OPTIMISATION OF CLOZAPINE USE DURING EARLY YEARS OF SCHIZOPHRENIA – CAN CLOZAPINE BE CONSIDERD A DISEASE MODIFYNG DRUG?

By

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ABSTRACT

Introduction

It is increasingly recognised that schizophrenia may be an immunological disease. Although there has been some progress in the management of schizophrenia by way of innovative service development over the last two decades, drug treatment has remained essentially static, with no major breakthroughs since the arrival of clozapine in the 1980s. Contrast this with the progress that has been made in the treatment of established immunological conditions such as rheumatoid arthritis, where the use of disease modifying drugs has transformed the therapeutic landscape. New insights into the immunology of schizophrenia provide potential for better understanding of how current treatments work and also a road map for developing novel compounds. Clozapine remains the treatment of choice for treatment resistant schizophrenia (TRS). It is possible that clozapine's superiority over traditional antipsychotics in TRS is down to its immunological effects. This raises the prospect that clozapine may be a disease modifying drug, capable of changing the course of disease, in which case earlier prescription of clozapine would be likely to produce better outcomes for patients. Clozapine is known to have effects on peripheral blood immune cells including neutrophils. Neutrophils have been shown to be elevated in schizophrenia, and there is much interest currently in the role of neutrophils in the pathogenesis of a number of immune conditions. A spike in neutrophils shortly after starting clozapine is commonly seen in clinical practice and has been reported in the literature. It is possible that these early changes in neutrophil counts following clozapine commencement may be linked with response to clozapine. In addition, clozapine has been found to be associated with secondary immunoglobulin deficiency which may also have value as a clinical

biomarker in the treatment of TRS. In this thesis I have explored whether there is evidence in the literature that earlier clozapine is beneficial in TRS and have conducted my own study into the timing of clozapine and its effectiveness. I have also examined neutrophil and immunoglobulin trajectories with clozapine use and looked for evidence of an association with clinical outcome.

Hypothesis and methods

I have set out to test the following three hypotheses.

Hypothesis 1. Clozapine will be more effective if used earlier in the course of course of TRS. I have investigated this by conducting a meta-regression of the effect of age (as a proxy for duration of illness) on clozapine response from randomised controlled trial data of clozapine in comparison to other antipsychotic drugs. I have then conducted an observational study of anonymised patient records, using ordinal logistic regression, to look for an association between duration of prior psychotic illness and clozapine response.

Hypothesis 2. Patients can be classified by neutrophil trajectory following clozapine commencement, and neutrophil trajectory can predict response to clozapine. I have taken the database that I created for paper 2, and linked this with ZTAS full blood count data, in order to conduct a latent class growth analysis (LCGA) of baseline and early neutrophil counts with clozapine and a logistic regression of neutrophil trajectory class against clinical outcome.

Hypothesis 3. Immunoglobulin levels fall with clozapine treatment and reduction in globulin level can predict response to clozapine. Combining my database from paper

2 with calculated globulin (CG) results obtained by a fellow CRIS researcher, I have compared CG counts pre and post clozapine prescription and have carried out a logistic regression of change in globulin score against clinical outcome.

Results

Paper 1 – Chapter 3

From a meta-analysis of 34 papers, meta-regression results failed to demonstrate an effect of age on clozapine response (p = 0.79, [95% CI -0.03 – 0.03]), however a linear regression of age against response using individual patient data from one study did show a significant effect of age, with younger age associated with greater response to clozapine (p=0.00, [95% CI -110.71 - -27.20]). Individual patient data from a second smaller study did not show a significant effect.

Paper 2 – Chapter 4

From a sample of 425 patients obtained using routine electronic clinical data, ordinal logistic regression results showed a significant association between duration of illness prior to commencing clozapine, and clozapine response (adjusted OR = 1.04 [95% CI 1.01 – 1.06]), indicating a 4% increase in the odds of a higher (worse) outcome CGI-S score per additional year of illness.

Paper 3 – Chapter 5

Using the same sample from paper 2, LCGA suggested 3 distinct classes of neutrophil trajectories, differing from outset with high, high-normal and low normal counts. Logistic

regression showed that high-normal neutrophils were associated with higher odds of clozapine response (adjusted OR = 2.10 [95% CI 1.31 – 3.36]).

Paper 4 – Chapter 6

From a sample of 343 patients, 149 had pre and post calculated globulin (CG) levels available whilst 341 had only post CG levels. Mean CG level fell significantly following clozapine treatment (t = 2.74 p = 0.007). Logistic regression showed no association between change in CG level and clozapine response (adjusted OR 1.02 95% CI [0.89-1.16]). There was a significant association between CG level 1 year post clozapine and clozapine response (adjusted OR 1.06 95% CI [1.00-1.12]), but the data was of poor quality. Sex and ethnicity differences were found in CG levels pre and post clozapine.

Conclusions

The results from papers 1 and 2 provide support for the hypothesis that earlier clozapine is associated with better response to clozapine. Whilst the meta-regression results were not significant, there was evidence from limited individual patient data of an association between younger age, and shorter duration of illness, and better response to clozapine. In the paper I provide a critique of the meta-regression, both in terms of the methodology and the data quality. Paper 2 in comparison provided clearer evidence of an association between shorter duration of prior illness and clozapine response, and whilst it was an observational rather than experimental study, it had the advantage of being a sample of real-world clinical data.

Paper 3 provided evidence in support of differing neutrophil trajectories following clozapine initiation. However, rather than variation between classes in terms of a spike

in neutrophil count with clozapine, neutrophil counts varied from the outset, with high normal neutrophil counts associated with a greater response to clozapine. These results are in keeping with a hypothesis that patients with high normal neutrophil counts, reflecting an ongoing inflammatory process, are more likely to respond to clozapine, and indicate that neutrophils may have utility as a biomarker to predict clozapine response in TRS.

Results from paper 4 support the hypothesis that immunoglobulin levels fall with clozapine treatment but failed to show an association between fall in CG level and response to clozapine. There were sex and ethnicity differences in CG levels both pre and post clozapine, with most patients who developed low CG levels on clozapine being white males. However, the data quality for this paper was poor and results need to be interpreted with caution.

In summary, the key findings from this thesis i.e. firstly, that earlier clozapine may be associated with better outcome in TRS and secondly, that neutrophil count can help predict response to clozapine, support the concept of clozapine acting as a disease modifier by reducing the burden of inflammation in TRS.

DEDICATION

I qualified as a doctor in 1992 and after an initial career in haematology commenced my psychiatric training in 1999. I became a consultant psychiatrist in 2007 and since that time have specialised in the treatment of psychosis. I spent my formative consultant years running an early intervention service for young people experiencing their first episode of psychosis. Whilst the prognosis for some people was unfortunately bleak, the use of clozapine was sometimes transformational, bringing people back to mainstream levels of wellbeing and functioning. However, commencing a medication like clozapine, with its need for lifelong monthly blood monitoring, was at times a hard sell for young people, who understandably wanted to live their life to the full. The prevailing literature was that stopping clozapine was ill advised, as prognosis was generally found to be poor. Despite medical advice however several of my young patients chose to come off clozapine and the results were surprising to me in that some of them fared much better than I had expected. This prompted me, along with my medical student, to carry out a retrospective analysis of clinical outcomes of patients on our caseload who had discontinued clozapine, and we found that over 50% remained out of hospital at 1 year, which indicated more positive outcomes than the literature would suggest. This discovery, along with the fact that clozapine appeared qualitatively different to other antipsychotics in terms of the response it produced, made me reflect on the possibility that clozapine acts differently to conventional antipsychotics. Relating this to my experience in the field of haematological oncology, in which powerful chemotherapy agents are used to achieve remission, then other less toxic drugs are given as maintenance therapy, I hypothesised that if we could use drugs

like clozapine in the early phase of illness, before the damage becomes set in, perhaps there would be scope, if remission was achieved, to either reduce doses or even change to less toxic drugs and maintain clinical recovery. In addition, with the burgeoning literature supporting an immunological basis for schizophrenia, I started to consider the parallels between schizophrenia and established autoimmune conditions, and the revolution that disease modifying drugs (DMDs) were achieving in the management of illnesses such as rheumatoid arthritis. I therefore decided to attempt to study whether clozapine itself may be an immunologically medicated DMD with scope to alter the prognosis of schizophrenia if given early enough in the course of illness.

I contacted various experts in the field including Professor David Taylor, Professor Robin Murray and Professor James MacCabe, as well as my supervisor Professor Rachel Upthegrove, all of whom were positive about my aspirations, and this led to me submitting my PhD proposal in 2017 and commencing my research part time alongside my consultant post. I am grateful to my supervisors Professor Upthegrove and Professor MacCabe for their patience with the speed (or lack of) with which I have undertaken this thesis. I have inevitably ended up working in fits and starts depending on the intensity of my other work and home commitments. Whilst travelling regularly to the Maudsley to carry out my data collection using their state-of-the-art anonymised patient record system, I met several very bright and ambitious young researchers who looked set to be trail blazers in their fields. I felt nostalgic about my youth not just in terms of wasted academic opportunities but also for how much time these young people had to focus on their projects in comparison to me. However, I remind myself that, at that stage in my career, I did not have the same experience, confidence or

burning interest that all my years in the field have brought to me, and that to me there is no greater motivator to carry out research than needing to know answers to difficult clinical questions.

In addition to the people mentioned above, I wish to thank a number of people for helping me both in my work as a psychiatrist and in conducting this research, Firstly I wish to thank my patients and colleagues from the South Birmingham Early Intervention Service. I also thank my co-authors Malcolm Price, Isabel Morales-Munoz, Adrian Shields, Megan Pritchard, Joyt Chandan, Graham Blackman, Sophie Legge and Daisy Kornblum. Thankyou to Debbie Cummings from the Institute of Psychiatry research nucleus for her assistance with my CRIS access and contracts, and to librarians Anna Cunningham and Anita Phul from the Barberry Library at BSMHFT for their assistance with my literature search. Finally, I wish to thank my good friend and colleague Dr Pavan Mallikarjun for his unwavering support throughout my research.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
ALS	Amyotrophic Lateral Sclerosis
AOR	Adjusted Odds Ratio
BEN	Benign Ethnic Neutropaenia
BIC	Bayesian Information Criterion
BPRS	Brief Psychiatric Rating Scale
BSMHFT	Birmingham and Solihull Mental Health Foundation Trust
CATIE	Clinical Antipsychotic Trials of Intervention Effectiveness
CD	Cluster of Differentiation
CG	Calculated Globulin
CGI	Clinical Global Impression
CGI-S	Clinical Global Impression - severity
CNO	Clozapine N Oxide
CNS	Central Nervous System
CRIS	Clinical Records Interactive Search
CRP	C-Reactive Protein
CSF	Colony Stimulating Factor
CSF	Cerebro-spinal fluid

CUtLASS	Cost Utility of the Latest Antipsychotic drugs in Schizophrenia Study
DMARD	Disease Modifying Anti-Rheumatic Drug
D	Dopamine
DAMPs	Damage-Associated Molecular Patterns
DMD	Disease Modifying Drug
DUP	Duration of Untreated Psychosis
ECT	Electro Convulsive Treatment
EPSE	Extra Pyramidal Side Effects
EULAR	European Alliance of Associations for Rheumatology
GATE	General Architecture for Text Engineering
GWAS	Genome Wide Association Study
H	Histamine
ICD	International Classification of Disease
IL	Interleukin
InterSePT	International Suicide Prevention Trial
LCGA	Latent Class Growth Analysis
LSD	Lysergic Acid Diethylamide
MCP-1	Monocyte Chemoattractant Protein -1
MHC	Major Histo-compatability Complex

MLR	Monocyte Lymphocyte Ratio
MS	Multiple Sclerosis
M	Muscarinic
NET	Neutrophil Extracellular Trap
NHS	National Health Service
NIHR	National Institute of Health Research
NK	Natural Killer
NLR	Neutrophil Lymphocyte Ratio
NMDA	N-Methyl D-Aspartate
NMDAR	N-Methyl D-Aspartate Receptor
NSab	Neuronal Surface antibody
nTRS	non-Treatment Resistant Schizophrenia
OR	Odds Ratio
PAMPs	Pathogen-Associated Molecular Patterns
PANSS	Positive and Negative Syndrome Scale
PLR	Platelet Lymphocyte Ratio
PRISMA	Preferred Reporting Items for Systemic Reviews and Meta-Analyses
PRRs	Pattern Recognition Receptors
RA	Rheumatoid Arthritis

RCT	Randomised Controlled Trial
ROS	Reactive Oxygen Species
RR	Relative Risk
SIRS	Schizophrenia International Research Society
SLE	Systemic Lupus Erythematosus
SMD	Standardised Mean Difference
STATA	Statistics and Data
T-reg	T-regulatory
T-h	T-helper
TRRIP	Treatment Response and Resistance in Psychosis
TNF	Tumour Necrosis Factor
TRS	Treatment Resistant Schizophrenia
VLMR	Vuong-Lo-Mendell-Reubin
ZTAS	Zaponex Treatment Access System
5HT	5 Hydroxy Tryptophan

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CHAPTER 1. INTRODUCTION

Schizophrenia is a severe and enduring mental illness characterised by positive symptoms, including hallucinations, delusions and disruption of thought processes, and negative symptoms such as blunting of affect, avolition and social withdrawal. In addition, patients commonly experience cognitive symptoms to the extent that Kraepelin originally described schizophrenia as dementia praecox (dementia of young age) (1).

Presentations of schizophrenia are heterogenous, with some patients presenting acutely with florid positive symptoms of the disease, and others having a more insidious onset with prominent negative symptoms and poor cognition. Clinical course and response to treatment also varies in schizophrenia. Most patients respond to first line antipsychotic medications (first or second generation), but approximately one quarter are treatment resistant from outset (2). Various factors increase the likelihood of early treatment resistance including early onset illness, male sex and poor premorbid function (2,3). Patients who have recovered from a first episode of illness have a high likelihood of relapse. Follow up studies of first episode patients vary in duration of follow up but report relapse rates ranging from 41% (4) to 97% (5), with longer studies generally showing higher relapse rates. Treatment with antipsychotics protects against relapse (6,7), but longer duration of treatment does not reduce the likelihood of relapse and patients often relapse quickly after treatment is stopped (8). With re-introduction of antipsychotic treatment most patients respond well but one in six show treatment failure with each relapse (8). Patients with treatment resistance,

either from outset or following relapse, are eligible for the antipsychotic medication clozapine, which is the only medication with proven efficacy in this patient group (9).

Aetiology of schizophrenia

The aetiology of schizophrenia is not as yet fully elucidated. Genetic and environmental factors are both known to play a role with recent studies suggesting heritability as high as 79% (10), and known environmental factors spanning in utero infections, early childhood adversity and more proximal events. Previous theories have centred around the idea of a two hit model for schizophrenia (11) with genetic and early life factors priming vulnerability to psychosis then a later environmental insult triggering onset of illness. However, as knowledge advances, it seems more likely that schizophrenia occurs as a result of multiple and cumulative genetic and environmental factors occurring at key times of neurodevelopment (12).

Immune abnormalities have been shown to be present in schizophrenia for decades (13), though some have been difficult to interpret due to confounding effects including antipsychotic treatment. The advent of genome wide association studies (GWAS), which have shown that multiple candidate genes for schizophrenia are located in areas of the genome known to be instrumental in the immune response (14,15), has pump primed research into the immunology of schizophrenia. Evidence for an immunological basis for schizophrenia now exists from a wide range of converging sources.

Immune system in health and disease

The immune system plays a vital role in protecting the body in the event of injury or infection. Put simply, it comprises innate immunity, which provides an immediate pro-inflammatory response when the body's defences are breached, and adaptive immunity, which is a slower targeted response involving specific antibodies to pathogens that have been encountered previously. In reality, the immune system is much more complex than this, and there are multiple cellular and humoral components involved. At the centre of immunity are a set of signalling proteins called cytokines, which orchestrate all aspects of the immune response including destruction of pathogens by phagocytes, stimulation of lymphocytes, activation of complement and coagulation cascades and restoration of homeostasis once the threat has resolved. Immune cells have been traditionally divided into innate cells such as macrophages and neutrophils, which directly destroy pathogens, and those involved in the adaptive immune response such as lymphocytes. However, this approach is reductive and in recent years understanding of the multifaceted roles of innate cells across the wider immune system has increased.

The natural process of immunity can be disrupted in a variety of ways including allergic reactions, malignancies and autoimmune conditions. In autoimmunity, the body triggers an immune response to self-antigens which it is unable to recognise as non-alien. Common examples include rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), in both of which an aberrant immune response causes damage to a number of different organs in the body. In addition to established autoimmune conditions, where autoantibodies have been identified, many other illnesses are

immune mediated but do not have specific autoantibody signatures. Examples include multiple sclerosis (MS) and a number of other neurodegenerative diseases.

Immunological basis for schizophrenia

There is now substantial evidence that schizophrenia may also be an immune disease. Linkage of national case registers have demonstrated increased rates of schizophrenia in patients with a history of autoimmune disorders (16). Large cohort studies have also shown clear links between infection rates, both in utero (17) and during childhood (18), and elevated schizophrenia risk. The diverse range of infections associated suggests that it is the inflammatory response to infectious pathogens that is likely to be the potential common mediator (19). There is also evidence for increased infection rates in patients diagnosed with schizophrenia, particularly during relapse (20). Cross-sectional studies have shown that peripheral blood cytokines are raised in patients with at risk mental state (21), first episode psychosis (22) and established schizophrenia (23). Additionally, raised cytokine levels in childhood (24), and polymorphisms of various cytokine genes (25), have been found to carry an increased risk of schizophrenia. More recently there has been much interest in innate immune system changes in schizophrenia including studies of complement factors (26) and innate immune cells, in particular monocytes and neutrophils (27). There have also been a number of papers looking at the composite measures neutrophil lymphocyte ratio (NLR) and monocyte lymphocyte ratio (MLR) in schizophrenia, as these measures have been found to have utility as an immune marker in a range of psychiatric and non-psychiatric illnesses (28).

Whilst the brain has traditionally been thought to be an immunologically privileged site, shielded from inflammation by the blood brain barrier, it is now recognised that there are complex interactions between the peripheral and central immune systems (29) and that peripheral immune cells can disrupt the blood brain barrier and also infiltrate the brain (30). In addition, studies of microglia, which are highly specific central nervous system macrophages, and a key component of neuroinflammation (31), appear to be show an increase in schizophrenia although results vary according to which outcome measure is used (32). Oxidative stress in the brain, caused by an imbalance of reactive oxygen species and antioxidants, is thought to be a significant factor in the causation of schizophrenia, as imbalances have been shown in patients with schizophrenia and in animal models, and environment insults associated with schizophrenia, such as maternal and obstetric stress, are known to cause oxidative stress (33). Studies of adjunctive treatment of schizophrenia with non-steroidal anti-inflammatory drugs have shown promising, albeit modest results (34,35) and the field of targeted cytokine-based immunotherapies in schizophrenia is just developing (36,37).

Could schizophrenia be an autoimmune disease?

In addition to its association with autoimmune illnesses, schizophrenia has clinical similarities to established autoimmune conditions, such as RA, including a familial pattern of inheritance, age and mode of onset, different phenotypes or frequencies in males and females, and a relapsing and remitting course (38,39). Furthermore, psychotic symptoms occur as part of the clinical syndrome of autoimmune illnesses such as systemic lupus erythematosus, sarcoidosis and neuronal surface antibody

(NSAb) CNS diseases such as anti-NMDAR encephalitis (40). A systematic review of evidence has shown increased prevalence of 20 known autoantibodies in schizophrenia (41). Autoantibodies against several brain structures have now been discovered in schizophrenia, the most well recognised being anti-NMDA receptor antibodies which have been found to be increased compared to controls in early psychosis (42). In addition, studies of lymphocytes have shown abnormalities, including alteration in CD4 to CD8 lymphocyte ratio in peripheral blood in patients with schizophrenia (43) and hippocampal lymphocyte infiltration in patients post mortem (44).

These findings raise the question of whether schizophrenia may be an autoimmune disease; a hypothesis which was first raised over fifty years ago by Burch et al (38). However, the bar for diagnosis of autoimmunity is set high. Criteria first proposed by Witebsky et al (45), which have since been modified by Rose and Bona (46), set out three levels of evidence for categorisation of an illness as an auto-immune condition. The highest level is direct evidence, i.e. proof that transmission of an antibody to an animal model or human subject will cause the symptoms of the disease. The next level is indirect evidence, for example replication of the disease in an experimental model or isolation of autoantibodies in the target organ. The lowest level of evidence is circumstantial, i.e. presence of phenotypes which are hallmarks of autoimmune disease. For schizophrenia, despite the lack of direct or indirect evidence of autoimmunity, there is compelling circumstantial evidence as detailed above, i.e., association with other autoimmune diseases in the same individual or family, presence of immune cells in the brain, association with MHC haplotypes and raised serum levels

of autoantibodies (39). There is also some, albeit modest, evidence for the effectiveness of immunosuppressive treatments (47).

Immune mediated versus autoimmune illness

In truth, there are many immune illnesses which do not at present meet the criteria for autoimmunity, as specific autoantibodies have not been identified. An example would be MS, which shares many similarities with schizophrenia. MS is considered an immune mediated illness, rather than an autoimmune one per se, whereby inflammation as a response to an, as yet, unknown trigger, appears to be the cause of the pathology seen in MS and treatment strategies are based on damping down the inflammatory response.

It would seem reasonable, based on current evidence, to hypothesise that schizophrenia is also an immune mediated illness, and to review what we know about the aberrant immune response in schizophrenia to see how we might better understand how current treatments, in particular clozapine, may be exerting their clinical effects. In addition, knowledge of immune abnormalities in schizophrenia may help identify targets for potential new treatment modalities and establish biomarkers for monitoring treatment response. It is also important to consider, if schizophrenia is indeed an immune mediated condition, how delays in treatment may be impacting on their effectiveness, and to consider how the clinical landscape for schizophrenia could change in the future if this is proved to be the case, with treatment paradigms of rapid and stepped immunological treatments to suppress an aberrant immune response, with measurable targets to reach.

Treatment resistant schizophrenia (TRS)

Treatment resistance in schizophrenia was originally defined by Kane as a 'therapeutic failure to respond to at least three treatment trials with full dose antipsychotics, using 400-600mg / day of chlorpromazine as reference' (48). Most modern treatment guidelines concur that patients should be considered to have treatment resistance if they have failed to respond adequately to two trials of antipsychotic medication of adequate dose and duration, however a systematic review by the Treatment Response and Resistance in Psychosis (TRRIP) working group demonstrated considerable variation in definition of TRS. The TRRIP group have published consensus guidelines in order to attempt to optimise definition of TRS, incorporating a minimum duration of symptoms, functional impairment, at least two antipsychotic treatment trials, monitoring of adherence, a prospective treatment trial and clear criteria to separate treatment resistance from response (49).

TRS is usually present from outset, though secondary treatment resistance can occur with increasing relapses (2). Approximately one third of patients with schizophrenia have treatment resistance (50). In view of the heterogeneity of schizophrenia TRS, particularly early TRS, may give a better paradigm for studying its aetiology.

Immunology of TRS

There is some evidence that TRS may be categorically distinct from non-treatment resistant schizophrenia (nTRS). A systematic review by Gillespie et al found that the factors which most distinguished between TRS and nTRS were presence of glutamate abnormalities, absence of dopamine abnormalities, decreases in grey matter volume

and higher familial loading (51). This suggests that immune processes may play a larger part in TRS than non TRS (nTRS), as glutamate is recognised to be a key immune modulator in the central nervous system (52) and familial loading suggests cumulative effects from a greater number of candidate genes, already known to congregate in the immune areas of the genome.

Further evidence to support an immune basis for TRS comes from study of cytokines. In an older study IL 6 levels were shown to be significantly higher in TRS but not nTRS patients, compared to healthy controls, though the study size was small (53). More recently, studies have found raised TNF cytokines and the chemokine MCP-1 (54), as well as raised IL 6 and other pro-inflammatory cytokines, in TRS as opposed to nTRS (55). Researchers have also looked longitudinally at first episode psychosis and showed that higher IL 6 and TNF gamma levels at illness onset were predictors of TRS (56). A recent narrative review has concluded that both an excess of pro-inflammatory cytokines and a deficit of anti-inflammatory cytokines are present in TRS, and the authors have proposed that it is the balance of pro and anti- inflammatory cytokines that determines response to treatment (57).

In addition to cytokine abnormalities there is some evidence that numbers of immune cells may differ in TRS and nTRS. A study of NLR and PLR in patients admitted to hospital with schizophrenia found that both ratios fell in patients who responded to treatment but did not fall in patients who were found to have TRS (58). Similarly, a study of immune cells and CRP in first episode psychosis has shown that a decline in neutrophils and CRP, and an increase in eosinophils, were associated with treatment response (59). There is also some evidence that TRS is associated with greater

oxidative stress, as shown by raised concentrations of lipid peroxidation by-products and neuron specific enolase (60).

The above findings support a hypothesis that TRS has different or additional immune phenotypes to nTRS. Given that first episode studies show that cytokines and immune cells are elevated in early schizophrenia, one plausible explanation is that the onset of psychosis marks an inflammatory phenomenon which fails to resolve in TRS, resulting in structural brain changes and manifesting in the development of negative or cognitive symptoms. A recent systematic review has shown that higher levels of mainly pro-inflammatory cytokines in first episode treatment naïve psychosis were associated with a greater degree of negative symptoms (22). T -regulatory (T-reg) cells may play a key role in terms of being able to control inflammation and a theory of hypofunctional T-reg cells in schizophrenia has recently been proposed (61). Studies of T-reg cells in schizophrenia have yielded mixed results and are hampered by small size and mixed groups of patient samples, however low levels of T-reg cells were found in 3 of the 8 studies including the only study specifically of TRS patients (62).

Clozapine and TRS

The drug clozapine was first discovered in 1959 by Wander Laboratories (63) and came into use firstly as a general antipsychotic medication for schizophrenia, being introduced to the European market in 1975 (64). Unfortunately, a series of 16 cases of agranulocytosis with clozapine occurred amongst patients in Finland, including 8 fatalities (65), which led to its license being withdrawn. Some use continued on compassionate and research grounds until it was re-introduced, specifically for the

treatment of TRS and with stringent blood monitoring requirements, following the seminal trials by Kane and Claghorn et al in the 1980s which confirmed its effectiveness in this patient group (48,66).

Clozapine remains the gold standard intervention for TRS and it has been shown to be effective in approximately 40% of TRS patients (67). However, it's place has not gone unchallenged. During the 1990s and 2000s there was a large increase in the use of second generation antipsychotics worldwide, whilst the rate of clozapine use remained low (68). The superiority of clozapine was questioned during these decades with several industry sponsored studies finding other second generation antipsychotics such as olanzapine and risperidone at least equally effective (69,70) and Cochrane reviews in 2009 and 2010 failing to show convincing benefit for clozapine (71,72). However, two large non industry funded trials, the Cost Utility of the Latest Antipsychotic drugs in Schizophrenia Study (CuTlaSS) trial (73) in 2005 and the Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) phase 2 E trial (74) in 2006, which were not included in the Cochrane reviews, were turning points, as they clearly demonstrated that clozapine was more effective than alternative antipsychotics in TRS. A more up to date meta-analysis of randomised controlled trials comparing clozapine with other antipsychotics in 2016 (75) found significantly in favour of clozapine, although a wider network meta-analysis, published in the same year, of all antipsychotic comparison data for TRS failed to do so (76). Most recently an updated network meta-analysis with broadened inclusion criteria has confirmed that clozapine is more effective than comparators (77).

Whilst randomized controlled trials can be criticized for not reflecting real life practice, there is also compelling evidence from observational data that clozapine is more

effective than any other antipsychotic medication in TRS. A large cohort study compared the outcomes of clozapine users, versus a propensity matched cohort who were not prescribed clozapine, and found clozapine to be associated with reduced hospital admission as well as lower index drug discontinuation and additional antipsychotic prescription (78). A meta-analysis of studies of hospital use by patients taking clozapine versus those on alternative antipsychotics demonstrated that clozapine reduced bed days (79), as did a subsequent large retrospective cohort study (80). Clozapine's longer-term benefits have also been convincingly shown in terms of reducing mortality rates, mortality being adopted as an outcome measure in schizophrenia relatively recently but considered to be the 'gold standard of clinical performance' (81). Several large database studies have demonstrated an association between clozapine prescription and reduced mortality, both from natural and unnatural causes (82–85).

Clozapine appears to be effective for both positive and negative symptoms, though the evidence for positive symptoms is more robust (75). Clozapine's effects on cognitive function have also been systematically reviewed with inconsistent results but more evidence of benefit in patients of younger age, higher educational attainment and greater improvement in positive symptoms, and also in studies with longer durations of follow up (86). Clozapine has an important advantage over other antipsychotics in terms of reducing suicide risk in schizophrenia. This was clearly demonstrated some years ago in the International Suicide Prevention Trial (InterSePT) (87) and has been confirmed in both a recent systematic review (88) and a population mortality data study (89). There is also convincing evidence that clozapine reduces behavioural

disturbance and aggression in schizophrenia (90) and considerable evidence that it reduces substance abuse (91).

Mechanism of action of clozapine

Clozapine is the prototype ‘atypical’ antipsychotic drug. The original dopamine theory of schizophrenia, on the back of the discovery that dopamine agonist drugs, such as amphetamines, cause psychosis, was based on the ability of typical antipsychotics to bind strongly to dopamine D2 receptors and to block dopaminergic transmission. The dopamine theory attributed positive psychotic symptoms to hyperactivity of subcortical mesolimbic dopaminergic projections and negative symptoms to hypoactivity in mesocortical dopaminergic projections to the prefrontal cortex. It was shown that there was a correlation between an antipsychotic’s D2 receptor potency and its clinical effect (92). The main side effect of first generation or ‘typical’ antipsychotic medications was of unwanted D2 blockade in nigrostriatal dopamine pathways causing extra-pyramidal side effects (EPSE). However, clozapine was the first antipsychotic in which its effectiveness appeared to be de-coupled from degree of D2 blockade, leading to its atypical definition. Whilst studies indicated that for first generation antipsychotics D2 blockade of at least 75% was needed for antipsychotic effect, for clozapine and for subsequent second generation (atypical) antipsychotics clinical effectiveness was shown to be occurring at less than 60% occupancy (93).

It was therefore recognised that the mechanism of action of clozapine, and the other second-generation antipsychotics, was not down to their potency at the D2 receptor. Rather, with second generation antipsychotics, antipsychotic effect appears to be due

to their ability to bind transiently to D2 receptors and then release, which usually prevents the development of EPSE at therapeutic doses. Second generation antipsychotics also block 5-HT 2a receptors which likely contributes to their antipsychotic potency, as it is known that 5HT2a agonist drugs, such as Lysergic Acid Diethylamide (LSD), can cause psychosis. Different second-generation antipsychotics vary in terms of their relative activity at D2 and 5HT2a receptors, and also their effects on various other receptors, leading to different side effect profiles (94).

Clozapine's effects on D2 and 5HT2a, however, do not account for its superiority over other second-generation antipsychotics in TRS. Clozapine is also known to act on many other receptors. It has high affinity for D4, 5HT 1a, 2b, 2c, 6 and 7, as well as adrenergic 1 and 2 receptors, histamine H1 receptors and muscarinic M1-5 muscarinic receptors. Some of these actions of clozapine can explain common side effects of clozapine, such as H1 receptor blockade causing sedation. It has been postulated that others may be relevant for its effectiveness e.g. noradrenergic alpha 2 blockade (95). However a clear mechanism of action has remained elusive (96). Recent hypotheses consider shared indications and possible mechanisms of clozapine with Electro-convulsive Therapy (ECT) (97) and also that its effectiveness in patients with co-morbid substance use may be down to its combination of weak D2 blockade, potent alpha 2 blockade and noradrenaline re-uptake inhibition (98).

Following on from the dopamine hypothesis various theories have emerged including that of a glutamate theory of schizophrenia, based on the ability of NMDA antagonist drugs, such as phencyclidine (PCP) and ketamine, to cause schizophrenia symptoms, along with increasing knowledge of disturbances of NMDA receptor gene expression in schizophrenia (99) and better understanding of neural connectivity. Both clozapine,

and one of its main metabolites, norclozapine, are known to activate NMDA receptors (97,100,101), which raises the possibility that clozapine's actions on the glutamatergic system may be driving its effectiveness.

Immune effects of clozapine

Clozapine causes a number of side effects which are not commonly observed with other antipsychotics. These may also provide clues as to its mechanism of action in TRS. On initiation of clozapine, patients frequently experience significant tachycardia, influenza like symptoms and fever, which are thought to be due to release of pro-inflammatory cytokines and acute phase proteins including IL6, TNF alpha and CRP (102–106). Clozapine also causes a range of blood dyscrasias, most notably agranulocytosis, which occurs in 0.8% patients (107) and is thought to have an immune basis (108,109). Clozapine can cause myocarditis in the first month of therapy, which again is thought to be due to release of pro-inflammatory cytokines (110), and can also cause a more insidious onset cardiomyopathy, possibly related to cytokine abnormalities but the fact that it can occur in the absence of prior myocarditis, and also evidence of asymptomatic cardiac dysfunction in approximately half of patients taking clozapine, suggest the cause may be direct clozapine cardiac toxicity (111). Clozapine is associated with increased risk of infections, in particular pneumonia (112,113), and also causes hypogammaglobulinemia (114,115). Recently clozapine has also been shown to be associated with increased rates of haematological malignancies (116).

Adverse effects of clozapine thus provide clear evidence of its immunomodulatory properties. It is possible that immunomodulation is related to clozapine's effects on

glutamatergic transmission and may also be the mechanism for its clinical benefit in TRS. There is some evidence supporting this hypothesis. It has been shown that pro-inflammatory cytokines modulate brain excitability by upregulating glutamatergic and down regulating GABA-ergic transmission (117). In addition, there is evidence that a metabolite of clozapine, clozapine N-oxide (CNO), may inhibit microglial neuroinflammation (118).

If clozapine is acting as an immunomodulatory drug in schizophrenia, this brings it into the realm of immunotherapy strategies that are in operation for more established immune illnesses. For a group of these illnesses, with RA at the fore, there has been a treatment revolution over the last few decades with the discovery of disease modifying drugs.

Concept of 'disease modification' and its application to schizophrenia

The term disease modification refers to treatments which can alter the course and prognosis of a chronic condition. The concept of disease modifying drugs was first reported in the literature by Gumpel in 1976 in relation to the use of gold, penicillamine and cyclophosphamide in the treatment of RA. He concluded that gold was the treatment of choice for RA in view of its effectiveness in reducing bone erosion and its absence of long-term toxicity. Evidence for disease modifying anti-rheumatic drugs (DMARDs) has continued to accumulate and current treatment guidelines for RA recommend first line treatment with a conventional DMARD, usually methotrexate, with second line treatments, based on risk stratification, comprising a number of newer agents which include targeted synthetic DMARDs or biological DMARDs (119).

EULAR (European Alliance for Associations of Rheumatology) guidelines for RA stress that treatment with DMARDs for newly diagnosed patients should begin as soon as possible, with a ‘treat to target approach’ (120).

Disease modifying drugs have been identified for a number of rheumatological and neurodegenerative diseases, and the concept has also been applied to the management of some chronic respiratory illnesses. A general definition for disease modification in rheumatological conditions is as follows- ‘disease modification is the improvement of symptoms (disease process) in conjunction with the change of the disease course (disease outcome)’ (121). For general neurodegenerative conditions disease modifying drugs have been defined as ‘an intervention that produces an enduring change in the trajectory of clinical decline of a neurodegenerative disorder by impacting the disease processes leading to nerve cell death’ (122). More specific definitions have been developed for individual conditions including RA (120), systemic sclerosis (123), Alzheimer’s disease (124), epilepsy (125), MS (126), chronic obstructive airways disease (127), emphysema due to alpha1 antitrypsin deficiency (128) and Parkinson’s disease (129). Guidelines are also being put forward for other conditions such as SLE (130). Some of these individual definitions make reference to halting or delaying organ damage or preventing cell death, but others include clinical rather than pathological outcomes as targets, for example the degree of seizure control in epilepsy (125). Perhaps the most similar illness to schizophrenia in the list above is MS. In this condition disease-modifying therapies are defined as 'drugs targeted to prevent relapses of the disease, and consequently, progression of disability’(126).

In translating these theories of disease modification to schizophrenia it is certainly possible that antipsychotic medication, in particular clozapine, could meet the criteria

of preventing relapse and progression of disability. The premise of early intervention in psychosis services is that there is a critical period during which the future course of illness is set. Early proponents of early intervention services noted that 'the course of psychosis is the most stormy at its onset and early in its manifest course...the first three years of treated or untreated illness offer a window of opportunity to prevent, or limit the potential decline in outcome'(131). Thinking at that time was based on evidence such as the International Study of Schizophrenia which followed up 1633 subjects with first episode schizophrenia and found that 2 year outcome was the strongest predictor of 15 year outcome (132). It was proposed that untreated psychosis was potentially neurotoxic based on evidence that clinical course often deteriorated rapidly then plateaued with greater treatment responsiveness at the start of the disorder and longer duration of untreated psychosis (DUP) was associated with poorer outcomes (133,134). McGlashan, 1999, noted that 'Overall, it appears that first-episode patients are healthier at onset than they are 2 to 5 years later and that, in the interim, something is lost'. The Newcastle Early Psychosis Declaration in 2002 (135) led to the setting up of UK wide early intervention services, and similar health policy initiatives in other countries, with a remit to provide comprehensive early detection and treatment of first episode psychosis. The evidence that reducing DUP has been effective in improving outcomes in schizophrenia has been hard to quantify, with various systematic reviews reporting modest effects at best (136–138), however a recent umbrella review of previous meta-analyses has been more positive (139).

Aside from the early intervention literature, which does not disentangle treatment effects from service design effects, there is clear evidence, starting from publication of a landmark trial by McEvoy et al in 1991 (140), that patients with first episode psychosis

respond to lower doses of medication than those with chronic illness (141). In addition patients are more likely to respond to treatment during a first episode of illness than during a second, and time taken to reach remission is shorter (142). Response rates in first episode schizophrenia have been analysed by meta-analysis which found an overall response rate of 81.3% for a 20% reduction in PANSS/BPRS and 51.9% for a 50% reduction (143), whereas figures in a separate meta-analysis of chronic schizophrenia reported rates of 53% and 23% respectively (144).

If duration of illness is indeed associated with degree of antipsychotic response, then it is reasonable to hypothesize that if clozapine is used earlier in the course of TRS, before treatment resistance has 'set in', it is likely to be more effective. There is some evidence in the literature to support this, though studies have been comparatively small (145–147), or indirect measures, such as previous hospitalisations, have been used (148). There is thus a need for more research into whether clozapine is more effective if used earlier in the course of schizophrenia, particularly as, at present, prescription of clozapine is often markedly delayed.

Delays in clozapine prescribing

The premise of disease modification relies on early administration of treatments to delay or prevent deterioration. However, despite research findings, and clear guidelines, the use of clozapine is often delayed for several years, and non-evidence based interventions including multiple antipsychotic trials, poly-pharmacy and prescribing above recommended limits are frequently resorted to prior to, or instead of, prescription of clozapine (149,150). In the UK, the 2020/2021 Royal College of

Psychiatrists report of the Early Intervention in Psychosis audit has indicated that only 50 % of patients who are eligible are being prescribed clozapine (151). In the US, data suggests that only 20% of people who are eligible receive clozapine (152). Reasons for delay are manifold, including obvious concern about haematological monitoring and potential for serious side effects, but the literature suggests that attitudes of clinicians are a significant barrier to increasing the use of clozapine (150). Patient and caregiver surveys generally report positive experiences of clozapine (153). A recent qualitative review by Jakobsen et al, involving interviews with psychiatrists looking after patients who were eligible for, but not prescribed clozapine, has highlighted that 'psychiatrists tended to accept quite high levels of symptom severity and quite low levels of functioning as patients being "stably ill" (and therefore too well-treated for clozapine) and/or "beyond clozapine treatment' (154). The interviews indicated that psychiatrists showed a desire to maintain stability and avoid exposing their patients to the risk of clozapine, and also had concern about organisational constraints. The paper included a quote from a participating psychiatrist stating "I think you should use clozapine when you have trialled all other antipsychotics (…) there are side effects to it, and…lots of blood tests (…) clozapine is like the last drug. Why should you trial the last choice as one of the first?"(154). This degree of therapeutic nihilism around clozapine may explain why delays in clozapine prescribing are continuing in this magnitude, and, as is reported by Jakobsen et al, 'when clozapine is introduced late in the treatment course, or even as a "last resort" treatment, the psychiatrists unintendedly end up confirming their own negative experiences with clozapine'.

This qualitative review chimes with my own experience as a practicing NHS consultant and previous Mental Health Act Review Tribunal medical member. Current clinical

practice remains divergent from clinical guidelines in relation to clozapine prescription and patients tend to be considered for clozapine late in the course of their illness, when other secondary disabilities have also set in, including homelessness, estrangement from families, drug and alcohol addiction and iatrogenic effects of antipsychotic medication. Excuses are frequently found for why a patient should not be considered for clozapine. These ongoing delays in clozapine prescribing means that it is difficult to predict how effective clozapine could be if it were to be started early in routine clinical practice.

Conclusion

I have presented evidence in support of an immune basis for schizophrenia, focussing particularly on the immunology of TRS and its only licensed treatment, clozapine. I have argued that TRS may be categorically distinct from nTRS, with evidence of different or additional immune markers, and have speculated that psychosis becomes treatment resistant when an inflammatory process fails to switch off. I have shown that clozapine has unique benefit in TRS, or at least a subset of TRS, and that there is evidence that it acts as an immunomodulator, causing an array of immune side effects, along with evidence that it affects glutamate transmission in the brain. I have argued that clozapine may be acting as a disease modifying drug in schizophrenia if it is exerting an antipsychotic effect via, at least in part, immunological mechanisms. I have presented criteria for disease modification in a number of other immune conditions and applied these to TRS. In this thesis I aim to investigate further for evidence of disease modification with clozapine. If there is clear evidence in support of this hypothesis then

this would have great value in terms of improving access to clozapine in routine clinical practice.

CHAPTER 2. AIMS AND HYPOTHESES

Aims

In this thesis I plan to investigate whether there is evidence from existing literature that would support a theory of clozapine being a disease modifying drug, and to design an observational study to investigate the same hypothesis using real world clinical data. I also plan to review in detail immune side effects of clozapine, focussing on two clinically relevant effects, namely neutrophil count changes and immunoglobin deficiency, in order to investigate whether these may have utility as disease or treatment biomarkers in TRS.

Hypotheses

I have set out to test the following hypotheses:

Hypothesis 1. Earlier use of clozapine in TRS is more effective than if its use is delayed.

Hypothesis 2. Patients can be classified by neutrophil trajectory in response to clozapine and neutrophil trajectory can predict clinical outcome.

Hypothesis 3. Immunoglobulin levels fall with clozapine use and reduction in immunoglobulin level is associated with improved clozapine response.

Paper 1. Effect of age on the relative efficacy of clozapine in schizophrenia

I have carried out a systematic review of all available randomised controlled trial data looking at the effectiveness of clozapine compared to alternative antipsychotic medications in non-treatment naïve schizophrenia. I have carried out a meta-analysis and meta-regression to see if there is evidence that age (as a proxy for duration of illness) is associated with clinical response. I have written a journal article which has been published.

Other author contributions for paper 1

Xianxin Liu carried out a review of Chinese language studies and collected data from papers which met inclusion criteria. I received supervision from Dr Malcolm Price on the use of STATA to carry out meta-analysis and meta-regression. Dr Price also provided some edits to the paper.

Paper 2. Duration of prior illness and clozapine response. An observational study using electronic health records

I have carried out an observational study of real-world clinical data, using anonymised electronic health records, to create a database of 425 patients who started clozapine between 2007 and 2016. I have rated their clinical status prior to clozapine and after 2 years of treatment. I have tracked through their clinical records to determine their date of onset of psychosis. I have then carried out a regression analysis to investigate for

an association between duration of psychosis prior to use of clozapine and clozapine response.

Other author contributions to paper 2

For this study I received support from Ms Megan Pritchard in carrying out data searches using the CRIS system. I also incorporated some data into my dataset from a previous CRIS dataset of patients starting clozapine, compiled by Dr Sophie Legge. I received supervision from Dr Malcolm Price on carrying out ordinal logistic regression using STATA and he also provided some edits to the paper. Dr Joyt Chandan reviewed the written paper and gave advice on some of the study limitations.

Paper 3 Early neutrophil trajectory may predict clozapine response – results from an observational study using electronic health records

I have linked my dataset from paper 2 with neutrophil data from the clozapine monitoring service. I have carried out a latent class growth analysis to examine trajectories of neutrophil counts from baseline for the first 6 weeks of clozapine therapy. I have then carried out logistic regression to look for an association between neutrophil trajectory and clozapine response.

Other author contributions to paper 3

Ms Megan Pritchard assisted with providing the neutrophil data. Dr Isabel Morales-Munoz provided supervision on the methodology of latent class growth analysis and

edited the paper. Professor Adrian Sheilds provided support with planning the study and also reviewed the completed paper. Dr Graeme Blackman also provided some edits to the paper.

Paper 4. Effect of clozapine on calculated globulin levels and association with treatment response. Results from a retrospective cohort study using electronic health records

I have combined my dataset from paper 2 with Immunoglobulin results from another researcher's database with an overlapping time period. I have compared CG levels (total protein minus albumin) pre-clozapine and 1 year post clozapine and have carried out logistic regressions to look for associations between 1) change in CG level and 2) one year CG level and clinical outcome. I have also examined demographic differences in CG levels.

Other author contributions to paper 4

Dr Risha Govind provided the data for CG results which I used for this study.

CHAPTER 3. PUBLICATION. EFFECT OF AGE ON THE RELATIVE EFFICACY OF CLOZAPINE IN SCHIZOPHRENIA

Introduction

In this paper I tested the hypothesis that earlier use of clozapine is more effective than if treatment is delayed. In order to do so I elected to perform a systematic review and meta-analysis of clozapine trial data from which I could analyse if there was any effect of duration of illness on the effectiveness of clozapine.

I decided to focus on randomised controlled trial (RCT) data only, as RCTs remain the gold standard of research into effectiveness of treatments (155), and would be more straightforward to use for a meta-regression. There have been several previous systematic reviews and meta-analyses of clozapine, but these have differed from each other significantly in terms of their methodology and inclusion criteria. In an early review Wahlbeck et al, 1999, found that clozapine was more effective than first generation antipsychotics (156) but a subsequent Cochrane review was more qualified in its conclusions (71). Both these reviews included all studies of schizophrenia rather than confining the inclusion criteria to TRS. A subsequent Cochrane review of clozapine versus second generation antipsychotics, again including studies of schizophrenia as a whole rather than just TRS, provided weak support only for clozapine's superiority (72). Systematic reviews which have looked specifically at the effectiveness of clozapine in TRS have been more positive. Chakos et al, 2001 reported that clozapine was more effective than typical antipsychotics in TRS but this review was of just 7 studies (157). Siskind et al, 2016, conducted a larger meta-analysis of TRS patients with results showing clear benefits for clozapine over first generation and second

generation antipsychotics (75). This review included studies of childhood onset schizophrenia and also included data from the CATIE study, which was not blinded for the clozapine arm. Finally, Samara et al, 2016 carried out a network meta-analysis of all antipsychotics for TRS which failed to show convincing benefit for clozapine (76).

The existing reviews which were of TRS patients only had quite stringent requirements for TRS diagnosis and on comparison of the demographics of patients in the non TRS versus the TRS studies the ages and lengths of illness were similar. Since clozapine is licensed for TRS rather than as a first line treatment for schizophrenia, it is probable that most patients in clozapine studies would be likely to be treatment resistant, unless the trial was specially of a first episode cohort. Therefore, for the purpose of my review I decided not to stipulate a TRS criteria, but rather to exclude studies which were of predominantly treatment naïve patients. I also decided to exclude childhood-onset schizophrenia, as the clinical course in this condition is often more severe than adult-onset disease.

My systematic review criteria were therefore as follows. I included all single or double blind RCT studies of clozapine versus any other single or multiple antipsychotic drugs, in patients with adult onset non treatment naïve schizophrenia. I was able to use all studies published in English but also in Chinese due to being able to collaborate with a visiting Chinese academic in the university department.

My original aim was to use data for duration of illness prior to clozapine use as a variable in a meta-regression to look at the effect of duration of prior illness on clozapine response (relative to alternative antipsychotics). However, on analysing the papers in the review, whilst the majority of papers did report duration of illness, this

was not defined in a standard way, and for some papers duration of illness referred to duration of hospital episode. I therefore decided instead to use mean age, as a proxy for duration of illness, in the meta-regression.

I also intended at the start of the review to carry out a meta-analysis of individual patient data if it were available. Unfortunately, the majority of the clozapine studies were old, with half being published before 2000. I contacted the authors of all the post 2000 studies but those who replied no longer had access to the study data. Two studies did publish their individual data and for those studies I was able to carry out regressions of patient age against treatment response.

The study was published in Acta Psychiatrica Scandinavica in 2020 with the title ‘Effect of age on the relative efficacy of clozapine in schizophrenia’. I have also presented the data in a poster presentation at the British Association of Psychopharmacology annual meeting in June 2019.

PUBLISHED VERSION. EFFECT OF AGE ON THE RELATIVE EFFICACY OF CLOZAPINE IN SCHIZOPHRENIA

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Abstract

Objective**:** Early treatment of schizophrenia improves outcomes. Clozapine appears to have unique benefit when other antipsychotic medication has failed. This systematic review and meta-analysis aims to assess clozapine's superiority over alternative antipsychotic medication and examine whether earlier use is associated with additional benefit.

Method**:** Systematic retrieval of blinded, randomized controlled trials comparing clozapine with alternative antipsychotics in adults with schizophrenia. The effect of mean age on relative clozapine response was examined using random effects meta-regression, and multiple linear regression on available patient data.

Results**:** 276 studies were retrieved. Thirty-four studies were included in the meta-analysis. Clozapine was significantly more effective than alternative antipsychotics in reducing psychotic symptoms and increasing response. However, meta-regression failed to show a more significant effect in younger patients (age on effect size (total psychotic symptoms) 0.00, p =0.79 CI -0.03 – 0.03). Individual patient data was available for 2 studies, the larger of which showed a significant interaction between younger age and superiority of clozapine.

Conclusion**:** The results support clozapine's superiority over other antipsychotics. A convincing effect of age on this effect was not demonstrated, although this was suggested in one study. In view of the age of many of the included studies, and changes in reporting practice over time, new clozapine RCTs, which include age of illness onset as well as age at trial time, would be welcome in order to provide meta-analysable data for future use.

Summations

- Clozapine is more effective than other antipsychotics both in terms of reducing psychotic symptoms and increasing rate of response.

- It is unclear whether clozapine's relative effectiveness is greater when started earlier in the course of illness.

Considerations

- Results need to be interpreted with caution in view of the heterogeneity of the data, narrow age range and the use of age as a proxy measure for duration of illness.

- There is an inherent risk of aggregation bias in meta-regression.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Introduction

Schizophrenia has a peak age of onset in adolescence and young adulthood, and early and effective treatment is crucial to limit long term disability - it has been acknowledged for some time that 'the course of psychosis is the most stormy at its onset and early in its manifest course...the first three years of treated or untreated illness offer a window of opportunity to prevent, or limit the potential decline in outcome' (1). This concept of a 'critical period' of illness in schizophrenia (2, 3), during which the future course of illness can be modified, is supported, albeit with qualification, by the literature. Studies have shown a clear association between shorter duration of untreated psychosis and more favourable clinical outcome (4-6). Prospective studies of 'services providing enhanced care' for first episode psychosis compared to 'treatment as usual' have also shown early clinical benefits (7, 8) although longer term follow-up has cast doubt on the degree to which these benefits are retained (9, 10).

Whilst the majority of people who develop schizophrenia respond well to standard antipsychotic medication, up to one third show treatment resistance (11-13), typically

defined as failure to respond adequately to two trials of antipsychotic medication of adequate dose and duration (14). The concept of treatment resistance in schizophrenia remains incompletely understood. A recent study of a first episode schizophrenia sample by Demjaha et al (12), found a high percentage of treatment-resistant cases (84%) to be treatment-resistant from the outset. However, a minority of cases had shown a previous good response to antipsychotic medication but had subsequently developed treatment resistance. Studies have demonstrated that patients in the early stages of psychotic illness require lower doses of antipsychotic medication (15), and have much higher rates of treatment response (16), compared to patients with multiple episodes of illness. These findings suggest that delay in effective treatment can increase the risk of treatment resistance.

Clozapine has been the gold standard intervention for treatment resistant schizophrenia (TRS) since the seminal trial by Kane and colleagues in the 1980s (17), and its use has generally been reserved for this indication due to its risk of agranulocytosis and the need for stringent blood monitoring. However clozapine's superiority in TRS has been questioned with some studies finding other second generation antipsychotics to be as effective (18, 19), and meta-analyses producing inconsistent results (20-23). One recent meta-analysis of randomised controlled trials (RCTs) (22) comparing clozapine to any other antipsychotic medication found in favour of clozapine in reducing total psychotic symptoms in short-term follow-up studies (standardized mean difference (smd) -0.39, 95% confidence interval (CI) -0.61 - -0.17, but in longer term follow-up studies the evidence was unclear (smd -0.11, 95% CI -0.31 – 0.09). For the same outcome a wider network meta-analysis of all antipsychotic comparison data (9 comparators) for TRS (23) did not find clozapine superior overall

with effect estimates ranging from -0.02 (-0.44 – 0.4) for clozapine compared to ziprasidone to -0.4 (-0.74 - -0.04) for clozapine compared to sertindole. There is, though, a sizeable evidence base for clozapine not included in these meta-analyses. Two large non industry funded trials, the CATIE phase 2 E study (24) and the CuTlaSS trial (25), have shown clear benefit of clozapine, as has evidence from observational data, suggesting improved clinical outcomes (26) such as hospital admission (26, 27) and reduced mortality rates (28-31) in people who had been prescribed clozapine compared to those prescribed alternative antipsychotics.

If duration of illness is associated with degree of antipsychotic response, then it is reasonable to hypothesize that if clozapine is used earlier in TRS, it may be even more effective compared to other antipsychotic medication than when given later in the illness course. There is some research to suggest that starting clozapine early in the course of TRS is beneficial compared to delaying clozapine (32-37). However, these findings are confined to retrospective data and do not assess the relative effectiveness of clozapine compared to alternative antipsychotics at different stages of illness.

Aim

To identify and synthesise RCT data comparing clozapine to any other antipsychotic medication in patients with schizophrenia and to evaluate whether they provide evidence that earlier use of clozapine is associated with greater efficacy. As previous definitions of treatment resistance used in clozapine trials have been broad, with only the more recent trials following the Kane criteria (17), we elected to include all trials of adult-onset schizophrenia, other than those of predominantly treatment naïve patients, rather than to rely on reported treatment resistance, in order to provide as large a

sample as possible for analysis. We hypothesized that, in studies that included adult participants with a younger age (suggesting shorter illness duration), improved response rates relative to alternative antipsychotics will be seen.

Methods

The systematic review protocol was registered with Prospero (CRD42017077910) in September 2017 and an updated literature search was conducted covering the period up to 9th July, 2018.Standard methods for systematic review following the PRISMA checklist were used.

Searches were carried out of PubMed, EMBASE and the Cochrane Schizophrenia Group's Trials Register and the WANGFANG database of Chinese medical literature.

The PubMed search terms used were- randomized controlled trial OR controlled clinical trial OR randomized OR placebo OR drug therapy OR randomly OR trial OR groups OR randomised (http://work.cochrane.org/pubmed). The Embase search terms used were - crossover procedure OR double-blind procedure OR randomized controlled trial OR single-blind procedure OR random* OR factorial* OR crossover* OR (cross adj1 over*) OR placebo* OR (doubl* adj1 blind*) OR (singl* adj1 blind*) OR assign* OR allocat* OR volunteer* (http://work.cochrane.org/embase).

The search terms used for clozapine were clozapine* OR clozaril OR zaponex OR denzapin* OR clopine OR leponex.

Secondary searches were carried out by examining references lists from included studies, past systematic reviews, citation searching of included studies, checking

online trial databases, hand-searching key journals and contacting authors who have published previously on clozapine and are recognized to be experts in the field.

Trials in Chinese identified from the searches were screened at abstract level then full text review of suitable studies was carried out by XL who also conducted the search of the WANFANG database.

Type of study

Any single or double-blind RCT comparing clozapine to one or more other antipsychotic drug. Only studies published in English or Chinese were included. In studies employing a cross-over design data were included for the first but not the cross-over phase of the study.

Population

Studies including predominantly treatment non-naive (>=60%) participants with diagnosis of schizophrenia or schizoaffective disorder. Studies of childhood-onset schizophrenia, or studies of clozapine to treat tardive dyskinesia symptoms, comorbid substance misuse or aggression were excluded.

Intervention and comparator

Comparison between clozapine and one or more other antipsychotic drug.

Outcomes

The two primary outcomes were 1) the effect on total psychotic symptoms as measured by a validated clinical scale, either the PANSS (Positive and Negative Syndrome Scale) total score or BPRS (Brief Psychiatric rating scale) total score, and 2) response

rate. Response was defined variably across the studies, therefore for the purpose of this review broad criteria were used, with response defined as at least a 20% reduction in PANSS or BPRS total score or by a CGI (clinical global impression) rating of improved or very much improved. Studies were included in the meta-analysis providing data could be extracted on either or both of the primary outcomes.

Secondary outcomes were:

1. positive symptoms of psychosis (PANSS or BPRS positive sub-scale score)
2. negative symptoms of psychosis (PANSS or BPRS negative sub-scale score or SANS score)
3. CGI-severity scores
4. all-cause discontinuation rate
5. discontinuation rate due to lack of efficacy

Variables chosen for meta-regression

Data was collected for both age and duration of illness when available. However, due to a lack of consistency in how the latter was defined, age was chosen for the primary analysis, with duration of illness as a secondary variable.

Study Selection

References were screened at title and abstract level by RJ. Full text review was completed by RJ with discussion of any uncertain articles with RU. Consensus was reached on all papers included in the final list.

Data extraction

Data extraction was carried out by RJ with input from RU. If data were only presented in graph form values were measured by both RJ and RU with the mean of the two data points recorded. In addition, RU independently extracted data on a random sample of 20% of papers. Missing data for standard deviations in a small number of early papers was inputted by taking the average values from the first half of studies (pre 2000) included in the review.

Data was extracted on the following: setting, interventions, number in each treatment arm, age, duration of illness, study duration and results of validated outcome measures.

For studies in which clozapine was compared to several comparator groups the total number of patients and events in each clozapine group was divided by the number of comparison groups in the study and rounded down to the nearest integer, to ensure that the effect size of clozapine was not given extra weight (38).

For rating scales, change scores were used when possible. When standard errors for change scores were missing these were estimated from p values when available. Otherwise, missing standard deviations were either inputted using methods referenced in the Cochrane handbook (38), or final scores were used instead. Standardized mean differences for each continuous outcome were used in the meta-analysis. For dichotomous outcomes proportions of responders were used.

For the meta-regression, data were extracted for mean age prior to commencement of clozapine. Four studies reported medians and ranges for these values rather than means and standard deviations. For these studies means were inputted from medians

as per methodology reported by Hozo et al (2005)(39). In 3 of these studies the sample size was sufficient to inpute medians directly for means. In the fourth study which was smaller the mean was estimated from the median.

Study Quality

The Cochrane risk of bias tool (38) was used to assess the quality of the included studies.

Solicitation of Individual Patient Data (IPD)

Individual patient data were requested by email from the corresponding authors of all papers published during or since the year 2000.

Statistical Analysis

Statistical analysis was conducted using STATA version 15 (40). Meta-analyses were carried out using the metan command. A random effects model was chosen in view of the known heterogeneity of the data, with comparisons between different drugs and dosages and studies of different durations. Heterogeneity was assessed using the I^2 statistic (41).

Sensitivity analyses were performed to exclude:

1 studies rated at high risk of bias in any category of the Cochrane risk of bias tool

2 non intention-to-treat studies

3 industry conducted or sponsored studies

4 studies with inputted standard deviations

Funnel plots were used to assess evidence of small study effects for both primary outcomes.

Random effects meta-regression models were fitted using the metareg command to look for possible effects of age/ duration of illness on relative treatment effects for each outcome measure.

Multiple linear regression was carried out on results from studies which reported individual patient data to look for evidence of interaction between age / duration of illness and treatment arm on outcome.

Results

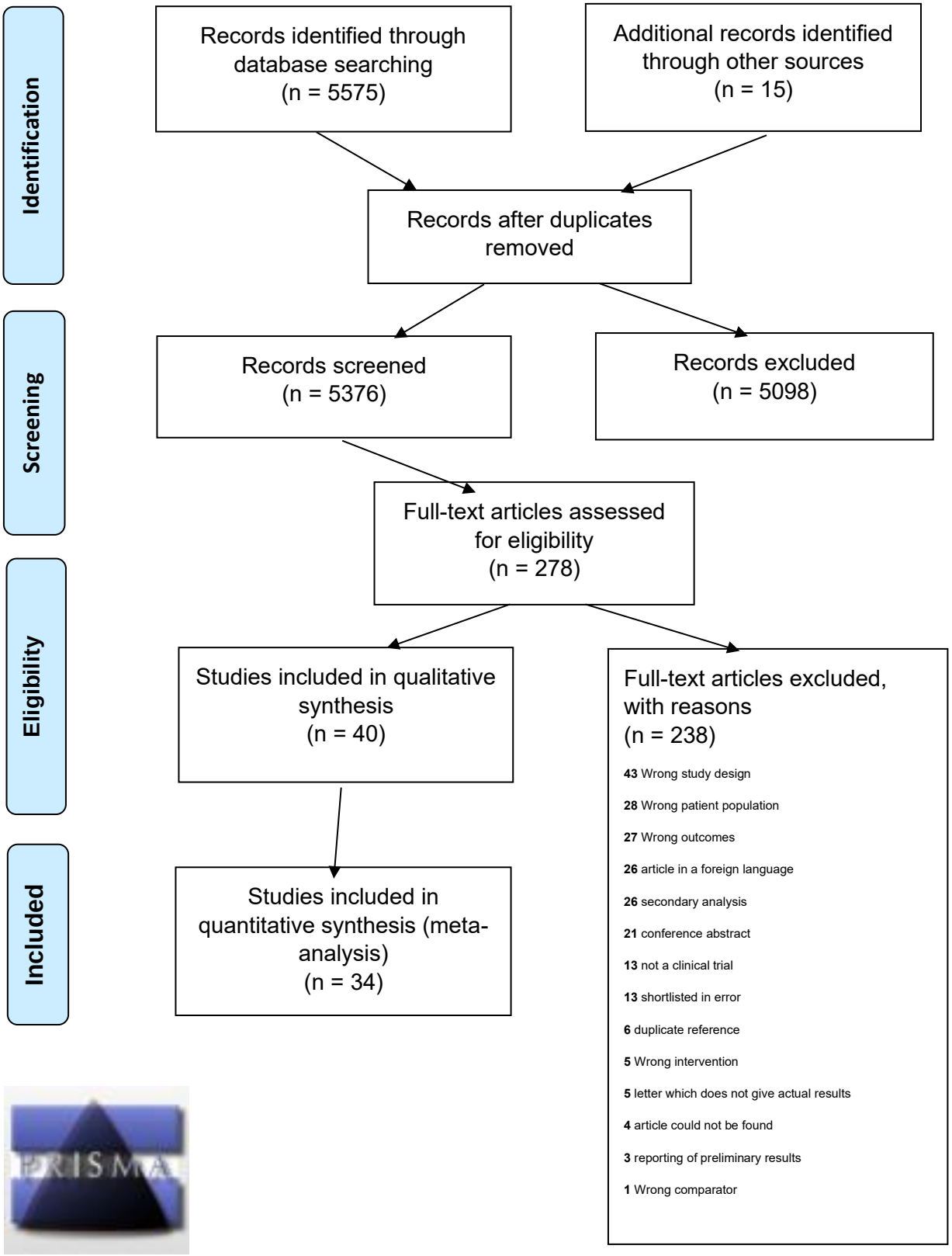
The initial search yielded 5575 studies for screening. A further 15 studies were identified by secondary search methods. Of these 278 papers were selected for full text review.

Full text review identified 40 studies which met the review inclusion criteria (17-19, 25, 42-77), but of these, 6 did not have any usable statistics (52, 54, 64, 66, 72, 77), therefore 34 studies were included in the statistical analyses (see Table S1 in the supplementary information for characteristics of included studies).

The Prisma flow diagram for the literature review is shown in figure 1.

Figure 1. PRISMA 2009 Flow diagram

From Moher D, Liberati A,Tetzladd J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement PLoS Med 6(7): e1000097/ doi 10.1371/journal.pmed1000097. For more information, visit www.prisma-statement.org



Characteristics of included studies

The majority of studies were reported as double blind (35 out of 40 studies) with sample sizes ranging from 10 – 423 participants. Most studies were of clozapine versus a single comparator group, with 5 studies having 2 or more comparators and one comparing clozapine to an alternative antipsychotic at two different dosages. Twenty-six of the 40 studies (24 of the 34 included in the statistical analyses) referred to patients being treatment resistant, though definitions of treatment resistance varied between studies.

Risk of Bias Review

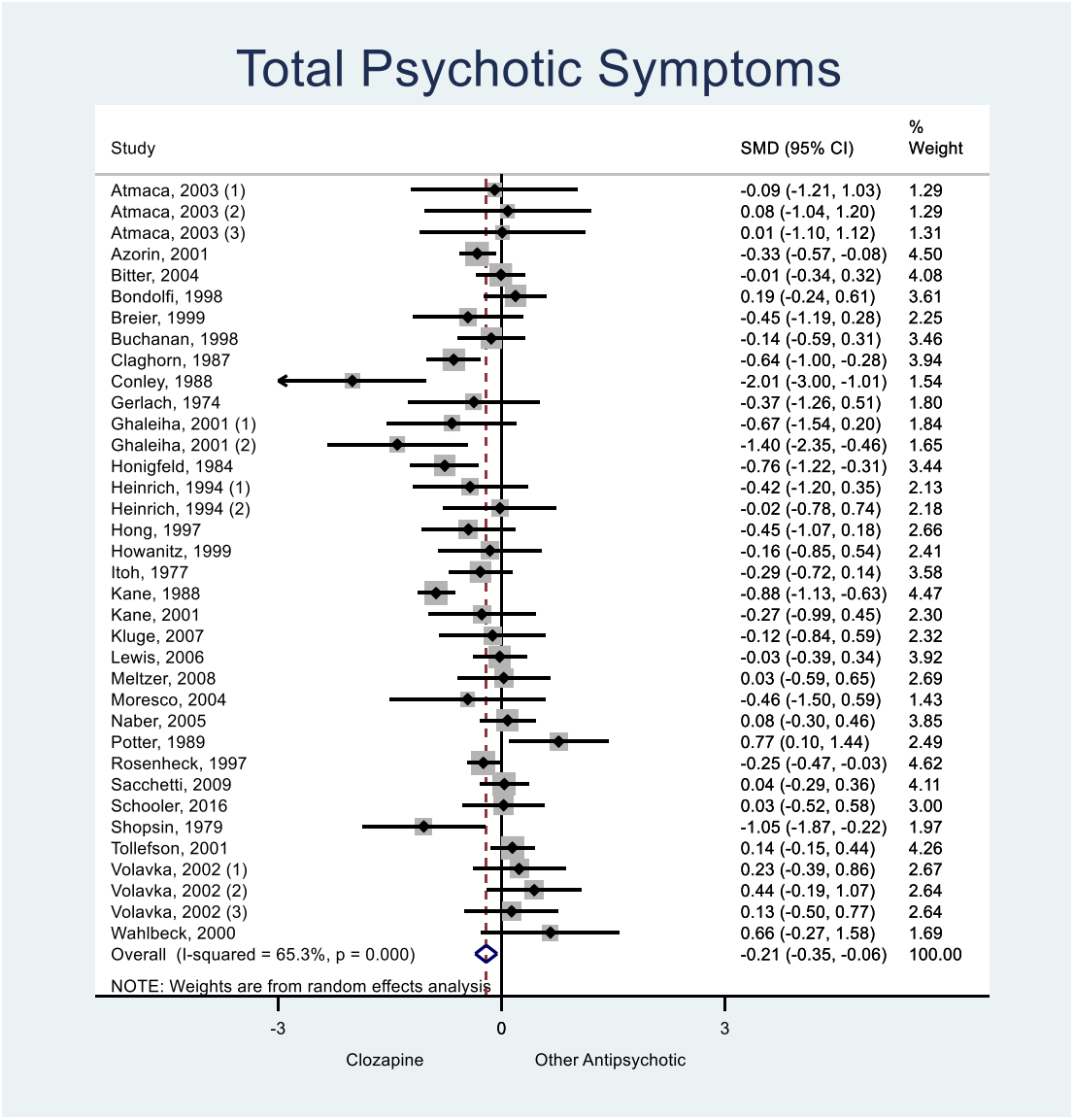
Using the Cochrane risk of bias tool, ten out of the 40 studies (six out of the 34 studies included in the meta-analysis) scored high on at least one domain. Few of the studies were recent, and 50% were published before the year 2000. The reporting of methodology was limited in the majority of studies. (see Table S2 in supplementary information for Cochrane risk of bias table).

Meta-analyses

Primary outcomes

Analysis of the complete set of 34 studies (40 treatment comparisons) showed that clozapine was on average superior to alternative antipsychotics for both the primary outcomes. The effect size for total psychotic symptoms was a standardized mean difference of -0.207. (-0.33, -0.06) I^2 65%. The effect size for response rate was a relative risk of 1.22 (CI 1.03, 1.44) I^2 55% (see Figure 2 (a and b)).

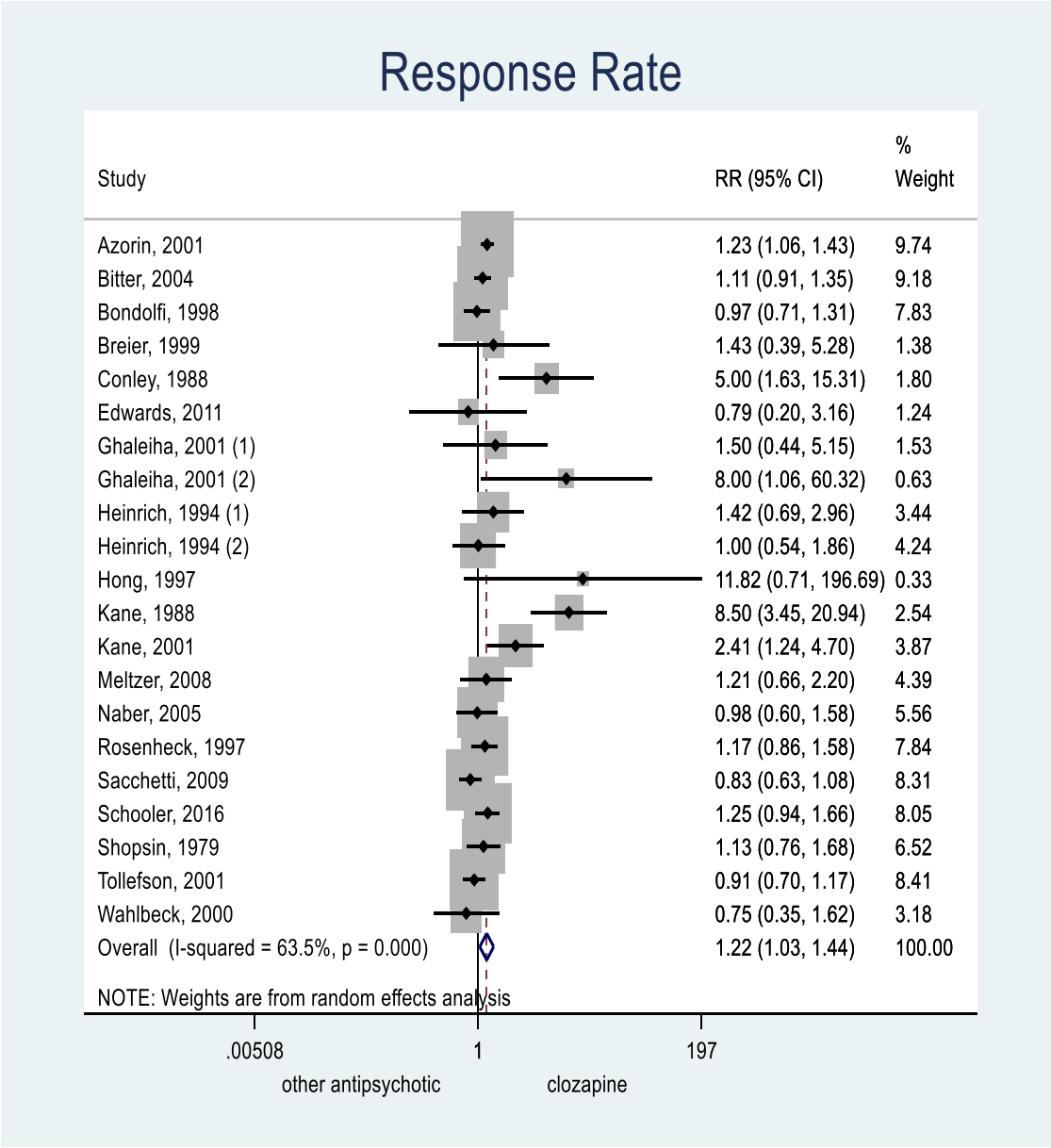
Figure 2a. Forest plot showing effect of clozapine compared to other antipsychotic medication on total psychotic symptoms



SMD – standardized mean difference

95% CI – 96% confidence intervals

Figure 2b. Forest plot showing effect of clozapine compared to other antipsychotic medication on response rate



RR – relative risk

95% CI – 95% confidence intervals

Secondary outcomes

There were significant differences in favour of clozapine in both reduction in CGI-S scores and lower discontinuation rates for lack of efficacy. Results for other secondary outcomes (positive psychotic symptoms, negative psychotic symptoms and all cause discontinuation rate) were not significant (see Table 3 supplementary information).

Sensitivity analyses

The results for the four planned sensitivity analyses are shown in figure S1 supplementary information. Effect sizes were broadly similar across the analyses and ranged from 0.18 to 0.21 for total psychotic symptoms and 1.19 to 1.38 for response rate.

Funnel plots for both primary outcomes showed no obvious evidence of small study effects. (Figures S2a and S2b in supplementary information).

Meta-regression

The median of the mean ages reported across the studies was 37 years (range 21 – 65 years), with an inter-quartile range of 34 –40 years.

Random effects meta-regressions did not show evidence of a relationship between age and clozapine response relative to alternative antipsychotic medication as measured by both primary and secondary outcomes. Neither was a relationship between duration of illness and relative response observed (table 1).

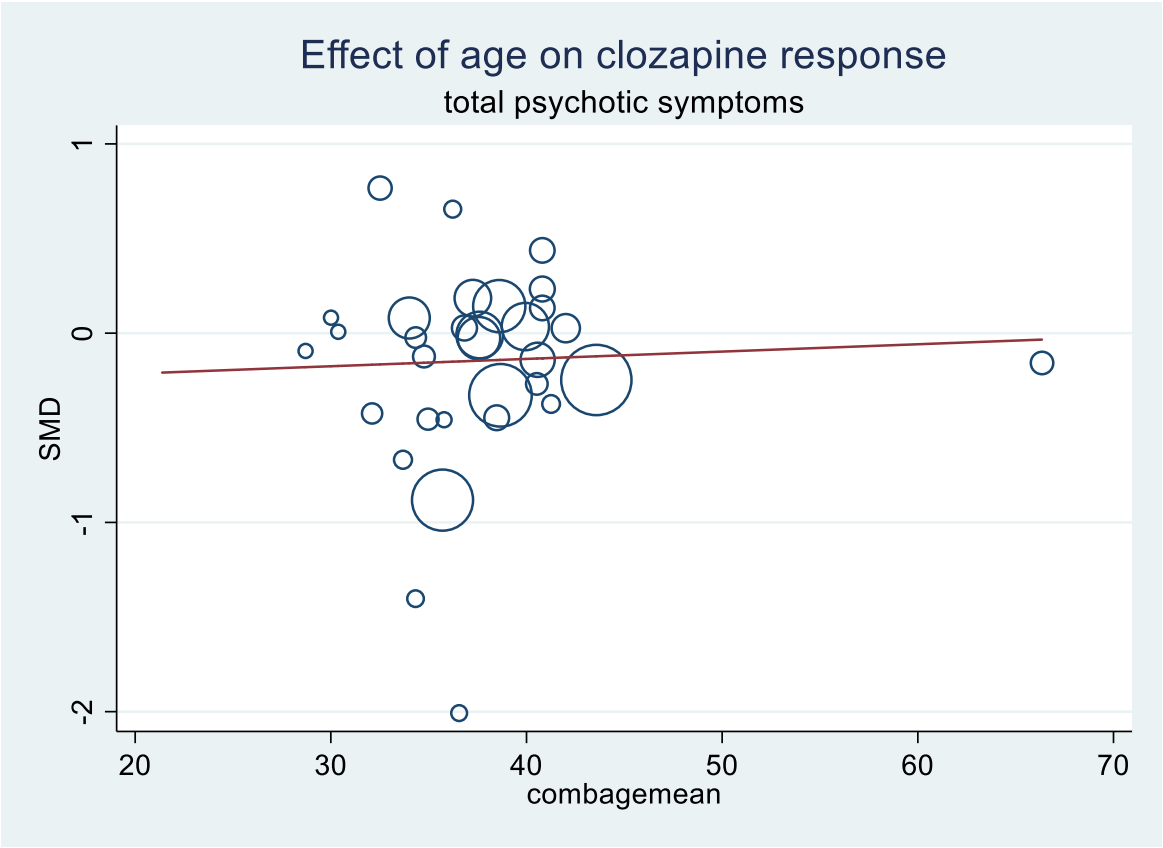
Table 1. Random effects meta-regression of the effects of age/ duration of illness on measures of clozapine response relative to alternative antipsychotics

	Age			Duration of illness		
Outcome measure	Mean age/ treatment interaction coefficient	P-value	95% Confidence Interval	Mean duration of illness /treatment interaction coefficient	p-value	95% confidence interval
Total psychotic symptoms	0.00	0.79	-0.03 – 0.03	-0.01	0.51	-0.04-0.02
Response rate	0.00	0.86	-0.03 – 0.04	0.01	0.75	-0.03-0.04
CGI-S*	-0.01	0.35	-0.04 – 0.02	-0.02	0.12	-0.05-0.01
Positive symptoms	0.01	0.44	-0.02 – 0.03	0.01	0.66	-0.02-0.03
Negative symptoms	0.00	0.78	-0.03 – 0.02	0.00	0.89	-0.02-0.03
All cause discontinuation	-0.03	0.08	-0.06 – 0.00	-0.02	0.11	-0.03-0.00
Discontinuation due to lack of efficacy	-0.09	0.06	-0.18 – 0.00	-0.05	0.10	-0.11-0.01

* CGI – S – Clinical Global Impression – severity scale

The results of the meta-regression for total psychotic symptoms are shown as a scatter plot in figure 3.

Figure 3. Scatter plot showing the effect of age on relative clozapine response as measured by total psychotic symptoms



SMD – standardized mean difference

Combagemean- combined mean age in studies

Individual patient data

Two studies (Hong 1997 and Wahlbeck 2000) reported individual patient data. Requests for individual patient data from other authors did not yield any additional data.

Hong et al, 1997 reported a 12-week study of 40 treatment-refractory patients comparing clozapine (mean dose 543mg) with chlorpromazine (mean dose 1163mg) in a double-blind randomized controlled study design. Six clozapine patients (28.6%) improved by more than 20% reduction in BPRS scores during the study, as compared to none from the chlorpromazine group. The percentage reduction in score for BPRS, PANSS and PANSS positive and general psychopathology subscales were all significantly more with clozapine than chlorpromazine. The effect of drug on PANSS negative subscale scores was not significant.

Wahlbeck et al, 2000 was a single-blind (raters only) trial of clozapine versus risperidone for 10 weeks. Mean doses were 385mg for clozapine and 7.8mg for risperidone. The study found no significant differences between the two groups in terms of PANSS total scores, positive and negative subscale scores, global scores or social functioning scores.

Multiple linear regression using age and drug as co-variables with the dependent variable as change in BPRS score showed significant interaction between age and drug in the Hong et al, 1997 study, with younger age associated with greater symptom reduction in the clozapine group. The results for the Wahlbeck et al, 2000 study were not significant (table 2).

Table 2. Multiple linear regression of interaction between age and treatment arm on change in BPRS scores from studies reporting individual patient data

	Hong et al 1997 n = 38 Adj R^2 = 0.34			Wahlbeck et al 2000 n = 19 Adj R^2 = 0.40		
Change in BPRS* total score	Regression co-efficient	p-value	95% Confidence interval	Regression co-efficient	p-value	95% Confidence interval
Clozapine / comparator drug	-68.95	0.00	-110.71 - -27.20	14.78	0.58	-40.80 - 70.34
Age	-1.30	0.00	-2.08 - -0.52	-0.84	0.1	-1.86 – 0.19
Drug / age interaction	1.38	0.01	0.33 – 2.42	-0.02	0.97	-1.51 – 1.45

*BPRS –Brief psychiatric rating scale

Similar results were found when duration of illness rather than age was used in the regression (see table S4 supplementary information).

Discussion

The results of this systematic review and meta-analysis showed clozapine to be on average superior to alternative antipsychotics in the treatment of non-treatment naïve schizophrenia in adults. These findings were consistent across a range of general measures of treatment response, but not in specific clusters of symptoms. The results were robust in sensitivity analyses. The results of the meta-regression found no evidence of an effect of mean age on the relative effectiveness of clozapine. Individual

patient data were only available from two studies, and multiple regression of age against drug effect yielded mixed results, with the larger trial showing an association between age and treatment arm.

In the light of recent meta-analyses of clozapine RCT data reporting contrasting results (22, 23), the current review helps provide clarity that clozapine has unique benefit for patients who have not responded to first-line treatment. As regards timing of clozapine the findings of the review do not provide an answer to our hypothesis as to whether earlier use of clozapine is beneficial. Individual patient data meta-analysis would be the optimum method for interrogating the question but unfortunately this was not available in sufficient quantity for this review.

This study has several strengths, in particular the larger number of studies than previous reviews. The removal of a criterion of treatment resistance increased the number of eligible studies without obviously increasing heterogeneity. The review by Siskind et al (22) included 21 randomised controlled trials of clozapine and that of Samara et al (23) twenty. All of the clozapine studies from the Samara et al clozapine analysis were included in this review, but six studies from the Siskind et al review were excluded, three because they were studies of childhood-onset schizophrenia, one as it was the phase two of the CATIE study (78), in which the clozapine arm was not blind, and two Chinese studies on the basis that they were either not considered to meet inclusion criteria or were unable to contact the authors for further information. Cochrane reviews were also of smaller study numbers and were limited to either comparing clozapine to typical (20) or atypical (21) antipsychotics. The inclusion of Chinese language studies is an additional strength, as most English-language reviews include only trials published in English.

The main limitations of the study are firstly those of the methodology of meta-regression itself. Meta-regression is prone to aggregation bias when examining patient level covariates and can produce misleading results. Thus, the lack of evidence of an effect of age in study-level data is not evidence of an absence of such an effect within studies, at the individual level. Indeed, where we were able to analyse individual patient data, we did see an effect of lower age on increased superiority of clozapine.

Secondly, the outcome in this meta-analysis is not response to clozapine, but the relative response compared to the comparator drug. The lack of a demonstrable effect of age on the superiority of clozapine compared to other antipsychotics does not mean that there is no effect of age on response rates to clozapine per se.

Thirdly, although the sample size of 35 studies is not atypical for meta-regression, the lack of variability in the mean age means that the lack of evidence of an effect is not surprising. Using duration of illness prior to clozapine prescription as a variable for meta-regression, rather than age, would have been optimal but whilst this was often reported in studies it was not consistently defined. Another potential confounder of using age as a proxy measure for duration of illness is the overlap between adult and child onset schizophrenia, with the latter often carrying a poorer prognosis. For this reason, studies of childhood onset illness were excluded. Other limitations of the clozapine RCT data in relation to potential methodological bias such as inadequate blinding, and the uncertain role of industry funding are unlikely to influence data in relation to age as an effect modifier.

Whilst this study did not find a specific effect of age on differential response to clozapine, this does not argue against the pressing need to reduce delays in clozapine

prescribing, which range in the literature from about 4 (14) to 10 years (79). In the UK despite the national roll-out of early intervention services, designed to optimize treatment of psychotic illnesses in the critical period of illness, clozapine is still only prescribed to less than half of those who are eligible (80). Under-use of clozapine remains an issue internationally, particularly in younger patients (81). The time until eligible patients receive a treatment trial of clozapine is marred by enduring psychotic symptoms and loss of social and occupational functioning. Risks during this period are high, including risk of self-harm, or suicide (82). Delay to clozapine prescribing has been shown to be associated with adverse outcomes in retrospective studies (83).

There is some support in the literature for the existence of a critical period for clozapine prescription. Whilst studies of first-line clozapine for treatment naive patients have been inconclusive (84-86) it has been suggested that lack of superiority of clozapine in the first episode population may be due to a ceiling effect, with response rates to antipsychotic medication as high as 90% reported (16). However bringing forward the use of clozapine to second line (87) or using clozapine earlier in the course of a first episode of illness may be more effective (88). It has also been shown that in first episode schizophrenia the response rate to a second antipsychotic drops dramatically then increases again with clozapine, suggesting that second line use of clozapine may well be more appropriate than third line (16).

There are many reported barriers to clozapine prescribing, including concerns over need for blood testing and potential for side effects but also clinician and patient attitudes to clozapine (89-91). Recent authors have highlighted the need to review stringent blood monitoring requirements for clozapine, which can lead to unnecessary treatment discontinuation (92). This review helps shore up the evidence base for the

use of clozapine in schizophrenia which has not responded to first line treatment and provides some qualified support for the hypothesis that using clozapine earlier in the course of illness is more effective, which it is hoped should help surmount some of these barriers.

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CONCLUDING REMARKS

The results of the meta-analysis showed a superior effect of clozapine, which was in keeping with the previous meta-analysis by Siskind et al (75), but included a larger number of studies. The results also support the substantial evidence of clozapine's superior effectiveness which has accrued from non RCT studies. The meta-regression did not show a significant effect of age; however, the individual patient data did suggest that age may be a significant factor in clozapine response. The lack of positive findings from the meta-regression may be due to the absence of any effect being present. However, it may also be due to the shortcomings of the methodology of meta-regression in a relatively small sample size and limited age range of studies. In addition, the restriction of data to RCTs, whilst rigorous, meant that the reporting of data was limited, particularly in the older studies.

I therefore decided for my second paper to reconsider my original hypothesis, by looking at real world clozapine data from the CRIS system, an anonymised patient data resource from the South London and Maudsley Trust. I attended meetings of the clozapine study group at the Maudsley hospital, chaired by Professor MacCabe, and with his assistance I designed a retrospective cohort study to look at clinical outcomes of patients who had started clozapine for the first time whilst under the Maudsley hospital, with a view to investigating whether duration of illness prior to commencing clozapine, was associated with response to clozapine.

CHAPTER 4. PUBLICATION. DURATION OF PRIOR PSYCHOTIC ILLNESS AND CLOZAPINE RESPONSE: A RETROSPECTIVE OBSERVATIONAL STUDY USING ELECTRONIC HEALTH RECORDS

Introduction

For this study the hypothesis being tested was again whether there is evidence that starting clozapine earlier rather than later improves its effectiveness.

I initially conducted a narrative review of previous studies which had reported an association between earlier clozapine use in TRS and clinical outcome. From this review there were several previous studies which had specifically looked at timing of clozapine use and outcome in TRS. An early study by Lieberman et al, 1994, looked at predictive variables for clozapine response in 84 patients with schizophrenia (approximately 80% had TRS and the remainder were treatment intolerant) and found poorer response was related to longer illness duration, though the measurement of duration was crudely divided into greater than or less than 9 years (145). Contrastingly, Umbricht et al, 2002 reported that age and duration of illness were not significant predictors of clozapine response in 37 patients who they described as ‘chronically psychotic and partially treatment refractory’ (158). In a prospective study of early clozapine use in first episode patients. Agid et al, 2007, showed that patients who agreed to start clozapine after failed trials of two previous antipsychotics fared better than those who refused, though numbers by this stage of the study were small (13 patients commenced clozapine compared to 9 who refused) (159).

From population-based studies there is also some evidence that earlier clozapine is more effective. Harrison et al (2010) reported that adoption of a government policy initiative in New Zealand, which shortened median delay to clozapine from 5.7 to 2.8 years, was associated with a reduction in hospitalization rates but the result was not statistically significant (160). Also, Nielsen et al (2012) investigated which clinical variables recorded in a population database were associated with better clozapine response. They found significant negative associations between number of previous hospitalisations and antipsychotic trials and markers of better clozapine response (148).

There have been more recent observational studies with larger patient numbers. Ucok et al, 2015, carried out a chart review of 162 patients who started clozapine and found that length of clozapine delay (i.e. time from classification as treatment resistant to commencement of clozapine) was shorter in clozapine responders (147). Gee et al, 2016, carried out a mirror image study of 102 patients who commenced clozapine, and analysed net change in bed days following initiation of clozapine in relation to duration of time that clozapine prescription had been delayed. Whilst they did not find that reduction in bed days post clozapine was related to the length of clozapine delay overall, they did show significantly greater reduction in bed days in younger patients and suggested that starting clozapine earlier was likely to have added benefit (161). Finally, Yoshimura looked at outcomes of 90 patients who had commenced clozapine and found that length of clozapine delay predicted outcome, reporting that there appeared to be a critical window for clozapine use of 2.8 years after diagnosis of treatment resistance (146).

Taking a different approach, Thien et al, 2018, have reported on a study in which they have optimized treatment of first episode schizophrenia, with 41 patients, out of a cohort of 544, commencing clozapine early (median delay of 44 weeks) and almost 80% of the group achieving remission. They report a higher remission rate with clozapine compared to that in patients considered eligible for, but not prescribed, clozapine. However, the difference was not statistically significant (162).

To summarise, there is data from observational studies which supports the hypothesis that earlier clozapine is more effective in TRS. However, the numbers in most of the studies have been low. I decided therefore to carry out an observational study using anonymized clinical records from the South London and Maudsley mental health trust, as this would enable me to use a rich source of real-world clinical data, in order to conduct a larger analysis than those which had been published to date.

There were several steps to the project. Firstly, I carried out a search to identify all patients aged between 18 and 65 years who may have commenced clozapine within the trust between 2007 (when the CRIS system became operational) and 2016 (in order to enable 2-year follow up data for all patients). From this list I reviewed patient records and identified which patients had commenced their first trial of clozapine during this time period and remained on clozapine for at least 6 weeks. From this manual search I identified a sample of 661 patients. For this sample I then established accurate start and stop dates, including re- starts if the patient discontinued clozapine, within a 2-year time window, and I recorded whether or not they were taking clozapine at the 2-year end mark. I also reviewed progress notes and correspondence in order to record the date of onset of psychotic symptoms.

For a subset of patients who were taking clozapine at 2 years (425 patients) I reviewed their notes prior to commencing clozapine, and separately at 2 years, in order to determine their level of symptoms, at both time points, using the Clinical Global Impression – Severity (CGI-S) scale. I also collected data for the whole sample on hospital bed days during the two year study period.

I carried out statistical analyses on both the larger sample (661 patients) and the CGI-S subset (425 patients) to look at the effect of illness duration on clozapine outcomes. For the whole sample I used linear regression to look for an effect of duration of illness on hospital bed days. The results showed no evidence of any effect (see supplementary information paper 2 – unpublished data table U1).

I then focussed on the CGI-S subset and carried out ordinal logistic regression to determine if there was an association between duration of illness and two year CGI-S score. The results for this analysis were significant and showed that longer duration of illness was associated with higher (i.e. worse) outcome scores.

I have written up the CGI-S results in a paper which was published in 2022, in the journal ‘Therapeutic Advances in Psychopharmacology’, with the title ‘Duration of prior psychotic illness and clozapine response: a retrospective observational study using electronic health records’. I have also presented the data in a poster presentation at the Schizophrenia International Research Society (SIRS) annual conference in April 2021.

Whilst I was writing the paper I also obtained additional follow up data for psychiatric bed nights for the cohort as due to time having elapsed during completion of the project it was by then possible to obtain psychiatric bed night data extending to 4 years for

each patient. I carried out a further linear regression of duration of illness against psychiatric hospital bed usage, but the results again were not significant (see supplementary information paper 2- table U2).

PUBLISHED VERSION - DURATION OF PRIOR PSYCHOTIC ILLNESS AND CLOZAPINE RESPONSE: A RETROSPECTIVE OBSERVATIONAL STUDY USING ELECTRONIC HEALTH RECORDS

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Ethics statement

Patient consent was not required for the study as it was a retrospective analysis of anonymized patient care records using the Maudsley Clinical Records Interactive Search (CRIS) system. CRIS has been approved by Oxfordshire Research Ethics Committee as an anonymized data resource for secondary analysis (08/H0606/71)

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Abstract

Background

Clozapine is the gold-standard medication for treatment-resistant schizophrenia (TRS) yet its initiation is often delayed.

Aims

To examine whether earlier initiation of clozapine in TRS is associated with lower clinical global impression-severity (CGI-S) scores at 2 years.

Methods

A retrospective cohort study from electronic health records of patients with first adequate trial of clozapine at the South London and Maudsley mental health service between 1st January 2007 and 31st December 2016. Dates of illness onset and clozapine commencement were manually extracted from anonymised case notes. CGI-S scores were rated blind to illness duration. Ordinal logistic regression was used to describe the association between illness duration at baseline and CGI-S outcome score at two years, following adjustment for CGI-S start score and other key covariates.

Results

Among the 401 patients included, there was an association between illness duration and CGI-S outcome score with a 4% increase in the odds of a higher (worse) outcome CGI-S score per year of illness (AOR = 1.04 95% CI 1.01 – 1.06). The association between illness duration and clozapine response was most marked at less than 4 years illness duration. There were too few clozapine initiations within the first two years of illness to draw any conclusions about early clozapine initiation.

Conclusions

Initiation of clozapine within 2-4 years of psychotic illness onset offers the best outcome for TRS, but the advantage, if any, of earlier initiation is unclear from these data.

1. Introduction

Schizophrenia most commonly manifests in late adolescence or early adult life, a time of significant growth in social and role functioning. Severe mental illness occurring in late adolescence and early adulthood can result in significant personal, family and societal burden. Early intervention may improve outcomes, and the ‘critical period hypothesis’ (1) argues that improving long term trajectory depends on the prompt

initiation of effective interventions during a critical window, potentially lasting three to five years after illness onset (2-7). Whilst some people with schizophrenia develop a relatively mild illness and recover most or all of their premorbid functioning, outcomes vary and around 25% of patients are found to be treatment resistant (8-11). Treatment-resistant schizophrenia (TRS) is typically defined as a failure to respond to two antipsychotic trials at an adequate dose for an adequate duration (12). Clozapine has long been the gold standard medication for TRS (13) and its superiority has been confirmed in randomized controlled trials (RCT) and meta-analyses thereof (14, 15) as well as in a number of large pharmacoepidemiological studies (16-21). However, despite the clear rationale for clozapine, its use continues to be delayed, often for decades (10, 22-26). Non evidence based treatments are frequently trialled ahead of clozapine, including prescribing antipsychotic drugs above their licensed limits, and antipsychotic poly-pharmacy (24, 27); both approaches are associated with potential for increased risk of adverse effects and questionable benefit (24, 28).

First episode treatment studies indicate that antipsychotic medication may be more effective when given earlier in the course of illness, with lower doses required for first episode schizophrenia compared with treatment of relapse (29-31). If clozapine were to be used earlier in the course of TRS, it is possible that it will be more effective than if its use is delayed. There is increasing interest in the concept of clozapine delay (time from onset of treatment resistance to treatment with clozapine) (32) and recent observational studies have found a relationship between duration of clozapine delay and outcome (33, 34). However there is evidence that treatment resistance is most often present from illness onset (9) and that a substantial proportion of patients may be treatment resistant on grounds of having persistent psychotic symptoms but fail to

meet the typical TRS threshold due to not being prescribed two antipsychotic medications (10). Also the point at which different patients would meet TRS criteria is likely to vary substantially depending on the duration of each antipsychotic treatment they receive. For these reasons the interval between the onset of psychotic symptoms to introduction of clozapine may be more clinically relevant than the interval between reaching criteria for treatment resistance and clozapine initiation.

The current study examines whether time from onset of psychotic symptoms to commencement of clozapine is associated with degree of response to clozapine. In keeping with the 'critical period hypothesis' we predict that earlier treatment with clozapine will be associated with a greater effect.

2. Material and methods

The study was a retrospective cohort study using data from the South London and Maudsley NHS Foundation Trust case register, which comprises complete anonymized patient electronic records from 1st January 2007 onwards. Data can be accessed by researchers using the Clinical Records Interactive Search (CRIS) system for which methodology has been described elsewhere (35, 36). The Maudsley serves a population of approximately 1.2 million people from the London boroughs of Lambeth, Croydon, Lewisham and Southwark. CRIS has been approved by Oxfordshire Research Ethics Committee as an anonymized data resource for secondary analysis (08/H0606/71).

This study was approved by the NIHR BRIC CRIS oversight committee (application no 1112).

2.1 Sample identification

Searches using a combination of structured data and free text were used in order to identify all patients aged between 18 and 65 years who may have initiated clozapine within the trust. Structured data fields used were the medication table from the patient record, which records drug name, start and stop dates, but is often incomplete, supplemented by information from the trust pharmacy databases which records dates and quantities of clozapine dispensed. In addition a natural language processing application was built using generalized architecture for text engineering (GATE) (for description of methodology see Hayes et al, 2015 (37)) to search free text for instances of clozapine with contextual information indicating actual use of clozapine at that time. Patients were included in the initial sample if the first clozapine instance was recorded between 1st January 2007 and 31st December 2016. They were excluded if their first clozapine instance was under the National Psychosis unit, as this is a specialist tertiary service focussing on treatment refractory or medically complex patients, drawn from a national catchment area, with follow-up typically outside the trust.

Records were manually searched by reading progress notes and correspondence. Clozapine start and stop dates were recorded to identify all patients who had their first adequate trial of clozapine during the defined study period.

A subset of patients had already been included in a previous dataset of first clozapine use (38) and for these patients clozapine start dates were taken from the existing database.

An adequate trial was defined as clozapine treatment duration of least 6 weeks in line with current NICE guidance for prescription of antipsychotic medication (39). To

determine whether this was a first clozapine trial, notes and correspondence were screened for any reference to previous use of clozapine. If clozapine had been prescribed previously the patient was excluded unless it was apparent that the clozapine had been given for less than six weeks. If patients under the care of the Maudsley subsequently commenced clozapine at a non-Maudsley site they were included if they remained under care-coordination by Maudsley clinicians.

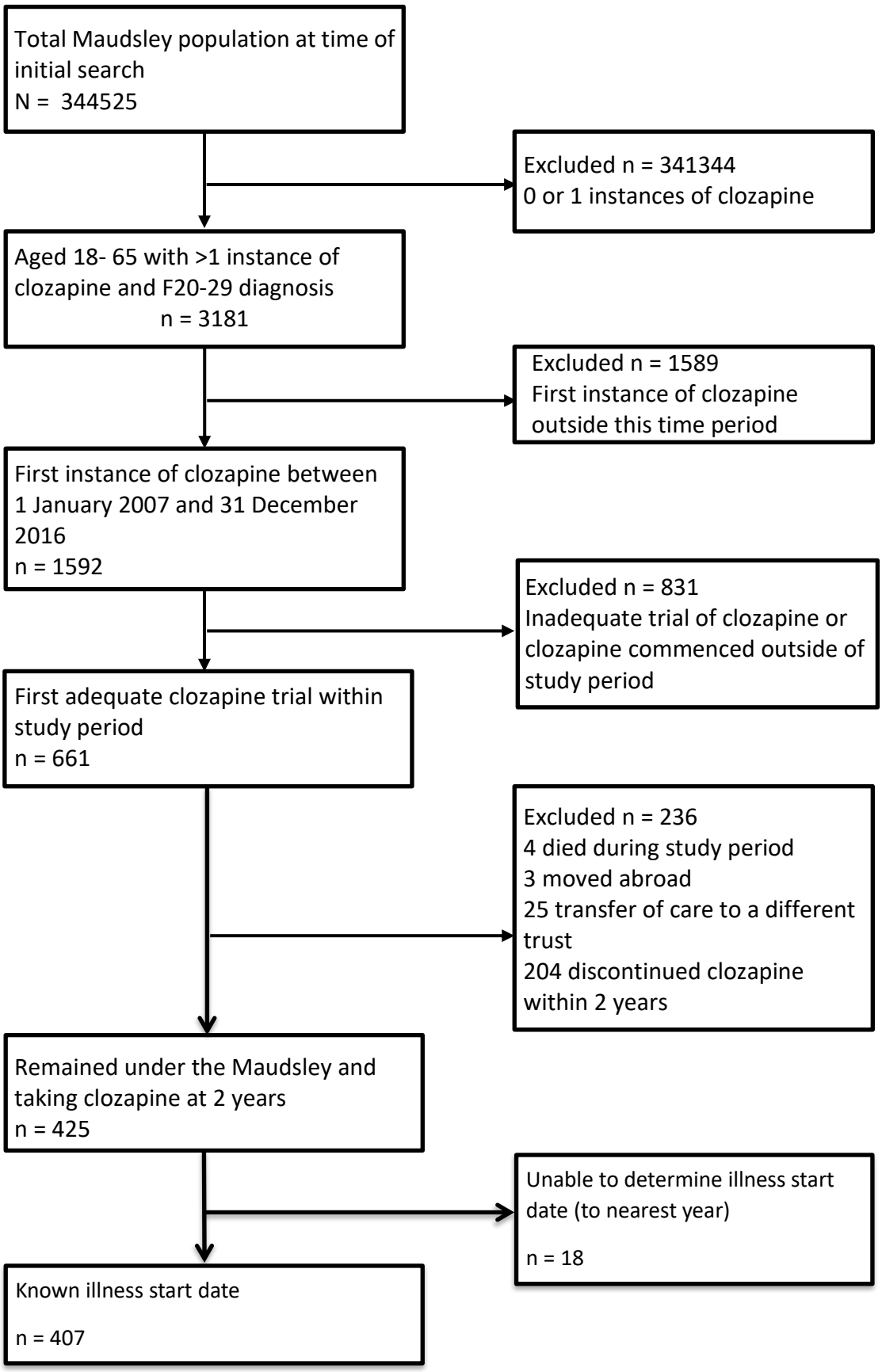
For each patient identified as having their first adequate clozapine trial, progress notes and correspondence were reviewed to ascertain whether they were still under Maudsley services and taking clozapine at 2 years. Patients who had discontinued clozapine, and had not restarted within 2 years, were excluded from the study, as were patients who had moved out of area or had died during this time period.

Date of first onset of psychosis was identified by examination of clinical notes and correspondence. Notes were scrutinised from first contact with SLAM onwards until a record of first date of contact with mental health services for a psychotic episode was found. Dates were recorded to the nearest month. A random number generator was used to assign a month if only the year of onset of psychosis was available.

Patients were excluded if the year of onset of psychosis was not recorded.

A summary of the sample identification process is provided below in Figure 1.

Figure 1. Identification of sample



2.3 Outcome

The outcome variable used in the study was the degree of illness severity at 2 years as measured by the Clinical Global Impression – Severity scale (CGI-S). (40) (for copy of scale see appendix 1 supplementary material). CGI-S is rated from 1 – 7 with lower scores indicating lower levels of symptomatology and a CGI-S score of 1 meaning that no symptoms of illness are present. CGI-S was assessed retrospectively by reviewing patient records. Scores were assessed at both the start and end of the study period, so that CGI-S outcome scores could be adjusted for start scores in the analysis.

Ratings were carried out by an experienced consultant psychiatrist blind to illness duration (RJ). Start and outcome CGI-S scores were rated at different sittings and using separate searches, with records for outcome scores restricted to the time period 6 months pre and 6 months post the two year end point.

2.4 Predictor Variables

The primary predictor variable for the study was the duration of psychotic illness prior to commencement of clozapine. This was obtained by subtracting illness start date from date of first clozapine prescription.

Additional predictor variables included:

(i) age at first presentation with psychosis,

(ii) sex,

(iii) ethnicity (UK census categories collapsed into four groups reflecting demographics of catchment area - white, black Caribbean, black other, mixed / other),

(iv) deprivation score, obtained by linkage of location variable (LSO A11) to Index of Multiple Deprivation 2015 (IMD15) (41) where a higher score indicates a greater level of deprivation,

(v) coded ICD-10 substance misuse diagnosis (F10-F19),

(vi) clozapine start date (by 2.5 year increments) to account for cohort effects during the 10 year inclusion period, and

(vii) number of medical hospital admissions during the follow up period (0,1 or >1) as an indicator of medical co-morbidity.

3. Statistical analysis

Stata version 15 was used for all analyses (42).

Ordinal logistic regression was carried out to test for an association between duration of illness prior to clozapine and CGI-S outcome score. The results were displayed as odds ratios to indicate the ratio of the odds at any cut-off of being in a higher versus lower CGI-S outcome score as the predictor variable changed. Two regression models were conducted, the first using illness duration as a continuous variable (time in years) and the second where duration was presented as a categorical variable (illness duration 0-2 years, 2-4 years, 4-6 years, 6-8 years, 8-10 years, 10-15 years and greater than 15 years). Both models were adjusted for illness severity at baseline (CGI-S baseline scores), age at illness onset, deprivation score, gender, substance disorder, ethnicity, clozapine start date and medical admissions during follow up. We compared the Akaike Information Criterion (AIC) (43) statistic to choose which to use as our primary model.

4. Results

Of the 407 patients included in the study, outcome data were available for 401 patients. The remaining 6 patients did not have sufficient notes available to complete either a pre or post CGI score and were excluded from the analysis.

Baseline characteristics of the sample are shown in Table 1.

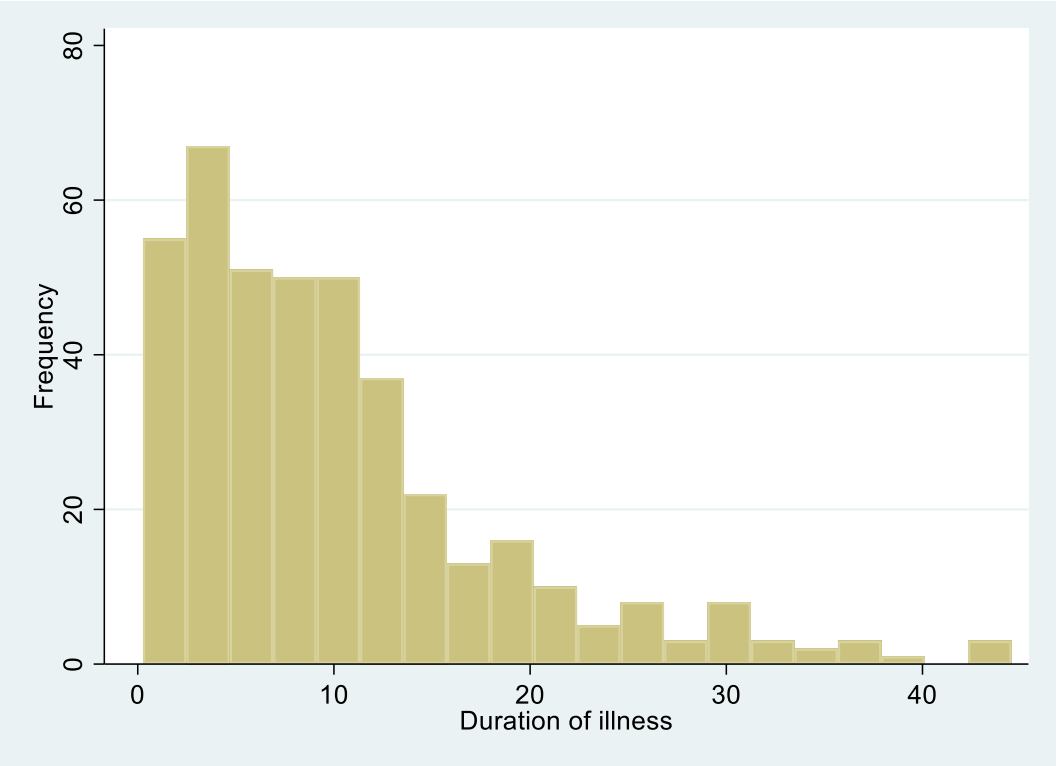
Table 1. Baseline Characteristics of sample (n = 407)

Characteristic	Descriptor	Number (percent)
Sex	Male	282 (69.3)
	Female	125 (30.7)
Ethnicity	White	154 (37.8)
	Black Caribbean	36 (8.9)
	Black other	147 (36.1)
	Mixed/ other	70 (17.2)
ICD substance disorder	Yes	50 (12.3)
	No	357 (87.7)
CGI-S score start	1	0 (0.0)
	2	1 (0.3)
	3	1 (0.3)
	4	39 (9.7)
	5	174 (43.1)
	6	171 (42.3)
	7	18 (4.5)
Number of medical hospital admissions	0	294 (72.2)
	1	72 (17.7)
	>1	41 (10.1)
Time period when clozapine commenced	1 Jan 2007 – 30 June 2009	113 (27.8)
	1 July 2009 – 31 Dec 2011	79 (19.4)
	1 Jan 2012 – 30 June 2014	106 (26.0)
	1 July 2014 – 31 Dec 2016	109 (26.8)
Duration of illness prior to clozapine	0 – 2 years	36 (8.8)
	2 – 4 years	65 (16.0)
	4 – 6 years	50 (12.3)
	6 – 8 years	52 (12.8)
	8 – 10 years	42 (10.3)
	10 – 15 years	78 (19.2)
	15 years +	84 (20.6)
Clozapine use during follow up period	Continued clozapine throughout	372 (91.4)
	Stopped and restarted clozapine	35 (8.60)
Characteristic		**Summary statistics**
Age at illness onset (years)		Median 22.32 IQR (19.08 – 28.41)
Age at clozapine initiation (years)		Median 33.19 IQR (26.08 – 41.78)
Deprivation score		Mean 29.70 SD 10.75

Categorical data has been presented using numbers and percentages (proportions) Normally distributed continuous data has been described using means and standard deviation (SD), whereas non-normal data has been presented as median and interquartile range (IQR)

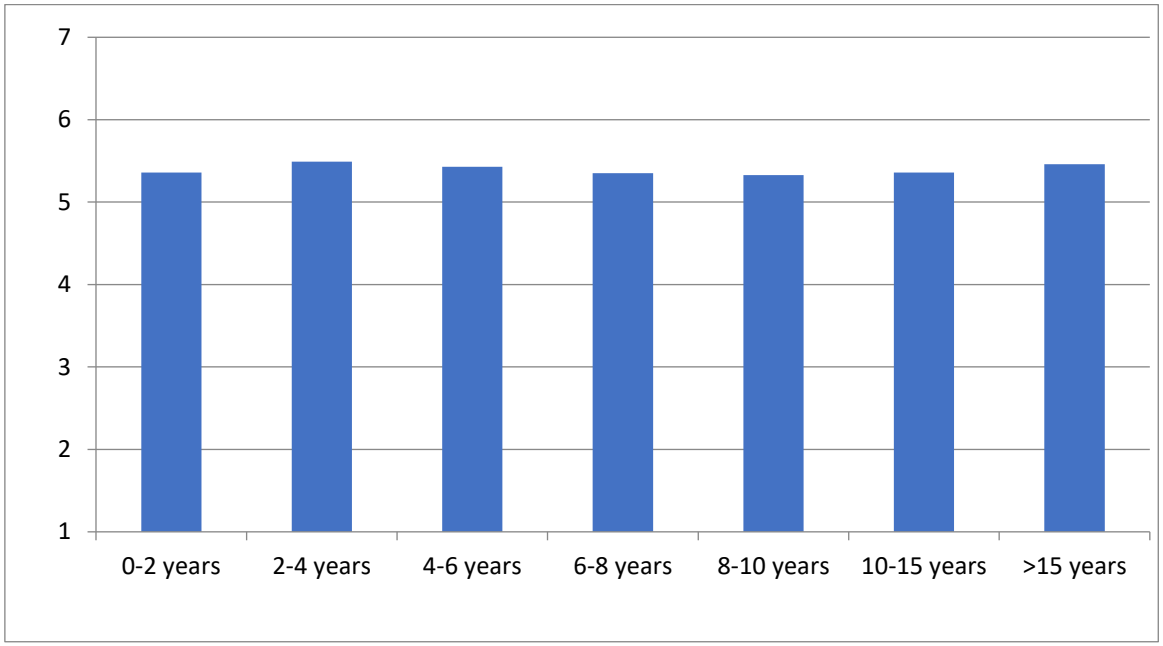
The median duration of illness prior to clozapine was 8 years (range 3 months to 44.5 years). The frequency distribution of duration of illness is shown in Figure 2.

Figure 2. Histogram of duration of illness prior to starting clozapine (years)



Most patients (89.9%) had CGI-S scores of 5 or above (5 = markedly unwell) at the time of clozapine commencement. Starting scores did not vary significantly with duration of illness (Figure 3).

Figure 3. Mean CGI-S starting scores by duration of psychotic illness



CGI-S Clinical Global Impression – severity- score

When treated as a continuous variable CGI-S outcome scores were on average 1.87 points lower than starting scores (paired t-test t = 31.56, df = 400, 95% CI 1.75 – 1.99).

Ordinal logistic regression analysis showed an association between illness duration and CGI-S outcome score. AIC scores were 1140.98 for the model using duration of illness as a continuous variable and 1153.98 for the model using duration of illness as a categorical variable, indicating that the continuous model gave a better fit to the data after accounting for parsimony.

The results for the continuous model are shown in Table 2.

Table 2. Model A. Illness duration as a continuous variable

Ordinal logistic regression of illness duration prior to clozapine and CGI-S outcome scores adjusted for age at illness onset, deprivation score, gender, co-morbid substance disorder, ethnicity, clozapine start date and medical admissions during follow up

Indicator Variables	Categories	Odds of a higher rather than lower CGI-S outcome score		
		Unadjusted OR	OR adjusted for CGI-S start score	Fully adjusted OR
Duration of illness prior to clozapine (years)		1.03 (1.01 – 1.05)	1.03 (1.01 – 1.05)	1.04 (1.01 – 1.06)*
Age at illness onset		0.98 (0.96 – 1.00)		0.99 (0.97 – 1.02)
Deprivation score		0.99 (0.97 – 1.01)		0.99 (0.97 – 1.00)
Male gender		1.51 (1.02 – 2.22) *		1.56 (1.04 – 2.36) *
Substance disorder		2.04 (1.20 – 3.48) *		2.14 (1.23 – 3.71) *
Ethnicity				
	White	Ref		Ref
	Black Caribbean	1.12 (0.58 – 2.14)		1.22 (0.62 – 2.38)
	Black other	1.34 (0.88 – 2.03)		1.67 (1.08 – 2.59)*
	Mixed / other	0.77 (0.46 – 1.29)		0.95 (0.56 – 1.63)
Clozapine start date				
	1 Jan 2007 – 30 June 2009	Ref		Ref
	1 July 2009 – 31 Dec 2011	1.10 (0.65 – 1.88)		0.98 (0.57 – 1.71)
	I Jan 2012 – 30 June 2014	0.92 (0.57 – 1.49)		0.84 (0.51 – 1.39)
	1 July 2014 – 31 Dec 2016	1.02 (0.63 – 1.65)		0.88 (0.54 – 1.45)
Medical admissions				
	0	Ref		Ref
	1	1.17 (0.73 – 1.87)		1.07 (0.66 – 1.73)
	>1	2.65 (1.43 – 4.93) *		2.90 (1.55 – 5.42) *

*significant result

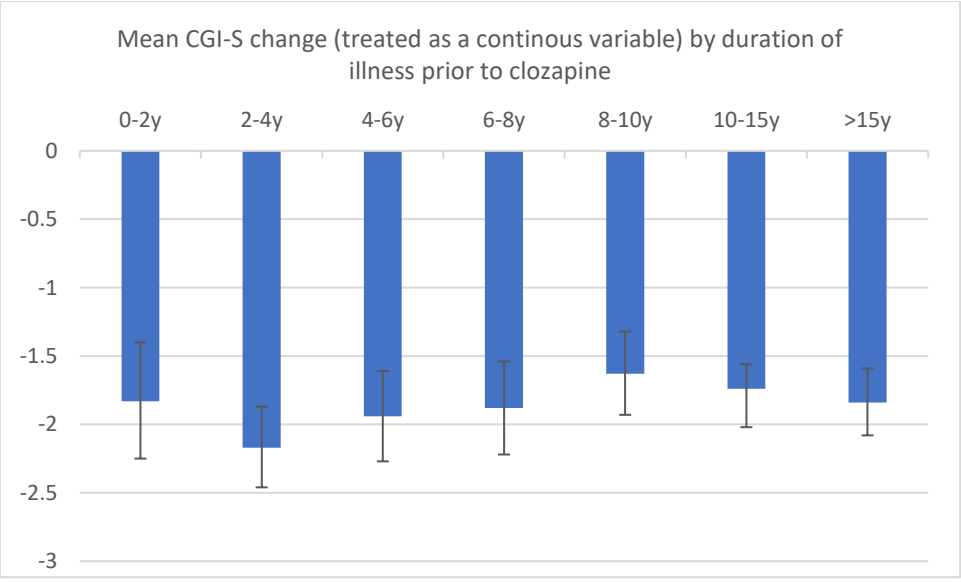
CGI-S score Clinical Global Impression – severity score OR – odds ratio

There was a significant association between duration of illness prior to clozapine and CGI-S outcome score at 2 years (fully adjusted OR 1.04 (1.01 – 1.06) indicating increased odds of a higher (worse) CGI-S outcome score as illness duration increased.

The regression using a categorical variable is included in the supplementary material (Supplementary Table 1).

A plot of change in CGI-S score (treated as a continuous variable (CGI-S start – CGI-S outcome)) against illness duration indicated that the largest change in CGI-S score occurred with an illness duration of 2-4 years with a gradual reduction of effect as illness duration increased further (Fig.4). There appeared to be a reduced effect when clozapine was started earlier than 2 years illness duration, however there were only 36 patients in this category.

Fig 4. Mean CGI-S change by duration of psychotic illness



CGI-S Clinical Global Impression – severity- score
CGI-S is annteger scale hence mean change in score used for illustrative purposes

5. Discussion

The study demonstrated a significant association between duration of psychotic illness prior to clozapine and severity of illness at follow up in patients who remained alive and were still taking clozapine under Maudsley services at 2 years. Overall the analysis showed that the odds of a higher (worse) rather than lower (better) outcome CGI-S score increased by 4% per year of psychosis prior to clozapine. The confidence interval indicated that the likely effect of illness duration on the odds was between 1 and 6% per year.

There was a lack of a clear association between starting clozapine earlier than 2 years and improved outcome. Whilst it is possible that clozapine is less effective when started this early, this finding could be due to the small sample size in this category, or could reflect a degree of confounding by indication, with more seriously unwell patients with limited prospects of recovery more likely to be offered clozapine earlier in the course of their illness.

The results are in keeping with a recent meta-analysis of observational studies which suggested that delaying clozapine may lead to poorer response (44). Studies with comparable methodology include Ucock et al (33) who analysed retrospective case records of 162 patients with TRS and found a significant association between shorter length of clozapine delay and better response and Yoshimura et al (34) who published similar findings for a sample of 90 patients with receiver operating characteristics (ROC) curve analysis indicating that 2.8 years delay from TRS diagnosis provided the best predictive cut off for response.

Key strengths of the current study are its larger size and also its generalisability, being a representative sample from an epidemiological clinical population (36, 45). By using

duration of illness as the predictor variable rather than duration of treatment resistance or clozapine delay, the results may be more easily replicated and more applicable to current service models in which resources are weighted towards the early years of psychosis. Similarly the use of direct rather than indirect clinical information in determining clinical response is a strength, with CGI-S being chosen as a well-established tool for assessing overall illness severity with good face validity. The application of the CGI rating scale by an experienced consultant psychiatrist provided scores with good clinical utility as close as possible to those that would be obtained by seeing the patient in real time.

The study has some clear limitations. The results are applicable only to patients who survived and remained on clozapine for at least a 2 year period and cannot be applied to patients who for whatever reason discontinued clozapine, who are likely to have had a less favourable treatment response. The use of CGI-S scores, applied retrospectively, is also a limitation in terms of the reliance on sufficient data being recorded in case notes to make an accurate assessment. In addition, CGI-S is not a continuous variable and therefore non integer values have little meaning. This is not an issue in the regression analysis but the use of change scores in figure 4 needs to be interpreted with caution.

The wide range in duration of illness prior to clozapine increases the likelihood of survivor biases in older patients. Whilst people who have lived for 10-15 years with schizophrenia might be expected to have less severe illness than those who have died, on the other hand those who have responded well to treatment may have been discharged to their GPs affecting the severity of disease/case-mix in the patients included in this cohort. Although the extent of these biases could not be measured,

CGI-S start scores did not reduce with age in the sample, suggesting that the overall effect of survivor bias was limited Also there was a clear cohort effect which affected data quality, with newer patients having more complete records of their first psychotic episode; for this reason the time period in which clozapine was commenced was controlled for in the analysis. However additional factors were not able to be controlled for, such as duration of psychosis prior to referral to mental health services and presence of negative symptoms. It is plausible that poor prognostic factors, such as prominent negative symptoms, may have led to clozapine being delayed in some patients, as highlighted in a recent systematic review of clozapine delay (32), and may account for the results obtained. Patients who are likely to respond well to antipsychotic treatment in general (ie those with prominent positive symptoms) may achieve a better response if treatment is given early in the course of schizophrenia, and this may also be the case with clozapine (46).

The time period from which clinical records were available for the study (2007 onwards) coincided with the national roll out of early intervention services in the UK, and therefore patients who started clozapine early in the course of their illness were often under the care of the Maudsley early intervention teams. Others were in the forensic system receiving intensive rehabilitation. It is quite possible that psychosocial support aspects of these services contributed to the improved outcomes seen with clozapine in the 2–4-year illness duration category. A longer duration of follow up would be required to see if clinical improvements following clozapine were sustained following transfer to generic services. However, whether or not clozapine is intrinsically more effective when started earlier or whether the therapeutic environment in which it is used

is key, there appears to be clear benefit in starting clozapine before a pattern of severe enduring mental illness is set.

Overall the results support the hypothesis that earlier clozapine initiation may be more effective in improving CGI-S scores, as beyond the first 4 years a clear pattern of diminishing effect over time did emerge, with 2- 4 years appearing to be the optimum period to commence clozapine. The use of clozapine during this time may improve outcomes by enabling patients to engage more in their recovery and rehabilitation, so that they have a better prospect of retaining or regaining a good level of functioning. Clozapine may also be intrinsically more effective if started earlier and there is the possibility, albeit speculative, that it may be disease modifying if it results in a change of trajectory of disease, for example by halting course to a deficit state. In recent years there has been much interest in the role of inflammation in the pathogenesis of schizophrenia and promising trials of anti-inflammatory and immunomodulatory drug treatments (47). Clozapine itself is known to have far reaching immunomodulatory effects (48) which may account for its unique antipsychotic efficacy in treatment resistant schizophrenia.

The low numbers of patients prescribed clozapine within the first 2 years in our sample is in keeping with clozapine prescribing elsewhere in the UK. A recent evaluation of prescribing patterns in the National Eden (National Evaluation of the Development and Impact of Early Intervention services) data looked at rates of treatment resistance and pathways to clozapine prescribing in a first episode psychosis sample of 1027 patients (10). Whilst the rate of treatment resistance over the course of one year follow up was found to be 18.1% there was a much lower rate of clozapine prescribing (2.4%) during the same time period. Likewise the UK National Clinical Audit of Psychosis continues

to show that clozapine is only offered to approximately 50% of patients in early intervention services who meet criteria for clozapine (49), reflecting missed opportunities to establish patients on clozapine and improve their prognosis.

Reasons behind clozapine delay may be multiple but include inadequate knowledge and skills of prescribers (25). Reluctance to prescribe may be well justified when there are legitimate concerns about adverse effects. However other commonly cited reasons not to use clozapine, such as a belief that an individual would be too chaotic to comply with a clozapine regime, may stem from a lack of knowledge of its effectiveness, since adherence commonly improves on clozapine. Another barrier may be tolerance of incomplete response to antipsychotic medication, particularly in patients below threshold for acute admission. Clinicians, particularly in early intervention services, have a responsibility to consider clozapine as soon as it is apparent that a patient is not responding adequately to first line treatments.

6. Conclusion

This study provides further evidence that earlier use of clozapine may be more effective in TRS. Clozapine prescription continues to be delayed across the UK. Reasons for clozapine delay should be explored and addressed to enable patients to benefit more from clozapine.

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CONCLUDING REMARKS

The results of this study showed convincingly that the shorter the duration of illness, the better the response to clozapine. The study was larger than those reported previously in the literature, and the results were clinically significant, with the odds of a good response to clozapine reducing by 4% per year.

The study provides support for the hypothesis that clozapine may be a disease modifying drug. However, there are also other reasons why clozapine may be more effective earlier, for example later clozapine means that people stay ill for longer and may develop other poor prognostic features, e.g. drug and alcohol abuse. Similarly, patients with inherent negative symptoms may have clozapine treatment delayed, particularly if they do not repeatedly get admitted to hospital with more florid positive symptoms.

In the absence of clinical biomarkers of TRS, at least outside of experimental paradigms, it is difficult to answer the question of whether drugs such as clozapine are having a disease modifying effect on the condition. Whilst neuroimaging, genetic and cytokine studies are progressing rapidly in the research field, they are not currently in general usage clinically. However, a relatively untapped source of information is the peripheral blood count which for clozapine is performed regularly as part of the mandated conditions of prescription. In view of recent advances in understanding of how the innate immune system may be involved in schizophrenia, I decided for my third project to examine how neutrophil counts vary with clozapine prescription and whether they can be used to predict clinical response.

CHAPTER 5. PUBLICATION. EARLY NEUTROPHIL TRAJECTORY FOLLOWING CLOZAPINE MAY PREDICT CLOZAPINE RESPONSE – RESULTS FROM AN OBSERVATIONAL STUDY USING ELECTRONIC HEALTH RECORDS

Introduction

In my first two papers I have been able to show evidence that supports my hypothesis that earlier clozapine use may be associated with greater response. In this project my aim was to investigate why this may be the case, and to return to the premise that clozapine may be acting by immunological mechanisms. As an introduction to this I will describe the main components of the immune response to then consider 1) how the innate immune response is thought to be affected in schizophrenia and 2) possible effects of clozapine on the immune system and potential targets for measuring against response.

Review of normal immunity

Immunity is typically described as two separate processes, namely an innate response and an adaptive response; however, in reality the immune response is a highly complex set of interacting cellular and humoral mechanisms with multiple feedback loops between innate and adaptive components.

The immune system needs to fulfil several key tasks; firstly, maintaining homeostasis and providing surveillance, secondly, generating an immediate pro-inflammatory response in the event of an invasion or injury, thirdly, triggering a specific targeted

response with long-lasting memory, and finally, recovery with restoration of the status quo (163).

Coordination of the immune response is achieved by signalling proteins called cytokines which are released by immune cells. They comprise five main groups. Chemokines direct cells to where they need to go. Interferons signal to cells to defend themselves against attack by viruses. Interleukins relay messages between cells (originally just thought to be between white cells, as per their name, but they are now known to communicate between a range of cells). Tumour necrosis factors (TNFs) regulate inflammation, and colony stimulating factors (CSFs) signal to haematopoietic stem cells to develop into particular types of blood cell.

The major components of the innate and adaptive immune responses are described in simplified terms below.

Innate immune response

The first line of defence, to infection or other bodily insult, consists of cells called monocytes, referred to as the fire fighters of the immune system. When they encounter pathogens, or damaged cells, they react by differentiating into dendritic cells and macrophages which can detect pathogen associated molecular patterns (PAMPS) or damage associated molecular patterns (DAMPS) on cell membranes via pattern recognition receptors (PRRs). Activated macrophages and dendritic cells release pro-inflammatory cytokines which trigger the release of acute phase proteins, including C-reactive protein (CRP) and fibrinogen, and also increase production of collagen and platelets. Macrophages also release chemokines which signal to neutrophils to migrate

to the site by chemotaxis (164,165). In uncomplicated inflammation macrophages respond to cues to switch to an anti-inflammatory phenotype, responsible for clearing away debris and restoring normality (164).

CRP binds to neutrophils and monocytes, triggering them to phagocytose pathogens and to release pro-inflammatory cytokines, in particular IL6 (166). Besides engulfing pathogens, neutrophils also release reactive oxygen species (ROS) and proteases, and produce neutrophil extra-cellular traps (NETs) which trap pathogens within their structures (167).

In addition, CRP activates the complement cascade via the classical pathway. The cascade can also be triggered by the alternative pathway (by direct contact with endotoxins from pathogens) and the lectin pathway (by recognition of carbohydrates on pathogens). Activation of the complement cascade leads to the production of a membrane attack complex. Activated neutrophils also release complement factors and enhance the cascade (168).

The complement system has three main functions in the immune system, opsonisation (tagging pathogens to mark them for destruction), chemotaxis and cell lysis. However, complement factors also play other roles including clearing immune complexes and apoptotic cells and increasing blood brain barrier permeability (168).

There are other immune cells involved in the innate response besides phagocytes. These include natural killer (NK) cells, which are large granular lymphocytes with natural ability to kill tumour cells without previous activation (169). They are activated by cytokines or by target cells that express ligands for NK cell receptors (170) and they produce a variety of cytokines and chemokines which destroy infected and

diseased cells. Also mast cells, traditionally known for their role in allergic responses, are now thought to act as sentinels in the innate immune response; they are activated by multiple mechanisms including PRRs and the complement cascade and are resident in skin, always and intestine so are well positioned as early defenders. They enhance recruitment of neutrophils by producing cytokines, in particular TNF, and release histamine and chemokines which increase vascular permeability thus enhancing availability of complement and phagocytes (171). Finally, B1 lymphocytes, which are part of the innate rather than adaptive immune system as they hold no memory, release natural antibodies, which are increasingly recognised to play a major part in fighting infections (172).

Adaptive immune response

Adaptive immunity is the hall mark of the immune response of higher animals, with precise and long-lasting antigen specific reactions which take days or weeks to develop (173). Once an adaptive response has been triggered, the immune system is primed to respond rapidly and effectively, should the same pathogen be encountered in the future. The main cells of adaptive immunity are T and B lymphocytes which carry antigen specific receptors. They encounter antigens either directly in the blood stream or they have antigens presented to them by antigen presenting cells (APCs) including dendritic cells, which present antigens to T lymphocytes by carrying them on surface molecules called the major histocompatibility complex (MHC).

There are three main types of T cells, T helper (Th) cells (carry CD4), T cytotoxic cells (carry CD8) and T regulatory cells. Th cells form two main types, Th1 cells recognise

antigens and release cytokines (IL2 and interferon gamma) which activate T cytotoxic cells and also macrophages and natural killer cells, and Th2 cells which release cytokines (IL4,5,6,10) which stimulate B cells to produce antibodies. T cytotoxic cells attack cells carrying their specific antigen, by inserting perforins which result in cell lysis. The third type of T cell, T regulatory cells, maintain homeostasis by regulating the activity of T helper and T cytotoxic cells though cytokine release.

B cells are divided into plasma cells and memory cells. Plasma cells produce antigen specific antibodies which neutralise pathogens by opsonisation, whereby the antibody binds to the antigen, prevents the affected cell from binding to its target, and sensitizes it to attack from T cytotoxic cells. B memory cells rapidly re-activate if the body re-encounters the same pathogen in order to mount a targeted immune response. It is also increasingly recognised that there are additional B cells with regulatory activity akin to T regulatory cells (174).

Innate immune system abnormalities and schizophrenia

Research into the immunology of schizophrenia suggests that there is overactivation of the innate immune response. The main strands of evidence for this are summarised below.

Pro-inflammatory cytokines

Pro-inflammatory cytokines are key in the amplification of the innate immune response. Cytokines have been extensively studied in schizophrenia, particularly IL6. A recent meta-analysis of 14 studies reported that IL6 levels were raised in schizophrenia and decreased after treatment (175) There is also convincing evidence from meta-

analyses of increased levels of other pro-inflammatory cytokines (23,25,176–180), along with evidence for the chemokine IL8 (178,181).

CRP

CRP binds to phagocytes and activates complement. Meta-analyses have shown that CRP levels are moderately raised in acute schizophrenia (182–184). A prevalence rate of 28% for an elevated CRP in schizophrenia has been reported (182). Meta-regressions of cross-sectional studies have shown a positive relationship between positive symptoms, but not negative symptoms, and CRP (183). A negative association has also been shown between CRP and age, suggesting that the early stages of schizophrenia may represent a particularly prominent inflammatory process (183,184).

Complement cascade

The complement system is the main orchestrator of the innate immune cellular response. There is now considerable evidence that the complement system is involved in the pathogenesis of schizophrenia.

A substantial proportion of the association between schizophrenia and the MHC shown in GWAS studies has been found to be due to variation in C4 genes (185). Longitudinal studies from childhood or of first episode psychosis cohorts show altered levels of many complement and coagulation factors years before psychosis develops (26). Whilst a meta-analysis of studies looking at serum complement factors in schizophrenia yielded mixed results (186), studies specifically of first episode psychosis have shown evidence of complement activation, and also a tentative association between complement levels and treatment response (187). Complement

levels have also been shown to be increased in CSF in schizophrenia (188). It has been theorised that dysregulation of the complement pathway by continuous activation either due to unresolved infection, or by inadequate regulation due to immune deficiency, may lead to psychosis (26).

Innate immune cells

Monocytes and macrophages

Monocytes have been shown by meta-analysis to be increased in schizophrenia compared to controls (27,28,189). In an older study macrophages have been shown to be increased in the CSF in acute schizophrenia (190). More recently evidence of increased macrophage markers have been found in the midbrain and frontal cortex post mortem (191,192).

Neutrophils

Abnormalities in white cell count in schizophrenia were first reported in 1930 (193) and a recent meta-analysis has confirmed that neutrophil counts are elevated in schizophrenia, including in first episode studies (27). Changes in neutrophil histology and function in schizophrenia have also been reported (194,195), as has increase in neutrophil ROS release (196), the latter showing correlation with negative symptoms of the disease. A recent study has also found that neutrophil count was associated with total PANSS score and reduced grey matter volume in patients with first episode psychosis (197)

Neutrophil Lymphocyte Ratio (NLR) and Monocyte Lymphocyte ratio (MLR)

These ratios, as a proxy of systemic inflammation, have been studied in a variety of illnesses, the theory being that, as each ratio represents two immune pathways, it is less likely to be affected by confounding conditions. A recent meta-analysis of NLR and MLR in schizophrenia has shown that both are increased in schizophrenia compared to controls (189).

Other innate immune cells

Although these have not been studied to the same extent, there are some findings of interest. Natural killer cells have been shown to be activated in patients with first episode psychosis compared to controls ((198) and mast cell activation has also been implicated in the causation of neuropsychiatric symptoms (199).

Clozapine and TRS

It is clear from the above that neutrophils play a central role in the immune response and that elevated neutrophil counts are present in schizophrenia. Clozapine, as the treatment of choice for TRS, has both unique efficacy and a unique side effect profile, which is largely attributable to its immune effects. Clozapine is known to affect neutrophil counts, both in terms of causing neutrophilia and neutropaenia to the extent of agranulocytosis, and it has previously been suggested that a neutrophil spike seen in the first weeks of clozapine treatment for TRS may be a predictor of a positive response (200), though this was not borne out in a more recent study (201). Using the database of clozapine patients from my previous study, which provided a larger sample than either of the previous studies, I decided to use latent class growth analysis (LCGA)

to investigate whether neutrophil trajectories with clozapine varied between different patients and if so whether this variation had an association with clozapine response.

The study has been published in 2023 in the journal ‘Brain, Behaviour and Immunity’ with the title ‘Early neutrophil trajectory following clozapine may predict clozapine response – results from an observational study using electronic health records’.

PUBLISHED VERSION – EARLY NEUTROPHIL TRAJECTORY MAY PREDICT CLOZAPINE RESPONSE - RESULTS FROM AN OBSERVATIONAL STUDY USING ELECTRONIC HEALTH RECORDS

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Abstract

Background

Clozapine has unique effectiveness in treatment-resistant schizophrenia and is known to cause immunological side-effects. A transient spike in neutrophils commonly occurs in the first weeks of clozapine therapy. There is contradictory evidence in the literature as to whether neutrophil changes with clozapine are linked to treatment response.

Aims

The current study aims to further examine the neutrophil changes in response to clozapine and explore any association between neutrophil trajectory and treatment response.

Methods

A retrospective cohort study of patients undergoing their first treatment with clozapine and continuing for at least 2 years identified 425 patients (69% male / 31% female). Neutrophil counts at baseline, 3 weeks and 1 month were obtained predominantly by linkage with data from the clozapine monitoring service. Clinical Global Impression-Severity (CGI-S) was rated from case notes at the time of clozapine initiation and at 2 years. Latent class growth analysis (LCGA) was performed to define distinct trajectories of neutrophil changes during the first month of treatment. Logistic regression was then conducted to investigate for association between the trajectory of neutrophil count changes in month 1 and clinical response at 2 years as well as between baseline neutrophil count and response.

Results

Of the original cohort, 397 (93%) patients had useable neutrophil data during the first 6 weeks of clozapine treatment. LCGA revealed significant differences in neutrophil trajectories with a three-class model being the most parsimonious. The classes had similar trajectory profiles but differed primarily on overall neutrophil count: with low, high-normal and high neutrophil classes, comprising 52%, 40% and 8% of the sample respectively. Membership of the high-normal group was associated with significantly increased odds of a positive response to clozapine, as compared to the low neutrophil group [Odds ratio (OR)= 2.10, p-value=0.002; 95% confidence interval (95% CI) = 1.31 – 3.36]. Baseline neutrophil count was a predictor of response to clozapine at 2 years, with counts of ≥ 5 x 10^9/l significantly associated with positive response (OR= 1.60, p-value=0.03; 95% CI = 1.03 – 2.49).

Conclusions

Our data are consistent with the hypothesis that patients with low-level inflammation, reflected in a high-normal neutrophil count, are more likely to respond to clozapine, raising the possibility that clozapine exerts its superior efficacy via immune mechanisms.

Introduction

The pathogenesis of schizophrenia is far from fully understood, but it has become clearer in recent years that inflammation may play a significant role (1). Until recently neutrophils have been considered primarily to be short-lived, non-specific cells which contribute to the innate immune response. However, interest in the role of neutrophils

has intensified and they are now understood to carry out a wide range of functions in the immune system (2). A recent meta-analysis has shown that neutrophil counts are elevated in schizophrenia compared to controls (3). Higher total white cell counts (of which neutrophils predominate) have been found to be associated with higher symptom levels in schizophrenia (4) and raised neutrophil counts have also been seen in first episode psychosis with an improvement of positive symptoms correlating with declining neutrophil scores (5). Patients with persistent positive symptoms may show a more pronounced inflammatory process (6). Treatment resistant schizophrenia (TRS) is defined as a failure to respond adequately to two adequate trials of antipsychotic medication (7). TRS may be categorically distinct from treatment responsive schizophrenia with abnormalities primarily in glutamate rather than in dopamine transmission (8), akin to more well-characterised neuro-immune disorders (9–11). Clozapine has superior efficacy to conventional antipsychotics in the management of positive symptoms in TRS (12) and on initiation can result in a wide range of immunologically mediated effects (13–15). Recent studies have shown that clozapine is associated with acquired immunoglobulin deficiency (16,17) which may explain why patients established on clozapine have higher rates of infections, particularly pneumonia (18–20), although pneumonia may also be caused by other adverse effects such as sedation and sialorrhea (21). Studies have consistently shown increases in pro-inflammatory cytokines, most notably interleukin (IL)-6, in patients prescribed clozapine (22–25), with limited longitudinal data indicating changes in cytokine levels related to clozapine response (26).

Clozapine is known to cause a range of blood dyscrasias, most notably agranulocytosis (27). However, a transient increase in neutrophils is more common

than a decrease (28–33), albeit with reported rates of leucocytosis varying widely. This variation appears to be mainly due to differences in classification of leucocytosis. The largest study to date included 2,404 patients (30) and reported that 7.7% of patients had a total white cell count greater than 15 x 10^9/l. Other studies report rates closer to 20%, using a lower threshold of neutrophils greater than 7 x 10^9/l (31,33). In most studies, a spike in neutrophils occurs early in the course of treatment, typically after two to three weeks, however other evidence suggests this may occur over six weeks (30–32,34). A systematic review of neutrophilia with clozapine therapy (35) concluded that the finding was likely an epiphonema and potentially related to smoking. However, it is also possible that an elevation in neutrophils may be directly related to treatment response. In a retrospective study Fabrazzo et al, 2017, reviewed the weekly blood counts of a sample of 135 patients who had commenced clozapine and found that the development of neutrophilia greater than 7 x 10^9/l was significantly associated with response to clozapine after 18 weeks of treatment (33). Another retrospective study by Blackman et al, 2021 found no association between peak neutrophil count and treatment response at 12 weeks in a sample of 188 patients (34). These conflicting findings may be explained by different outcome measures and response rates in the two studies as well as by the relatively small sample sizes and differences in patient demographics. Given the clear evidence in support of clozapine causing an increase in neutrophil count in the early phase of treatment, which coincides with its clinical efficacy, further study of the potential role of neutrophils in clozapine response is warranted. Early neutrophil count would be a particularly useful biomarker for predicting response to clozapine, as it is already routinely monitored in clinical practice.

In summary, there is evidence that immune dysfunction may be relevant in some patients with schizophrenia, including involvement of innate actors such as neutrophils. The immunomodulatory effects of clozapine alongside its superior efficacy in TRS may indicate the presence of a subgroup of patients with immune dysregulation with reduced responsiveness to conventional antipsychotics. Neutrophil changes have been shown to occur in the first weeks of clozapine treatment and there is tentative evidence to suggest an association between its immunomodulatory effects and its clinical efficacy. As such, early neutrophil counts may be an accessible potential marker of clozapine response.

Using clinically representative data, we aimed to explore:

1) the early longitudinal neutrophil response to clozapine exposure.

2) the association between neutrophil trajectory and clinical outcome.

We hypothesised that early elevated neutrophil response would be associated with greater clinical improvement.

Methods

Participants

This study is based on a retrospective cohort study using data from the South London and Maudsley (subsequently referred to as 'The Maudsley') NHS Foundation Trust case register, which comprises complete anonymized patient electronic records from 1st January 2007 onwards. Data was accessed using the Clinical Records Interactive Search (CRIS) system, for which methodology has been published previously (36,37).

The Maudsley is the largest mental health trust in the UK, serving a predominantly inner-city population of approximately 1.3 million people. Use of CRIS as an anonymised resource for secondary analysis has been approved by the Oxfordshire Research Ethics Committee (08/H0606/71). The current study was approved by the NIHR BRC CRIS oversight committee (application number 21-073).

Participants in this study came from a previously identified retrospective cohort of n= 661 patients who commenced clozapine over a 10-year period between 1st January 2007 and 31st December 2016. A full description of how the cohort was identified can be found elsewhere (38). From the original cohort a sub-sample of patients were identified (n = 425) who were still taking clozapine 2 years later. Clinical Global Impression – severity (CGI-S) scores (39) were recorded at baseline and at 2 year follow up for this group. CGI-S scores were rated retrospectively by reviewing patient records. Ratings were carried out by an experienced consultant psychiatrist (RJ). Baseline and 2-year scores were rated separately, with records for outcome scores restricted to the period 6 months pre and 6 months post the 2-year end point.

The final cohort consisted of all patients who were still taking clozapine at 2 years, had sufficient clinical data available to reliably complete CGI scores pre clozapine and at two years, and who had useable neutrophil data available during the first 6 weeks of clozapine treatment (see flowchart figure 1).

Measures

Neutrophil counts for the cohort were primarily extracted using linkage with Zaponex Treatment Access System (ZTAS) data, ZTAS being the clozapine monitoring service used by the Maudsley throughout the time period of the cohort (ZAPONEX; Teva UK, Harlow, United Kingdom). For patients for whom ZTAS data was not available,

neutrophil counts were extracted from CRIS data using structured lab results data. As this was only available for patients from 2013 onwards, a manual review of free text data was conducted to extract neutrophil counts if data from ZTAS and structured lab results were unavailable.

Neutrophil counts were collected at three time points: at baseline (before clozapine was commenced), 3 weeks, and at 1 month post clozapine initiation, with tolerance of +/- 1 week. These time points were chosen in accordance with the literature available on the timing of the peak neutrophil count following clozapine (34).

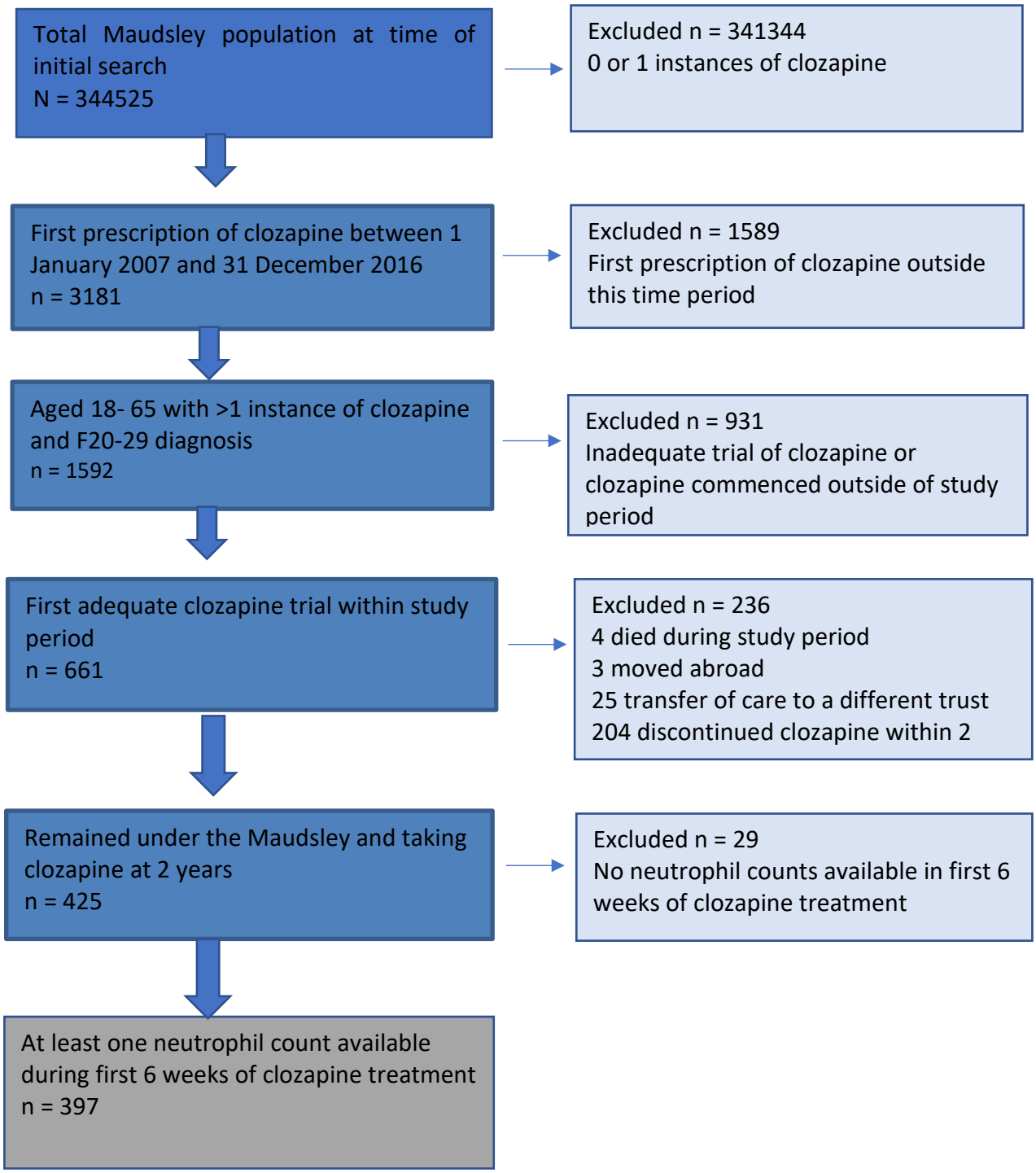
The main outcome measure used for the study was response to clozapine at 2 years, defined as a reduction in CGI-S score by at least 2 points from the start of the study period. CGI-S uses a Likert scale from 1 to 7 to assess overall illness severity, with lower scores indicating lower levels of symptoms.

Outcome data was collected as part of the earlier study, which took place prior to the linkage with ZTAS, therefore CGI-S ratings were conducted blind to neutrophil scores.

Covariates

Data for significant covariates, which could affect both neutrophil count and outcome scores, were also collected. Covariates included 1) age, 2) sex, 3) ethnicity (UK census categories collapsed into four groups - white, black Caribbean, black other, mixed / other – to reflect the demographics of the study catchment area) and 4) medical illness (number of medical admissions during the two-year study period; 0, 1 or more than 1).

Fig. 1. Cohort identification



Statistical Analysis

First, Latent Class Growth Analyses (LCGA) (40) was conducted using M plus v8 (41), to detect trajectories of neutrophil response to clozapine across the time points (baseline, 3 weeks post clozapine and 1 month post clozapine). Five models were fitted, testing performance of two to six classes. The best fitting classification model was chosen according to fit indices (i.e., Bayesian Information Criteria [BIC] (42) and Vuong-Lo-Mendell-Rubin [VLMR] test) (43). Lower BIC values suggest a better model fit. A significant VLMR value ($p < 0.05$) suggests that a K-class model fits the data better than a (K-1) class model. Entropy, a measure of the degree of separation between classes (44), was also used to select the best model fit; entropy with values approaching 1 indicates clear delineation of classes. Finally, to decide the optimal class solution, an emphasis was placed on large enough group sizes (i.e., >2% of the sample) and clinically relevant and informative interpretation. Missing values due to attrition were handled by the Full Information Maximum Likelihood estimation method (45).

Secondly, logistic regression was performed using STATA version 15 (46), to investigate the associations between neutrophil classes (obtained with LCGA-as the exposure) and treatment response (as the outcome), controlling for age, sex, ethnicity and medical comorbidity. Class 1 was used as the reference group to which the other two classes were compared.

Finally, logistic regression was performed to investigate for association between baseline neutrophil count (prior to clozapine initiation) and treatment response, adjusted for age, sex, ethnicity and medical comorbidity. Baseline neutrophil count was

recorded as a dichotomous variable with higher or lower neutrophil groups, using a neutrophil count ≥5 x10^9/l as the cut-off, to include patients with high - normal counts in the higher group.

Results

Summary statistics for the sample are shown in table 1.

Table 1. Characteristics of the sample (n = 397)

Characteristic	Descriptor	Number (percent)
Sex	Male Female	273 (68.8) 124 (31.2)
Ethnicity	White Black Caribbean Black other Mixed/ other	153 (38.5) 34 (8.6) 144 (36.3) 66 (16.6)
CGI-S score start	1 - 4 5 6 7	42 (10.6) 170 (42.8) 168 (42.3) 17 (4.3)
CGI-S score end	1 2 3 4 5 6 - 7	12 (3.0) 40 (10.1) 141 (35.5) 141 (35.5) 55 (13.9) 8 (2.0)
Medical admissions during study	0 1 >1	278 (70.0) 75 (18.9) 44 (11.1)
Baseline neutrophil count (x10^9/l)	≥5 <5	139 (35.0) 258 (65.0)
Characteristic	Descriptor	Mean (standard deviation / range)
Age	Years	35.3 (10.8)
Neutrophil count	Pre-clozapine 3 weeks 1 month	4.6 (2.0 / 1.4 – 14.9) 5.1 (2.3 / 1.1 – 14.4)) 4.9 (2.2 / 1.5 – 12.2)

CGI – S. Clinical Global Impression – Severity scale. 1=normal, not at all ill; 2=borderline mentally ill; 3=mildly ill; 4=moderately ill; 5=markedly ill; 6=severely ill; 7=among the most extremely ill patients.

Note Some cells have been collapsed due to CRIS rules which state that no cells <5 can be reported without special permission to avoid identification.

Latent classes of neutrophils across time points

There was minimal missing data in the study (0.4%).

Table 2 shows VLMR, BIC, and entropy for all models assessed (2 to 6 classes), tested for neutrophil counts at baseline, 3 weeks and 1 month. The 3-class model was selected based on goodness of fit indices. The 2-class and 3-class models were the only models reporting significant VLMR p-values, which is one of the requirements for the selection of the model. In this case, a significant VLMR p-value for 3-class model indicated that the three-class model gives significant improvement in model fit over the 2-class model. Furthermore, the BIC value for the 3-class model was lower than the 2-class. Finally, all the classes from the 3-class model included a sample size greater than 2%. The selection of the 3-class model was reviewed by a clinical immunologist (AS), who corroborated its clinical validity.

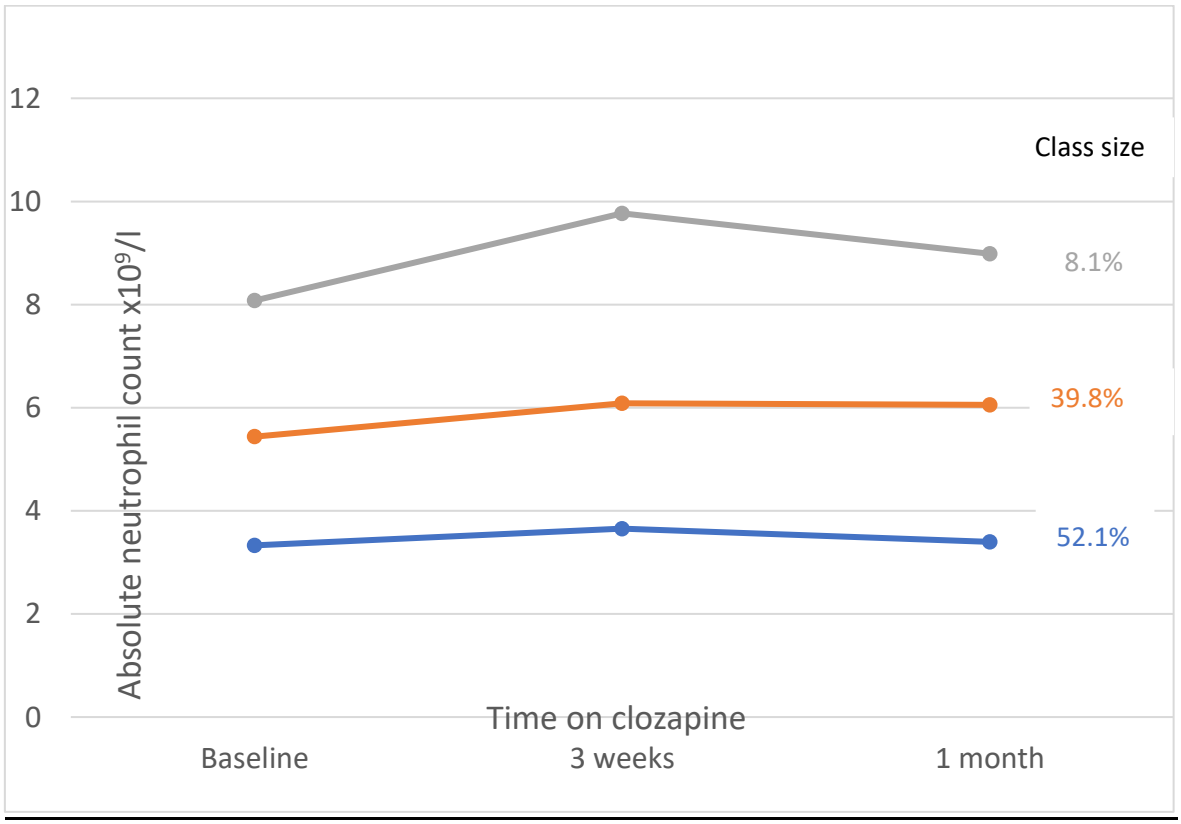
The 3 derived classes of neutrophil counts from the 3-class model, and the sample size for each class are shown in Figure 2. Briefly, Class 1 (blue) represented low to normal neutrophil counts and included 207 patients (52.1%), Class 2 (green) represented high-normal neutrophil counts of around $6x10^{-9}$/L and included 158 patients (39.8%) and Class 3 (red) represented high neutrophil counts and included 32 patients (8.1%).

Table 2. Model selection

Model	BIC	VLMR-P	Entropy
2 classes	4899.712	<0.001	0.810
3 classes	**4804.528**	**0.005**	**0.793**
4 classes	4766.063	0.264	0.846
5 classes	4751.748	0.731	0.861
6 classes	4732.532	0.064	0.838

BIC – Bayesian Information Criteria. VLMR-P - Vuong-Lo-Mendell-Rubin test

Fig. 2. Class sizes and mean neutrophil counts for each class for the three-class model, across time points



The demographics of the three classes are shown in table 3. There were significant differences between the three classes in relation to age, ethnicity, medical co-morbidity and baseline CGI-S scores.

Table 3. Characteristics of the three classes derived by LCGA (Latent Class Growth Analysis) Class 1 = low neutrophils. Class 2 = high-normal neutrophils Class 3 = high neutrophils

	Class 1 n = 207	Class 2 n = 158	Class 3 n = 32	F	p
Mean age	33.3	36.8	40.5	9.10	0.00
Male Sex	71%	66%	69%	0.56	0.57
White ethnicity	27.5%	51.3%	46.9%	11.74	0.00
1 or more medical admissions	25.1%	33.5%	43.7%	3.11	0.05
Baseline CGI-S score	5.30 (5.20 – 5.41)	5.51 (5.40 – 5.62)	5.4 (5.22 – 5.65)	3.28	0.04

Regression Model of neutrophil classes and clozapine response

Logistic regression found that the odds of a positive response to clozapine was significantly increased in patients in class 2 (medium neutrophil group) compared to class 1 (low neutrophil group) [Odds ratio (OR) = 2.10, p-value = 0.002; 95% confidence interval (95% CI) = 1.31 – 3.36]. The odds of a positive response also increased for class 3 (high neutrophil group) compared to class 1, but the results were not significant (Table 4).

Table 4. Logistic regression of neutrophil class on clozapine response (n = 397). Adjusted values are adjusted for age, sex, ethnicity and medical admissions

		Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Class	1				
	2 versus 1	**2.04 (1.32 – 3.16)**	**0.00**	**2.10 (1.31 – 3.36)**	**0.00**
	3 versus 1	1.11 (0.53 – 2.35)	0.78	1.42 (0.63 – 3.18)	0.40
Age		0.98 (0.96 – 1.00)	0.05	0.97 (0.95 – 0.99)	0.01
Sex		0.69 (0.43 – 1.04)	0.08	0.57 (0.35 – 0.91)	0.02
Ethnicity	White				
	Black Caribbean	1 (0.46 – 2.17)	1	1.02 (0.45 – 2.30)	0.97
	Black other	0.66 (0.42 – 1.06)	0.08	0.67 (0.40 – 1.11)	0.12
	Mixed other	0.46 (0.46 – 1.52)	0.56	0.88 (0.47 – 1.63)	0.68
Medical admissions	0				
	1	1.03 (0.61 – 1.75)	0.90	0.95 (0.55 – 1.64)	0.86
	>1	0.56 (0.30 – 1.07)	0.08	0.60 (0.30 – 1.19)	0.14

OR – odds ratio

Regression Model of baseline neutrophil count and clozapine response

Using baseline neutrophil count as a dichotomous variable (higher versus lower neutrophil count) with a cut off value of ≥5 x$10^{9/l}$, logistic regression analysis showed that a higher neutrophil count prior to clozapine initiation was associated with greater clozapine response, adjusted for age, sex, ethnicity and medical admissions [Odds ratio (OR) = 1.68, p-value =0.03; 95% confidence interval (95% CI) = 1.06 – 2.06]. Overall, 68% of the higher neutrophil group responded to clozapine compared to 56% in the lower neutrophil group.

Discussion

The study examined neutrophil trajectories with clozapine exposure across the first month of treatment and their association with outcome. Our hypothesis was that neutrophil changes in response to clozapine may be driving clinical outcome, however this was not upheld. Rather, using latent class growth analysis, we identified three stable classes of neutrophil counts detected across the time points. Neutrophil trajectory was divided into low, medium or high values, with differences present from baseline. Using class 1 (low neutrophils) as the reference group, the likelihood of a positive long-term response to clozapine, as measured by CGI-S score at 2 years, was significantly increased by membership of class 2 (high-normal neutrophils). A smaller effect was seen with class 3 (high neutrophils), and this effect was not significant. The effect size for class 2 was clinically significant with the odds of a positive response to clozapine more than doubled by membership of the high-normal group. Furthermore, baseline neutrophil counts alone were significantly associated with response to clozapine, with higher counts more likely to be associated with a positive response. However, response to clozapine was not limited to patients with higher baseline neutrophil counts, and therefore neutrophil count alone would not be sufficient to usefully predict outcome.

The improved response to clozapine in the high-normal compared to the low neutrophil group did not appear to be explained by a specific early spike in neutrophil count, as the rise in neutrophils in classes 2 was modest. This is in keeping with the study by Blackman et al, 2021 (34), which found no association between early increases in neutrophil and subsequent treatment response. It should be noted that the sample in Blackman et al was a subsample of that reported in the present study, although

Blackman et al studied baseline and peak neutrophil counts as opposed to trajectories as in this study. In our study the differences in neutrophil count between the classes appear to be set prior to clozapine initiation, consistent with the hypothesis that a proportion of TRS patients have a low-grade inflammation, reflected in high-normal neutrophil counts, and that these patients are especially responsive to clozapine. The lesser effect seen in the high neutrophil group, as compared to the high- normal group, may be due to the reduced precision of the estimate in this smaller group. Also, patients in this group were more likely to have co-morbid medical illnesses, and were older, both of which may have affected their response to clozapine. The early transient increase in neutrophils seen across all groups may represent mobilisation of neutrophils from the bone marrow as part of the immunological response to clozapine. The short half-life of neutrophils (hours to days) may explain the transient nature of this increase.

The precise role neutrophils may be playing in inflammation in TRS is unclear, but there are a number of potential candidate mechanisms. For example, neutrophils are known to interact closely with the complement system, which is activated as part of the initial immune response. Complement activation triggers chemotaxis of neutrophils to the site of injury and promotes direct cell lysis. Stimulated neutrophils themselves release complement factors which further activate the cascade via the alternative and lectin pathways (47). The complement system has been shown to be activated in psychosis (48), and Susai et al, (49), have shown that levels of complement factors are associated with response to antipsychotic treatment in first episode psychosis. Stimulated neutrophils also release pro and anti- inflammatory cytokines, including TNFα, IL-1β, IL-1ra, IL-6 (50), several of which have been found to be elevated in

psychosis (1). In addition, there appears to be a complex interplay between neutrophils and T-cells and recent work has indicated that regulatory T-cells (Tregs), which are key to maintaining immune homeostasis, may be hypofunctional in psychosis (51). Neutrophils also produce traps called NETs (neutrophil extra-cellular traps) which ensnare pathogens and activate antigen presenting cells, which promote the differentiation of T-helper cells (2). Recent animal models and clinical studies have implicated neutrophils in the pathophysiology of a number of neuro-immune conditions including multiple sclerosis (MS) (52), immune anti-NMDAR encephalitis (53) and amyotrophic lateral sclerosis (ALS) (54,55); all conditions with known glutaminergic dysfunction. Glutamate is the main excitatory neurotransmitter in the CNS and is also an important immunomodulator (56). The hypothesis that TRS is categorically distinct from treatment responsive illness and is driven by glutaminergic, rather than dopaminergic dysfunction (8), may indicate similarities between TRS and these illnesses and shed light on the neutrophil changes seen in TRS. For example, it has been suggested that neutrophils may play a role in the development of neuroinflammation in MS as a direct result of NETosis negatively impacting on the ability of the blood brain barrier to control the influx of immune cells into the brain (52). NETs have been directly shown to be present in a number of auto-immune conditions (57) and they have also recently been found in the plasma of patients with early schizophrenia (51).

There may also be functional and/or phenotypic differences in neutrophils associated with disease severity or treatment responsiveness. This has been shown to be the case in both MS and ALS (52,54). Phenotypic studies in MS have shown that activated neutrophils (with enhanced ROS production compared to normal neutrophils) may be

involved in MS immunopathology and that granulocytic myeloid derived suppressor cells, which are neutrophils with an immunosuppressive function, may participate in the recovery phase (52). In addition, current MS treatments have effects on neutrophils either by reducing their numbers or altering their functioning (52). Studies in ALS have shown that CD16 expression on neutrophils was increased in patients with more severe disease (54). Studies of neutrophil phenotypes as possible markers of disease activity and treatment responsiveness in TRS could help further understanding of the role of neutrophils in TRS pathology and treatment response.

The finding that neutrophil count is associated with treatment response may not be specific to clozapine, nor to TRS, as raised counts have been linked with symptom severity and treatment response in non-treatment resistant illness (4,5). Whilst the unique efficacy of clozapine and its clear immunomodulatory properties, alongside the specific characteristics of TRS, suggest that clozapine may work differently to other antipsychotics in this patient group, studies looking at neutrophil counts and treatment response to alternative antipsychotics would be helpful.

Our study has several strengths. It is the largest study to our knowledge to demonstrate a significant relationship between neutrophil count and response to clozapine. The methodology used (LCGA) fits well with a heterogenous condition such as TRS. Another strength is the generalisability of the study, being representative of a large epidemiological population. The use of CGI-S as an outcome measure has good validity and ratings were carried out by an experienced consultant psychiatrist. The main limitations of the study are its retrospective design and that patients who discontinued clozapine before two years were excluded. Another limitation is the lack of information regarding serum clozapine levels in the cohort.; it is plausible that

patients with lower neutrophils achieved lower clozapine doses, with sub-therapeutic clozapine levels, as clinicians were hesitant about more rapid increases, leading to reduced or delayed treatment effectiveness. The lower neutrophil group also had lower illness severity scores at baseline, which could mean that the effect size with clozapine was reduced in this group. It is also noteworthy that patients of black ethnicities were over-represented in the low neutrophil group, although we controlled for ethnicity in the analysis. This may indicate that the group included patients with benign ethnic neutropaenia (BEN) (58), which occurs in people of African, Middle Eastern and West Indian ethnicities; the significance of which for the hypothesis being studied is unknown.

To conclude, our results are consistent with the hypothesis that patients with low-level inflammation, reflected in a high-normal neutrophil count, are more likely to respond to clozapine, raising the possibility that clozapine exerts its superior efficacy via immune mechanisms.

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CONCLUDING REMARKS

In this study there were two main findings of interest. Firstly, the trajectories of neutrophil counts following clozapine initiation differed from the outset and the neutrophil spike seen in previous studies did not appear to be a significant predictor of response. Secondly, patients differed significantly in terms of their baseline neutrophil counts and those with high normal counts at the time of commencing clozapine appeared more likely to show a good response. This result is in keeping with the literature indicating subtle sustained inflammation in schizophrenia and provides tentative support for the hypothesis that clozapine is acting as an immunomodulator, with patients with higher levels of inflammation, as measured by a simple neutrophil count, responding better to clozapine than those with lower counts.

CHAPTER 6. EFFECT OF CLOZAPINE ON CALCULATED GLOBULIN LEVELS AND ASSOICATION WITH TREATMENT RESPONSE. RESULTS FROM A RETROSPECTIVE COHORT STUDY USING ELECTRONIC HEALTH RECORDS

INTRODUCTION

For my final paper I decided to use my dataset from paper 2 to investigate whether Immunoglobulin levels were affected by clozapine treatment, in view of recent high-profile publications reporting an excess of clozapine patients presenting to a national immunodeficiency service with antibody deficiency (115,202) and also evidence that clozapine is associated with increased rates of lymphoid malignancies (116). Both these findings point towards clozapine acting as an immunomodulator and thus provide indirect support for my overarching hypothesis that clozapine may be a disease modifying drug. On review of the literature regarding clozapine and immunoglobulin levels, I identified two case-control studies showing an association between clozapine prescription and lower immunoglobulin levels (203,204), and also a recent small prospective study which showed a reduction in immunoglobulin levels during treatment which appeared to be associated with treatment response (205). As my database comprised a larger number of patients than the published study, and I had corresponding outcome data already collected, I decided that it would be a useful exercise to combine my results with that of a fellow researcher who had compiled a dataset of complete calculated globulin (CG) levels for patients who had received clozapine during an overlapping time period to my study. I manually extracted CG results for all the patients in my dataset for whom data were available (unfortunately

electronic records for blood results were not available in the CRIS system until 2012) at two time points, firstly a CG level pre clozapine prescription as close as possible to the clozapine start date, and secondly the closest CG level to 1 year post clozapine commencement. I compared pre and post clozapine CG levels using an unpaired t-test and carried out logistic regression to investigate for associations between change in CG level and clozapine response, and also post clozapine CG level and clozapine response.

PREPARED FOR SUBMISSION FOR PUBLICATION

EFFECT OF CLOZAPINE ON IMMUNOGLOBULIN LEVELS AND ASSOCIATION WITH TREATMENT RESPONSE. RESULTS FROM A RETROSPECTIVE COHORT STUDY USING ELECTRONIC RECORDS

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Abstract

Introduction

Clozapine is the treatment of choice for treatment resistant schizophrenia. Its exact mechanism of action is unclear but it is known to have an array of immunological side effects. It is possible that some of the immune effects of clozapine may be responsible for its efficacy in TRS. Clozapine has recently been shown to be associated with immunoglobulin deficiency. The current study aims to investigate whether reduction in globulin levels during treatment with clozapine are associated with response to clozapine.

Methods

A retrospective cohort of patients undergoing first treatment with clozapine was assessed for illness severity at baseline and after 2 years of treatment using the Clinical Global Impression – Severity (CGI-S) rating scale. Calculated globulin levels were obtained prior to clozapine commencement and post clozapine commencement at one year (or as close as possible to this date). Logistic regression was carried out

to look for associations between 1) change in globulin score, and 2) one year globulin count, and response to clozapine, defined as at least a 2-point reduction in CGI-S score.

Results

341 patients from the original cohort had at least one globulin count available and formed the study sample. A minority of the sample (43%) had a globulin score pre clozapine, whereas 99% had a post globulin count. Globulin levels fell significantly following clozapine, particularly in male patients. Logistic regression showed no relationship between change in globulin score and clozapine outcome. In a separate analysis of 1 year outcome scores and clozapine response, the results were significant but in the opposite direction to the null hypothesis.

Discussion

The study provides clear evidence, using real world clinical data, that immunoglobulin levels fall with clozapine treatment. The finding that clozapine levels disproportionately fall in male patients appears to be novel. The small but highly significant association between post clozapine globulin count and response to clozapine suggests that higher globulin scores are associated with better response to clozapine but limitations with the data quality prevent definitive conclusions being drawn.

Introduction

Schizophrenia is a serious mental illness with high rates of morbidity and significant mortality. Approximately 25% of people with schizophrenia are found to be treatment resistant (1). Clozapine has unique efficacy in treatment resistant schizophrenia (TRS) (2–4) and has also been shown to reduce all-cause mortality (5). Clozapine's mechanism of action in TRS is not fully clear although it is known to act on a wide range of neurotransmitter receptors (6). Clozapine also has effects on the immune system, with activation of pro-inflammatory cytokines on initiation commonly causing tachycardia and pyrexia, and other significant adverse effects, such as myocarditis, neutropaenia and gut stasis believed to have an immunological basis (7). There is emerging evidence of an association between clozapine's actions on immune markers and its therapeutic effect, and it is plausible that its capacity to act as an immunomodulator may explain its superior effectiveness in TRS.

Clozapine treatment is associated with increased rates of infection (8), particularly pneumonia (9–11), and there is increasing interest into the effect of clozapine on immunoglobulin levels. An early study of 16 patients commencing clozapine found a significant increase in IgG levels after 6 weeks of clozapine therapy, in line with increases in pro-inflammatory cytokines during the first weeks of clozapine treatment (12). A more recent study of similar size found IgG levels were significantly elevated after 12 weeks treatment compared to baseline (13). More robust evidence indicates a progressive reduction in immunoglobulin levels as clozapine treatment progresses. A survey of immunoglobulin results from biochemistry labs across Wales found that 13% of samples from across primary care found to have low IgG levels (IgG <4 g/l) were recorded as being prescribed clozapine (14). A retrospective study of referrals

to the Immunology centre for Wales found that, of 23 patients with a diagnosis of schizophrenia, 17 were taking clozapine, and in relation to those patients who went on to require immunoglobulin replacement therapy, 6 out of 7 were on clozapine. In addition the patients taking clozapine showed a marked reduction in class switched memory cells and plasmablasts, akin to a common variable immunodeficiency picture, compared to healthy age-matched controls (15). Similarly, a case series of 17 patients on clozapine presenting to immunology clinics with pan hypogammaglobulinemia, of whom 40% required immunoglobulin replacement, has been reported in the North- West of England (16). A case control study comparing 123 patients on clozapine with 111 clozapine naïve patients showed reduction in all immunoglobulin groups in the clozapine treated sample, with an association between longer duration of clozapine treatment and lower immunoglobulin levels (17). A smaller case control study of 33 patients on clozapine and 67 psychiatric controls found that clozapine use was associated with IgM deficiency (18).

There is tentative evidence that immunoglobulin changes with clozapine may be related to clinical effectiveness. A recent prospective study of 56 TRS patients demonstrated a reduction in immunoglobulin levels at 12 and 24-week time points following commencement of clozapine which correlated with reduction in PANSS scores in the 32 patients who had outcome scores recorded (19). The use of calculated globulin (CG) (total protein minus albumin) as a measure of total immunoglobulin (14) has made screening for immunoglobulin deficiency more accessible as total protein and albumin levels are checked as standard in many liver function test panels. Patients are designated to have low immunoglobulins if CG level is 20 g/l or less. The present study therefore aims to use CG scores, obtained from

routine clinical monitoring, to investigate immunoglobulin levels in a larger cohort of patients undergoing clozapine treatment in order to answer the following questions:

1) is there evidence of CG levels changing with clozapine in a larger sample of patients compared to those reported in previous studies?

2) is there is a relationship between change in CG level, pre and post clozapine, and clinical response?

We hypothesised that, as in the majority of previous studies, CG levels would reduce with clozapine treatment and that reduction in CG level would be associated with increased rate of response to clozapine.

Methods

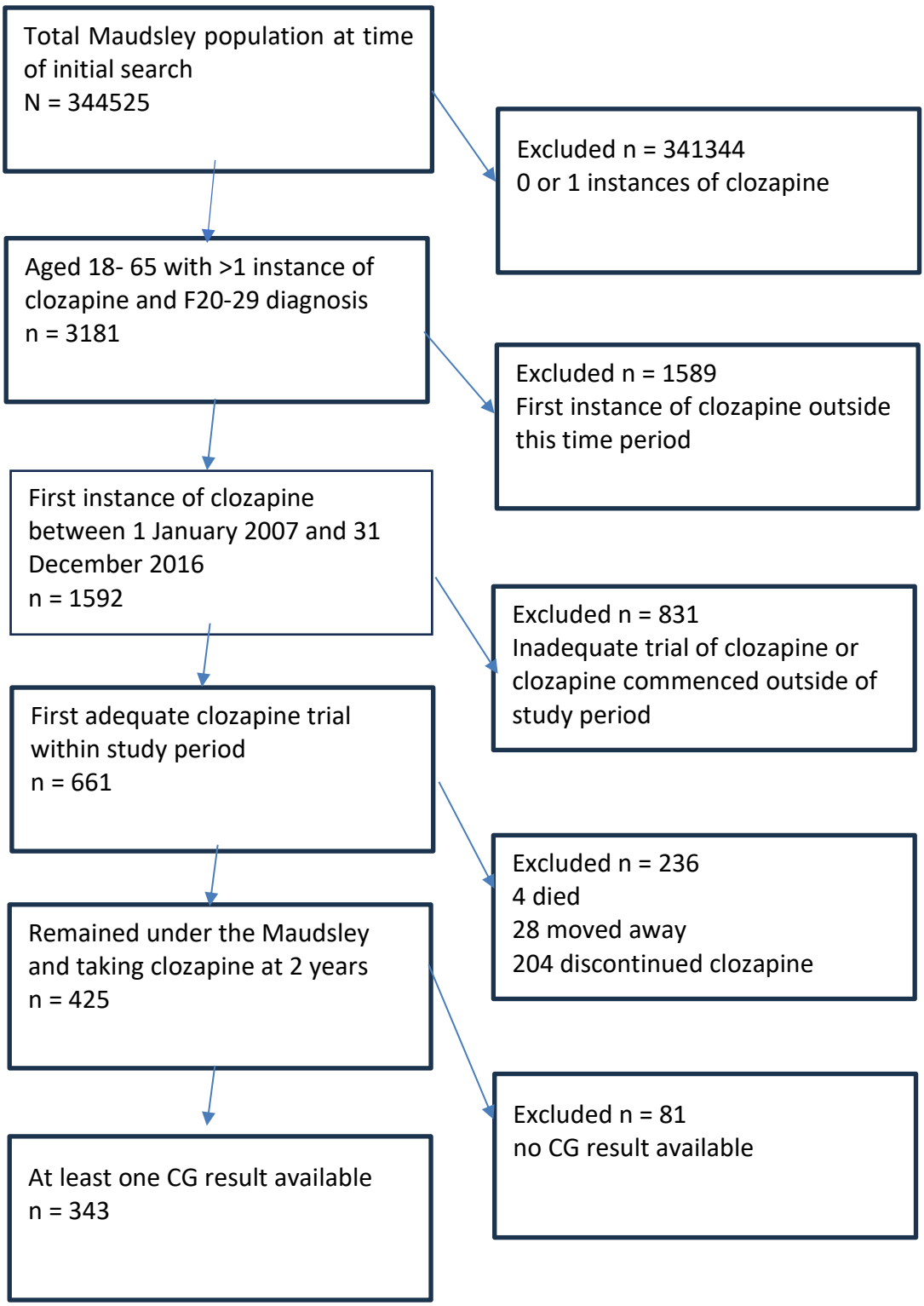
Sample

The study used data from the South London and Maudsley ('the Maudsley') case register which consists of complete anonymised electronic patient records dating back to 1st January 2007. Data was accessed using the Clinical Records Interactive Search (CRIS) system, for which methodology has been described elsewhere (20,21). Use of CRIS as an anonymised resource for secondary analysis has been approved by the Oxfordshire Research Ethics Committee (08/H0606/71). The study was approved by the NIHR BRC CRIS oversight committee (application number 21–073).

A retrospective cohort of 425 patients from a previous study, who had commenced their first adequate trial of clozapine whilst under the care of the Maudsley between

1st January 2007 and 31st December 2016, was followed up for a 2-year period. A detailed description of how the cohort was identified has been published previously (22). In this study, a rating of illness severity, the Clinical Global Impression – severity score (CGI-S) was recorded retrospectively at two time points, firstly prior to commencement of clozapine and secondly at 2-year follow-up providing the patient was still taking clozapine. From the original cohort, CG data was available for a subset of 344 patients, obtained from a separate CRIS search by a fellow researcher (RG) (electronic health records at the Maudsley only incorporated investigation results from 2012 onwards hence the lower number of patients with data available). CG levels were extracted manually from the available data at two planned time points, pre-clozapine and one year post commencement of clozapine. For pre-clozapine levels the closest CG result prior to initiation was used, and for post-clozapine levels the result closest to 1 year after commencement was used. This time point was chosen in line with previous evidence that immunoglobulins appear to fall with sustained clozapine treatment. A flow diagram showing how the cohort was identified is shown in figure 1.

Figure 1 Flow diagram – identification of cohort



Outcome measures

Outcome measures used in the study were 1) change in CG level with clozapine, 2) rates of low CG levels pre and post clozapine, and 3) clinical response to clozapine at 2 years.

Change in CG level following clozapine was measured by subtracting the post-clozapine CG level from the pre-clozapine CG level.

Patients were recorded as having low CG levels if the level was 20 g/l or less.

Clinical response to clozapine at 2 year follow up was defined as a reduction of at least 2 points between baseline CGI-S score and CGI-S score at 2 years.

Covariates

Data was also collected for possible confounders, namely sex, age, ethnicity (UK census categories collapsed into four groups - white, black Caribbean, black other, mixed/other – reflecting the demographics of the local catchment area) and medical co-morbidity (recorded number of medical admissions during study period).

Statistical Analysis

Stata 15 was used for all analyses (23).

CG levels pre and post clozapine were compared using unpaired t-tests.

Logistic regression analysis, controlled for confounding variables, was carried out to investigate for an association between change in CG level and clozapine response.

As there were only limited numbers of pre-clozapine CG levels available, a second logistic regression analysis of 1-year CG level and clozapine response was also conducted.

Linear regression was performed to look for effects of sex and ethnicity on pre and post clozapine CG levels.

Results

343 patients in total had at least one CG result available and made up the study sample.

Due to the lack of blood investigation data prior to 2012 only a minority of patients had pre clozapine CG results available. This led to a much larger number of patients having post-clozapine CG levels than pre-clozapine ones.

Clinical characteristics of the sample are shown in table 1.

Table 1 Characteristics of the sample (n = 343)

Characteristic	Descriptor	Number (percent)
Sex	Male	227 (66.2)
	Female	116 (33.8)
Ethnicity	White	131 (38.2)
	Black Caribbean	26 (7.6)
	Black other	126 (36.7)
	Mixed other	60 (17.5)
Hospital admissions during study period	0	241 (70.3)
	1	64 (18.7)
	>1	38 (11.1)
		Mean (standard deviation)
Age	years	35.3 (10.7)
Mean CG level pre-clozapine (g/l)	n = 149 (43.4%)	28.57 (4.28)
Mean CG level post-clozapine (g/l)	n = 341 (99.4%)	27.50 (3.91)

149 patients (43.4%) had a CG level pre clozapine. 341 patients (99.4%) had a post clozapine CG level performed. Many tests were taken much later than one year after commencing clozapine, and less than half of the values were within the two-year study period (47.5%).

Mean CG level fell following clozapine treatment. The mean pre clozapine CG level was 28.57 (standard deviation 4.28). The mean post clozapine CG level was 27.50 (standard deviation 3.91). The fall in CG level was statistically significant (two sample t-test t = 2.74 p = 0.007).

2/149 patients (1.34%) had clinically low CG levels (CG <21) prior to clozapine therapy, whilst 10/341 (2.93%) had low CG levels after starting clozapine.

Logistic regression was performed to look for an association between change in CG level and clozapine response, adjusted for covariates. No effect was seen, though the sample size was relatively small (n = 147) (table 2).

Table 2. Logistic regression of change in CG level on clozapine response (n = 147)

Scores adjusted for age, sex, ethnicity and medical admissions

	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Change in CG level	1.00 (0.89-1.13)	0.94	1.02 (0.89-1.16)	0.91
Age	0.98 (0.97-1.00)	0.14	0.94 (0.91-0.98)	0.01
Sex	0.79 (0.50-1.25)	0.31	0.98 (0.44-2.20)	0.96
Ethnicity				
White (reference group)				
Black Caribbean	1.34 (0.54-3.32)	0.52	0.53 (0.12-2.37)	0.41
Black other	0.72 (0.44-1.19)	0.20	0.54 (0.21-1.38)	0.20
Mixed other	0.96 (0.51-1.80)	0.90	0.60 (0.22-1.66)	0.33
Medical admissions				
0 (reference group)				
1	1.24 (0.46-2.21)	0.46	3.38 (1.05-10.92)	0.04
>1	0.59 (029-1.16)	0.13	0.81 (0.29-2.28)	0.69

A further logistic regression was performed to look for an association between post clozapine CG levels and clozapine response, adjusted for the same covariates. A larger sample was available for this analysis (n = 341) (Table 3). The results showed an association between post clozapine CG level and response to clozapine, with higher post clozapine CG levels associated with increased odds of response to clozapine (adjusted OR 1.09. 95% CI [1.02 – 1.16].

Table 3. Logistic regression of post clozapine CG level on clozapine response (n = 341) Scores adjusted for age, sex, ethnicity and medical admissions

	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Post clozapine CG level	**1.06 (1.00-1.12)**	**0.05**	**1.09 (1.02-1.16)**	**0.01**
Age	0.98 (0.97-1.00)	0.14	0.98 (0.96-1.00)	0.09
Sex	0.79 (0.50-1.25)	0.31	0.81 (0.49-1.33)	0.41
Ethnicity				
White (reference group)				
Black Caribbean	1.34 (0.54-3.32)	0.52	0.99 (0.39-2.54)	0.99
Black other	0.72 (0.44-1.19)	0.20	0.48 (0.27-0.86)	0.01
Mixed other	0.96 (0.51-1.80)	0.90	0.79 (0.40-1.54)	0.48
Medical admissions				
0 (reference group)				
1	1.24 (0.70-2.21)	0.46	1.15 (0.64-2.07)	0.65
>1	0.13 (0.29-1.16)	0.13	0.64 (0.31-1.32)	0.23

Further exploration of the results revealed that there were significant sex differences in CG levels. Males were found to have lower CG levels than females both pre and post clozapine therapy. The majority of patients who developed clinically low CG

levels post clozapine were male (80% versus 20%). Similarly, there were notable differences between white and non-white ethnic groups with white patients having lower CG levels and 70% of patients developing low CG levels being white. Sex and ethnicity differences in mean CG levels are shown in table 4.

Table 4 Mean CG levels for 1) males vs females and 2) white vs non white ethnicities pre and post clozapine therapy

	Sex		Ethnicity	
	Male	female	white	Non white
Mean baseline CG n = 149	27.67 [26.94 – 28.40]	30.41 [29.04 – 31.77]	26.17 [25.30 – 27.04]	29.90 [29.04 – 30.74]
Mean one year CG n = 341	26.91 [26.43 – 27.38]	28.64 [27.87 – 29.41]	25.44 [24.91 – 25.98]	28.78 [28.26 – 29.29]

CG levels were plotted against sex and ethnicity demonstrating a clear left shift in CG levels for males versus females (Figure 2a) and white versus non white ethnicity (Figure 2b).

Figure 2a. Clustered column chart showing one year CG levels post clozapine by sex

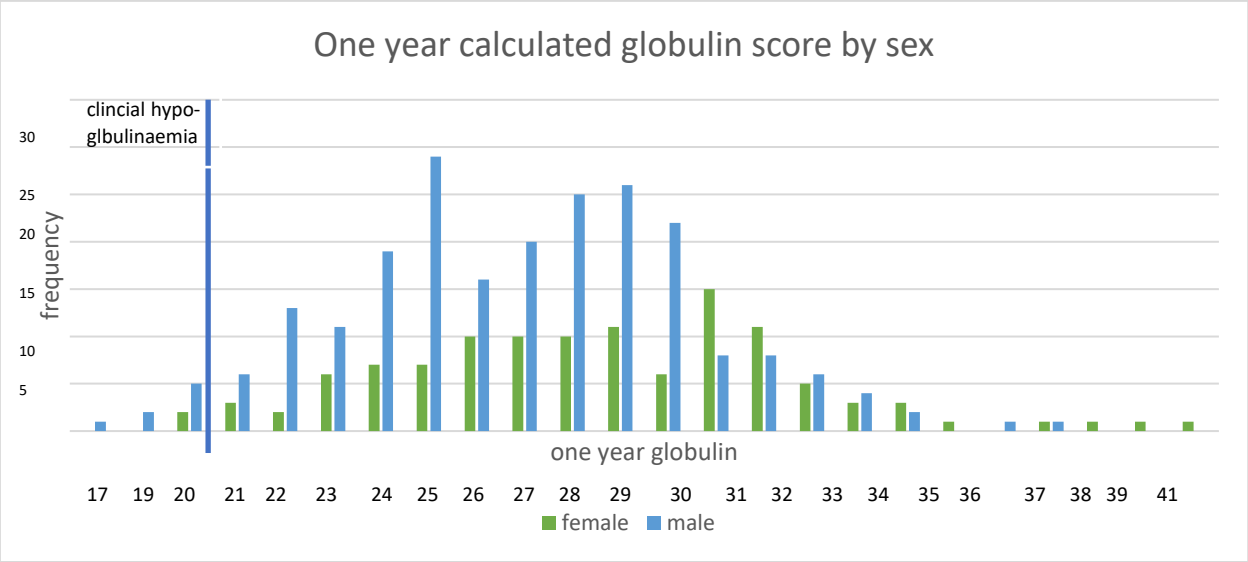
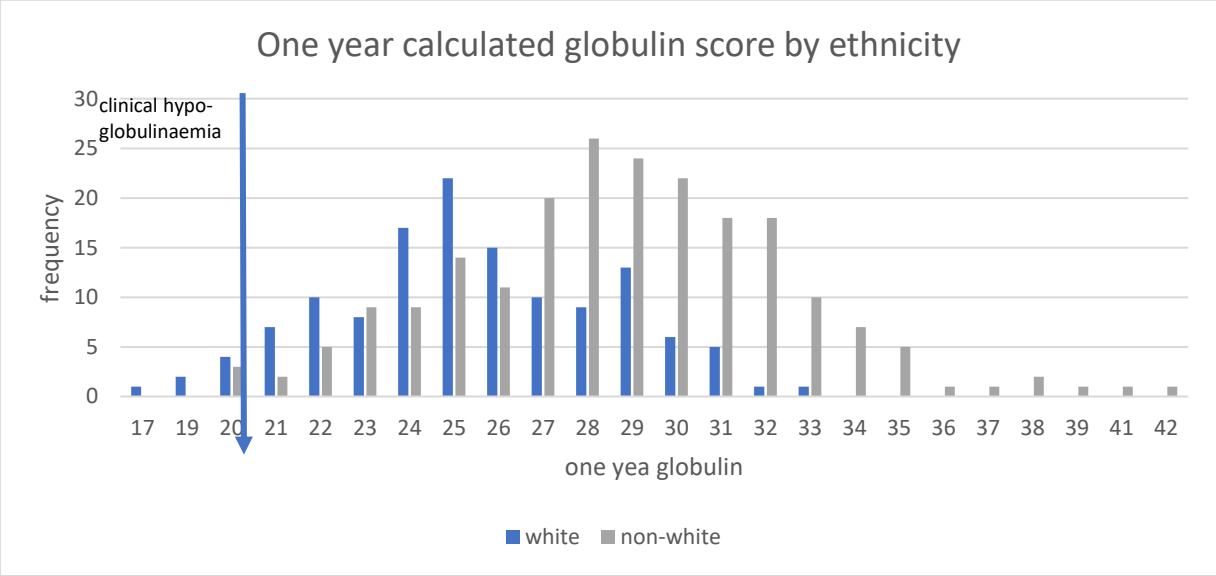


Figure 2b. Clustered column chart showing one year CG levels post clozapine by ethnicity



Linear regression analysis of effect of sex and ethnicity, adjusted for duration of illness, deprivation score and medical co-morbidity, on pre and post clozapine CG scores showed a significant association at both time points (Table 5).

Table 5. Linear regression of effects of sex and ethnicity on pre clozapine CG levels (n = 149) and post clozapine CG levels (n = 341). Scores adjusted for duration of illness, deprivation score and medical admissions

	Baseline globulin n = 149			One year globulin n = 341		
	Co-efficient	p-value	[95% CI]	Co-efficient	p-value	[95% CI]
Male sex	**-2.02**	**0.00**	**-3.39 - -0.74**	**-1.46**	**0.00**	**-2.25 - -0.67**
Ethnicity						
White (reference group)						
Black Caribbean	**3.02**	**0.02**	**0.51 – 5.53**	**2.63**	**0.00**	**1.14 – 4.11**
Black other	**3.90**	**0.00**	**2.37 – 5.44**	**3.58**	**0.00**	**2.70 – 4.45**
Mixed other	**2.19**	**0.01**	**0.49 – 3.89**	**2.44**	**0.00**	**1.36 – 3.52**
Duration of illness	-0.01	0.89	-0.08 – 0.07	-0.14	0.54	-0.59 – 0.03
Deprivation score	0.02	0.43	-0.04 – 0.09	0.02	0.33	-0.02 – 0.53
Medical admissions						
0 (reference group)						
1	-0.68	0.41	-2.29 – 0.93	0.69	0.16	-0.28 – 1.65
>1	-0.14	0.88	-1.91 – 1.64	0.38	0.54	-0.84 – 1.60

Discussion

The result of the study supports the hypothesis that there is a fall in CG levels with clozapine treatment. 2.93% of patients had clinically low CG levels post clozapine versus 1.34% pre clozapine. The risk of clinical immunodeficiency with clozapine use is apparent in clinical practice with an excess of patients on clozapine referred to immunology services and requiring immunoglobulin replacement therapy.

No association was found between change in CG level and clozapine response. However, a significant association was found between CG level post clozapine and clozapine response. The findings suggest that higher rather than lower CG levels are associated with better response to clozapine, which was in the opposite direction to the effect observed by Griffiths et al (19). This may be due to the variation in time points at which CG levels were taken post clozapine, as compared to the previous study.

The study also illuminated clinically relevant sex and ethnicity differences in CG levels, with male sex and white ethnicity both showing association with lower CG levels. No significant effect of duration of illness on CG levels was observed. Whilst the differences were present pre as well as post clozapine, the results suggest that clozapine may accentuate previously low levels as the majority of the patients who developed clinically low CG levels with clozapine were white males.

Sex and ethnicity differences in immunodeficiency related to clozapine do not appear to have been reported previously, however, there is literature, dating back to the 1960s, demonstrating sex and ethnicity differences in immunoglobulin levels in healthy and clinical populations (24,25). The more robust findings appear to be in

relation to ethnicity. Caucasian populations have been shown to have lower levels of Ig A, Ig G and Ig M (24) than non-white populations. It has been suggested that these differences are due to a higher infective burden in non-white populations, as typified by experience of the COVID 19 pandemic where socioeconomic factors are believed to have led to greater viral exposure in these groups. However, detailed studies of immune responses to COVID 19 have shown that non-white ethnicity is independently associated with increased immunoglobulin levels (26) and there is also evidence of differential immune responses to vaccination, without prior exposure to infection (27), these findings suggesting genetic factors may be a significant cause. Studies of sex differences in immunoglobulin levels have yielded more nuanced results than those of race but indicate higher Ig A and lower Ig M levels in males than females but no difference in Ig G levels. A number of other factors such as age, smoking, alcohol and metabolic factors may also be exerting effects (24). The literature as a whole suggests that sex and ethnicity differences in CG levels in the current study are likely to be robust findings and increased risk of clinical immunodeficiency in male and white patients is possible,

The main advantages of the study were its use of real-world clinical data and its larger sample size compared to previous studies. The diversity of the study was also key in terms of its large number of non-white patients, which may explain why novel findings in relation to ethnic differences in CG levels were observed. However, there were significant limitations to the study, notably less than half of the sample had immunoglobulin levels tested prior to commencing clozapine meaning that the sample size was smaller than planned. The post clozapine CG levels were taken at widely discrepant time points. Due to these limitations the results of the analyses

need to be interpreted with caution as to whether changes in CG level are associated with clozapine response.

Alongside knowledge of immunological deficiency related to clozapine, the possibility of increased haematological malignancies amongst patients prescribed clozapine has been mooted for some time and there has been recent confirmation of a small but increased risk (28,29). Both these discoveries indicate that continuing clozapine treatment is associated with progressive alterations in lymphocyte function and support a hypothesis that clozapine is an immunosuppressant drug. Haematological malignancy is a well-recognised complication of established immunosuppressant therapies, most strikingly in the field of transplant medicine (30,31). Immunosuppressant drugs are thought to increase cancer risk either by weakening host immune system surveillance for tumour cells, or by reducing the body's ability to fight off infections, particularly viruses, which cause cancers (32,33). More research into possible effects of clozapine on tumour susceptibility is required.

In conclusion, whilst the current study did not substantially address the question of whether immunoglobulin changes following clozapine affect clinical response to clozapine, it does provide convincing evidence of reducing immunoglobulin levels (as measured by CG) with clozapine treatment, in a large diverse real world patient population. The risk of immunodeficiency may be higher in male and white populations. Current national guidelines do not reflect this increased risk and need to be modified, in order to better educate patients and clinicians of the risks so that patients can be closely monitored. In addition, guidelines should consider whether specific intervention programmes, such as vaccination, should be implemented in order to reduce risk of overwhelming infection and mortality.

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CONCLUDING REMARKS

The results of the study showed that CG levels did fall significantly with clozapine treatment, with a more than doubling in the percentage of patients who had clinically low levels, though numbers were small (1.3% to 2.9%). Regression analysis did not show an association between change in CG level and clozapine response, but there was a significant association between one year CG levels and response, with higher levels associated with improved odds of response. This finding was in the opposite direction to the null hypothesis.

On review of demographic factors, differences were noted in CG levels, both pre and post clozapine, between males and females and white and non-white populations, and of the small number of patients found to have clinically low CG levels on clozapine, the majority were white males. However, the quality of the data was poor. Only approximately one third of patients had a pre clozapine CG result available and the CG levels post clozapine were often taken many years after clozapine was commenced. In view of the shortfalls in the data quality, results of the study need to be interpreted with caution.

CHAPTER 7. CONCLUSION

Synopsis of results

For paper 1, I carried out a systematic review of the randomised controlled trial literature comparing clozapine to alternative antipsychotics, in patients with non-treatment naïve TRS. I performed a meta-analysis which showed convincingly that clozapine was superior to other antipsychotic medications in reducing psychotic symptoms and improving rate of response. I then carried out a meta-regression to look for an effect of age on clozapine's effectiveness which did not show a significant effect. However, two studies reported individual patient data on which I was able to perform regression analyses looking at the effect of age, and duration of illness, on response to clozapine, and in the larger of the two studies I found that that both indicators were significantly associated with clozapine response.

For paper 2, I carried out an observational study, using a retrospective cohort of patients at the Maudsley hospital who were undergoing their first treatment trial of clozapine, to look for an effect of duration of prior illness on clozapine response. I hypothesised that a shorter duration of prior illness would be associated with improved response to clozapine. I carried out an ordinal logistic regression using illness severity scores at 2 years as the primary outcome, with hospital bed days and psychiatric re-admissions as secondary outcomes. I adjusted for known confounders including sex, ethnicity, deprivation, substance abuse and age in the analyses. The study showed that duration of illness was significantly associated with illness severity outcome score, with a shorter duration of illness associated with better outcome, confirming my initial

hypothesis. There was no significant effect on the secondary outcome measures. In hindsight the study methodology was not optimum to look at these outcomes, which would be better studied using a mirror image design comparing admission rates and bed use pre and post clozapine use.

For paper 3, I moved to the field of immunology, examining neutrophil trajectories following clozapine initiation in a second observational study incorporating the database I created for paper 1 along with data obtained via linkage with ZTAS full blood count data. I hypothesised that there would be an early spike in neutrophil count with clozapine, in line with previous reported clinical findings, that would be associated with clinical response. This hypothesis was in fact not proven; rather I showed that neutrophil trajectories appeared to be set from baseline with patients clustering into low, high-normal and high neutrophil counts. Patients with high-normal neutrophil counts had a better response to clozapine, suggesting that a degree of inflammation at baseline, as reflected in the neutrophil count, was associated with better clozapine response.

For paper 4, I considered recent literature reporting an excess of cases of immunoglobulin deficiency in patients prescribed clozapine. I linked my clozapine database with that of a fellow CRIS researcher who had collected all available globulin results for patients prescribed clozapine between the years of 2012 to 2021. I extracted globulin results for patients in my database, pre clozapine and 1 year post clozapine, to test the hypotheses that, firstly, globulin levels would fall with clozapine treatment, as per the available literature, and secondly, that fall in globulin would be associated with clinical response. The study was limited by the finding that pre-clozapine globulin levels were only available in a minority of the patients, and post globulin levels were

often taken many years later than the planned 1-year time point. In addition, the data collected did not always include whether the patients were still taking clozapine at the time of the globulin result. Despite this limitation a significant fall in globulin level was observed with clozapine treatment. There was no significant effect of change in globulin score on clozapine response, though the sample size was small. There was however a small but significant effect of 1- year globulin score on clinical outcome, with higher globulin scores indicating a higher likelihood of clozapine response. This effect was in the opposite direction to the initial hypothesis and should be interpreted with the caution in the light of size of this effect and the significant limitations of the study.

Considering the results of these four studies, the conclusions drawn are essentially as follows. Firstly, the results from the systematic review and new data are in keeping with previous evidence that clozapine is superior to other antipsychotics in TRS, and suggests that there is potentially a greater chance of response to clozapine if it is given earlier in the illness course, in line with the concept of disease modification. Secondly, they suggest that clozapine is more effective in patients with higher rather than lower neutrophil counts, and that clozapine treatment is associated with a fall in immunoglobulin levels, supporting a theory that clozapine is acting as an immune modulator in TRS.

Since the completion of these studies there have been some significant publications in the literature which provide further support to these hypotheses. The first published meta-review (a quantitative review of all meta-analyses) of clozapine authored by Wagner et al, 2021 (206) has shown that clozapine is superior to other first and second generation antipsychotics in terms of effectiveness, hospitalisation, mortality and all cause discontinuation. Chan et al, 2021 (207) have published a large cohort study of

first episode schizophrenia in which they found that longer delay to clozapine was associated with greater rates of clozapine resistance. Similarly, Griffiths et al, 2021 (208) have carried out a meta-analysis of clozapine outcome data and shown that a longer delay to clozapine prescribing predicts clozapine resistance.

Focus on immune markers

In this thesis I have homed in on two simple immune markers, namely neutrophil count and calculated globulin, for several reasons. Firstly, as a practicing psychiatrist I am most interested in research which is clinically applicable to a real-world setting and both these tests can be routinely performed in day-to-day practice. Secondly, neutrophils appear to be centre stage in the literature concerning the immune pathogenesis of a number of illnesses which share characteristics with schizophrenia, including multiple sclerosis and systemic lupus erythematosus, and there is potential to apply knowledge from study of these disorders to that of schizophrenia. Thirdly, a clearer understanding of the effects of schizophrenia, and clozapine, on neutrophils and immunoglobulins has very tangible clinical benefit. Enhanced ability to predict neutrophil response to clozapine may decrease the risk of agranulocytosis, which would not only reduce risk but could eventually lead to a transition away from the current heavy burden of blood monitoring associated with clozapine therapy. Similarly, clearer elucidation of the risk of immunoglobulin deficiency with clozapine will enable clinicians to monitor and mitigate, eg by pneumonia vaccination programmes. Finally, it is possible that a simple neutrophil count, or globulin level, may have utility as a biomarker of either schizophrenia severity or response to treatment, most likely in

combination with other clinical characteristics. The advent of machine learning techniques now makes this possibility appear much more credible and such combination approaches are already starting to bear fruit (209).

Potential roles of neutrophils in the pathology of schizophrenia

Although reports of neutrophil abnormalities in schizophrenia are not new, neutrophils have remained somewhat overlooked in the literature regarding the immunology of schizophrenia until recently. This may be partly because neutrophils have previously been viewed as a fairly blunt tool in the immune system, thought of simply as 'suicidal killers which cause collateral tissue damage' (210), and also due to the belief that the blood brain barrier rendered the brain an immune privileged site, through which peripheral immune cells could not pass (211). However, neutrophils are now understood to be at the centre of a complex system of signalling operations which make up the immune response, both peripheral and central, and there are therefore many potential mechanisms by which they may be involved in neuroinflammation in schizophrenia, including disruption of the blood brain barrier, direct cell damage, activation of complement and cytokine systems and also effects on other immune cells.

It is now known that the blood brain barrier becomes disrupted in schizophrenia (30,212,213), and that neutrophils may play a key role in this process, as they have been shown to increase the permeability of the blood brain barrier, and infiltrate the brain, in a number of other illnesses such as Alzheimer's disease, stroke and multiple sclerosis (214). Neutrophil extra-cellular traps (NETs) have been shown to disrupt the blood brain barrier in multiple sclerosis, rendering it permeable to immune cell

infiltration (215). Activated neutrophils may cause direct nerve cell damage by NET formation and release of reactive oxygen species (ROS), matrix metalloproteases (MMPs) and other cytotoxins (214) and neutrophils have been linked with disease progression in animal models of multiple sclerosis (216).

Brain neutrophil infiltrates have been found to be present in schizophrenia (30), as have NETs (217). Cortical grey matter depletion in schizophrenia has been shown to be associated with neuro-inflammation (218). Neutrophil count has also been shown to be associated with reduced grey matter volume and PANSS score (197).

Neutrophils may also cause brain inflammation via their effects on complement factors and cytokines. Activation of the complement cascade releases chemokines which causes chemotaxis of neutrophils. Stimulated neutrophils themselves further activate the complement pathway. Complement activation has been shown to be present in patients with schizophrenia (26,187). As regards cytokines, neutrophils release both pro and anti-inflammatory cytokines which are involved in orchestrating the immune response. There is now very established evidence of various cytokine alterations in schizophrenia (19).

Cross-talk between neutrophils and other immune cells may also be relevant in schizophrenia. Neutrophils have been shown to interact with T-regulatory cells which are key in maintaining immune homeostasis and may be hypofunctional in schizophrenia (61). Neutrophils also regulate T h17 cells (219) which have been implicated in the immune pathogenesis of schizophrenia (220).

In summary these multiple strands of evidence place neutrophils at the centre of immune dysfunction in schizophrenia. Further research should investigate whether

neutrophils in schizophrenia show specific phenotypes, and parallel studies of neutrophil function in other immune disorders.

Treatment resistant schizophrenia (TRS) and clozapine – evidence in support of TRS being a chronic immune mediated disease

Clozapine is superior to other antipsychotics in TRS and appears to have a novel mechanism of action unrelated to dopamine blockade. This suggests that TRS may be qualitatively different to non-treatment resistant schizophrenia (nTRS), albeit still with significant heterogeneity, the latter evident from the challenge with achieving consensus on TRS criteria and the fact that not all patients with TRS will respond to clozapine (49). Patients with TRS have been shown to have additional immunological abnormalities when compared with nTRS including greater elevation of cytokines (55) and greater oxidative stress (60). Whilst in nTRS elevated dopamine synthesis has been shown to be present, this does not appear to be the case in TRS (221), and it has been proposed that glutamatergic dysfunction may be more relevant in this patient group (51,222,223). Glutamate dysfunction is a key feature of various immune mediated illnesses including multiple sclerosis (224), amyotrophic lateral sclerosis (225) and anti-NMDAR encephalitis (226) and it now appears that glutamate dysfunction occurs in a wide range of neuropsychiatric diseases (227). Earlier trials of glutamate modulators in TRS failed to show benefit (228), however experimental studies of more targeted glutamatergic agents on healthy volunteers experiencing psychotic symptoms in response to ketamine, have been more promising (229). There is evidence that clozapine activates NMDA glutamate receptors (97), and clozapine's

role in glutamate transmission may explain not just its clinical superiority in TRS but also its apparent efficacy across a range of other neuropsychiatric disorders (97).

TRS thus appears to sit comfortably within a paradigm of an immune mediated illness, with clozapine acting at least in part as an immunomodulatory drug. Combining neuroscience findings with clinical evidence of similarities between TRS and established immune mediated illnesses suggests that treatment paradigms and immune biomarkers for treatment of these conditions may also be relevant for TRS. Whilst treatment options for TRS other than clozapine remain limited at present, development of biomarkers is becoming more plausible and there may be significant learning in this regard from the field of study of other immune conditions. Neutrophil counts are one such marker, and there is much interest currently in the role of neutrophils in the pathogenesis of MS, ALS and anti-NMDAR encephalitis and in monitoring neutrophil response to treatment in these conditions (215,230–232).

Disease modification in schizophrenia – can the course of disease be altered?

The literature regarding disease modification in schizophrenia is limited and the concept appears to be late in its introduction to the field (233). There are obvious challenges in terms of heterogeneity and establishing clear biomarkers of disease progression, but there is also a degree of therapeutic nihilism regarding available treatments, with the perception that they control symptoms rather than the course of the disease (233). However, others point out that early intervention paradigms have consistently shown that earlier use of antipsychotic drugs in schizophrenia improves long term outcome, and maintenance treatments alter disease course by reducing the

number of relapses (234). Evidence from this thesis and other recent publications indicating that earlier use of clozapine is associated with better recovery from TRS also supports the concept of disease modification. Couple this with the strong possibility that clozapine is an immunomodulator, and there seems no reason why a set of parameters to assess treatment response and end organ damage, incorporating neuroimaging evidence, cytokine levels, neutrophil counts and clinical ratings of psychotic or cognitive symptoms, cannot be developed for TRS, in line with those that have been published for other neurodegenerative conditions (122).

The vexed question of whether improvements would be maintained if treatment were to be discontinued, applies not only to TRS but to established immune conditions, with expectation that treatment will often be required long term. However, treatments are rapidly advancing in immunology and in rheumatoid arthritis for example, treatment paradigms have been revolutionised by a move from sequential step up treatments to early use of TNF inhibitors with evidence that many patients can stop these treatments subsequently and remain disease free for long periods (235). At present evidence suggests that clozapine should be lifelong therapy, but this is in the absence of a repertoire of drugs which a patient could potentially step down to. In addition, the majority of the literature on clozapine discontinuation is likely to consist of patients who encountered significant delay before being prescribed clozapine. It is possible that earlier clozapine use, before treatment resistance has ‘set in’, may mean that it would be easier to step down to other agents after remission has been achieved, or to reduce doses to a lower maintenance level. In a retrospective study of first episode patients who discontinued clozapine, which we carried out before I embarked on this thesis, we were able to show that outcomes in this patient group were more positive than would

be expected from the literature (236). Whilst for first line antipsychotics there have been studies of discontinuation that show that duration of treatment does not reduce chances of relapse (237), I am not aware of similar studies being carried out for clozapine, nor whether a shorter delay before starting clozapine might mean that clozapine could subsequently be stopped.

Use of clozapine early in schizophrenia when there are biomarkers of TRS

Whilst the evidence is now very clear in classical immune illnesses such as rheumatoid arthritis for intensive treatment in the early stage of illnesses, the situation with schizophrenia is more complex. This is due to clinical heterogeneity, particularly at disease onset, along with a lack of clear biomarkers. Whilst it is now well established that clozapine is more effective than other antipsychotics when used as a third line treatment in patients who have failed previous treatments, this does not appear to be the case when clozapine is used first line in schizophrenia (238–240), albeit study of first line clozapine has been limited due to its significant side effect profile. Clozapine may be no more effective in this population group because most patients respond adequately to first antipsychotic treatment, leaving little room for added benefit with clozapine. Some patients presenting with a first episode of psychosis and receiving antipsychotic treatment, would not in fact have schizophrenia at all.

There is interest in the use of clozapine as a second line agent in schizophrenia rather than third line, and there is compelling data to support this from the study of rates of treatment response to first and second treatment trials of antipsychotic medication, with the response to the second trial tailing off dramatically (from 75.4% – 16.7% in

one quoted study) (241). A recent systematic review has concluded that clozapine may be more effective than other antipsychotics as a first or second line agent, but that large clinical trials were needed (242).

Increased knowledge of neurological and immunological markers in schizophrenia may help co-ordinate a process of stratifying first episode schizophrenia patients as to their risk of TRS, to enable earlier use of clozapine in patients at risk of TRS. For example, in the OPTiMiSE trial patients with first episode schizophrenia were stratified into four classes with the class with higher inflammatory markers showing a poorer response to first line treatments (243). More recently, researchers have been able to predict the need for clozapine from routinely available demographic and clinical markers namely age, sex, ethnicity, triglycerides, alkaline phosphatase levels, lymphocyte count, neutrophil count, smoking status, body mass index, and random glucose levels (209). In addition, machine learning has been used identify different subsets of schizophrenia based on their inflammatory profiles. The study identified 5 distinct groups namely, low Inflammation, elevated CRP, elevated IL-6/IL-8, elevated IFN-γ, and elevated IL-10 (244). Further research is needed to see whether these groups translate into clinically distinct categories of illness and are able to predict treatment resistance or response to clozapine.

Potential immunological side effects of clozapine – clinical implications

Clozapine's many immunological side effects again add weight to the theory that clozapine is acting as an immunomodulatory drug in TRS. Whilst some of these effects are transient and are thought due to a cytokine storm on clozapine initiation (102),

serious adverse events can occur with clozapine, such as agranulocytosis, myocarditis, cardiomyopathy and bowel pseudo-obstruction, which require careful management. More recently evidence has emerged of significant secondary immunodeficiency related to clozapine treatment (114,115), and of increased risk of lymphoid malignancy (116), which are yet to fully find their way into the clinical sphere. In chapter 5 of this thesis I was able to confirm a significant reduction in immunoglobulin levels in a large cohort of patients prescribed clozapine, though the rates of clinical immunoglobulin deficiency in the sample was low. However, treatment protocols for use of clozapine, taking account of these newly identified risks, need to be developed at pace, incorporating screening both for immunodeficiency and malignancy, and appropriate interventions for example with vaccines, antibiotics, and referral to specialist services.

Refocussing early intervention teams – getting back to medicine

Since their advent, early intervention teams have been hailed as the gold standard model for delivery of services for patients with first episode psychosis, of whom a significant minority will have TRS. The early intervention model promises early recognition and treatment of psychosis, through medicines and psychological and social interventions; and their perpetuity in the climate of severe budget cuts and service restraints since the 2008 financial crisis is testament to how much they are valued by patients, families and those who commission services.

However, despite their obvious advantages of accessibility and holistic care, it could be argued that early intervention teams are falling somewhat short in the treatment of

TRS. Rates of clozapine use remain stubbornly low in early intervention teams (151,245) Teams may provide assertive outreach to manage patients with TRS, for example with intensive support and medication supervision, only for this approach to falter when the patient is stepped down to mainstream services after their commissioned time with early intervention teams has come to an end.

As knowledge of an immunological basis for TRS increases, a refocussing of early intervention teams could start to address this treatment and outcome gap for TRS. Borrowing from the treatment of a condition such as rheumatoid arthritis, services for TRS could be revolutionised if a more ambitious treatment paradigm was adopted. Patients with first episode psychosis could be rapidly assessed and stratified according to their clinical presentation and risk factors, and those recognised to be high risk of TRS could then receive urgent work-up with neuroimaging, blood and spinal fluid examination. Confirmed high risk patients could then commence clozapine whilst non confirmed received a first line antipsychotic medication, with an early switch to clozapine if they failed to respond to first line treatments as measured by both clinical and immunological markers.

Such an approach would bring psychiatry squarely back into the field of medicine, with an emphasis on investigations, staging of illness, and robust treatment paradigms. As a useful parallel, psychiatrists will have experience of managing suspected cases of anti-NMDAR encephalitis, and thus know the importance of recognising this condition quickly, so that they can receive rapid neurological assessment and instigation of immunological treatments such as plasma exchange. If we can treat first episode psychosis with high risk of TRS using a similar framework, then we will be able to truly see whether current drugs such as clozapine, or future compounds found to have

benefit in this patient group, are able to provide significant disease modification in TRS, in the way that DMDRs have revolutionised treatment of rheumatoid arthritis. Whilst planning this thesis I spoke to a rheumatologist who advised that one striking observation from his work was the change in rheumatology waiting rooms over the last two decades, as notably fewer patients now attended in a wheelchair compared to before. Perhaps the landscape of psychiatry could similarly change in the future, with far fewer patients with chronic schizophrenia either incarcerated in long term care or living very vulnerable and restricted lives in the community, though with present levels of funding into psychiatric services at least in the UK, the idea of such a transformation seems more of a pipe dream than a realistic prospect.

SUPPLEMENTARY INFORMATION

Supplementary Information for Paper 1

Table S1. Characteristics of Included Studies

Study	Sample size	Comparison	Doses	Study Duration	Blinding	Outcomes included in meta-analysis	Intention to Treat analysis	Sponsorship
Atmaca et al, 2003	56	Quetiapine Olanzapine Risperidone	Clozapine mean 207mg Quetiapine mean 535.7mg Olanzapine mean 15.7mg Risperidone mean 6.7mg	6 weeks	Single blind-raters only	PANSS	No	None
Azorin et al, 2001	273	Risperidone	Clozapine median 600mg Risperidone median 9mg	12 weeks	Double blind	BPRS, PANSS including subscales, CGI-S, response as per KANE criteria, response as per BPRS reduction greater than 20%, ADR, DLE	Yes	Novartis
Bitter et al, 2004	147	Olanzapine	Clozapine mean 216.2mg	18 weeks	Double blind	CGI – S, PANSS including subscale	Yes	Lilly

			Olanzapine mean 17.2mg			s, response as per PANSS reduction greater than 20%, ACD, DLE		
Bondolfi et al 1998	86	Risperidone	Clozapine mean 294.2mg Risperidone mean 6.4mg	8 weeks	Double blind	CGI-S, CGI – change, PANSS including subscales, response as per PANSS reduction >20%, ACD, DLE	Yes	Janssen-Cilag
Breier et al, 1999	29	Risperidone	Clozapine 200-600mg Risperidone 2-9mg	6 weeks	Double blind	BPRS including subscales, SANS, response as per BPRS reduction >20%	No attrition	Lilly
Buchanan et al 1998	75	Haloperidol	Clozapine mean 413.2mg Haloperidol mean 26mg	10 weeks	Double blind	BPRS including subscales, SANS, ACD, DLE	Yes	Sandoz
Chiu et al, 1976	64	Chlorpromazine	Clozapine 300mg Chlorpromazine 300g	6 weeks	Double blind	ACD, DLE Other outcomes cannot be used as were just measured on 9 matched	No	Sandoz

						pairs of study completers		
Claghorn et al, 1987	151 (125 in Honigfels ITT analysis	chlorpromazine	Clozapine mean 417mg Chlorpromazine mean 795mg	4 weeks	Double blind	CGI change, BPRS, ACD, DLE	ITT analysis of data published subsequently by Honigfeld 1984	None
Conley et al, 1988	24	Chlorpromazine	Clozapine up to 900mg Chlorpromazine up to 1800mg	6 weeks	Single blind	BPRS, response as per BPRS reduction >20%	No attrition	Sandoz
Conley et al, 2003	13	Olanzapine	Clozapine 450mg Olanzapine 50mg	8 weeks (cross over trial)	Double blind	ACD	No	Zenith-Goldline
Edwards et al, 2011	48 (25 in meta-analysis)	Thioridazine Thioridazine +CBT Clozapine +CBT	Clozapine mean 315.15mg Thioridazine mean 268.65mg	12 weeks	Single blind	BPRS positive symptoms score, SANS, CGI-S, response as per BPRS mild on each item plus CGI mild or less	Yes	Novartis
Ekblom et al, 1974	41	Chlorpromazine	Clozapine range 279-338mg Chlorpromazine range 320-410mg	6 weeks	Double blind	No usable data	No attrition	Sandoz

Fischer-Cornelsen et al, 1976	223	Chlorpromazine	Clozapine median 300mg Chlorpromazine median 350mg	6 weeks	Double blind	ACD	No	Sandoz
Gelenberg et al, 1979	15	Chlorpromazine	Clozapine mean 279mg Chlorpromazine mean 606mg	4 weeks	Double blind	No usable data	No	Sandoz
Gerlach et al, 1974	20	Haloperidol	Clozapine median 200mg Haloperidol median 10mg	12 weeks	Single blind – raters only	BPRS	No attrition	Sandoz
Ghaleiha et al, 2011	51	Risperidone haloperidol	Clozapine 300mg Risperidone 6mg Haloperidol 15mg	8 weeks	Double blind	PANSS including subscales, response as per PANSS reduction >50%, ACD	No	None
Heinrich et al,1994	59	Risperidone 8mg Risperidone 4mg	Clozapine 400mg Risperidone 8mg Risperidone 4 mg	4 weeks	Double blind	BPRS, ACD, DLE, response as per CGI improved or very much improved	Yes	None
Hong et al, 1997	40	Chlorpromazine	Clozapine mean 543mg Chlorpromazine mean 1163mg	12 weeks	Double blind	BPRS, PANSS including subscales, response as per BPRS reduction	No	None

						>20%, CGI-S, ACD		
Honigfeld et al 1984	79	Haloperidol	Clozapine mean 397mg Haloperidol mean 7.6mg	6 weeks	Double blind	BPRS, ACD	Yes	Sandoz
Howanitz et al, 1999	42	Chlorpromazine	Clozapine mean 300mg Chlorpromazine mean 600mg	5 weeks	Double blind	CGI-S, PANSS including subscales	No	none
Itoh et al, 1977	88	Haloperidol	Clozapine up to 500mg Haloperidol up to 15mg	12 weeks	Double blind	BPRS, ACD	No	No
Kane et al, 1988	268	Chlorpromazine	Clozapine mean 600mg Chlorpromazine mean 1200mg	6 weeks	Double blind	BPRS including subscales, CGI-S, response as per Kane criteria, ACD	Yes	Sandoz
Kane et al, 2001	71	Haloperidol	Clozapine mean 523mg Haloperidol mean 18.9mg	29 weeks	Double blind	BPRS including subscales, CGI-S, CGI-improvement, response as per Kane criteria, ACD, DLE	Yes	Novartis
Kluge et al, 2007	30	Olanzapine	Clozapine mean 266.7mg Olanzapine mean 21.2mg	6 weeks	Double blind	BPRS including subscales, CGI-S, ACD	Yes	Lilly

Leon 1979	50	Chlorpromazine	Clozapine mean 600mg Chlorpromazine mean 600mg	6 weeks	Double blind	No usable outcomes	No attrition	Sandoz
Lewis et al, 2006	136	Other SGA	Clozapine mean 333mg Amisulpiride 683mg Olanzapine 19mg Quetiapine 525mg Risperidone 6mg	12 months	Single blind – raters only	PANSS including subscales, ACD	Yes	none
Meltzer et al, 2008	40	olanzapine	Clozapine mean 564mg Olanzapine mean 33.6mg	6 months	Double blind	CGI-S, PANSS including subscales, SANS, SAPS, GAF, response as per PANSS reduction >20%, ACD	No	Lilly
Meyer-Lindenberg et al, 1997	50	Zotepine	Clozapine up to 450mg Zotepine up to 450mg	6 weeks	Double blind	No usable outcomes – high drop-out rate-analysed results of matched pairs who completed	No	Klinge Pharma
Morescco et al, 2004	15	Olanzapine	Clozapine mean 325.4mg Olanzapine mean 18.3mg	8 weeks	Double blind	BPRS, CGI-S, PANSS including subscales, ACD	No	Lilly

Naber et al, 2005	114	Olanzapine	Clozapine mean 209 mg Olanzapine mean 16.2mg	6 months	Double blind	BPRS, CGI change, PANSS including subscales, response CGI much or very much improved, ACD DLE	Yes	Lilly
Potter et al, 1989	57 37 in meta-analysis	Chlorpromazine Clozapine plus Chlorpromazine	Clozapine up to 600mg Chlorpromazine up to 600mg	8 weeks	Double blind	BPRS	No	None
Rosenheck et al 1997	423	Haloperidol	Clozapine mean 552mg Haloperidol mean 28mg	12 months	Double blind	Response as per PANSS reduction >20%, ACD, DLE	Yes	Sandoz
Sacchetti et al 2009	147	Ziprasidone	Clozapine mean 346mg Ziprasidone mean 130mg	18 weeks	Double blind	PANSS including subscales, CGI-S, response as per PANSS reduction >20%, ACD, DLE	Yes	Pfizer
Salganik et al 1998	10	Haloperidol	Clozapine mean 120mg Haloperidol mean 18mg	10 weeks	Double blind cross over	No usable results	No	None
Schooler et al 2016	107	Risperidone	Clozapine mean 456.7mg	6 months	Double blind	BPRS including subscale	No	Lilly

			Risperidone mean 6.8mg			s, CI-S, CGI-I, response as per BPRS reduction >20%, ACD, DLE		
Shopsin et al 1979	3125 in meta-analysis	Chlorpromazine Placebo	Clozapine up to 900mg Chlorpromazine up to 1600mg	5 weeks	Double blind	CGI – much or very much improved, unable to use any other results	No attrition	'drug company monitor'
Tollefson et al 2001	180	Olanzapine	Clozapine mean 303.6mg Olanzapine mean 20.5mg	18 weeks	Double blind	BPRS, CGI-S, PANSS including subscales, response as per PANSS reduction >20%, ACD, DLE	Yes	Lilly
Volavka et al 2002	157	Olanzapine Risperidone Haloperidol	Clozapine mean 526.6mg Olanzapine mean 30.4mg Risperidone mean 11.6mg Haloperidol mean 25.7g	3 months	Double blind	PANSS including subscales, ACD, DLE	Yes	Multiple drug companies
Wahlbeck et al 2000	19	Risperidone	Clozapine mean 385mg Risperidone mean 7.8mg	10 weeks	Double blind	PANSS including subscales, response as per PANSS	Yes	no

						reduction >20%, CGI-S, ACD, DLE		

Table S2. Cochrane Risk of Bias Tool for included studies

Study	Sequence Generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessors	Incomplete outcome data	Selective Outcome reporting	Other sources of bias
Atmaca et al, 2003	Unclear No details given	Unclear No details given	Participants were not blinded	Unclear No details given	Low All but 3 completed study, one dropped out from each patient group	Low All planned outcomes reported	Unclear Primary aim of study was to assess change in leptin and tricglyceride levels
Azorin et al, 2001	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Low Attrition rate 26% and similar in both groups	Low All planned outcomes reported	Low
Bitter et al, 2004	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Low Low attrition 7/147 ITT analysis using LOCF	Unclear Some PANSS subscales not reported	Low
Bondolfi et al, 1998	Unclear No details given	Unclear No details given	Low 'medications were started according to a double blind, double dummy protocol'	Low 'medications were started according to a double blind, double dummy protocol'	Low Attrition equal in both groups (21%) Reasons for attrition reported ITT analysis using LOCF	Unclear One outcome measure (UKU) not reported	Low

Breier et al, 1999	Unclear No details given	Unclear No details given	Unclear No details given	Low 'Symptom ratings were conducted by blinded psychiatrists'	Low No attrition	Low All planned outcomes reported	Low Low
Buchanan et al, 1998	Unclear No details given	Unclear No details given	Unclear Anticholinergic or placebo were given as part of study design	Low 'All raters were blind except side effects and vital sgns were ascertained by a non-blind research nurse'	Low 8/38 clozapine and 3/37 risperidone patients did not complete, reasons for drop outs given No statistical difference between those who competed and those who dropped out ITT analysis carried out	Low All planned outcomes reported	Low
Chiu et al, 1976	Unclear No details given	Unclear No details given	Low 'Identical capsules continuing 50mg or 100mg clozapine or chlorpromazine were prepared'	High Reporters were blind but no further details given, Dosage regime led to high rates of sedation in clozapine group with 4 withdrawing in first	High High withdrawal rate 33.3% of clozapine patients 54.8% chlorpromazine Reasons for withdrawals were given	Low Planned outcomes were reported but just on matched pairs of completers	High Analysis of matched pairs of completers only which comprised 18 out of original 64 patients Clozapine dose restricted to maximum

				48 hours therefore likely to have affected the blind			dose of 300mg
Clagho rn et al, 1987	Unclear No details given	Unclear No details given	Low 'Medications were identical in appearance and packaging'	Unclear Marked difference between the two groups in side effects reported	High High attrition rate (48%) No ITT analysis performed	Low All planned outcom es reported	High Study reported as an 8 week study but after 4 weeks participan ts voluntaril y continued if they had has a therapeut ic effect
Conley et al, 1988	Unclear No details given	Unclear No details given	Low 'medications administered in identical unmarked blue capsules' Use of benztropine and placebo	Low 'medicatio ns administer ed in identical unmarked blue capsules' use of benztropin e and placebo	Low No attrition	Unclear One planned outcom e (NOSIE) not reported	Low
Conley et al, 2003	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Unclear Attrition in first arm 23 % olanzapin e, 0% clozapine	Low All planned outcom es reported	Unclear Small study of 13 particpan ts
Edward s et al, 2011	Unclear No details given	Unclear No details given	Single blind trial involving psychotherap y as well as medication in two arms	Unclear No details given	Unclear 3 patients were not compliant – does not report	Low All planned outcom es reported	Unclear Study ran over 5 years

					which group. Missing data handled by multiple imputation		
Ekblom et al, 1974	Unclear No details given	Low 'on breaking the code it was observed that 20 patients had been treated with clozapine and 21 with chlorpromazine'	Low 'drugs were given in identical capsules'	Low 'blind final subjective evaluation'	Low 3 clozapine patients and 2 chlorpromazine patients dropped out Reasons for drop outs given	Low All planned outcomes reported	Low
Fischer-Cornelsen et al, 1976	Unclear No details given	Unclear No details given	Low 'identical capsules given in a fixed –flexible – fixed schedule'	Unclear No details given	Low Attrition rate similar in both arms approx 10%	Low All planned outcomes reported	Low
Gelenberg et al, 1979	Unclear No details given	Unclear No details given	Unclear no details given	Unclear No details given	High Study terminated early Clozapine patients were in the study over twice as long	Low All planned outcomes reported	High Trial length was not fixed – states 4 to 8 weeks
Gerlach et al, 1974	Unclear No details given	Unclear No details given	Single blind	Low 'Evaluation of therapeutic effect and registration of side effects	Low No attrition	Low All planned outcomes reported	Low

				were carried out by different persons, in this way it was possible to perform a blind evaluation of the therapeutic effect'			
Ghaleiha et al, 2011	Low 'randomised to receive haloperidol or clozapine or risperidone in a 1:1:1 ration using a computer generated code	Unclear No details given	Unclear No details given	Unclear No details given	Low Low attrition rate 1 patient from each group	Low All planned outcomes reported	Unclear Primary aim of study was to correlate adenosinergic activity with drug efficacy
Hong et al, 1997	Low 'randomisation accomplished by using a table of random numbers'	Unclear No details given	Low 'both medications were identical in appearance and packaged uniformly'	Unclear No details given	Unclear 2 patients dropped out of clozapine arm early were not included in analysis 2 patients dropped out late from chlorpromazine arm were included	Low All planned outcomes reported	Low
Honigfeld et al, 1984	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Low Attrition rate 38%	Unclear Planned outcom	unclear methodology

					haloperidol, 21% clozapine, reasons for attrition reported, ITT analysis performed	es not clearly reported	reported elsewhere (not available in English language)
Howanitz et al, 1999	Unclear No details given	Unclear No details given	Low 'Drugs packaged in identical capsules' 'psychiatrist who was blinded to the patients treatment status administered baseline PANSS and CGI' Chlorpromazine chosen as comparison due to similar side effect profile	Unclear Does not state who conducted outcome measures	High Results reported for patients who completed at least 5 weeks of stable dose medication, rather than those who completed whole study Reasons for drop outs not accurately recorded	Unclear One planned outcome (AIMS) not reported	High Study was designed to last 12 weeks including titration phase but analysis was based on patients who received at least 5 weeks of stable treatment
Itoh et al, 1977	Unclear No details given	Unclear No details given	Low 'those physicians who had conducted the trial or those related to the pharmaceutical company were excluded from the controllers who coded the double blind trial and supervised	Low 'those physicians who had conducted the trial or those related to the pharmaceutical company were excluded from the controllers who coded the	Low Low attrition rate 4/47 clozapine 0/41 haloperidol Reasons for attrition given	Unclear One planned outcome (behavioural rating scale) was not reported	High No baseline demographic data

			the entire experiment'	double blind trial and supervised the entire experiment'			
Kane et al, 1988	Unclear No details given	Unclear No details given	Low 'all medications coded and administered under double blind conditions' Use of benztropine plus placebo Choice of chlorpromazine as comparator	Low 'Nursing staff blind to treatment assignment'	Low Low attrition rate 12% clozapine 13% chlorpromazine Reasons for drop outs given ITT analysis using LOCF	Low All planned outcomes reported	Low
Kane et al, 2001	Low 'computer generated block randomisation'	Low 'sealed envelopes with treatment assignment'	Low 'Identical capsules' Use of benztropine / placebo All patients had weekly blood count	Low 'Outcomes assessed by research psychiatrists'	Unclear High attrition rate 66.7% haloperidol 35.1% clozapine ITT analysis performed	Low All planned outcomes reported	Low
Kluge et al, 2007	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Low Low attrition rate 3/15 clozapine 1/15 olanzapine Reasons for attrition given ITT analysis	Low All planned outcomes reported	Unclear Primary outcomes for study were food craving, binge eating and BMI Does escalation faster with

					using LOCF		olanzapine
Leon 1979	Unclear No details given	Unclear No details given	Low 'Drugs provided in capsules of identical appearance'	Unclear No details given	Low No attrition	Low Planned outcome reported	Unclear Mistake in study and doses of clozapine were double planned doses
Lewis et al, 2006	Low 'method of allocation was randomised, permuted bocks'	Low 'Randomisation was undertaken by a remote telephone service'	Single blind	Low 'Outcomes were assessed blind to treatment allocation. Measures were taken to protect the blind, and cases where blind was broken were reported'	Low Follow up interview rate 87% Study Reasons for attrition given	Low All planned outcomes reported	low
Meltzer et al, 2008	Low 'Randomly assigned using a previously generation randomisation list for each site'	Unclear No details given	Low 'Study medications were packaged by an off site pharmacist according to batch numbers that corresponded to patient id codes' All patients had blood count monitored weekly Double dummy	low	Unclear High attrition rate 40% Reasons for attrition given No difference between the 2 groups in discontinuation	Low All planned outcomes reported	low

			method was used				
Meyer-Lindenberg et al, 1997	Unclear No details given	Unclear No details given	Unclear 'double blind design'	Unclear Initial dose of 150mg in both drugs would likely have caused significant side effects in clozapine group	High 10/25 zotepine and 7/25 clozapine dropped out ITT analysis not done	High Analysis of a subset only	High Changed study design and used a subsample of matched pairs of 26 patients who completed the study
Morescso et al, 2004	Unclear No details given	Low 'Randomisation was blind to personnel at study site, expect in emergency in which case blind code was broken'	Low'Randomisation was blind to personnel at study site, expect in emergency in which case blind code was broken'	Unclear No details given	High High attrition rate 2/11 olanzapine 6/12 clozapine ITT analysis not done	Low All planned outcomes reported	Unclear Primary outcomes were D2 and 5HT2 receptor occupancy
Naber et al, 2004	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Unclear High attrition rate 36/57 olanzapine 35/57 clozapine ITT analysis performed using LOCF	Low All planned outcomes reported	Low
Potter et al, 1989	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Low No attrition reported	Low All planned outcom	High Unclear treatment histories of

						es reported	participants Unclear if baseline differences in illness severity are significant
Rosenheck et al 1997	Unclear No details given	Unclear No details given	Low Both groups had blood tests and benztropine or placebo	Unclear No details given	Unclear Large attrition rate 40% discontinued clozapine 72% discontinued haloperidol ITT analysis performed	Low All planned outcomes reported	low
Sacchetti et al, 2009	Unclear No details given	Low 'Randomisation took place on a centralised basis'	Unclear States double blind, double dummy but no details given	Unclear No details given	Low 38.4% discontinued in both groups Reasons for drop outs reported	Low All planned outcomes reported	Unclear Short timescale to last exposure with depot
Salganik et al 1998	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	High attrition 41% in small study	High Results not reported Just states no significant differences	Low

Schooler et al 2016	Unclear No details given	Unclear No details given	Low 'medication was administered under double blind double dummy design as matching tablets for clozapine and risperidone were not available' Weekly blood tests Gradual titration	Low	Unclear 47% clozapine and 29.6% risperidone discontinued Not ITT analysis	Low Planned outcomes reported	Unclear Long delay before publication Differences between sites in speed of initial titration
Shopsin et al 1979	Unclear No details given	Unclear No details reported	Low 'medications were dispensed in capsules that were indentical in size, shape and colour'	Low 'all staff involved in ratings were unaware of the medications used'	Low No attrition	Low All planned outcomes reported	Unclear Had additional placebo arm which was prematurely terminated
Tollefson et al 2001	Unclear No details given	Unclear No details given	Unclear No details given	Unclear No details given	Unclear High attrition rate clozapine 41.1%, olanzapine 40%, reasons for discontinuation reported, ITT analysis	Low All planned outcomes reported	Unclear Study did not get planned number of participants Reduced sample size was unmasked before blinding
Volavka et al 2002	Unclear No details given	Unclear No details given	Low Weekly bloods for all patients	Low 'raters blind to treatment group	Unclear 42% drop out rate	Low Planned outcomes reported	Unclear Originally a 3 arm study, olanzapin

			‘all tablets looked alike’ ‘psychiatrists blind to treatment group assignment could change the doses by prescribing various levels of medication’ Benztropine or placebo used	performed all clinical research assessments’	ITT analysis performed		e arm subsequently added in
Wahlbeck et al 2000	Low ‘Computer generated randomisation’	Low ‘after receiving consent, treating physician contacted senior investigator who provided allocation information’	Unclear No details given	Low ‘independent assessor was blind to treatment to ensure blindness’	Unclear High attrition rate in small study 50% clozapine 11% risperidone ITT analysis performed	Low Al planned outcomes reported	Low

Table S3 Effect of clozapine versus alternative antipsychotics on secondary outcomes

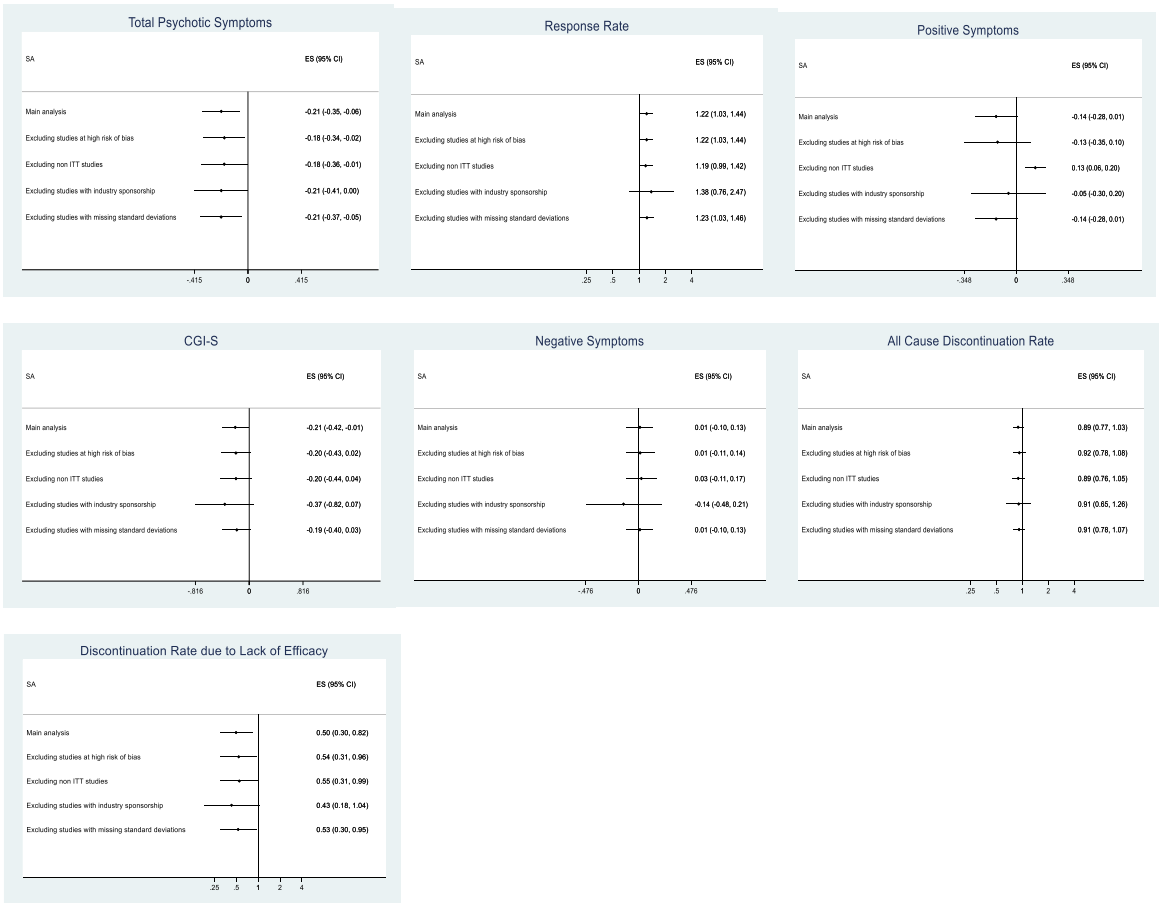
Outcome measure	Effect size	Confidence intervals	I^2	Significant
CGI-S	Smd = -0.21	-0.42 - -0.01	70.9%	yes
Positive symptoms	Smd = -0.13	-0.28 – 0.01	643.9%	No
Negative symptoms	Smd = 0.01	-0.11 – 0.13	17.5%	No
All cause discontinuation (ACD)	RR = 0.89	0.77-1.03	41.4%	No
Discontinuation due to lack of efficacy (DLE)	RR = 0.5	0.3-0.83	60.6%	yes

Table S4. Multiple linear regression of interaction between age and treatment arm on change in BPRS scores from studies reporting individual patient data

	Hong et al 1997 n = 38 Adj R^2 = 0.41			Wahlbeck et al 2000 n = 19 Adj R^2 = 0.40		
Change in BPRS* total score	Regression co-efficient	p-value	95% Confidence interval	Regression co-efficient	p-value	95% Confidence interval
Clozapine / comparator drug	-29.02	0.00	-44.96 - -13.10	7.55	0.53	-17.70 – 32.8
Duration of hospitalisation	-1.30	0.00	-2.08 - -0.52	-0.07	0.17	-0.16 – 0.03
Drug / duration of hospitalisation interaction	0.15	0.02	0.03 – 0.27	0.04	0.56	-0.09 – 0/17

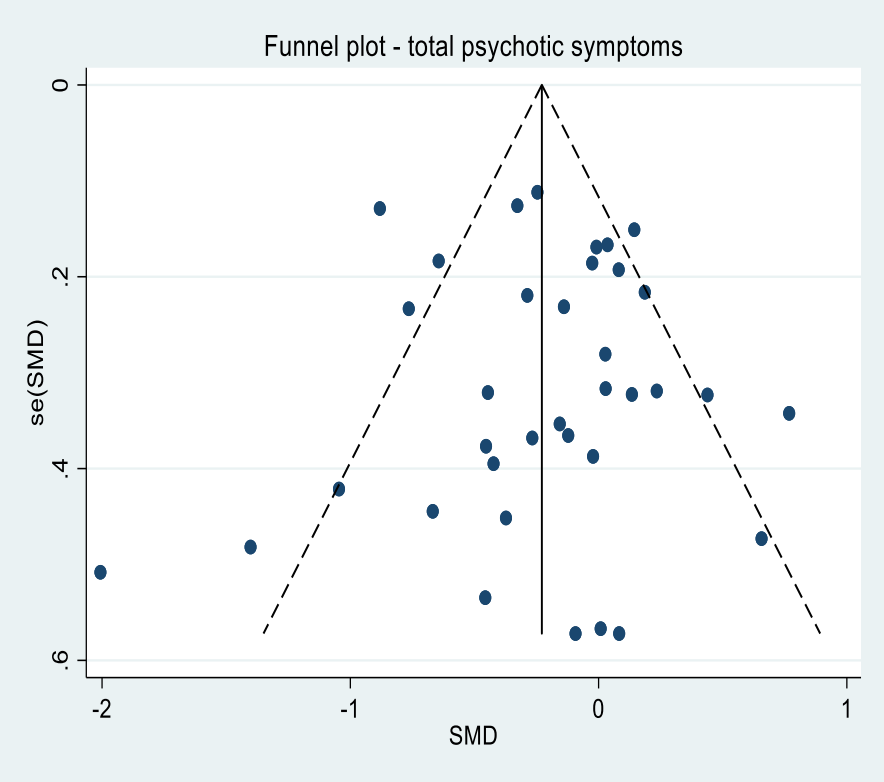
*BPRS –Brief psychiatric rating scale

Figure S1. Results of planned sensitivity analyses for primary and secondary outcomes



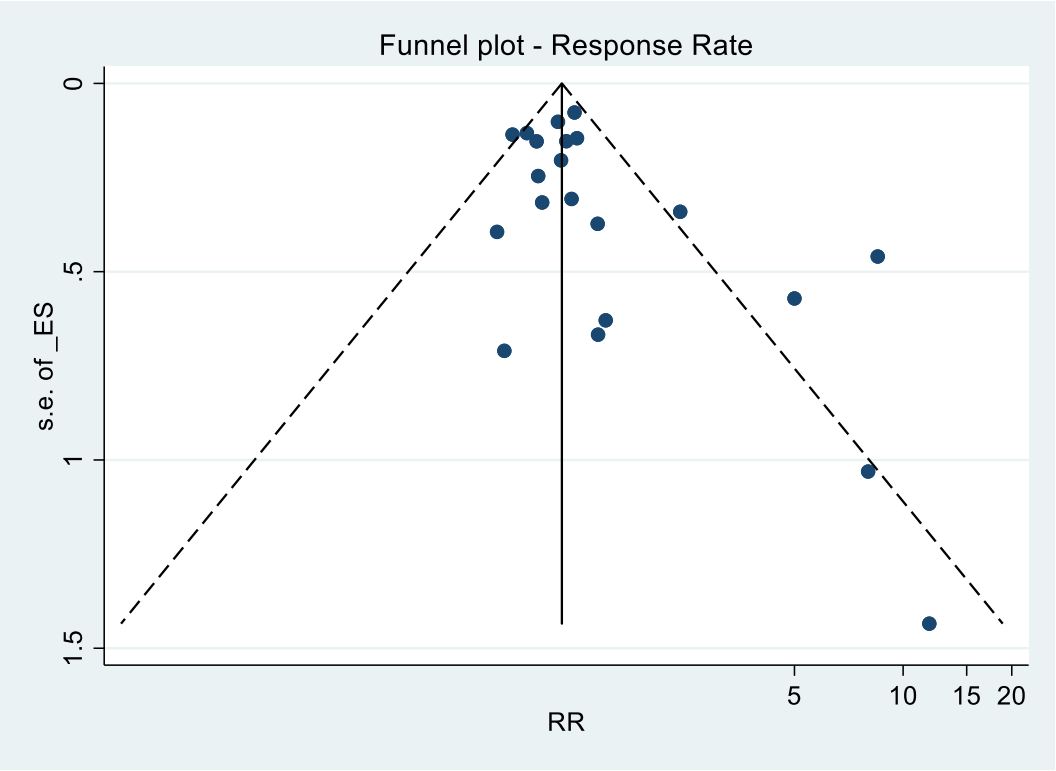
SA sensitivity analysis, CGI – S . Clinical Global impression – severity scale, SMD – standardised mean difference

Figure S2a Funnel plot – total psychotic symptoms



SMD – standardized mean difference

Figure S2b Funnel plot – response rate



RR – response rate

Supplementary information for Paper 2

Unpublished data

Table U1 unpublished data – Linear regression of duration of prior illness and total number of psychiatric bed nights over 2 year study period, adjusted for sex, ethnicity, co-morbid substance use, deprivation score and presence of a restriction order (n = 661)

	Coefficient	p-value	95% Confidence Interval
Duration of illness	0.74	0.45	-1.15 – 2.63
Deprivation score	-1.93	0.02	-3.51 - -0.36
Substance disorder	38.49	0.11	-8.29 - 85.28
Restriction order	337.71	0.00	274.75 – 400.68
Sex	25.84	0.16	-9.76 – 61.45
Ethnicity White (reference group) Black Caribbean Black other Mixed other	 21.98 35.92 -14.27	 0.47 0.07 0.58	 -37.04 – 80.99 -3.06 – 74.89 -64.87 – 36.34

Table U2 unpublished data – Linear regression of duration of prior illness and total number of psychiatric bed nights over 2 year study period plus additional 2 years follow up, adjusted for sex, ethnicity, co-morbid substance use, deprivation score and presence of a restriction order (n = 661)

	Coefficient	p-value	95% Confidence Interval
Duration of illness	1.57	0.25	-1.11 – 4.26
Deprivation score	-1.36	0.23	-3.59 – 0.87
Substance disorder	58.40	0.08	-7.86 – 124.66
Restriction order	561.16	0.00	471.98 – 650.34
Sex	31.26	0.22	-19.17 – 81.69
Ethnicity White (reference group) Black Caribbean Black other Mixed other	 26.02 24.91 -9.04	 0.54 0.38 0.80	 -57.57 – 109.60 -30.29 – 80.10 -80.71 – 62.62

Published data

Table S1. Model B. Illness duration as a categorical variable

Ordinal logistic regression of illness duration prior to clozapine and CGI-S outcome scores adjusted for age at illness onset, deprivation score, gender, co-morbid substance disorder, ethnicity, clozapine start date and medical admissions during follow up.

Indicator variables	Categories	Unadjusted OR	OR adjusted for CGI-S start score	Fully adjusted OR
Duration of illness prior to clozapine				
	15 years +		Ref	Ref
	10 – 15 years		1.08 (0.62 – 1.90)	0.95 (0.53 – 1.70)
	8 – 10 years		1.25 (0.64 – 2.42)	0.98 (0.49 – 1.94)
	6 – 8 years		0.74 (0.39 – 1.41)	0.67 (0.34 – 1.30)
	4 – 6 years		0.77 (0.40 – 1.49)	0.64 (0.32 – 1.26)
	2 – 4 years		0.59 (0.33 – 1.07)	0.52 (0.28 – 0.97) *
	0 – 2 years		0.87 (0.42 – 1.78)	0.84 (0.4 – 1.74)
Age at illness onset		0.98 (0.96 – 1.00)		0.99 (0.96 – 1.01)
Deprivation score		0.99 (0.97 – 1.01)		0.99 (0.97 – 1.00)
Male gender		1.51 (1.02 – 2.22) *		1.48 (0.98 – 2.25) *
Substance disorder		2.04 (1.20 – 3.48) *		1.97 (1.13 – 3.44) *
Ethnicity				
	White	Ref		Ref

	Black Caribbean	1.12 (0.58 – 2.14)	1.18 (0.60 – 2.33)
	Black other	1.34 (0.88 – 2.03)	1.53 (0.99 – 2.37)
	Mixed / other	0.77 (0.46 – 1.29)	0.94 (0.55 – 1.61)
Clozapine start date			
	1 Jan 2007 – 30 June 2009	Ref	Ref
	1 July 2009 – 31 Dec 2011	1.10 (0.65 – 1.88)	1.04 (0.60 – 1.80)
	I Jan 2012 – 30 June 2014	0.92 (0.57 – 1.49)	0.90 (0.55 – 1.49)
	1 July 2014 – 31 Dec 2016	1.02 (0.63 – 1.65)	0.90 (0.55 – 1.48)
Medical admissions			
	0	Ref	Ref
	1	1.17 (0.73 – 1.87)	1.09 (0.67 – 1.77)
	>1	2.65 (1.43 – 4.93) *	2.98 (1.58 – 5.60) *

*significant result

CGI-S score - Clinical Global Impression – severity score

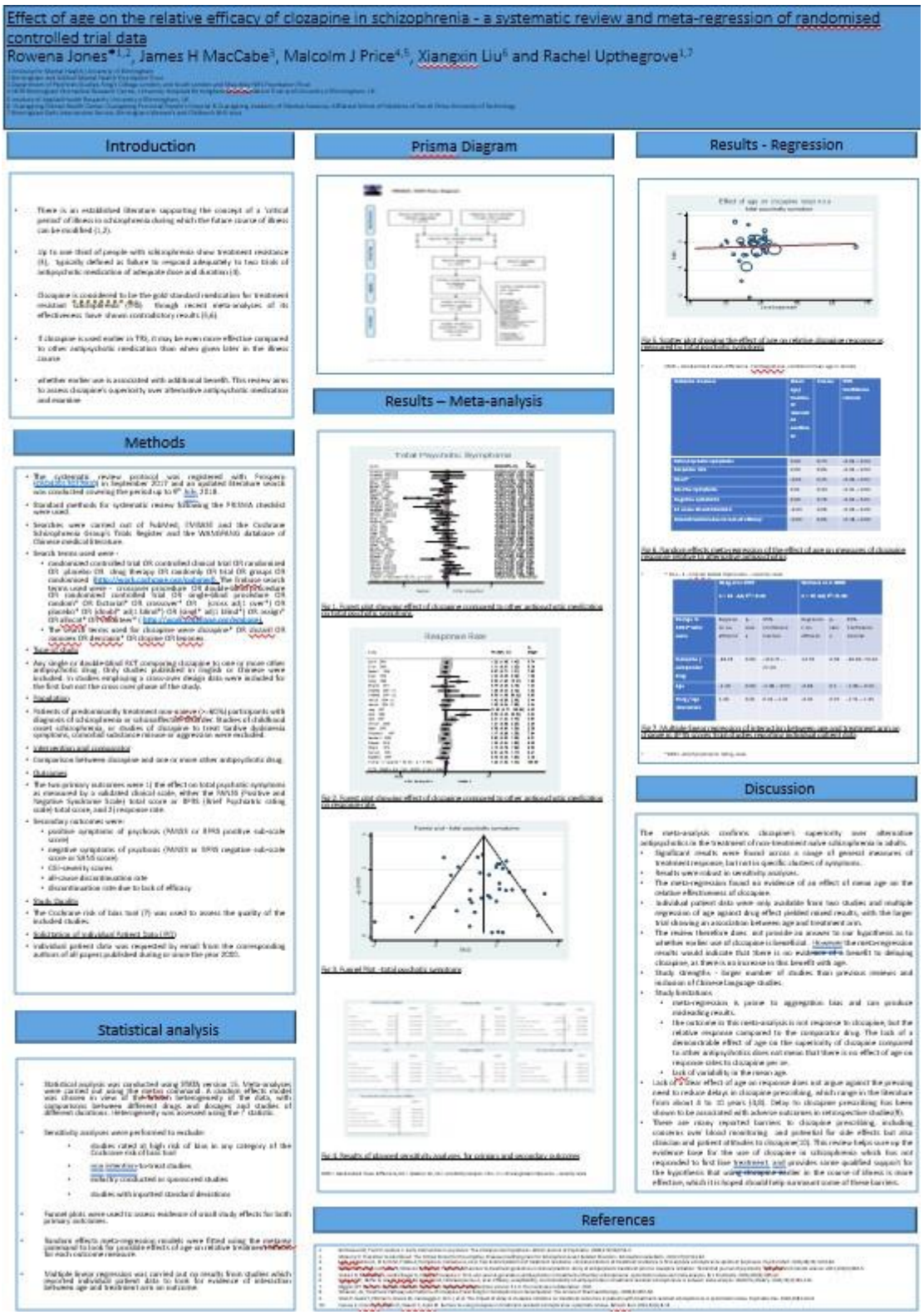
OR – odds ratio

APPENDICES

Appendix 1. CGI-S Score Guide

1 = Normal—not at all ill, symptoms of disorder not present past seven days
2 = Borderline mentally ill—subtle or suspected pathology
3 = Mildly ill—clearly established symptoms with minimal, if any, distress or difficulty in social and occupational function
4 = Moderately ill—overt symptoms causing noticeable, but modest, functional impairment or distress; symptom level may warrant medication
5 = Markedly ill—intrusive symptoms that distinctly impair social/occupational function or cause intrusive levels of distress
6 = Severely ill—disruptive pathology, behavior and function are frequently influenced by symptoms, may require assistance from others
7 = Among the most extremely ill patients—pathology drastically interferes in many life functions; may be hospitalized
Adapted from Kay SR. Positive and negative symptoms in schizophrenia: Assessment and research. Clin Exp Psychiatry Monograph No 5. Brunner/Mazel, 1991.

Appendix 2. Poster for BAP Conference June 2019



Appendix 3. Poster for SIRS conference. April 2021

Effect of duration of prior psychotic illness on clozapine response: a retrospective cohort study using electronic health records

Rowena Jones[1,2], Rachel Upthegrove[1], Malcolm J Price[3,4], Megan Pritchard[5], Sophie Legge[6] and James H MacCabe[7]

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Background

- There is evidence that early effective treatment of schizophrenia improves outcomes
- It has been hypothesized that there is a 'critical period' in schizophrenia of up to five years during which the course of future illness is set
- Clozapine is the gold standard medication for treatment resistant schizophrenia yet its use continues to be delayed beyond this period
- The aim of the study was to compare clinical outcomes of patients who started clozapine within 5 years of illness onset with patients who started clozapine after this point

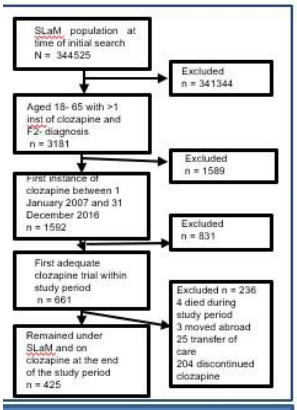

Fig.1 Flow diagram showing identification of sample

Methods

- The study was a 2 year observational study of 425 patients who had their first adequate trial of clozapine whilst under the care of South London and Maudsley (SLaM) mental health services between 1st January 2007 and 31st December 2016 and were taking clozapine at the end of the study period (see flow chart Fig.1)
- Data from electronic health records were extracted using the SLaM clinical records interactive search (CRIS) system. Dates of illness onset and clozapine commencement were manually extracted from anonymised case notes and correspondence. Clinical Global Improvement – Severity (CGI-S) scores were rated at the time of starting clozapine and at 2 years
- Ordinal logistic regression was performed using stata 15 to look for an association between illness duration prior to clozapine commencement and end CGI-S scores, adjusted for starting scores and other potential confounders

Results

- The mean duration of illness prior to clozapine was 10.33 years (SD = 8.40)
- 128 (30.12%) of patients started clozapine within 5 years
- Most patients (389 (91.53%)) remained on clozapine throughout the 2 year follow up.
- CGI-S start scores remained fairly constant across the range of illness duration . CGI-S end scores were lower during the first 5 years of illness (Fig.1)
- There was a clear effect of illness duration on CGI-end scores (Adj OR clozapine within 5 years 0.60 [95% CI 0.40, 0.89]) (Table 2)

CGI-S final score	Unadjusted OR	OR adjusted for CGI-S start score	OR fully adjusted
Cloz < 1 y		0.85 (0.26 – 2.80)	0.95 (0.28 – 3.25)
Cloz < 3 y		0.74 (0.47 – 1.17)	0.78 (0.49 – 1.25)
Cloz < 5 y		0.62 (0.43 – 0.91)	0.60 (0.40 – 0.89)
Cloz< 7 y		0.68 (0.48 – 0.97)	0.67 (0.46 – 0.96)
Cloz < 10y		0.76 (0.53 – 1.09)	0.72 (0.50 – 1.04)
			Cloz < 5y
Age at illness onset	0.99 (0.96 – 1.01)		0.99 (0.97 – 1.02)
Deprivation score	0.99 (0.98 – 1.01)		0.99 (0.97-1.00)
Male gender	1.45 (1.00 – 2.11)		1.46 (0.98 – 2.16)
Substance disorder	1.96 (1.15 – 3.34)		1.85 (1.08 – 3.18)
Ethnicity: White			
Black Carib	0.99 (0.52-1.89)		0.98 (0.50 – 1.90)
Black other	1.30 (0.87-1.96)		1.45 (0.95 – 2.21)
Mixed/ other	0.80 (0.48-1.33)		0.91 (0.54 – 1.52)
Clozapine start date			
Jan 2007 – June 2009			
July 2009 – Dec 2011	1.15 (0.68 – 1.93)		1.06 (0.62 – 1.80)
Jan 2012 – June 2014	0.97 (0.61 – 1.55)		0.94 (0.58 – 1.51)
July 2014 – Dec 2016	1.04 (0.65 – 1.67)		0.91 (0.56 – 1.48)
Medical admissions			
0			
1	1.26 (0.80 – 1.99)		1.19 (0.75 – 1.89)
>1	2.95 (1.62 – 4.58)		3.07 (1.68 – 5.64)

Table.2 Effect of starting clozapine within 1,3,5,7, and 10 y of first psychotic episode on final CGI-S scores

Discussion

- The study demonstrated a significant relationship between duration of psychotic illness and response to clozapine
- The effect size was substantial particularly when an illness cut-off of 5 years was used, with final CGI-S scores nearly twice as likely to be lower when clozapine was commenced within 5 years of diagnosis
- A key strength of the study is that it is a representative patient sample i.e. ratings are from anonymised clinical records. Other strengths are its large size relative to previous studies and the use of direct clinical ratings
- Limitations include a number of possible biases, however treatment by indication bias would likely act in the opposite direction to the effect seen and a range of possible confounders were included in the analysis
- The results provide support to the concept of a critical period in schizophrenia, during which clozapine can exert an optimum effect

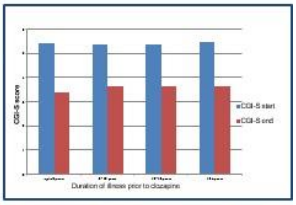

Fig.2 CGI-S start and end scores by duration of illness prior to clozapine

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