Review

Clozapine. Pharmacodynamic Mechanisms, Potential Pharmacogenetic Biomarkers, and Risks in Schizophrenia. Particularities in the Context of Covid-19. An Updated Overview

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Abstract: *Background:* Clozapine (CLZ) use is precarious due to its side effects - neurological, cardiovascular, and hematological; however, it is the gold standard in the therapy-resistant schizophrenia (TRS) in adults and harshly underused. *Objective:* Our primary outcome examined the most recent data regarding clozapine in order to update the knowledge in (a) side effects to optimize its use in the context of a little and scarce of resources pathology, with regard on recent (b) pharmacological mechanisms, (c) therapy benefits and (d) particularities in the COVID-19 pandemic. *Data sources:* We performed an accurate search in the primary sources of Databases (PubMed, Web of Science) with specific keywords: "clozapine" and "schizophrenia," "side effects," agranulocytosis," "TRS" "bipolar affective disorder (BAF)" "pregnancy" "early-onset schizophrenia" "resistance" on a period of ten years (up to 15th Aug 2020). Two authors independently selected studies and extracted data. *Study eligibility criteria:* We included clinical trials and reviews on adults with acute symptoms of schizophrenia or related disorders; we extracted information regarding drug treatment, side effects profile, and efficacy for each trial; *Results:* Of all the searched data, we selected 45 studies: RCT's, clinical trials, systematic reviews, and meta-analyses centered on six main topics in the search area: (a) CLZ in schizophrenia, (b) CLZ in bipolar disorder, (c) side effects during the clozapine therapy - agranulocytosis, dysmetabolic side effects, pharmacogenetic severity markers, pulmonary embolism, seizure risk – (d) safety of CLZ in pregnancy (e) safety of CLZ in Early Onset Schizophrenia (f) clozapine therapy and COVID-19 infection. *Limitations:* We considered RCT's, CT's, SR, MA from two databases, published in the last ten years, limited to the topics above. *Conclusions and implications of key findings: (a)* The genetic vulnerability postulates predictors of adverse reactions severity so clozapine doses should be personalised for each patient based on pharmacogenetic testing; patients with a lower genetic risk may benefit from a more relaxed hematological monitoring schedule; (b) Pulmonary embolism associated with clozapine has a mortality rate of 36.36%, prophylactic measures for venous thromboembolism for six months after initiating therapy is mandatory; (c) Convulsive episodes are not an indication for stopping the treatment, side effect (s.e.) incidence increases with the dose, the plasma concentration of clozapine (1300 ng/ml) it is a better s. e. predictor than the dosage; (d) clozapine refractory improves up to 69% early-onset schizophrenia, assesed by the Brief Psychiatric Rating Scale (BPRS) (e) more pharmacogenetic studies of the Romanian schizophrenic patients are needed in relation with the clozapine therapy in order to define more precise safety margins; (f) COVID-19 infection may

enhance clozapine toxicity generating an increased risk of pneumonia therapy must be continued with proper monitoring of the white blood count and with the decrease of the clozapine dose by half until three days after the subside of the fever; psychiatrists and healthcare providers must act togheder. As in the past four decades, research has failed to generate effective novel psycho-pharmaceuticals, there is an urgent need to enhance the access to clozapine for people with TRS at the worldwide level. The progress of pharmacogenetic researches, endocrinology, genetic testing - offer the psychiatrists nowadays the chance to use this drug at its highest potential in a personalized manner for every patient - minimizing the adverse side-effects.

Keywords: clozapine; schizophrenia; pharmacogenetic; early-onset; pregnancy; bipolar affective disorder; agranulocytosis; Romania; COVID-19

1. Introduction

Controversies are frequent in psychiatric therapy, and the consensus is hard to find [1]; however, there is a full widespread consensus regarding the unique place that clozapine occupies in treating severe mental illnesses refractory schizophrenia [2]. Schizophrenia is a major mental illness, having a lifelong impact on the patients and their caregivers. The precise etiopathology of schizophrenia is unknown and most likely multifactorial [3], implying neurodevelopmental (hypoxia, maternal infection and stress), genetic (family history), and environmental factors (social and cannabis use) [4].

The second-generation antipsychotics are the first line of treatment of acute psychotic episodes, and they are currently prescribed for long-term management of schizophrenia, affective disorders, and some dementia-related symptoms. They are considered atypical when comparing their clinical profile with the first-generation antipsychotics and have a better response regarding the negative symptoms of schizophrenia. Extrapyramidal side effects are less common than in the case of typical ones [5,6]. Risperidone, ziprasidone, paliperidone, and aripiprazole are potent antagonists of D2 receptors of dopamine; quetiapine, clozapine, are weak antagonists of D2 and antagonists for 5-HT 2A and agonists for 5-HT 1A receptors.

The most potent molecules that bind to the alpha-adrenergic receptors are clozapine, iloperidone, and risperidone. Clozapine, olanzapine, and quetiapine also bind to muscarinic cholinergic receptors [7,8]. The less common incidence of extrapyramidal side effects made the atypical antipsychotics very popular among psychiatrists [9]. However, they still carry a risk of different types of side effects that must be monitored that includes metabolic disorders (type 2 diabetes, weight gain, dyslipidemia) and cardiovascular disorders like the prolongation of the QT interval [10], neurological and hematological (agranulocytosis) complications.

The life expectancy of patients diagnosed with schizophrenia and affective bipolar disorder is between 11 and 20 years shorter, patients being vulnerable and continuously in need of medical and social care - to prolong their life expectancy [11]. One-third of patients respond to "typical" antipsychotics (e.g., chlorpromazine and haloperidol) [12]; the remaining of two - thirds need the second strategy. Clozapine is established as the gold-standard treatment for treatment-resistant schizophrenia (TRS), 32% on the short term and almost 40% on the long term therapy TRS patients - respond to clozapine [13-15], the absolute reduction in overall Positive and Negative Symptom Scale (PANSS) scores being clinically significant.

Considering the terrible burden that schizophrenia brings to the lives of patients and their families, the discovery of clozapine, the first atypical antipsychotic, was a substantial pharmacological and clinical milestone. The significant therapeutically effect of clozapine compared with other classes of drugs, and the reduced incidence of extrapyramidal side effects - which added more stigma to the psychiatric patients - brought hope in the most severe cases of schizophrenia.

At this moment, clozapine is the most effective antipsychotic drug in the therapy-resistant schizophrenia TRS [16-18], listed on the WHO Model List of Essential Medicines [19], superior to other drugs in the class due to: (1) lower risk of suicide; (2) lower risk for tardive dyskinesia (3) improvement of cognition able to lead to improved quality of life; (4) decreased relapse. Clozapine is

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specific in psychiatric therapy due to its effectiveness but also due to a pharmacodynamic conundrum. With a progressive prescription trend during 2005 - 2014 (a relative increase of 7.8% up to 197.2%), it is underused due to its specific adverse reactions – hematological (agranulocytosis), cardiovascular and neurological side effects. Despite the benefits, clozapine remains underutilized in most countries- up to two-thirds of TRS cases, as revealed in a study in Australia in 2017 [20]. Less we know about clozapine use in Romania.

An update to the actual clinical experience will reinforce the therapy benefit considering increased safety in use due to the screening, early detection of the side effects, and rigorous choice of the potential beneficiary patient – to treat within the margins of safety and not underuse the drug.

Purpose of the review: To examine the latest research regarding: (a) potential pharmacogenetic markers predictors of adverse reactions associated with clozapine treatment, registered in the last ten years, (b) side effects not as isolated events, but as a network of interdependent elements managed as a whole (c) use in younger patients or during pregnancy; (d) strategies in clozapine resistance pathology; (e) particularities of clozapine therapy in the COVID-19 pandemic.

2. Materials and Methods/Data Search

We performed a systematic qualitative review according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [Moher, 2009] searching published original papers or reviews on clozapine on human use, with data filter on the current use of clozapine in younger patients, during pregnancy, bipolar specter disorder, COVID-19, and associated side effects, published in a scholarly peer-reviewed journal, written in English, French and Romanian languages, with no country restriction, during the last ten years.

We established the review methods before the conduct of the review. The initial review protocol assumed a Google Scholar search; due to the diversity of the document types, the search was resumed on two significant databases, PubMed®/MEDLINE, and Web of Science Core Collection. The report had any other significant deviations from the initial study plan.

Information sources: We searched databases - PubMed®/MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed), Web of Science, for clozapine, side effects, and related keywords. **Search:** We did an accurate search in the primary sources of Databases (filters applied: Clinical Trial, Meta-Analysis, Randomized Controlled Trial, Systematic Review, on Humans, in the last ten years) with the keywords: "clozapine" AND "side effects" OR "schizophrenia" OR "bipolar affective disorder" OR "TRS" OR "Treatment-resistant bipolar disorder" OR "agranulocytosis" OR "obesity or metabolic" OR Pharmacogenetic "OR "Pulmonary embolism" OR "seizures," OR "COVID 19" OR "pregnancy" OR "early-onset schizophrenia," OR "Romania." We restrained the search to articles written in English, French, Romanian; the last updated search was done on 15.08.2020.

Study selection: Inclusion criteria were: (1) patients with a diagnosis of schizophrenia or a related disorder, **on clozapine therapy** or with indication of (2) clozapine therapy (3) non-pregnant or (4) pregnant adult or (5) child, in early-onset schizophrenia – compared to control or other antipsychotics; (6) patients on clozapine therapy with COVID-19 AND related side effects (neutropenia, agranulocytosis, pneumonia, TEP, seizures, obesity, and weight gain) due to therapy registered in Clinical trials (RCT) or reviews - Systematic reviews (SR), Meta-analysis (MA) on a ten years period.

Data extraction: We extracted the following data: authorship, year of publication, country of the study, aim of the study, study design, assessments, and main results. Of all the searched data and retrieved on the database sources, we selected RCT's, CTs and systematic reviews, and meta-analyses presented below. Two independent investigators extracted the data, selected a sample of eligible studies achieving good agreement. Firstly, the authors screened articles by title and abstract, and then by full-text. We did snowball searches of key papers. Duplicates, simple review articles, and articles not fulfilling the search criteria were excluded.

Data analysis was conducted by three authors (A.M.D., A.L.P., N.B.A.). Over three thousand studies with schizophrenia specter disorder or bipolar specter disorders in therapy with clozapine, with or without a control group, were identified and screened for eligibility by the two examinators.

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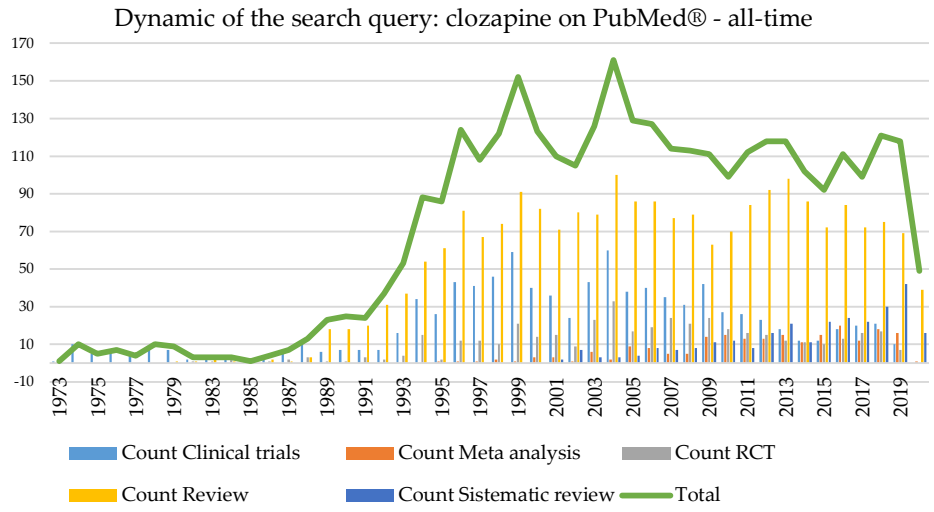
Data extracted included demographic variables, number of participants in the study, treatment, side effects profile, associated comorbidities according to the topic search. We completed the data collection in August 2020. The quality of the studies selected for review was evaluated.
We included forty-five studies in the present study, centered on seven main topics included in the search. Statistical analysis was performed using Microsoft Excel® 2010 (Microsoft® Corporation).

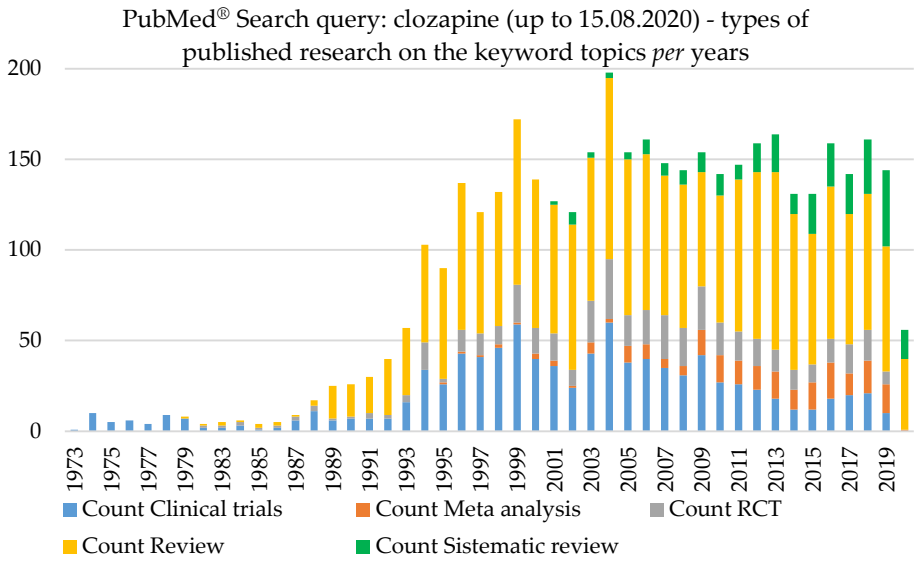
3. Results

3.1 Study selection

PubMed®/MEDLINE
"Clozapine" keyword search (from inception until 15.08.2020) retrieved over 3075 results, 937 Clinical Trials, 211 Meta-analysis, 277 Systematic reviews, 428 Randomised Clinical trials showing a persistent interest in the widening of the use of clozapine as no new alternative therapies are available yet.



(a)



(b)

Figure no. 1 Systematic search of keyword "clozapine" on PubMed® database (all-time topic) retrieved over 4000 results (a) total (b) by type of study

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For the association 'CLZ' and 'schizophrenia', search refined to RCT/CT or SR/MA retrieved 127 results; CLZ" and "Treatment resistant schizofrenia" 19 results, "CLZ" and "resistance" 4 results, "CLZ" and "bipolar disorders" 38 results, "CLZ" and "Treatment resistant bipolar disorder" 7 results, CLZ" and "agranulocytosis" 68 results, "CLZ" and "Pharmacogenetic" 6 results, "CLZ" and "obesity" 12 results, "CLZ" and "pulmonary embolism" 7 results, "CLZ" and "seizure" 23 results, "CLZ" and "COVID 19" retrieved 5 published papers, "CLZ" and "pregnancy" 7 results, and "CLZ" and "early-onset schizophrenia" 12 results; CLZ" and "Romania" (TOPIC) three results.

Web of Science:

We searched for: TITLE: (Clozapine) Refined by: TITLE: (side effects) AND DOCUMENT TYPES: (ARTICLE OR REVIEW) Timespan: 2010-2020. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC. Results: 794 published papers (595 articles and 199 reviews);

For the association 'CLZ' and 'schizophrenia', search retrieved 21 results; CLZ" and "Treatment resistant schizofrenia", "CLZ" and "resistance" 21 results, "CLZ" and "bipolar disorders" 38 results, "CLZ" and "Treatment resistant bipolar disorder" 7 results, CLZ" and "agranulocytosis" 68 results, "CLZ" and "Pharmacogenetic" 6 results, "CLZ" and "obesity" 12 results, "CLZ" and "pulmonary embolism" 7 results, "CLZ" and "seizure" 23 results, "CLZ" and "COVID 19" retrieved 5 published papers, "CLZ" and "pregnancy" 7 results, and "CLZ" and "early-onset schizophrenia" 12 results; CLZ" and "Romania" (TOPIC) three results.

From the databases retrieving 331 articles initially screened by title and abstract or *in extenso* to match the search criteria and excluding the duplicates, we selected 45 studies for full-text reading, centered on six main topics in the search area: (a) treatment-resistant schizophrenia, (b) use in bipolar disorder, (c) side effects during the clozapine therapy - agranulocytosis, metabolic side effects, pharmacogenetic severity markers, dysmetabolic side effects, pulmonary embolism, seizure risk – (d) safety of clozapine in particular situations - pregnancy and early-onset schizophrenia, (e) clozapine resistance and ECT augmentation, (f) clozapine therapy and COVID-19 infection.

From the selected papers, we identified seven main topics on the search area: (a) treatment-resistant schizophrenia, (b) use in bipolar disorder, (c) side effects during the clozapine therapy - agranulocytosis, metabolic side effects, pharmacogenetic severity markers, dysmetabolic side effects, pulmonary embolism, seizure risk – (d) safety of clozapine in pregnancy, (e) clozapine resistance and ECT augmentation, (f) clozapine therapy and COVID-19 infection. More detailed information regarding the selection process is presented The PRISMA flow diagram (Figure 2).

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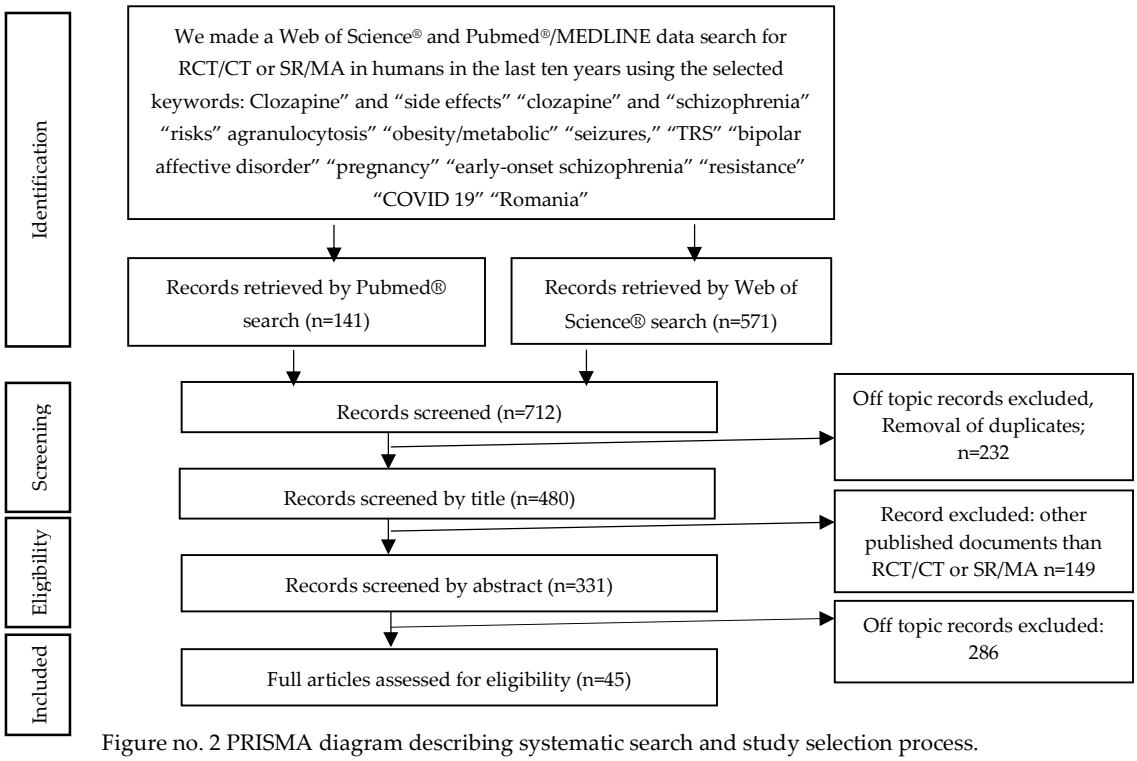

Figure no. 2 PRISMA diagram describing systematic search and study selection process.

3.2 Clozapine

Clozapine $C_{18}H_{19}ClN_4$ (Figure no. 2) is the only atypical antipsychotic agent approved to manage treatment-resistant schizophrenia. From a pharmacological point of view, clozapine is a tricyclic dibenzodiazepine which binds to several receptors of the central nervous system (Figure 3).

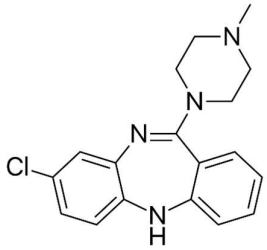

Figure. no. 3 Clozapine chemical structure (IUPAC - C 3-chloro-6-(4-methylpiperazin-1-yl)-11*H*-benzo[b][1,4]benzodiazepine)

This antipsychotic agent has a unique profile by binding to 5-HT 2A/2C receptors, being a serotonin antagonist. Clozapine also has an affinity for dopaminergic receptors, but when it comes to the dopamine D2 receptor (which is known to modulate neuroleptic effects), it displays only a weak antagonistic effect.

Recent studies highlight that the causative abnormalities in schizophrenia may lie in non-dopaminergic pathways, particularly for TRS, involving the GABAergic system [21, 22]. The X-ray crystal structure and molecular docking of clozapine in the extracellular domain of the $GABA_B$ receptor support the recent clinical and experimental findings of the direct interaction of clozapine with the $GABA_B$ receptor [23].

Wander Pharmaceutical Company synthesized clozapine in 1956 in Switzerland; In the mid-1960s, clozapine was one of the three compounds that Wander offered to researchers in Berlin to study to establish the chemical formulas that differentiated the tricyclic substances for those molecules to be either neuroleptics-antipsychotics or antidepressants. The result of the research was that clozapine

should have been an antidepressant, but the clinical studies revealed its neuroleptic-antipsychotic effects [24].

More clinical studies showed clozapine's antipsychotic properties and the safer profile regarding extrapyramidal side effects, so in the 1970's it was released on the European market. Soon after, it was withdrawn from the pharmacies after some Finnish psychiatrists reported seven deaths related to a high incidence of agranulocytosis among the elderly patients treated with clozapine (25,26). In 1990, clozapine became available again in therapy, with a strict blood concentration monitoring protocol.

A short time later, two other second-generation antipsychotic agents were introduced: risperidone and olanzapine, compounds whose administration did not reveal any associated hematological risks, but none have shown efficacy for TRS.

The indications for the use of clozapine in therapy are treatment-resistant schizophrenia (TRS) and suicidal behavior in schizophrenia or schizoaffective disorder. A series of off label uses are mentioned in references: treatment-resistant bipolar disorder and psychosis/agitation associated with dementia, psychosis in Parkinson's disease [27].

However, at this moment, regulations of clozapine prescribing and monitoring vary widely worldwide, lacking worldwide update and harmonization [28]). In most countries evaluated for regulatory reasons - China, Denmark, Ireland, Japan, The Netherlands, New Zealand, Romania, the UK, and the US – (Nielsen, 2016), exists a mandatory hematological monitoring registrar, the dispensing of clozapine is not allowed without acceptable white blood count and absolute neutrophil count results, except for Romania but also Denmark, and The Netherlands. The Risk Evaluation and Mitigation Strategy (REMS) in the US monitoring program's motto is "No Blood, No Drug." The baseline risk of severe neutropenia for clozapine is approximately 1.3% overall, with peak risk around one month after initiation with a substantial reduction in risk at 18 weeks [29]; Neutropenia increases the risk of infection, mainly when the ANC is <500/microL; the COVID-19 pandemic has consideration for novel ANC testing via point-of-care devices [30]. More than that, the local guidelines in New Zealand recommend echocardiography and routine troponin during the initial phases of treatment with clozapine.

3.3 Pharmacogenetic severity markers as potential biomarkers in clozapine therapy

The pharmacological recommended doses are estimated as an average dose for an ideal average patient, but pharmacologists identified genetic outliers: poor metabolizers (PMs) and ultrarapid metabolizers (UM's). The type of metabolizer is generated by personal (genetic and metabolic) factors also environmental. So, estimating the drug clearance (concentration-to-dose (C/D) ratio), a meager C/D ratio indicates a UM, high C/D ratio indicates a PM. Clozapine C/D ratios range from 0.6 (male smokers) to 1.2 (female non-smokers) ng/ml per mg/day in the US [31] with a double value in East Asians. Clozapine C/D ratios can be enhanced in interaction with inhibitors (including fluvoxamine, oral contraceptives) or an inflammatory state.

Two hundred four studies were published on clozapine and pharmacogenetic topic on the PubMed® database starting to the year 1994, with 57 reviews, 12 Clinical trials, RCT, and three systematic reviews and meta-analyses, described below.

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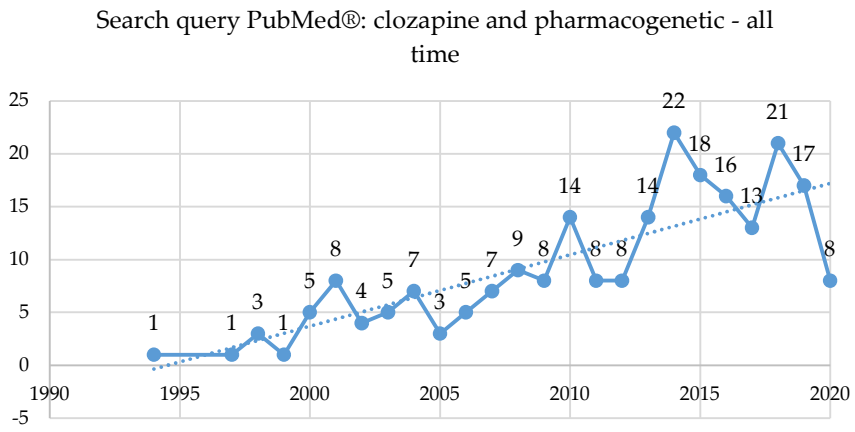

Figure no. 4 Search query PubMed®: clozapine and pharmacogenetic - all time – up to 15.08.2020

The genetic vulnerability is correlated with metabolic side effects with a higher prevalence of adverse metabolic reactions in clozapine treated patients and postulates predictors of severity – pharmacogenetics markers such as CYP2C19, LEP, LEPR, and HTR2C receptors [32]. Clozapine's metabolism, clearance, and response were evaluated by genotyping specific enzymes, such as CYP1A2, CYO2C19, and by measuring HTR2C serotonin receptors and leptin receptor, taking into account concomitant medication if present. The result showed that metabolic syndrome was correlated with higher levels of Clozapine and CYP2C19*2 and LEPR c.668 G alleles. Individuals who metabolize clozapine slower are at higher risk for this type of disorder.

The antipsychotic-related weight gain antipsychotic-related weight gain is polygenic and associated with specific genetic variants - 13 SNPs from 9 genes (Adrenoceptor Alpha-2A [ADRA2A], Adrenoceptor Beta 3 [ADRB3], Brain-Derived Neurotrophic Factor [BDNF], Dopamine Receptor D2 [DRD2], Guanine Nucleotide Binding Protein [GNB3], 5-Hydroxytryptamine (Serotonin) Receptor 2C [HTR2C], Insulin-induced gene 2 [INSIG2], Melanocortin-4 Receptor [MC4R], and Synaptosomal-associated protein, 25kDa [SNAP25]), (Zhang *et al.*, 2016)

Gressier *et al.* (2015) found in meta-analyses three genetic variants within serotonin genes associated with clozapine response: rs6313 and rs6314 within HTR2A gene and rs1062613 within HT3A gene, suggesting a possible serotonergic modulation of clozapine clinical response but no link with the weight gain on these genes.

DiGeorge syndrome or 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion in humans (one in 4000) [33] and is associated with high rates of attention-deficit/hyperactivity disorder (ADHD), psychotic spectrum disorders, and mood and anxiety disorders [34]. Late adolescence and early adult years are characterized by the emergence of psychotic disorders with rates of up to 41% of schizophrenia spectrum disorders in adults with 22q11.2DS over 25 [9, 24, 25]. Much of the published literature in 22q11.2DS is of treatment-resistant schizophrenia, and the therapy with clozapine is the gold standard for it. The medical comorbidities are common in individuals with 22q11.2DS that may complicate the administration of pharmacotherapy, mainly antipsychotics and stimulants. Specific side effects of medications seem to be more common in 22q11.2DS than in typical individuals.

In clinical practice, pharmacogenetic testing is widely available, and psychiatrists should be able to adjust and personalize clozapine doses for each patient, decreasing the risks of metabolic side effects to a minimum level.

3.4 Treatment - resistant schizophrenia

A number of 1,422 studies were published on clozapine and treatment-resistant schizophrenia on the PubMed® database, of which 443 results during the past five years, with four randomized controlled trials and ten systematic reviews and meta-analyses, described below.

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Treatment-resistant schizophrenia is described as the persistence of symptomatology after two trials of 2 different antipsychotic drugs, inappropriate dosage, and duration, with proven medication compliance [35]. Although the persistent symptoms may be from any of the three areas of the disorder (negative, positive, cognitive), treatment-resistant schizophrenia is usually characterized by persistent positive symptoms [36].

The lack of response to antipsychotic medication is not enough to diagnose treatment-resistant schizophrenia, as clinicians must differentiate it from pseudo-resistance.

A pivotal study (US Clozaril Study) on patients who failed to respond to three previous antipsychotic drugs, showed the efficacy of clozapine over chlorpromazine in a 6-week trial of, [37] that led to FDA approval of clozapine for TRS (not a first-line) due to side effects (agranulocytosis). A 2018 meta-analysis done in 2018 showed that clozapine might be more effective than other antipsychotics even when used as a first or second line of treatment [38]

The CUtLASS 2 study showed that patients who did not respond to at least two antipsychotic drugs had significantly improved outcomes after one year on clozapine [39].

The phase 2E CATIE study showed that clozapine had a better therapeutic response than risperidone and quetiapine. The researchers postulated that patients whose symptoms do not improve with a second-generation antipsychotic would benefit if prescribed clozapine rather than if they were treated with another second-generation antipsychotic drug [40]. A recent meta-analysis also has revealed the superior efficacy of clozapine when compared to olanzapine [41].

3.7.1. agranulocytosis and the nitrenium ion. Potential leukocytes autoimmune biomarkers

Agranulocytosis is the most dangerous adverse effect linked to clozapine administration in the psychiatric population. Agranulocytosis/granulocytopenia induced by clozapine is not very common, but it can have a fatal outcome. The pathogenesis is not understood. Recent research postulates that this type of agranulocytosis is linked to an autoimmune response of the organism. The nitrenium ion can be activated biochemically by clozapine. The CYP3A4, CYP2D6, and myeloperoxidase system in white blood cells synthesize the nitrenium ion. Therefore, the main component in the pathogenesis of clozapine-induced agranulocytosis might be a genetic aberration in the antigen genes of the leukocytes and some genes related to apoptosis [42].

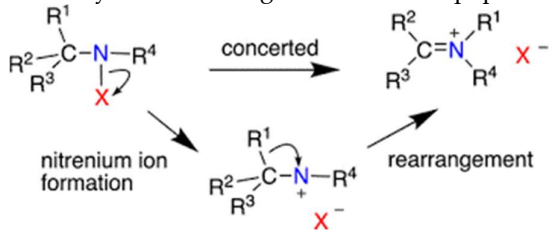

Figure no. 5 Computational Studies of Non-Aryl Nitrenium Ions and Their Rearrangements (after Falvey, 2018) [43]

One genetic study observed that reactive oxidative species could oxidize clozapine metabolites to nitrenium ions and concluded that this adverse reaction to clozapine is more likely a complex polygenic trait [44].

Agranulocytosis is diagnosed when the absolute neutrophil count is <100/mm3, associated with an infectious disease. A study conducted postulates the risk of agranulocytosis and neutropenia at 0.38% of patient treated with clozapine in the Indian population, which is at the highest level in the first six months after initiation of treatment and remains significantly high for 18 months, with few reported cases after this period [45].

Another study, conducted in 2016, surprisingly concluded that neutropenia incidence during treatment with clozapine is not related to the drug itself and that patients diagnosed with schizophrenia treated with other antipsychotics had the same risk of neutropenia. The research measured blood counts 18 weeks after the initiation of treatment, every 25 days, and after this period, the median was 124 days. After an average observation time of 9.2 years, there were found 34 cases

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of neutropenia developed under clozapine treatment. Twenty-four patients only had mild cases of neutropenia (1500 – 1900 neutrophils/mm^3), which did not progress to agranulocytosis. The other ten patients developed more severe neutropenia (500-1400/mm^3), and among them, only one progressed towards agranulocytosis. Three other patients discontinued clozapine, and six others remained on the drug for at least one year without any hematological side effects [46].

The risk of developing agranulocytosis is under 1% in patients who take clozapine, and this may be independent of dosing [47, 48]. It occures early in the treatment, within six weeks to six months, and require extensive monitoring of blood absolute neutrophil counts [49]. Many have tried to find the link between clozapine and agranulocytosis by attributing this adverse effect on drug interactions with the immune system and genetic predisposition [61]. A study in 2015 analyzed the benefits of pharmacogenetic testing and how it may affect monitoring in patients at risk for clozapine-induced agranulocytosis [50]. Patients with a lower genetic risk may benefit from a more relaxed hematological monitoring schedule. Risk factors include old age, female, genetics, and concurrent treatment with other drugs known to cause agranulocytosis. Clinicians must place patients taking clozapine on a national registry. Granulocyte colony-stimulating factor may be an option to increase levels of white blood cells [51].

Clozapine associated neutropenia is thought to occur due to selective neutrophil toxicity mediated by clozapine N-oxide metabolites [52] or an immune response mediated by a hapten-based mechanism [53] occurred early in an exposure.

3.5 Clozapine pseudo-resistance

A review published in the current year suggests some clinical situations which prove pseudo resistance [54]. The authors mention that while pharmacodynamic factors cause the treatment-resistant schizophrenia, the pseudo-resistance is underlined by clinical or dependent on pharmacokinetics. Also, the pseudo-resistance might mask an inaccurate diagnosis, dose, or duration of treatment; also, it might be caused by insufficient levels of medication in the serum, limited compliance, or comorbidities (including substance use). In many studies, the mortality rate of patients undergoing clozapine treatment proved lower than that of patients on first-generation antipsychotic drugs and atypical ones like quetiapine and risperidone [55 – 57].

The literature is paucity regarding clozapine use in late life; a particular approach is needed due to their physical comorbidities and increased risk of adverse effects; nevertheless, recent reviews highlight a definite benefit even in the elderly if used with proper care and monitoring [58].

3.6 Clozapine resistance and ECT augmentation

In the population of psychiatric patients diagnosed with treatment-resistant schizophrenia, 40 -70% of the individuals are estimated to have incomplete or no response to clozapine, with low improvements on psychometric scores (lower than 20% from baseline) [59]. 12% to 20% of clozapine patients with schizophrenia will be ultra-resistant [20]. The term clozapine-resistant schizophrenia was introduced by Mouffak *et* al. [60]. The following criteria were proposed in defining this category of patients: Brief Psychiatric Rating Scale (BPRS) improvement less than 20% after a trial with clozapine at least eight weeks long, no stable period of proper social and occupational functioning for at least five years, Global Assessment of Functioning (GAF) score lower than 40, BPRS score higher than 45, Clinical Global Impression (CGI) score higher or equal to four, and a score of at least four on two out of four positive symptoms.

Twenty-eight studies were published on clozapine and ECT augmentation on the PubMed® database with 12 systematic reviews and meta-analyses, one randomized controlled trial [61], and 12 systematic reviews and meta-analyses, described below.

ECT appears to be an effective treatment combined with clozapine in the context of treatment-resistant schizophrenia spectrum disorders [62]; the practice of ECT still lacks consensual protocols. The first reported success in the augmentation of clozapine treatment by electroconvulsive therapy was first described in the early 1990s. Since then, this therapeutical combination has been used as the

last line of treatment, with excellent results that show clozapine's superior efficacy when compared to other antipsychotics in combination with ECT.

A metanalysis published in 2017 compared the effect of the combined treatment with Clozapine and ECT and other non-clozapine antipsychotics like flupenthixol, chlorpromazine, risperidone, sulpiride, olanzapine, and loxapine [63]. This paper analyzed 1179 patients from 23 studies, which reported antipsychotic treatment augmented with ECT. The patients were assessed using two standardized psychiatric methods: The Brief Psychiatric Rating Scale (BPRS) and the Positive and Negative Symptom Scale (PANSS). The results showed improvement in psychometric scores, with clozapine use and ECT, resulting in the best outcome. The other potent combination was flupenthixol augmented with ECT.

A paper from 2015 also studied the therapeutical response of severe cases of schizophrenia treated with clozapine and ECT, concluding that the combination is safe, potent, and should be used on patients with refractory symptoms who do not respond to clozapine alone [64]. Nevertheless, the prescription of the drug should be reviewed when ECT treatment is initiated [65].

3.7 Clozapine in treatment-refractory bipolar disorder

Treatment-refractory bipolar disorder (TRBD) can be defined as a bipolar disorder that does not respond to at least two trials of different treatments of adequate dose and duration [66].

543 studies were published on clozapine and bipolar disorder on PubMed® Database with 175 systematic reviews and meta-analyses, during the past ten years - five randomized controlled trials and 24 bipolar disorder systematic reviews and meta-analyses, described below.

In the review mentioned above, we found 15 trials (open-label retrospective studies, prospective and two randomized controlled) with 1044 patients. The authors concluded that clozapine could be useful for mood-related symptoms and rapid cycling patients, and also for psychotic symptomatology associated with bipolar disorder. It could also improve the number of episodes necessitating hospital admission and the number of drugs in the therapeutical plan. Good results were also found in the area of social functioning, hetero-agresivity, and suicidal thoughts.

It was demonstrated that clozapine has an antisuicidal effect not related to its antipsychotic action. The International Suicide Prevention Trial concluded that those properties could be useful in bipolar disorder as they are in schizophrenia [67].

A recent paper shows that clozapine has been used to treat TRBD for over 30 years with excellent results. It positively influences the suicidal ideation and the aggressivity, only 1.5% of the bipolar patients are getting it prescribed [68]. Clozapine's efficacy was similar to other antipsychotics in manic episodes and is superior to other antipsychotics among treatment-resistant bipolar disorder (TRBD) patients.

3.8 Side effects during the clozapine therapy

The side effects associated to clozapine therapy are frequent (>10%) cardiovascular: tachycardia orthostatic hypotension, hypertension, gastrointestinal - constipation, dyspepsia, nausea, sialorrhea, vomiting - weight gain, nervous system (dizziness, drowsiness, insomnia, sedated state, vertigo, fever); less common (below 10%) side effects are agranulocytosis, myocarditis, metabolic, seizures, sialorrhea, pulmonary embolism. Other side effects may include fever, dizziness, headache, syncope, diaphoresis, nausea, vomiting, weight gain, sedation, sexual dysfunction, and urinary retention. The black box warnings mention: severe neutropenia, orthostatic hypotension, bradycardia, syncope, seizures, myocarditis, cardiomyopathy, and mitral valve incompetence and increased mortality in elderly patients with dementia-related psychosis (Drugs.com, 2020) [69]

3.8.1. Metabolic side effects of clozapine

Antipsychotic agents are linked to metabolic disorders, weight gain, diabetes mellitus, and dyslipidemia, leading to increased cardiovascular risk. Clozapine has the worst metabolic profile of all antipsychotics mediated by an effect on glucagon-like peptide (GLP-1) (Siskind, 2016).

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A meta-analysis of 100 randomized controlled trials involving 25.952 patients exposed to 18 antipsychotics concluded that olanzapine and clozapine determine the most metabolic disturbances. On the other hand, aripiprazole, brexpiprazole, and cariprazine had the least metabolic side effects, being the safest to be used. The risk factors for adverse metabolic reactions included: high BMI at the beginning of the treatment, male gender, and non-white ethnicity. Improvement of psychiatric symptoms is associated with dire metabolic side effects [70].

The dysmetabolic side effects of clozapine differ based on gender difference. Their prevalence is more frequent among females who reach a higher plasmatic clozapine concentration, on average 17% greater than the plasmatic concentration in men. The retrospective survey also observed that the BMI and the plasmatic glucose levels of females were higher than in men [71]. Since clozapine is a lipophilic drug, and females usually have more adipose tissue than men, it was expected that women would show lower concentrations in their plasma at a given dose. More studies show the opposite, and the reasons underlining this fact might be the gender differences in pharmacokinetic factors, such as faster renal clearance in men [72]. The study quoted above found no significant gender differences regarding the concentration of norclozapine (the major active metabolite of clozapine); this further adds to the observation that women are at higher risk of clozapine accumulation and side effects (because the ratio between clozapine and norclozapine is increased).

Clozapine, like other antipsychotic drugs, quetiapine, haloperidol, trifluoperazine, risperidone, aripiprazole, olanzapine [73] increased the body weight significantly with ≥7% from baseline as showed in a recent meta-analysis. Obesity is associated with a series of comorbidities that decreases life expectancy; in the context of increased use of the antipsychotic drugs at a global level, the specific weight gains adverse reaction must be surveilled during the clinical course of the psychiatric disease for which there is a need in further cohort studies with higher sample sizes and longer durations of treatment for this purpose [74]. However, as higher energy intake was found in antidepressant users compared to non-users, this may partially explain the increase in body weight [75]. A protocol (COMET) for a randomized controlled trial with metformin therapy of as an adjunctive treatment to attenuate weight gain and metabolic syndrome in patients with schizophrenia newly initiated on clozapine [76].

3.8.2 Clozapine risk of pulmonary embolism and pneumonia

Venous thromboembolism is considered to be rare among the side effects of clozapine, but compared to other atypical antipsychotic drugs, the risk is more elevated [77]. The development of PE in clozapine users is linked with several risk factors such as age, obesity, smoking, increased risk of cardiovascular diseases in schizophrenic patients.

There are many theories regarding the etiology of pulmonary embolism in patients treated with clozapine. Another hematological side effect might be at the root of the venous thromboembolism in the patients treated with clozapine - the increases in adhesion and aggregation of blood platelets [78]. A recent in vitro study revealed that clozapine binding affected fibrin formation by reducing coagulation speed and thickness of fibrin fibers by fibrinogen developing thrombogenic characteristics, dose-dependent, increasing the risk for development thrombosis in patients on clozapine treatment [79].

Patients diagnosed with schizophrenia are at higher risk of obesity and cardiovascular diseases; this observation being another pathophysiological explanation of the increased risk of pulmonary embolism [80] but also of aspiration pneumonia [81]. Sedation is another significant and widespread side effect of clozapine administration that can be related to Body Mass Index (BMI) modification, and also can be linked with sedentary and development of venous stasis.

A recent review showed that 55.57% of the patients with pulmonary embolism went to the hospital complaining of dyspnea/tachypnea and were prescribed anticoagulant treatment in 80% of the cases. Of these, three died upon presentation, another nine died in the follow-up period, with a mortality rate of 36.36% [82].

Another review of the case studies of pulmonary embolism associated with clozapine treatment concluded that this side effect, though rare, is lethal. In general, this adverse reaction has an early

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onset, and it is not dependent on the dose, bringing into discussion the opportunity of prophylactic measures for venous thromboembolism for six months after initiating clozapine [83].

3.8.3. clozapine and seizure risk

Clozapine is the most frequent atypical antipsychotic agent associated with seizures. It lowers the seizure threshold, depending on the dose. 300-600 mg/day resulted in a seizure prevalence of 1.8%, which increased at 4.4% at doses higher than 600 mg/day [84].
One proposed etiological mechanism of clozapine-induced seizures claims that this drug has an affinity for the mesolimbic dopamine receptors, while typical antipsychotics usually bind to the striatonigral dopamine receptors. The mesolimbic structures represent a frequent site of seizure onset, and this might be an argument for the high epileptogenicity of clozapine compared to other antipsychotic medications [85].
Given the high efficiency of clozapine in managing psychiatric disorders, the convulsive episodes are not an indication for stopping the treatment in most cases. The available antiepileptic drugs such as valproate, topiramate, or lamotrigine proved effective in treating this particular side effect [86].
A retrospective study examined seven years of the medical history of 222 patients after starting the clozapine treatment to evaluate the incidence of seizure before and after the treatment. The results showed that 6% percent of the patients had seizures, increasing with the dose [87].
Some clinicians recommend anticonvulsant therapy in association with clozapine as a contraceptive method. However, it was not proved that this brings a positive outcome in every circumstance. Some authors postulate the fact that plasma concentration of clozapine (1300 ng/ml) is a better predictor than the dosage when it comes to the seizure risk. Also, it has to be considered that the concentration of clozapine is correlated with age, gender, BMI, and genotype variations; anticonvulsants associated with clozapine may lead to a higher risk of severe side-effects or may interfere with the therapeutic response to the clinical psychiatrist must take into consideration drug interactions [88].

3.9 Clozapine COVID-19 infection

"Clozapine" and "COVID 19" search retrieved 22 results in PubMed® Database search and 26 results in Google Scholar® Database, of which two reviews with topics on toxicity and side effects, management of clozapine monitoring and therapy during SARS-COV-2 quarantine, COVID 19 patients, clozapine and agranulocytosis - among which mentioned the PROSPERO (CRD42020178819) systematic review regarding the impact of COVID-19 on mental health patients and clinical mental health staff [89].
Patients with COVID-19 infection frequently experience lymphopenia, but not neutropenia [90]. COVID -19 infection may cause clozapine intoxication by dramatically increase serum clozapine levels, involving cytokine release downregulating the metabolism of clozapine in the P450 system through CYP 1A2 [91], as revealed by recent studies and case reports [92, 93], posing an increased risk of pneumonia, clozapine toxicity, even need for intervention in a critical care unit, disruption to clozapine treatment by COVID-19 induced lymphopenia [94 - 97].
Nevertheless, the therapy must be continued [98] with the following recommendations (Siskind & al., 2020): (1) ANC may be reduced to every three months, with a dispensation of up to a 90-day supply (if it can be safely stored) for people fulfilling all of the following criteria: • continuous clozapine treatment for more than one year • have never had an ANC below 2000/μL • any safe or practical access to ANC testing.
(2) patients on clozapine with any symptoms of infection (including for severe acute respiratory syndrome coronavirus 2 [SARSCoV-2] such as cough, fever and chills, sore throat, or other flu-like symptoms), an urgent physician assessment, a complete blood count (with ANC) should be done, in person or by telehealth based on local protocols.
(3) If patients on clozapine become symptomatic with fever and flu-like symptoms, the debut of signs and symptoms of clozapine toxicity may require to decrease the dose of clozapine by as much as half – by the clinician, up to three days post-hyperthermia, then the clozapine dose is reestablished

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progressive to the pre-fever dose. Where available, clozapine levels could backup the clinical decision.

A recent cohort study on 6309 participants, of which 102 were positive for SARS-CoV-12, clozapine treatment is associated with an increased risk of COVID-19 infection compared to other anty-psychotics, an association that needs further research to be confirmed [99].

Psychiatrists and healthcare providers involved in monitoring the absolute neutrophil count (ANC) and dispensing the prescription must be aware of the increased risks in COVID-19 – clozapine treated patients, communicate and strengthen the side effects surveillance, like the duration, the full impact of the COVID-19 pandemic is still unknown [100].

3.10 Safety of clozapine in pregnancy

We found 129 studies published on clozapine and pregnancy on the PubMed® database beginning with 1978, with 12 systematic reviews and meta-analyses, two randomized controlled trials [101], and 12 systematic reviews and meta-analyses, described below.

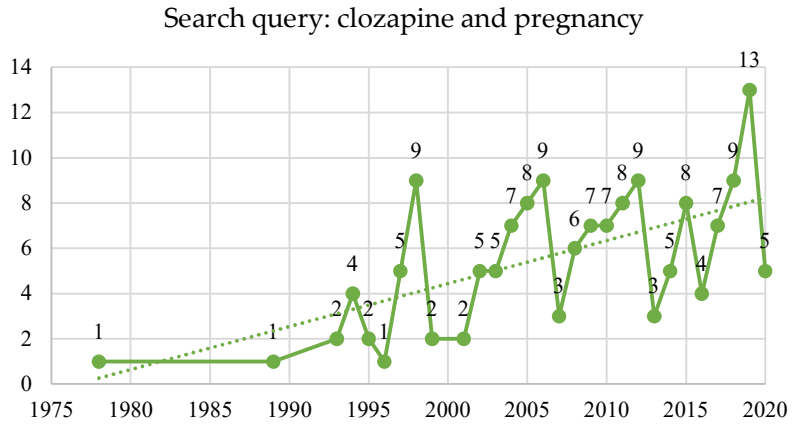

Figure no. 6 Search query: clozapine and pregnancy

A review from 2017 showed that there is not much data about clozapine treatment during pregnancy. The lack of control regarding the treatment doses, duration, and exposure of the fetus does not allow us to observe an accurate picture of the effects of clozapine in-utero.

A systematic review of the literature search in PubMed® and EMBASE conducted in 2018 analyzed forty-nine studies, found the penetration ratios (antipsychotic concentrations in the target matrix (i.e., amniotic fluid, umbilical cord blood or breast milk) /maternal concentration) in the amniotic fluid were estimated with a mean of 0.56 in the range 0.31-0.82 for clozapine, while in the breast milk a mean of 3.19, within the range 2.79 - 4.32, advising the need for measuring antipsychotic concentrations in maternal blood to estimate of fetal/infant exposure [102].

However, several case reports showed its teratogenic effects and associated disorders ranging from congenital malformations to metabolic and neurological disorders and unwanted side effects for the pregnant woman [103].

Several case reports show that clozapine increases the risk of developing gestational diabetes [104, 105], but a recent systematic review in 2020 [106] suggests no significant relationship between antipsychotic drugs, including clozapine, and the risk of gestational diabetes mellitus (GDM). When analyzed the clozapine and olanzapine treatment, the results indicated a higher risk for macrocephaly.

A follow-up study conducted by a pharmaceutical company found malformations in the children of 4.2% of mothers treated with clozapine during pregnancy [107]. Other papers reported that 7 out of 84 women under clozapine treatment suffered spontaneous abortions. There were also mentions of shoulder dystocia [108, 109], atrial septum defect, and ectopic anus [110], floppy infant syndrome, EEG abnormalities [111], seizures, and digestive tract related disorders have been observed.

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Regarding the long-term effect on children born by mothers who were treated with clozapine during pregnancy, we found one single study that concluded that the children had a lower score in the Bayley III scale of adaptative behavior when compared with children whose mothers received treatment with other atypical antipsychotics [112].

3.11. Safety of clozapine in very early-onset schizophrenia or childhood-onset schizophrenia

Early-onset schizophrenia occurs before age 18; very early-onset schizophrenia (EOS) or childhood-onset schizophrenia (COS) onsets under 13 years old and is extremely rare [113]. It is well known that EOS has a poorer prognosis and more severe symptoms, as it affects the individual before the brain and personality are fully developed. The risks and benefits of clozapine administration in adults were well assessed as reviewed above, but clinicians are reluctant to prescribe clozapine for younger psychiatric patients.

A number of 125 studies were published on clozapine and early-onset schizophrenia or childhood-onset schizophrenia PubMed® database beginning with 1994, six randomized controlled trials, six systematic reviews, and three meta-analyses, described below.

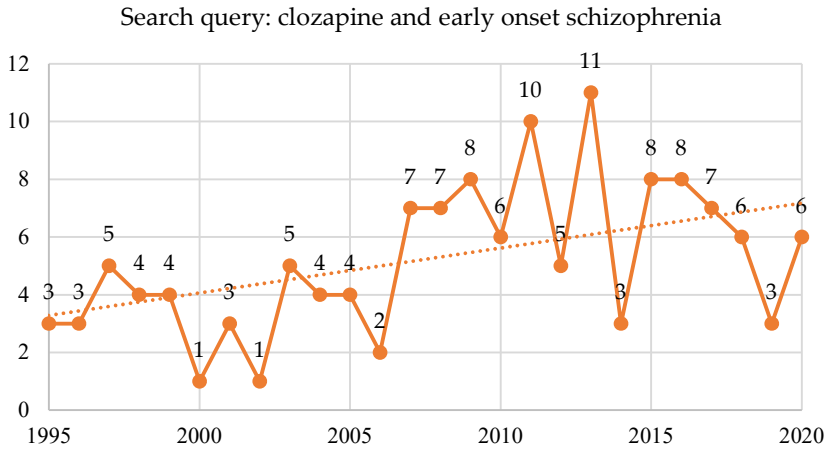

Figure no. 7 Search query: clozapine and early-onset schizophrenia PubMed® Count – total studies

The present protocols advise against prescribing clozapine in Early Onset Schizophrenia after two trials of different antipsychotics, which showed no improvement and to schedule regular follow-ups to check for adverse reactions. As in adults, clozapine also showed its superior efficacy in treatment-resistant early-onset schizophrenia. Clinical trials resulted in an improvement of up to 69% in assessments with the Brief Psychiatric Rating Scale that was maintained up to 9 years. More than 90% of the patients complained of sedation and sialorrhea. Enuresis, intestinal transit disorders, weight gain, and EEG abnormalities were reported by 10-60% of the patients. 1 - 30% complained of akathisia, blood pressure abnormalities, and tachycardia; 6 - 15% developed transient neutropenia. Agranulocytosis incidence was under 0.1%. In 8 - 22% of the cases were found metabolic disorders, but diabetes had a less than 6% incidence. Between 3 - 6% of the patients discontinued the use of clozapine.

The scientific data proves that clozapine is an effective and safe treatment for refractory early-onset schizophrenia [115].

3.12. Clozapine use in Romania

The data search on clozapine and Romania retrieved all-time 14 results on PubMed® five results on Web of Science® all Databases (WOS, BCI, CCC, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC, Search language=All), no clinical trials or RCT found on PubMed® and one in Web of Science® database (Figure no. 8).

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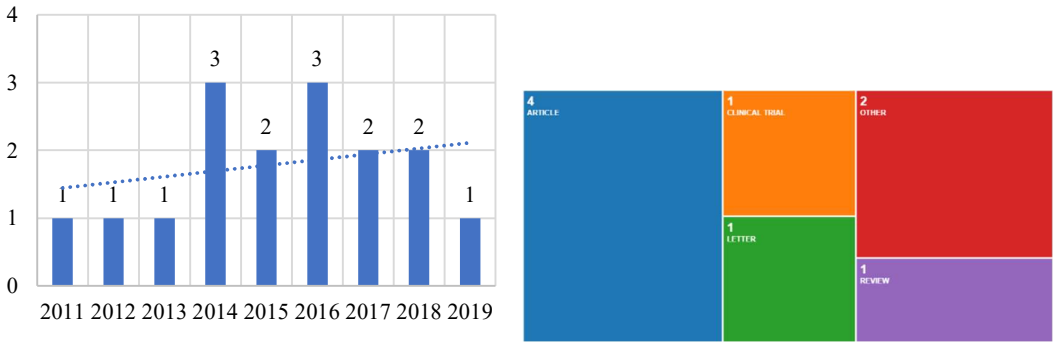

Figure no. 8 Data search on clozapine and Romania search query (a) on PubMed® – all-time papers published by year (n=14) (b) Web of Science® - five papers during the last five years

In one clinical trial (Ifteni, 2013)[116] was evaluated the safety and effectiveness of clozapine in patients who received CLZ after lack of response to other antipsychotics, showing rapid clozapine titration to be safe and effective in the therapy of schizophrenia, with an initiation time of 7.1 ± 4.8 days [117] but a study performed in Romania, Iasi showed that rapid titration of clozapine increases the risk of miocarditis in the first 2-8 weeks after the initiation of the therapy (Mitu *et al.* 2018)[118].
A cross-sectional study in Brasov, Romania (Ifteni, 2020) [119] was conducted on four years on 115 patients with schizophrenia (age = 39.7 ± 11.1 years; male = 59%) involuntarily admitted and restrained due to violence, suggest an early anti-aggressive effect of clozapine during the immediate use of clozapine in highly problematic patients previously showed in a eficacy study on 337 patients (Teodorescu, 2018) [120].
Study conducted by Ifteni *et al*., 2017, on the clinical efficacy of clozapine in bipolar disorder showed that switching from clozapine to another antipsychotic may increase the risk of relapse [126].
Teodorescu *et al.* (2020) published a recent paper on clozapine for Treatment-Refractory Aggressive Behavior made on a 504 patients admitted in the Clinical Hospital of Psychiatry and Neurology of Brasov; CLZ was effective and safe in cases of patients with treatment-refractory aggressive behavior [121].
More studies are needed in order to highlight the safety profiles and use in certain population groups due to the specific profile of the geographical population.

3.13 Publication Bias

There were insufficient studies to test for publication bias.

4. Discussion

There is an urgent need to enhance the access to clozapine for people with TRS at the worldwide level [122]; however, 40% of people with TRS will fail to respond to clozapine, suggesting that 12% to 20% of all people with schizophrenia will be ultra-resistant (failure to respond to adequate trials of two antipsychotics and clozapine) [123].

- The genetic vulnerability is correlated with metabolic side effects and postulates predictors of side effects severity; individuals who metabolize clozapine slower are at higher risk for this type of disorders. DiGeorge syndrome or 22q11.2 deletion syndrome encounters rates of up to 41% of schizophrenia spectrum disorders, much of the published literature in 22q11.2DS is on treatment-resistant schizophrenia. The medical comorbidities may complicate the administration of pharmacotherapy; psychiatrists should be able to adjust/ personalize clozapine doses for each patient based on pharmacogenetic testing as a potential biomarker in the severity of potential side effects.
- Agranulocytosis and neutropenia occurs in less than 1% in patients who take clozapine, is at the highest level in the first six months after initiation of treatment, and remains significantly high for 18 months; patients diagnosed with schizophrenia treated with other antipsychotics had the same risk of neutropenia, patients with a lower genetic risk may benefit from a more relaxed hematological

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monitoring schedule. Granulocyte colony-stimulating factor may be an option to reduce the impact of agranulocytosis.

- Olanzapine and clozapine determine the most metabolic disturbances that differ based on gender difference, more frequent among females.
- Pulmonary embolism associated with clozapine treatment, though rare, is lethal (a mortality rate of 36.36%), affirming the opportunity of prophylactic measures for venous thromboembolism for six months after initiating clozapine.
- Convulsive episodes are not an indication for stopping the treatment, 6% percent of the patients had seizures, with the incidence increasing with the dose, the plasma concentration of clozapine (1300 ng/ml) it is a better predictor than the dosage, anticonvulsants associated with clozapine may lead to a higher risk of severe side-effects or may interfere.
- There is not much data about clozapine treatment during pregnancy, risk of developing gestational diabetes, spontaneous abortions, teratogenic effects, and associated disorders (4.2% malformations), higher risk for macrocephaly, lower score in Bayley III scale of adaptative behavior.
- Clozapine remains an effective and safe treatment for refractory early-onset schizophrenia, improvement of up to 69% in assessments with the Brief Psychiatric Rating Scale, agranulocytosis incidence was under 0.1%.
- Few papers are published in indexed databases on the topic in the specific area Romania; more studies are needed in order to highlight the experience and pharmacogenetic characteristics of this specific geographical population in relation to the clozapine therapy.
- COVID-19 infection may enhance clozapine toxicity by significantly increase serum clozapine levels by CYP 450 system, generating an increased risk of pneumonia, even the need for intervention in a critical care unit. Nevertheless, the therapy must be continued with proper monitoring of the white blood count; positive COVID-19 patients may require a decrease of the clozapine dose by half until three days after the subside of the fever and reestablish the initial dosage gradually. Psychiatrists and involved healthcare providers must be aware of the increased risks in COVID-19 – clozapine treated patients, communicate, and strengthen the side effects surveillance.

5. Limitations

We selected CT/RCT and MA/SR from PubMed and Web of Science Core Collection Databases, searched by title and abstract topic; our study has not analyzed the simple reviews and case presentations, also papers present in other databases.

6. Conclusions

Even though clozapine is known and used for an extended period, as that in the past three or four decades has failed to generate effectively, mechanistically novel psychopharmaceuticals, and the limited prospects of the novel, more effective antipsychotics in the short to medium term [124], there is a need to maximize the access to the clozapine therapy and to investigate drugs that increase the effects of in resistant cases.

The progress of pharmacogenetic researches, the discoveries in the area of endocrinology, genetic testing, and other interdisciplinary approaches offer the psychiatrists nowadays the chance to use this drug at its highest potential, in a personalized manner for every patient, minimizing the adverse side-effects and maybe decreasing the rate of clozapine resistance by correctly identifying the clinical situation and the neurobiology of the resistance.

Legend:

- ADHD - Attention-Deficit/Hyperactivity Disorder
- ANC - Absolute Neutrophil Count
- BAF - Bipolar Affective Disorder
- BMI - Body Mass Index
- BPRS - Brief Psychiatric Rating Scale

- CT's - Controlled Trials
- C/D ratio - Concentration-to-dose ratio
- COS - Childhood-onset schizophrenia
- ECT – Electroconvulsive Therapy Augmentation
- EEG – Electro Encefalgram
- EOS - Early-onset schizophrenia
- GDM - Gestational diabetes mellitus
- PANSS - Positive and Negative Symptom Scale
- PRISMA - Preferred Reporting Items for Systematic Review and Meta-Analysis
- RCT's - Randomised Controlled Trials
- SARSCoV-2 - Severe acute respiratory syndrome coronavirus 2
- TRS - Therapy Resistant Schizophrenia
- TRBD - Treatment resistant bipolar disorder
- TEP – Pulmonary TrombEmbolia
- WHO – World Health Organisation

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Supplementary Materials: Figure S1: search of keyword "clozapine" on PubMed® database, Figure S2: PRISMA diagram, Table S1: Databases search results by numbers, Table S2: Pharmacogenetic biomarkers in clozapine treatment selected papers, Table S3: Clozapine associated pulmonary embolism selected papers, Table S4: Clozapine and COVID-19 selected papers, Table S5: Safety of clozapine use during pregnancy selected papers, Table S6: Safety of clozapine use in Children and Adolescents with Early-Onset Schizophrenia selected papers, Table S7: Clozapine for Treatment-Refractory Aggressive Behavior selected papers, Table S8: Clozapine resistance. Augmentation strategies - selected papers.

Conflicts of Interest: The authors declare no conflict of interest

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