Pharmacology and toxicology

Clozapine, agranulocytosis, and benign ethnic neutropenia

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Current knowledge and clinical implications

Clozapine is an atypical antipsychotic that is effective in treatment resistant schizophrenia.[1] The National Institute for Health and Clinical Excellence (NICE) guidelines for schizophrenia specify that "in individuals with evidence of treatment resistant schizophrenia, clozapine should be introduced at the earliest opportunity".[2]

A severe adverse effect of clozapine that limits its more widespread use is agranulocytosis. Patients who are taking clozapine need to have their full blood counts (FBC) monitored regularly, and if the total white cell and/or neutrophil counts indicate agranulocytosis, clozapine prescription must be terminated. Among certain ethnic groups, a significant proportion of people have a low baseline neutrophil count. This is called benign ethnic neutropenia (BEN). This editorial looks at the important issues associated with agranulocytosis and BEN in patients receiving clozapine.

CLOZAPINE AND AGRANULOCYTOSIS

Agranulocytosis occurs in about 1% of patients taking clozapine.[3 4] Neutropenia is seen in about 3%.[4] The risk of both agranulocytosis and neutropenia is highest between 6 weeks and 18 weeks after starting clozapine treatment.[4] Hence, in the United Kingdom and Ireland, weekly FBC monitoring is mandatory for the first 18 weeks, after which it is done fortnightly until the end of the first year, and every four weeks thereafter. In the USA, FBC is monitored weekly for the first six months and fortnightly thereafter.

Not all risk factors are the same for agranulocytosis and neutropenia; this implies that there may be distinct mechanisms for the two disorders. A low baseline white cell count has been associated with future neutropenia but not agranulocytosis.[5] The risk of agranulocytosis increases with age,[3 6] while that of neutropenia decreases with age.[6] Agranulocytosis is more common in women.[3] It is more than twice as frequent in Asians as in the white population.[6] Neutropenia, but not agranulocytosis, is more common in black people.[6] A white cell count spike of 15% or more above the immediately preceding measurement may predict agranulocytosis within the next 75 days.[7] However, as these differences between the risk factors for agranulocytosis and neutropenia have been extrapolated primarily from epidemiological studies, they may be subject to change as further evidence, from even larger studies, come to light.

The exact mechanism of clozapine induced agranulocytosis is unclear. It has been postulated that clozapine is metabolised to a nitrenium ion.[8] The binding of this ion to neutrophils may result in agranulocytosis. Antineutrophil antibodies may be involved in mediating agranulocytosis.[9] Some human leucocyte antigen (HLA) alleles, for example the HLA B38 phenotype in Ashkenazi Jews,[10] have been shown to be associated with clozapine induced agranulocytosis.

OTHER HAEMATOLOGICAL ABNORMALITIES

Clozapine is associated with increased risk of eosinophilia, particularly in women.[11] Eosinophilia typically occurs between weeks 3 and 5 of treatment and resolves spontaneously without need for specific treatment. Clozapine is also associated with anaemia, lymphopenia, leucocytosis, and thrombocytopenia.[8]

BENIGN ETHNIC NEUTROPENIA

BEN has been defined as "the occurrence of neutropenia, defined by normative data in white populations, in individuals of other ethnic groups who are otherwise healthy and who do not have repeated or severe infections".[12] About 25% to 50% of Africans and some ethnic groups in the Middle East, including Yemenite Jews and Jordanians, have BEN.[12 13] BEN has only been reported in ethnic groups that have tanned or dark skin.[13] Subjects with BEN do not show increased incidence of infections, and their response to infections is similar to those without BEN.[13]

CLINICAL IMPLICATIONS

In the United Kingdom and Ireland, the Clozaril patient monitoring service (CPMS) supervises the prescribing of clozapine and the haematological testing (Clozaril is the brand name of clozapine). The CPMS uses a lower cut off point for patients with BEN than for the general population (table 1). A "green" alert indicates satisfactory count, an "amber" alert requires a repeat FBC test while clozapine can be continued, and a "red" alert warrants immediate cessation of clozapine.

It is important for eligible subjects to be registered with the CPMS under the BEN category, so that patients belonging to certain ethnic groups do not have to stop clozapine unnecessarily. This has great clinical ramifications, as there is no other antipsychotic that has comparable efficacy to clozapine in the treatment of resistant schizophrenia. In addition, there is evidence that some ethnic groups, particularly black people, may be less likely, even in the first place, to be prescribed clozapine.[14] These factors may combine to further worsen the prognosis of an already severely debilitating illness in this group of patients.

As clozapine induced agranulocytosis is an idiosyncratic reaction,[8] it is difficult to predict and to identify high risk patients. Also, as it is a comparatively rare phenomenon occurring in less than 1% of subjects, the number of reported cases is not adequate to clearly identify specific risk factors; general risk factors such as increasing age, female sex, etc, are not robust enough to change decision making in individual patients. Therefore, clinicians should continue to remain vigilant against this potentially fatal side effect of clozapine in all the patients prescribed this drug, especially in the first few months of treatment.

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Table 1 CPMS alert ranges for subjects with BEN (ranges for non-BEN subjects)

Alert colour	WCC $\times 10^9$/l	Neutrophils $\times 10^9$/l
Green	>3.0 (>3.5)	>1.5 (>2.0)
Amber	2.5–3.0 (3.0–3.5)	1.0–1.5 (1.5–2.0)
Red	<2.5 (<3.0)	<1.0 (<1.5)

CPMS, Clozaril patient monitoring service; BEN, benign ethnic neutropenia; WCC, white cell count.

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Single subject design

Should the single subject design be regarded as a valid alternative to the randomised controlled trial?

R G Newcombe

For debate.

In an accompanying article Janine Janosky sets out the case for the use of single subject designs.[1] I was asked by my colleague Dr John Mayberry, the editor of the journal, to referee this paper, but felt it would be more appropriate to respond to it, largely to stimulate debate on this issue. I would suggest that the proper applicability of single subject designs is much narrower than this article would imply. I would furthermore warn readers of the dangers of a view that if left to grow unchecked could result in an important undermining of the dominance of the multi-patient randomised clinical trial that is now, with very strong justification, accepted as the cornerstone of evidence based clinical practice—with serious consequences for the choice of appropriate management for future patients. The two key issues are equivocation regarding the ambit of the single subject design, and the robustness of the inference to be drawn from data such as figure 1 in Janosky's paper.

It is well accepted that clinical expertise is needed to apply the findings of large clinical trials to the individual patient. The doctor's initial training, ongoing CPD, and clinical experience facilitate the recognition of patients who are not ''average'' and for whom current evidence based guidelines, which are optimised for patient populations, may not be optimal. How to decide on management for a specific patient may be problematic. When the issue relates to maintenance treatment, the single patient design certainly has a role. For example, the patient may have two coexisting conditions for which the therapeutic requirements conflict. Another context is polypharmacy—perhaps the patient is currently taking four drugs, and the clinician suspects that one could be withdrawn without diminution of therapeutic effect.

In some parts of the article, including the six listed ''possible research questions'', Dr Janosky clearly implies that the research issue only applies to a specific patient. In other places, a broader scope is implied by phrases such as ''unique study populations'', ''choosing the patient to participate'', and ''typical in terms of the practice demographics (and) for disease presentation and progression''. Dr Janosky concedes that there is an issue of limited generalisability. I would argue that a study of this kind cannot provide any reassurance that we can extrapolate the findings to other patients. One could say, to other similar patients, but what does similar mean in this context? Demographic, physiological, and diagnostic similarity are of little relevance here, the only similarity that matters relates to propensity to respond to the treatment in question, and this can neither be observed nor ensured. Conversely, the conventional large clinical trial relates to patients drawn from a population defined by well defined eligibility criteria, and random allocation ensures groups are comparable within limits of chance variation in respect of all possible variables, including counterfactual treatment response. This is what justifies applying the conclusions of the trial to patients at large who fulfil the eligibility criteria used in the trial.

The other key issue relates to drawing an ''obvious'' conclusion from a limited dataset. This is shaky on two counts, relating to clinical lability and statistical methodology. Dr Janosky refers to the patient ''in need of lower fasting blood glucose values''—but there is such a thing as regression towards the mean (strictly, a misnomer, regression towards the mode would be a more apt description). The inference that the ''switch'' in figure 1 is real is strongly dependent on a presupposition that patients don't just ''switch'' spontaneously in this way. Perhaps this is reasonable in diabetes—it would not be for remitting/relapsing conditions such as inflammatory bowel disease or multiple sclerosis, and certainly not for thyroid disease or bipolar disorder. What Dr Janosky terms the ''primary A-B single subject design'', as used here,

is particularly vulnerable to criticism—while it is the simplest within subject design, it is the least adequately controlled, and effective blinding is unlikely to be achieved. The methodological issues arising in the familiar multi-subject crossover design are well known. Borrowing terminology commonly used in that context, the observed treatment difference could equally be interpreted as a period effect, or could be distorted by carry-over. In the example given, the treatment difference could be considerably confounded by seasonal differences.

Furthermore, with regard to statistical methodology, what is the implied cut off between a "real" difference and one that could be attributable to chance? For the data as shown, an unpaired two sample *t* test would give a highly significant p value, around 0.0001 here, but we do not have sufficient evidence to decide whether an assumption of Gaussian distributional form is reasonable, without considerable extrapolation from data on others. The non-parametric Mann-Whitney test is robust, and yields a two sided exact p value of 1 in 35 or 0.029. This is below 0.05, but much less extreme, normally a p value of this magnitude would not be regarded as strongly convincing. A decision rule approach is more relevant. This might relate to a pre-agreed clinically importantly large difference—although this would share the non-robustness problem. Alternatively, one could abandon conventional hypothesis testing with a low α level and opt for a "pick the winner" approach with implied equal α and β rates. This corresponds more closely to the less formalised "trial and error" course of events that commonly occurs in clinical practice.

Dr Janosky's stance contrasts quite sharply with that taken by Guyatt *et al*.[2] This highly informative review of the appropriate use of single subject trials was restricted to double blind, randomised, multiple crossover designs aiming to optimise management of a specific patient. Decisions about efficacy were based on a combination of a signed standardised difference measure of effect size D and a single tailed p value. Although my reservation above concerning unquestioned use of parametric methods still applies. Furthermore, an effect size criterion expressed in absolute terms (for example, fasting blood glucose units) would be much more directly interpretable for clinical importance than a relative measure such as D.

Clinicians are forever "trying" patients with different treatments. Use of a single subject design, with additional rigour ensured by multiple periods, randomisation of treatments to periods and blinding, and perhaps some statistical analysis, is certainly one stage more formal and rigorous. But we should not imagine it is anything more than that: we can only validly draw conclusions about that one patient, in their present state, it would be very risky to extrapolate to ostensibly "similar" cases.

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Single subject design

Single subject trials in primary care

N A Francis

Lack of generalisability limits use.

From a GP's perspective, the accompanying article by Janine Janosky on the single subject design is both interesting and stimulating.[1] This type of design, as Dr Janosky highlights, is infrequently used in research and has some potential advantages. Most notably, it is the only type of design that can provide information about effects at an individual level. There are obvious benefits in formalising what all GPs do on a day to day basis, namely observing the effects of individual treatments on individual patients. However, the article suggests a scope and potential for the n = 1 trial that I would take issue with, and the author fails to adequately describe the limits and disadvantages of this type of design.

While single subject designs have the potential of examining effects at an individual level, they do not provide data that can readily be applied to others. The author does mention that the generalisability of results from this type of study is limited, but goes on to suggest that if a subject that is "representative of the general type of patients for which this intervention would be used" then the results become more generalisable. A person can be chosen that has a certain disease at a certain stage and with certain sociodemographic characteristics. But is this person really representative? How do we know exactly which variables are relevant to the effect being shown? And how can we judge "biological representation". For example, are there aspects of a person's metabolism that influences the way they respond to a drug? Furthermore, demonstrating an effect in a person, even if the person is similar to a population, provides little evidence about the probable effects of an intervention on a population, or the effects on another, inevitably unique, person.

Other potential problems with single subject trials are problems of bias and the determination of statistical significance. Effects that are likely to lead to bias in this type of trial include regression to the mean and "carry-over" effects. Values towards the extremes are likely to normalise on repetition for statistical reasons, an effect that is described as regression to the mean. This will occur without any clinical change in the subject, and in a single subject trial is likely to lead to a false impression of treatment effect when none may be present. Secondly, the author describes using a washout period between different phases of treatment. However, treatments can have lasting effects and it can be difficult to distinguish whether any prior treatments or indeed the rotation of treatments plays a part in any observed effect. Furthermore, once a subject has "changed", how can you ever really know what you are comparing to? Can change revert back to its original state in every way? After all, you can only ever be a virgin once!

Ways of attempting to minimise bias in n = 1 trials, as the author points out,

include randomisation, blinding, and multiple treatment phases. An effect that is observed by a blinded observer, in a blinded patient, each time a treatment is randomly introduced, and that disappears each time the treatment is withdrawn, is more likely to represent a true treatment effect. However, trials that entail randomisation, blinding, and multiple phases are likely to be more difficult to implement in primary care, and more costly. Larson *et al* estimate the costs of formal n = 1 trials at $450 to $500 per patient.[2] This is much less than large scale RCTs, but still a significant cost for a primary care clinician interested in evaluating a treatment on a patient. Furthermore, if the single subject trial is repeated in a number of patients to try and prove generalisability, the costs could easily add up to the sort of amount that a small scale RCT would cost. A series of n = 1 trials combined could thus be seen as a long, drawn out, ''sequential RCT''. Some comments from the author on the feasibility and methods for combining the results of multiple single subject trials, and the applicable lessons that have been learnt from cross over trials, would prove very useful for those interested in conducting studies of this kind.

The working example given by the author shows fasting blood glucose values before and after a comprehensive intervention for diabetes management. This is a two phase, A-B, design that shows fasting blood glucose values after the intervention as being lower than those before the intervention. The author concludes that, ''it seems that the intervention was effective in lowering the measured fasting blood glucose in this subject.'' Is this a fair conclusion from this type of study? Is it not possible that some other, unmeasured variable changed during the course of this study, and that this was the cause of the change in measured fasting blood glucose? For example, the subject may have independently taken up a new form of exercise.

In conclusion, Janosky's paper on single subject trials has relevance to both GP clinicians and researchers. In primary care, making treatment decisions with patients and then monitoring their response to those treatments is a daily occurrence. This, on occasion, entails a number of phases, where either different treatments or no treatment are tried. Formalising this process, and consideration of the use of blinding or placebo treatments, provides opportunities for primary care clinicians to assess the effectiveness of treatments on a person in a more comprehensive and rigorous fashion. Conducting formal n = 1 studies, compared with the informal treatment trial, can however, have significant time, cost, and ethical considerations. Given these constraints, it is unlikely that this type of trial will have wide applicability in everyday general practice. Furthermore, it must be emphasised that the results from this type of study can generally not be applied to anyone other than the person that has been studied, and that this design is particularly vulnerable to certain types of bias as described above.

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AUTHOR'S COMMENTS

I am pleased to read the commentaries that accompany my article (see page 549). Each of the commentaries raises relevant issues that serve to illuminate the strengths and limitations of single subject research designs. As with all methodological designs, each affords strengths and limitations to answer tailored research questions. The research questions best answered, through the application of a single subject research design, are questions that garner their interest in the potential to have high internal validity while acknowledging that the external validity is most probably weak. The single subject design can be easily contrasted with a clinical trial or true randomised experimental study that aims to examine effectiveness where the strength of the design is its strong external validity. The results of these clinical trials provide conclusions regarding effectiveness on the average treatment effects for only the studied populations; however at times, the results from these clinical trials might not always be applicable in determining the most effective treatment for an individual patient. A single subject research design, although limited for external validity, provides an opportunity to examine the applicability of the study findings to a specific patient. As methodologists, having an arsenal of research designs with strengths and limitations identified for each affords us a comprehensive means to answer diverse research questions.