessment of efficacy in terms of schizophrenia symptoms, substance use and behavior, as well as clozapine side effects, should be assessed.

Concluding, amisulpride, when added to clozapine therapy, may improve negative, positive, and general symptoms in patients with treatment-resistant schizophrenia, compared to placebo. The authors advise future randomized controlled trials with larger sample sizes to ascertain the role of amisulpride as an add-on to clozapine.

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Appendix

Table 1 General Search Key

General Search Key	
#1	(schizophrenia[MeshTerms/INDEXTERMS] OR schizophrenia[Title] OR schizophrenia[Keywords] OR psychosis[MeshTerms/INDEXTERMS] OR psychosis[Title] OR psychosis[Keywords])
#2	(amisulpride[MeshTerms/INDEXTERMS] OR amisulpride[Title] OR amisulpride[Keywords])
#3	(clozapine[MeshTerms/INDEXTERMS] OR clozapine[Title] OR clozapine[Keywords])
#4	(schizophrenia[Title] OR psychosis[Title]) AND amisulpride[Title] AND clozapine[Title]
#1 AND #2 AND #3 OR #4	

Table 2 Search Keys (General Key and Only Title Key) used in each Database, according to its rules

Database	Search Keys
Cochrane Library	#1 MeSH descriptor: [Schizophrenia] explode all trees 7467 #2 Schizophrenia:ti 9765 #3 Schizophrenia:kw 10976 #4 MeSH descriptor: [Psychotic Disorders] explode all trees 2944 #5 Psyhcosis:ti 2111 #6 Psychosis:kw 2741 #7 MeSH descriptor: [Amisulpride] explode all trees 131 #8 Amisulpride:ti 242 #9 Amisulpride:kw 204 #10 MeSH descriptor: [Clozapine] explode all trees 510 #11 Clozapine:ti 841 #12 Clozapine:kw 683 (#1 OR #2 OR #3 OR #4 OR #5 OR #6) AND (#7 OR #8 OR #9) AND (#10 OR #11 OR #12) 41 #13 Schizophrenia:ti #14 Psychosis:ti #15 Amisulpride:ti #16 Clozapine:ti (#13 OR #14) AND #15 AND #16
Pubmed (MEDLINE)	#1 schizophrenia[MeSH Terms] OR schizophrenia[Title] OR schizophrenia[Other Term] #2 psychosis[MeSH Terms] OR psychosis[Title] OR psychosis[Other Term] #3 amisulpride[MeSH Terms] OR amisulpride[Title] OR amisulpride[Other Term] #4 clozapine[MeSH Terms] OR clozapine[Title] OR clozapine[Other Term] (#1 OR #2) AND #3 AND #4 #5 (schizophrenia[Title] OR psychosis[Title]) AND amisulpride[Title] AND clozapine[Title] #5
SCOPUS	#1 INDEXTERMS (schizophrenia) OR TITLE (schizophrenia) OR KEY (schizophrenia) #2 INDEXTERMS (psychosis) OR TITLE (psychosis) OR KEY (psychosis) #3 INDEXTERMS (amisulpride) OR TITLE (amisulpride) OR KEY (amisulpride) #4 INDEXTERMS (clozapine) OR TITLE (clozapine) OR KEY (clozapine) #5 INDEX (medline) (#1 OR #2) AND #3 AND #4 AND NOT #5 #6 (TITLE (schizophrenia) OR TITLE (psychosis)) AND TITLE (amisulpride) AND TITLE (clozapine) #6

Table 3 Studies Quality Assessment by GRADE

Certainty assessment							№ of patients		Effect		Certainty	Impor-tance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Amisulpride augmentation	Placebo and Quetiapine augmentation	Relative (95% CI)	Absolute (95% CI)		
Randomized Controlled Trials (Interventional Studies) (follow up: range 1 weeks to 12 weeks; assessed with: PANSS and BPRS)												
4	Randomised trials	not serious [a]	serious [b]	not serious	serious [c]	dose response gradient	96	78	-	not estimable	⊕⊕⊕◯ MODERATE	Important
Pre-Post Interventional Trials (follow up: range 8 weeks to 12 months; assessed with: PANSS and BPRS)												
3	observational studies	serious [d]	very serious [e]	not serious	serious [f]	all plausible residual confounding would reduce the demonstrated effect dose response gradient	68		-	see comment	⊕◯◯◯ VERY LOW	Important
Observational Study (follow up: mean 20 weeks; assessed with: CGI)												

Certainty assessment							№ of patients		Effect		Certainty	Impor-tance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Amisulpride augmentation	Placebo and Quetiapine augmentation	Relative (95% CI)	Absolute (95% CI)		
1	observational studies	serious [d]	very serious [e]	serious [g]	serious [h]	all plausible residual confounding would reduce the demonstrated effect dose response gradient	14		-	**0** (0 to 0)	⨁◯◯◯ VERY LOW	Not Important
Descriptive Studies (follow up: range 4 weeks to 46 months; assessed with: PANSS or non-specified questionnaires.)												
5	observational studies	very serious [i]	very serious [e]	very serious [g,i]	very serious [j]	all plausible residual confounding would reduce the demonstrated effect	12/-	not pooled	not pooled	see comment	⨁◯◯◯ VERY LOW	Not Important

Certainty assessment							№ of patients		Effect		Certainty	Impor-tance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Amisulpride augmentation	Placebo and Quetiapine augmentation	Relative (95% CI)	Absolute (95% CI)		

CI: Confidence interval; SMD: Standardized mean difference

Explanations:

a. The allocation sequence of all studies was random. An appropriate analysis was used to estimate the effect of assignment to intervention, once there were patients' dropouts. Data for this outcome were available for all, or nearly all, participants randomized. Measurement or ascertainment of the outcome could have differed between intervention groups, once in 2 studies PANSS was used and in the other 2 studies BPRS was used. The numerical result being assessed is likely to have been selected, on the basis of the results, from multiple outcome measurements within the outcome domain, once there was several patients' assessments throughout the study.

b. For Barnes et al. and Kreinin et al. studies, the measure for inconsistency was Chi2= 0.01, df=1, P=0.93, with I2=0%, meaning the variability in effect estimates due to heterogeneity rather than sampling error was not important. For the other 2 studies, the inconsistency was not calculated due to missing data.

c. The sample size does not meet the optimal information size: per example, as stated by Barnes et al., to detect the criterion response in 30% of participants in amisulpride group and 10% in placebo group, with 90% power and an alfa=0.05, would require 92 participants per group to complete the study and the authors only had 68 patients randomized, with only 52 completing their assigned treatment. None of the other 3 studies had larger sample sizes than Barnes et al. study.

d. Inpatients and outpatients were selected, based on DSM-IV criteria for schizophrenia and inclusion criteria, from clinical centers or hospitals. Non-parametrical statistical procedures were used due to small samples sizes and ordinal scales.

e. Inconsistency and heterogeneity was not assessed.

f. Small sample sizes. Only one study reported the important benefit threshold as 20% or more improvement in BPRS score (Munro).

g. No sufficient data was provided regarding the results of primary outcome measures.

h. Small sample size. No threshold of important benefit value was defined.

i. The majority of provided information regarding patients is qualitative and based on health records and case notes.

j. No threshold of important benefit value was defined. The majority of reported outcome results were qualitative.

Table 4 Side Effects Profile

Side Effects	Amisulpride NTotal = 190		Placebo NTotal = 50	
	n	%	n	%
Cardiac Symptoms (Dyspnea, Dizziness, Arrhythmia, Tachycardia, Postural diziness)	21	11.05	10	20.00
Bradykinesia	4	2.11	1	2.00
Tremor	2	1.05	1	2.00
Hyperprolactinaemia	27 + 13 + 9 + 1	26.32	___	___
Sialorrhea or Flunctuation in Sialorrhea Severity	4 + 1 + 1	3.16	___	___
Sedation (Daytime Fatigue)	3 + 2	2.63	___	___
Akathisia	3	1.58	___	___
Electroencephalogram Changes	3	1.58	___	___
Elevated Liver Enzymes	3	1.58	___	___
Weight Gain	3	1.58	___	___
Extrapiramidal Effects	1	0.53	___	___
Joint Stiffness	1	0.53	___	___
Constipation	1	0.53	___	___

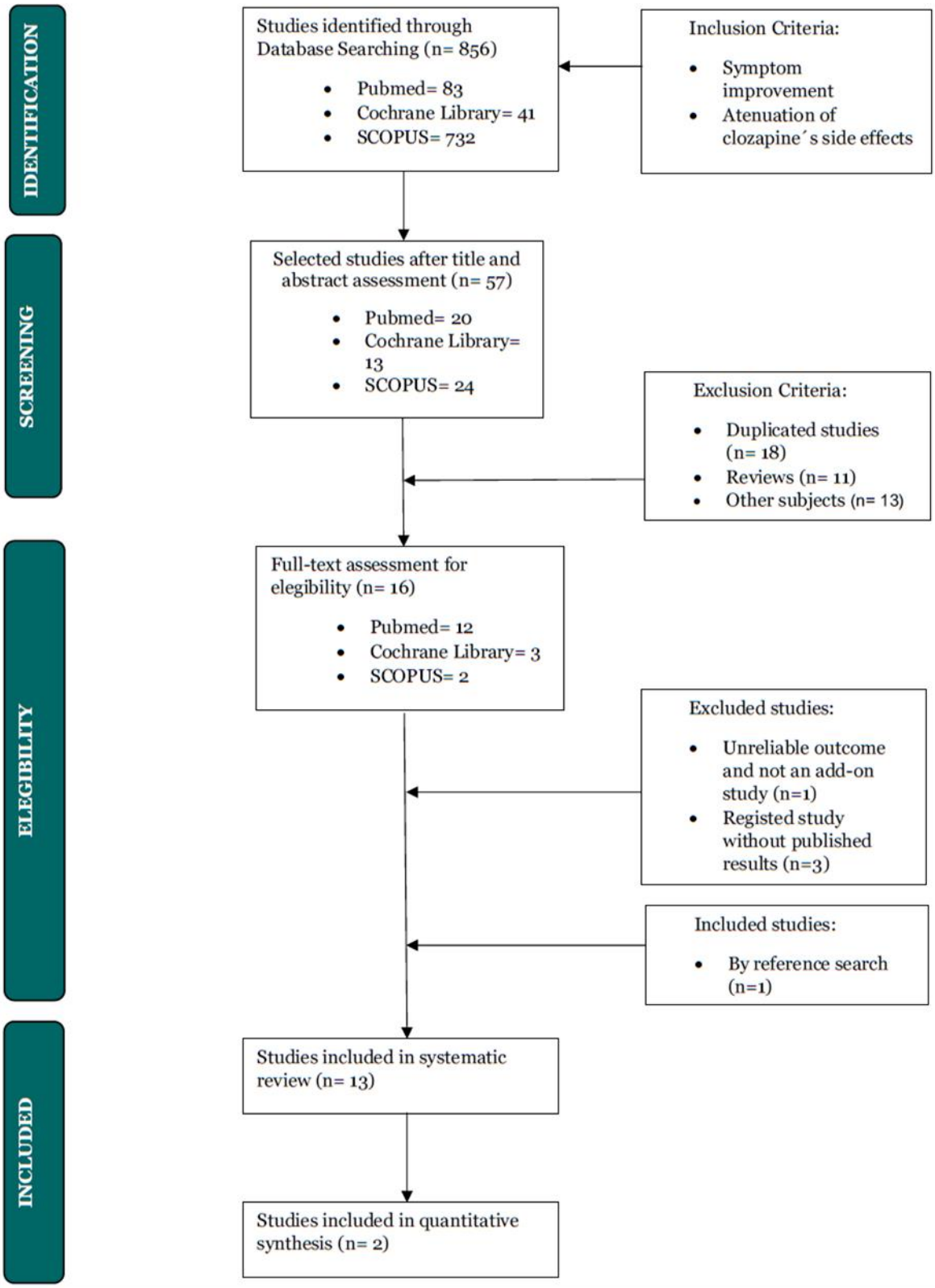

Figure 2 Fluxogram of the search, study selection and extraction process

Author, Year, Country	Study Design	Population *	Follow-up **	Comparator	Assessments ***	Quality	Main Findings ****
Amisulpride efficacy as an adjuntive to clozapine in treatment-resistant schizophrenia or psychosis in Interventional Studies							
Barnes, 2018, United Kingdom	RCT	68 Inpatients with treatment-resistant schizophrenia - Amisulpride Group (n= 35) - Placebo Group (n= 33)	4th week 6th week 12th week	Add-on 400 mg/day Amisulpride or Add-on Placebo	**PANSS**; SES; CDSS; SOFAS; CGI; SAI; ANNSERS-E; BARS; AIMS; EPSE	Moderate ⊕⊕⊕◯	*Amisulpride augmentation was not more effective than placebo.* No significant differences between groups were achieved on the primary and secondary outcome measure scales. At 12 weeks assessment, amisulpride intervention more likely improved clinical response (**PANSS**: OR=1.17 with 95%CI [0.40 - 3.42]) and had more side effects.
Assion, 2008, Germany	RCT	30 Patients with treatment-resistant schizophrenia: - Amisulpride 400 mg/day Group (n=12) - Amisulpride 600 mg/day Group (n=12) - Placebo Group (n=6)	3rd week 6th week	Add-on Placebo or Add-on 400 mg/day Amisulpride or Add-on 600 mg/day Amisulpride	**BPRS**; CGI; MADRS; GAF; SF-36; ESRS	Moderate ⊕⊕⊕◯	*Amisulpride augmentation was not more effective than placebo.* There was a tendency to favour amisulpride 600mg group in **BPRS** subscore activity (P=0,073), but in BPRS total score no clinical amelioration was attained. Only secondary outcomes showed a clinical improvement. - **BPRS** total score: amisulpride 600mg= 18.33 vs amisulpride 400mg=4.14 vs placebo= 6.67 - CGI score: amisulpride 600mg= 2.17 vs amisulpride 400mg= 0.71 vs placebo= 0 - MADRS score: amisulpride 600mg= 13.5 vs amisulpride 400mg= 5.29 vs placebo= 1.67 - GAF score: amisulpride 600mg= 20.67 vs amisulpride 400mg= 10.71 vs placebo= 5 All groups had similar results in ESRS score and no severe side effects ocurred.
Genç, 2007, Turkey	RCT	56 Inpatients and outpatients with treatment-resistant schizophrenia: - Quetiapine Group (n=28) - Amisulpride Group (n=28)	1st week 3rd week 6th week 8th week	Add-on 437.03 mg/day ([SD]=104.32) Amisulpride or Add-on 595.65 mg/day (SD=125.21) Quetiapine	**BPRS**; **SANS**; **SAPS**; CGI; SAS; UKU	Moderate ⊕⊕⊕◯	*Amisulpride augmentation appeared to be more effective than the quetiapine intervention.* º Higher decrease in the **BPRS** score (F=8.59; df=4; P<0.001) º Higher decrease in the **SANS** score (F=4.74; df=4; P=0.003) º Higher decrease in the **SAPS** score (F=7.79; df=4; P<0.001) º Higher increase in the CGI score (F=3.806; df=4; P=0.01) º UKU Side Effects Rating scale showed similar results between groups (F=1.544; df=4; P=0.206)
Kreinin, 2006, Israel	RCT with crossover	20 Inpatients with treatment-resistant schizophrenia and CIH: - Amisulpride Group (n=9) - Placebo Group (n=11)	7 weeks (3 weeks + 1 week for washout + 3 weeks of alternative treatment)	Add-on 400 mg/ day (up-titrated from 100 mg/day) Amisulpride or Add-on Placebo	**NHRS**; PANSS; CGI; SAS	Moderate ⊕⊕⊕◯	*Amisulpride augmentation appeared to be more effective than placebo.* In amisulpride augmentation (mean±SD): - **NHRS** index was lower than placebo (1.40 ± 1.10 vs 2.60 ± 1.35), F= 5.36 and P= 0.026 - PANSS was lower than placebo (27.65±4.50 vs 29.55 ± 6.20), F= 0.77 and P= 0.40 - CGI severity was lower than placebo (4.55±0.94 vs 4.75 ± 0.79), F= 0.42 and P= 0.52 - CGI change score was lower than placebo (3.65±0.81 vs 3.80± 0.62), F= 0.30 and P= 0.59

Figure 3 Characteristics of included studies assessing the clinical efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia or psychosis (continues)

Ziegenbein, 2006, Germany	PP	15 Patients (6 outpatients and 9 inpatients) with treatment-resistant schizophrenia	3rd month 6th month 12th month	Add-on 600.0 ± 100.0 mg/day (range from 350 mg/day to 750 mg/day) Amisulpride	**BPRS**	Very Low ⊕○○○	*Amisulpride augmentation appeared to be effective in 73.3% of patients.* At 12th month assessment with amisulpride augmentation: - Mean improvement in **BPRS Total** score= 11.9 ± 5.0, range 5 to 23, P<0.001 - Mean reduction of **BPRS Positive** subscale= 5.7±4.1, range 0 to 13, P<0.01
Munro, 2004, United Kingdom	PP	33 Outpatients and inpatients with treatment-resistant schizophrenia	3rd month 6th month	Add-on up to a maximum of 800 mg/day Amisulpride	**PANSS**; **BRPS**; **SANS**; CAS; CDS; GAS; SAS; BARS; AIMS; CAERS; EPSE	Very Low ⊕○○○	*Amisulpride augmentation appeared to be effective in 71% of patients.* There was a significant clinical improvement, reflected on the decrease of means and range scores of: - **PANSS**: F=55.11, P<0.0001 - **BPRS**: F= 41.47, P<0.0001 - **SANS**: F= 30.40, P<0.0001 No significant changes in side effects ratings.
Koen, 2006, Cape Town	PP	20 Inpatients: - 16 Inpatients with treatment-resistant schizophrenia - 4 Inpatients with schizoaffective disorder	8th week	Add-on 400 mg/day Amisulpride	**PANSS**	Very Low ⊕○○○	*Amisulpride augmentation appeared to be effective.* There was a significant clinical improvement in **PANSS**: t=3.49, df=18, P=0.003 There was no correlation between the improvement in negative symptoms and the improvement in the depression factor (N = 19, Spearman's r = 0.40, t (N−2) = 1.81, P= 0.09).
Amisulpride efficacy as an adjuntive to clozapine in treatment-resistant schizophrenia or psychosis in Observational Study							
Kampf, 2003, Germany	RPP	14 Patients: - 8 Patients with schizophrenia - 6 Patients with a schizoaffective disorder	20±22 weeks	Add-on 514±235 mg/day Amisulpride	**CGI**	Very Low ⊕○○○	*Amisulpride augmentation appeared to be effective.* **CGI** decreased from 5.6±0.5 to 3.9±1.0, P<0.01 The improvement was not associated with treatment settings.

Figure 3 Characteristics of included studies assessing the clinical efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia or psychosis (continuation)

Amisulpride efficacy as an adjuntive to clozapine in treatment-resistant schizophrenia or psychosis in Descriptive Studies							
Hotham, 2013, United Kingdom	CS	8 Inpatients with treatment-resistant schizophrenia and violence history	From 1.2 months to 45.5 months	Add-on 667 mg/day (ranging from 400 mg/day to 1000 mg/day) Amisulpride	**CGI**	Very low ⊕○○○	*Amisulpride augmentation appeared to be effective.* 5/6 of patients reported side effects as unchanged or improved.
Bogorni, 2015, Brazil	CR	1 Inpatient with treatment-resistant schizophrenia	4th week 10th week	Add-on up to 50mg/day Amisulpride	**BPRS**; **NHRS;** HDRS;	Very low ⊕○○○	*Amisulpride augmentation appeared to be effective.* **BPRS positive** symptoms remained in remission. **NHRS** improved from 4 to 1 (at 4weeks) but then worsened (at 10 weeks).
Porcelli, 2014, Italy	CR	1 Patient with treatment-resistant schizophrenia	3rd month 5th month	Add-on of up to 6 mg/day on Haloperidol as a first approach and then gradually switched to up to 1000 mg/day of Amisulpride	**PANSS**	Very low ⊕○○○	*Amisulpride augmentation appeared to be effective.* At 5 months assessment, the patient showed improvement in his clinical condition, exhibited by a reduction in several subscores: - **PANSS Total** score from 97 to 69 - **PANSS Positive** subscale from 21 to 17 - **PANSS Negative** subscale from 31 to 20 - **PANSS General** subscale from 45 to 32
Chiu, 2011, Taiwan	CR	1 Inpatient with treatment-resistant schizophrenia	_____ ___	Add-on 600 mg/day Amisulpride	**PANSS**	Very low ⊕○○○	*Amisulpride augmentation appeared to be effective.*
Dervaux, 2007, France	CR	1 Patient with treatment-resistant schizophrenia (paranoid type)	3 months	Add-on 600 mg/day Amisulpride	_____	Very low ⊕○○○	*Amisulpride augmentation appeared to be effective.* Treatment tolerability was well achieved, once there was not a worsening in patient's diabetes or weight gain.

AIMS: Abnormal Involuntary Movement Scale; ANNSERS-E: Antipsychotic Non-Neurological Side-Effects Rating Scale; BARS: Barnes Akathisia Rating Scale; BPRS: Brief Psychiatric Rating Scale; CAERS: Clozapine Adverse Effects Rating Scale; CAS: Calgary Anxiety Scale; CS: Case Series; CDSS, Calgary Depression Rating Scale for Schizophrenia; CGI: Clinical Global Impression; CI: Confidence Interval; CR: Case Report; EPSE: Extrapyramidal Side-effects Rating Scale; F: Fisher-Snedecor F Distribution; GAF: Global Assessment of Functioning; GAS: Global Assessment Scale; HDRS: Hamilton Depression Rating Scale; IQR: interquartile range; MADRS: Montgomery-Asberg Depression Rating Scale; NHRS: Nocturnal Hypersalivation Rating Scale; OR: odds ratio; P: p-value; PANSS: Positive and Negative Syndrome Scale; PP: Pre-post; RCT: Randomized Controlled Trial; RPP: Retrospective Pre-post; SAI, Schedule for the Assessment of Insight; SANS: Scale for the Assessment of Negative Symptoms; SAPS: Scale for the Assessment of Positive Symptoms; SAS: Simpson–Angus Scale; SES: Service Engagement Scale; SD: standard deviation; SOFAS: Social and Occupational Functioning Assessment Scale

* Intention-to-treat population
** All patients were first assessed at baseline.
*** Assessments: on **bold** reflect the **primary outcome measures**; underlined relfect the secondary outcome measures; simple font reflect the side effect measures.
**** Main Findings: All results on **bold** reflect the **primary outcome measures** and all scores are measured in points.

Figure 3 Characteristics of included studies assessing the clinical efficacy of amisulpride as an adjunctive to clozapine in treatment-resistant schizophrenia or psychosis (continuation)