Invest Clin 57(4): 352 - 363, 2016

Clozapine-induced myocarditis in observational cross-sectional and follow-up evaluations: comparison with other antipsychotics in naturalistic settings.

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Key words: atypical antipsychotics; heart toxicity; clozapine titration.

Abstract. Myocarditis occurs more frequently during clozapine (CLZ) administration than during treatment with other antipsychotic drugs (APs). In this observational study, we transversally screened outpatients for myocarditis by comparing a CLZ group of 132 subjects, with a non-CLZ group taking other APs (n = 371) only, and in 21 CLZ-treated patients and 18 subjects treated with other APs who had been followed for more than one year. The protocol included a) assessment of symptoms such as dyspnea, tachycardia, chest discomfort, fever, cough, and edema, b) blood pressure and heart auscultation; c) a standard electrocardiogram after a 5-minute rest, d) white cell count, and qualitative determination of troponin I, crea-

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tine-kinase-MB and myoglobin, and e) a cardiologist evaluation of subjects with suspected myocarditis. Only one case of myocarditis was detected, providing an approximation of the frequency of myocarditis of 1.6% in the first month of treatment. This was a 30-year-old man with schizophrenia who developed symptoms at day 6 after starting a treatment with 200 mg of CLZ a day without titration. Myocarditis was not observed during prolonged CLZ or other AP administration. These results support the proposal of starting CLZ treatment with a low dose and the feasibility of a simple protocol for myocarditis detection in psychiatry primary care.

Miocarditis inducida por clozapina durante la evaluación observacional, transversal y longitudinal: comparación con otros antipsicóticos en ambientes naturalísticos.

Invest Clin 2016; 57(4): 352 - 363

Palabras clave: antipsicóticos atípicos; clozapina; dosis; titulación; toxicidad cardiovascular.

Resumen. El desarrollo de miocarditis ocurre con más frecuencia durante el tratamiento con clozapina (CLZ) que durante el uso de otros antipsicóticos (APs). En el presente estudio observacional evaluamos la presencia de miocarditis mediante un protocolo transversal comparando 132 sujetos tratados con CLZ con 371 pacientes tratados con otro AP, y en 21 sujetos tratados con CLZ y 18 pacientes tratados con otro AP en un protocolo longitudinal mayor 1 año de duración. La evaluación incluyó: a) detección de síntomas como disnea, taquicardia, malestar torácico, fiebre, tos y edema; b) presión arterial y auscultación cardiaca; c) electrocardiograma estándar luego de un reposo de 5 minutos; d) contaje de glóbulos blancos y determinación cualitativa de troponina I, creatin-kinasa-MB y mioglobina, y e) evaluación por un cardiólogo en sujetos sospechosos para miocarditis. Detectamos un solo caso de miocarditis, lo que permite una aproximación sobre la frecuencia de miocarditis de 1,6 % durante el primer mes de tratamiento. Se trató de un sujeto masculino con esquizofrenia que desarrolló síntomas durante el día 6 después de haber iniciado el tratamiento con CLZ a la dosis de 200 mg por día sin titulación. No se detectaron sujetos sospechosos de miocarditis durante el tratamiento prolongado con CLZ u otro AP. Estos resultados sustentan la recomendación de comenzar el tratamiento con clozapina a dosis bajas, y la factibilidad de utilizar un protocolo sencillo para detectar miocarditis en la atención psiquiátrica primaria.

Recibido: 09-03-2016 . Aceptado: 30-06-2016

INTRODUCTION

Myocarditis occurs more frequently during clozapine (CLZ) administration than with any other typical or atypical antipsychotic drugs (APs) (1). Even though the clinical relevance of this association was established by Kilian et al., in 1999 (2), the interest of the medical community on this topic has increased considerably in the last few years.

The incidence of CLZ-associated myocarditis is controversial, with values of around 3% in Australia and of ≤1% elsewhere (3-7). In any case, these values are considerably higher than the estimated incidence of all types of myocarditis, which ranges from 0.0003 to 0.01% (6,7). No studies on this subject in Venezuelan populations have been published.

The difference in the prevalence of CLZ-associated myocarditis between Australia and other countries has been attributed to greater genetic susceptibility, inadequate dose titration, advanced age in the CLZ-treated Australian population and high case ascertainment (6). None of this explanations has been definitively ruled out, but Ronaldson et al., (6) proposed that the last one (high case ascertainment) appeared to be the most plausible.

Myocarditis may be acute or chronic, local or diffused in the myocardium (8). It may display minimal symptoms that can be easily overlooked or confused with signs of infectious illness and mild CLZ side effects, such as mild fever and tachycardia, or it can have a lethal course. Moreover, acute myocarditis may evolve to a chronic stage of cardiomyopathy. Diagnosing myocarditis in psychiatric patients is complex and requires considering diverse etiologies, mostly infectious, but it can also be associated with toxins, drug-induced hypersensitivity or autoimmune activation (1).

The risk of CLZ-associated myocarditis is higher at the beginning of the treatment. In a review of 59 cases (6), myocarditis developed in 54% of the patients within the first three weeks after starting the treatment (range: 4-21 days), and 81% of the cases developed within the first three months. By contrast, only one among those 59 cases developed myocarditis after prolonged (8 years) CLZ administration (9).

The rate of CLZ titration after starting the treatment appears to influence the risk of myocarditis. Ronaldson et al., (10) found that such a risk increased by 26% for each additional 250 mg of CLZ administered in the first nine days of drug titration. However, Ifteni et al., (11,12) did not report a single case of myocarditis in two studies using fast CLZ titration, involving either patients with schizophrenia or with treatment-refractory bipolar disorder.

Considering drug interactions, Ronaldson et al., (10) found that the concomitant use of valproate doubled the risk of myocarditis, and each successive decade increased the chance of developing myocarditis in 31%. Concomitant use of serotonin reuptake inhibitors also increased the risk of myocarditis (7).

The rate of CLZ-related-myocarditis in Venezuelan patients is unknown. Besides, in this country there is no formal consensus about CLZ dosage at different periods of the drug administration. However, our research group has recommended starting treatment with low CLZ doses, whenever this is possible (4).

In this observational study, we screened for myocarditis in two naturalistic settings: 1) a cross-sectional assessment of patients receiving prolonged CLZ or other AP administration, and 2) a group of subjects who started CLZ or any other AP treatment and who had been followed for over one year.

PATIENTS AND METHODS

Subjects

This was an observational study conducted in a non-probabilistic sample of out- and inpatients, including all the CLZ-treated subjects at two Venezuelan psychiatric institutions: the Center for Attention of Patients with Schizophrenia and their Families (Centro para la atención del paciente esquizofrénico y su familia, CATESFAM, Maracaibo, Zulia) and the Psychiatry and Psychology Unit (Unidad Andina de Psiquiatría y Psicología, Merida Clinic, Mérida) between January 2014 and May 2015. The control group

consisted of subjects receiving acute or chronic treatment with other APs. The Ethics Committees from CATESFAM (Maracaibo) and from La Universidad de los Andes (Mérida) approved the protocol. Patients or the responsible caretaker signed an informed consent of voluntary participation. The psychiatric diagnoses were conducted according to the DSM-IV-TR criteria (13).

The subjects were evaluated during their regular appointments in their respective institutions and were referred by their treating psychiatrist. As an observational protocol (14), the research team did not impose any specific treatment or dose on any patient, and did not discriminate by medication brand name. Patients directly purchased clozapine from Novartis Venezuela.

The inclusion criteria were: 1) voluntary participation in the study, and 2) to be undergoing acute (< 4 weeks) or chronic treatment (> 4 weeks) for any medical condition requiring CLZ or any other AP. This time-set was designed to encompass the period of highest risk for myocarditis (6). In the case of CLZ, it implied numerous off-label indications.

Subjects with infectious illnesses, with known heart diseases such as myocardial infarction, cardiomyopathy or valve dysfunction, or patients under chemotherapy were excluded.

Procedure

The study had two arms that were organized after data collection (Table 1). The cross-sectional one consisted of patients assessed in a single occasion at different times after starting CLZ or other AP treatments. The follow-up group consisted of subjects evaluated several times after starting any of the above-mentioned treatments.

The following information was requested from patients and/or care-takers: demographic data, psychiatric and medical diagnoses, past and current psychopharmacological treatment, and physical disease symptoms, such as fever, cough, dyspnea, chest/abdominal discomfort or pain, and lower extremity edema. A physical examination was performed by the treating physician, usually a primary-care psychiatrist who focused on blood pressure, heart rate, body temperature, lung congestion, and abnormal heart sounds.

A standard electrocardiogram (ECG) was recorded after a 5-minute resting period. A peripheral venous blood sample was then obtained for white blood count and qualitative troponin I determination. Troponin I was only qualitatively assessed (Cardiac Triple Test, Humasis Co., Ltd). This test has sensitivity and specificity of 95% and 97%, respectively, compared to quantitative evaluation. The test also screens for creatine kinase isoenzyme-MB (CK-MB) (sensitivity 98%, specificity 97%) and myoglobin (sensitivity > 99%, specificity 90%).

Furthermore, any subject with persistent tachycardia, chest discomfort, fever, positive troponin I and/or CK-MB, abnormal ECG (inverted wave, elevated/depressed ST segment) was followed and assessed by a cardiologist who conducted an exhaustive clinical and laboratory examination when necessary, including an echocardiogram. Major criteria for the diagnosis of myocarditis were positive troponin I and EEG abnormalities, such as T wave inversion and/or ST interval elevation or depression.

Our protocol did not include the routine evaluation of liver or kidney function

ECG quality assessment

Twenty randomly selected ECGs from patients with a negative diagnosis of myocarditis and the ECG of the single positive subject were also analyzed by a specialist in Internal Medicine who was blind to the pharmacological treatments. In all the cases, his analyses agreed with the psychiatrist's electrocardiographic diagnoses.

RESULTS

Demographic and clinical information

The whole sample consisted of 153 CLZ-treated subjects (2 inpatients) and 389 subjects (19 inpatients) receiving other APs (Table I). The CLZ group comprised more subjects with schizophrenia than the Other AP group (p = 0.000), and the gender distribution did not differ between both treatment groups (p = 0.3). Women were significantly older than males in both the CLZ and Other AP groups (p = 0.000) (Table II). No case of neutropenia or agranulocytosis was detected.

Cross-sectional arm

The CLZ group consisted of 132 patients, 104 (79%) of which were on monotherapy with CLZ. The remaining 28 subjects (21%) also received other APs. The non-CLZ group consisted of 371 patients treated with other APs (Table I). The type of other AP in both treatment groups is described in Table III.

TABLE I
STUDY DESCRIPTION

	Cross-sectional arm (*)			
Treatment (number of subjects)	Number of subjects and daily dose in parenthesis Time after starting treatment < 1 month	1 month-1 year	> 1 year	Total number of samples
Clozapine (n = 132)	43 (33.2 ± 30.4)	12 (109.3 ± 116.7)	77 (143.0 ± 93.9)	132
Other APs (n = 371)	177 (5.6 ± 5.0)	119 (7.3 ± 9.9)	75 (7.2 ± 6.5)	371
	Follow-up arm (**)			
Clozapine (n = 21)	21 (59.8 ± 66.9)	18 (49.9 ± 43.1)	7 (116.1 ± 138.2)	46
Other APs (n = 18)	12 (6.6 ± 6.4)	14 (6.1 ± 7.6)	7 (3.4 ± 1.6)	33

(*) Each subject was evaluated on a single occasion. (**) Each subject was evaluated on at least two ocassions. The clozapine daily dose is expressed as mg/day. The Other AP group daily dose is expressed as olanzapine equivalents.
Range of time in treatment after 1 year: cross sectional arm: clozapine group: 1-22 years.
Other APs group: 1-25 years; follow-up arm: clozapine group: 1.07-3.0 y; other AP group: 1.4-2.58 years.

TABLE II
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Treatment	Diagnosis n (%) (a)	Gender n (%)(b)	Mean age ± SD years [range] (c)
Clozapine n = 153	Schizophrenia 63 (41.2)	Female 76 (49.7)	52.7 ± 19.4 [7-87] (*)
	Bipolar disorder 20 (13.1)		
	Other 70 (45.8)	Male 77 (50.3)	42.5 ± 14.9 [16-85]
Other antipsychotics n = 389	Schizophrenia 33 (8.5)	Female 214 (55.1)	49.5 ± 17.8 [8-89] (*)
	Bipolar disorder 91 (23.4)		
	Other 265 (68.1)	Male 175 (44.9)	37.9± 16.7 [7-86]

SD: standard deviation.
CLZ vs. Other AP group: (a) $\chi 2$ (2) = 82.5, p = 0.000; (b) $\chi 2$ (1) = 0.1, p = 0.3;
CLZ and Other AP in a single analysis: (c) = f (3, 540) = 20, p = 0.000:
(*) = significantly higher than the male groups, $p < 0.01$.

Follow-up arm

The CLZ group consisted of 21 patients and the non-CLZ included 18 subjects. All the CLZ-treated patients were evaluated within the first month after starting the treatment. During this period no extra-cardiovascular side effects were reported. Eighteen subjects were re-evaluated between one month and one year after starting the treatment, and 7 subjects were re-evaluated after one year. From the 18 patients in the non-CLZ or other AP group, 12 were evaluated during the first month, 14 between one month, and one year and 7 after one year (Table I).

Three patients in the CLZ group (14 %) were also treated with other APs: 2 were taking aripiprazole and 1 received levomepromazine.

Diagnoses of myocarditis

Only one subject qualified as positive for myocarditis. This was a 30 year-old- man with schizophrenia who was receiving CLZ 200 mg/day without titration as a single treatment for a severe psychotic episode. He had been treated with that CLZ dose for 10 years, but he requested his treatment to be changed against the opinion of his treating physician. He remained under olanzapine (10 mg/day) and clonazepam (2 mg/day) administration for 6 months, but CLZ had to be administered again due to a severe relapse. The patient developed mild dyspnea and chest discomfort at day 6 without fever. At day 7, positive troponin I, ST segment elevation, normal c-reactive protein (CRP) and eosinophil count, negative CK-MB and myoglobin and normal chest X-ray were observed. CLZ

was withdrawn, and the symptoms improved within the next 24 hours. Troponin I remained positive until day 12. An echocardiogram and an exhaustive clinical evaluation conducted by a cardiologist were normal at day 15. This case is described elsewhere in detail (16). No other case of myocarditis was detected at any period in any of the two treatment groups.

The first three weeks after starting CLZ treatment appear to be the period of highest risk for myocarditis (6), and such a risk seems to be proportional to the CLZ dose and to the simultaneous use of drugs, such as valproate (10). We thus conducted a detailed analysis during the first month of treatment (Table IV). The subject who developed myocarditis was the only patient who was evaluated twice in the first week of treatment. He also was the only patient who had received the highest dose (200 mg/day) of CLZ from the beginning of the treatment.

The CLZ doses in the whole sample were as follows: < 25 mg/day (60% of patients), 25-50 mg/day (25 % of patients), 75 mg/day (3.3% of patients), and 100-200 mg/day (11.6% of patients). Valproate (250 mg/day) was used only in one CLZ-treated subject with schizophrenia.

DISCUSSION

Clozapine is approved for treatment-resistant schizophrenia, very often in severely aggressive and disturbed subjects (17). However, CLZ is also used for off-label indications, such as bipolar disorder (18), polydipsia and severe aggressive behavior and behavioral disorders in subjects with intellectual disability (19). Hence, CLZ is a key drug in psychiatric therapeutics, whose safety is of primary interest for clinicians.

Frequency of myocarditis

Only one patient out of 64 evaluated during the first month of CLZ administration was positive for myocarditis. By integrating the cross-sectional and the follow-up groups, this

TABLE III
TYPE OF OTHER ANTIPSYCHOTIC IN THE CROSS-SECTIONAL ARM

Type of antipsychotic drug	Clozapine group n (%)	Other antipsychotic-drug group n (%)
Olanzapine	0	101 (27.2)
Quetiapine	7 (25.0)	97 (26.1)
Risperidone	13 (46.4)	142 (38.2)
Ziprazidone	1 (3.6)	1 (0.3)
Aripiprazole	0	5 (1.4)
Paliperidone	1 (3.6)	5 (1.4)
Typical antipsychotics	6 (21.4)	2 (0.5)
Combination of atypical antipsychotics	0	18 (4.9)
Total	28 (100)	371 (100)

TABLE IV
DAILY AND CUMULATIVE DOSES OF CLOZAPINE OR OTHER ANTIPSYCHOTICS DURING THE FIRST FOUR WEEKS OF TREATMENT

Treatment	Week	Number of subjects	Daily dose (mean ± SD, range)	Accumulated dose (mean ± SD, range)
Clozapine (n = 60)	1	6	87 ± 79; 13-200 (a) 37 ± 18; 13-50 (b)	611 ± 554; 88-1400 (a) 261 ± 124; 88-350 (b)
	2	7	45 ± 20; 13-75	625 ± 283; 175-1050
	3	25	26 ± 27; 6-100	552 ± 563; 131-2100
	4	22	35 ± 31; 6-100	992 ± 868;¸175-2800
Other antipsychotics (n = 190)	1	4	6 ± 3; 3-10	42 ± 23; 23-70
	2	22	8 ± 7; 3-23	117 ± 93; 35-327
	3	87	6 ± 5; 1-28	121 ± 103; 14-595
	4	77	5 ± 5; 0.3-20.0	137 ± 134;¸ 9-560

Each subject was assessed on a single occasion, except the positive patient for myocarditis who was assessed twice in the first week.
SD = standard deviations.
Clozapine dose is expressed in milligrams; the other AP dose represents olanzapine equivalents (15).
(a,b) Including or excluding the case positive for myocarditis who received a clozapine dose of 200 mg/day

represents a value of 1.6 % as an approximation to the frequency of CLZ-associated myocarditis in the first 30 days of treatment. A pre-treatment evaluation was not conducted in any treatment group. This, along with the relatively small sample size in the first-month administration CLZ group, weakens the strength of this result.

In this study, we also screened for myocarditis after one month of treatment in 89 CLZ-treated subjects in the cross-sectional branch, and we also retested the whole group of 21 subjects in the follow-up group. Moreover, we screened 389 patients treated with other APs at different periods of time after beginning the treatment, and we retested 18 subjects in the follow-up branch. To our knowledge, this is the first study that screened for myocarditis during prolonged CLZ or other AP treatment, and not a single case was detected.

Titration of CLZ administration

Interestingly, the only positive case of myocarditis was found in a subject who started his CLZ treatment with a relatively high dose of 200 mg without titration. His myocarditis was mild and transient and did not require any specific intervention apart from CLZ withdrawal.

The safety of rapid CLZ dose titration at the beginning of the treatment is a controversial issue. On the one hand, the Dutch guidelines (20) and the pharmacokinetic guidelines (21) recommend CLZ doses of 6.2-25 mg on the first day

with progressive increments to no more than 200 mg/day by the end of the second week of treatment and to 300 mg/day by the end of the third treatment week. In Australia, Ronaldson et al., (10) also recommend CLZ titration. Specifically, these authors reported that cumulative CLZ doses above 920 mg during the first 1–9 days of treatment doubled the risk of myocarditis compared to cumulative CLZ doses lower than 500 mg (adjusted OR 2.31; 95% CI 0.98–5.48; p=0.06). Besides, according to these authors, each additional 250 mg of CLZ over days 1–9 increased the risk of myocarditis by 26% (adjusted OR 1.26; 95% CI 1.02–1.55; p=0.03). In our study, a cumulative dose above 925 mg was administered only in the fourth week of treatment. Hence, the majority of our patients started the treatment at a relatively safe CLZ dose.

Other research groups advocate for high CLZ doses from the beginning of the treatment. In their two research studies conducted in Romania, Ifteni et al., (11,12) administered high doses of CLZ from the start of the treatment. In their first study, they treated 111 patients with severe schizophrenia, 73 with previous exposure to CLZ and 38 CLZ-naïve subjects. The average CLZ dose during the first 24 h was 129 ± 75 mg (range 25–400 mg). An adequate symptom control was obtained on average with 371.9 ± 181.2 mg/day after 5.1 ± 4.0 days. In the second study, the authors compared two protocols in treatment-refractory bipolar disorder. In one protocol, CLZ was started at 25 mg followed by 25–50 mg as needed every 6h (maximum = 100mg/day) on day 1, followed by increases of 25–100mg/day. In the other protocol, CLZ was administered as 25 mg in day 1, followed by increases of 25–50 mg/day. Not a single case of myocarditis was reported in any of these studies.

Our naturalistic study reflects the typical CLZ use at our two institutions. Almost half (46%) of the CLZ-treated patients had diagnoses other than schizophrenia with extreme age ranges from a 7-year-old child with severe autism disorder to an 87-year-old patient with dementia. With such clinical diversity, a single protocol for all CLZ users would be inappropriate.

Diagnosing myocarditis

The fact that the only patient who developed myocarditis did not display eosinophilia and that his CRP and CK-MB were negative raise the issue of CLZ-associated myocarditis heterogeneity, as also emphasized by other authors (5,22-26). This drug-associated toxicity has been generally related to an acute, Ig-E mediated, type I immune reaction at the beginning of the treatment (5,22-24). However, the power of individual diagnostic variables in diagnosing CLZ-associated myocarditis is controversial (22-24).

Preliminary evidence, mainly from animal studies, suggests a direct CLZ cardiovascular toxicity as an additional mechanism. Catecholamine-associated myocardial inflammation may be one of those putative mechanisms since, in a murine model the degree of myocardial inflammation significantly correlates with CLZ dose and blood catecholamine levels (25). The relevance of catecholamine-mediated toxicity and its relationship to oxidative stress and other immunological pathways in CLZ-induced heart inflammation needs further clarification (26). In any case, the possible involvement of catecholamines opens the possibility of prevention or early treatment of CLZ-induced myocarditis with beta-adrenergic blocking agents or angiotensin converting inhibitors (16) in selected patients.

The extension of myocardial inflammation may also influence the type and magnitude of laboratory abnormalities (8). Hence, in mild and transient myocarditis, a smaller diversity and severity of the test abnormalities are expected than in more severe cases.

Use of clozapine in low-income settings

Experts recommend screening for myocarditis during CLZ administration without necessarily implementing mandatory, sophisticated, and, therefore, expensive protocols (5). Otherwise, low-income patients and those attended at public institutions could be denied a potentially life-saving treatment.

Ronaldson et al., (27) recommend obtaining baseline troponin I/T, CRP and echocardiography, and monitoring troponin and CRP on days 7, 14, 21 and 28. We contend that such a protocol is adequate for specialized CLZ clinics where follow-ups can be optimally achieved. The problem is that such clinics are rare not only in Venezuela but also all over Latin America, and perhaps, all over the developing world, too.

This is why we followed a simplified protocol that could be conducted at least one time during the first month of CLZ administration, including clinical examination, ECG, troponin and white blood cell count. This procedure can be realistically implemented in our populations, particularly in economically-deprived ones. The clinical examination proposed here can be conducted by a general physician or by the treating psychiatrist. The ECG can be interpreted by the primary physician as well. What is more, the white cell count and the qualitative enzyme tests require minimal technological support. Patients and caretakers should be instructed as well to monitor symptoms associated with myocarditis such as fever, chest discomfort and tachycardia, which would require additional evaluations.

Strengths and limitations

Our study has the limitation that the follow-up sample size is very small, and so we cannot be confident from our data that myocarditis doesn't occur after the first 4 weeks of clozapine. All the subjects were evaluated for myocarditis at least on one occasion during the first month of CLZ administration. Even though the patients were instructed to report myocarditis-associated symptoms, we cannot rule out that some cases could have been detected with a more rigorous protocol, for example, by conducting several evaluations within the first four weeks.

Other limitations were that it was not possible to evaluate the patients at baseline and that the cross-sectional protocol in most subjects may not allow the detection of brief and mild myocarditis or any other subclinical myocardium dysfunction (28). This limitation weakens the strength of our conclusion regarding the approximate value of myocarditis incidence. Finally, most of our patients started with low doses of CLZ. Hence, the myocarditis incidence reported here may differ from that of patients who start their treatment with high doses of CLZ.

The strength of this study is that it has used a uniform and sensitive testing procedure in naturalistic settings with a relatively large sample size in the cross-sectional group.

In conclusion, in agreement with studies conducted in other countries, the risk of myocarditis seems to be restricted to the first weeks of treatment, and it may be detected by a simple protocol. We plan to apply the procedure herein described at other Venezuelan institutions in order to increase the sample size and explore other risk factors.

We found one case only of myocarditis during the first month of CLZ administration, which represents a frequency of 1.6%. This positive subject had not received CLZ titration and he had started his treatment with relatively high doses of CLZ (200mg/day). Not a single case of myocarditis was detected after prolonged CLZ or other AP administration. Since CLZ-associated myocarditis appears to be a heterogeneous condition, further longitudinal studies are needed that explore risk factors in some subjects,

other than those evaluated by Ronaldson et al., (10), such as an enhanced response to catecholamines (15,25) and the constituting variables of the metabolic syndrome, inflammatory markers and natriuretic peptide, which have been implicated in subclinical cardiomyopathy (29). These studies, by exploring additional risk factors, may aid in clarifying the divergent results of Ronaldson et al., (10) and Ifteni et al., (11, 12) when considering the role of rapid CLZ titration at the beginning of treatment. In the meantime, we recommend introducing CLZ by slow dose titration and avoiding the simultaneous use of valproate at the beginning of treatment whenever it is possible.

ACKNOWLEDGMENTS

This study was funded by Grant 2013-440 from the "Fonacit, (Fondo Nacional de Ciencia y ecnología) Programa de Investigación Universitaria" (Venezuelan Government University Research Program). The authors are grateful to Diego Dávila-Spinetti, MD (the consultant cardiologist who passed away before the publication of this article), to Françoise Salager-Meyer, a medical linguist, for her editorial assistance, and to the treating physicians for allowing us to assess their patients, also providing the required information.

REFERENCES

1. **Merrill DB, William G, Goff DC.** Adverse cardiac effects associated with clozapine. J Clin Psychopharm 2005; 25: 32-41.
2. **Kilian JG, Kerr K, Lawrence C, Celermajer DS.** Myocarditis and cardiomyopathy associated with clozapine. Lancet 1999; 354: 1841-1845.
3. **Cohen D, Bogers JP, von Dijk D, Bakker B, Schulte PF.** Beyond white blood cell monitoring: screening in the initial phase of clozapine therapy. J Clin Psychiat 2012; 73: 1307-1312.
4. **de Leon J, Tang Y-L, Baptista T, Cohen D, Schulte PFJ.** Titrating clozapine amidst recommendations proposing high myocarditis risk and rapid titrations. Acta Psychiat Scand 2015; 132: 242-243.
5. **Freudenreich O, Henderson DC, Sanders KM, Goff DC. T**raining in a clozapine clinic for psychiatry residents: a plea and suggestions for implementation. Acad Psychiatr 2013; 37:27-30.
6. Ronaldson KJ, Fitzgerald P, McNeil JJ. Clozapine-induced myocarditis, a widely overlooked adverse reaction. Acta Psychiat Scand 2015; 1: 1-10**.**
7. **Youssef DL, Narayanan P, Gill N.** Incidence and risk factors for clozapine-induced myocarditis and cardiomyopathy at a regional mental health service in Australia. Australas Psychiatry 2016; 24: 176-180.
8. **Liu P, Baughman KL. Myocarditis. I**n: Braunwald's Heart Disease: a Textbook of Cardiovascular Medicine. (Bonow, R.O., Mann, D.L., Zipes, D.P., Libby, P, eds.) Elsevier Saunders, Philadelphia, PA, 2012. P. 1595-1609.
9. **Lang UE, Willbring M, von Golitschek R, Schmeisser A., Matchke K, Malte Tugtekin S.** Clozapine-related myocarditis after long-term treatment: case presentation and clinical perspectives. J Psychopharmacol 2008; 22: 576-580.
10. **Ronaldson KJ, Fitzgerald PB, Taylor AJ, Topliss DJ, Wolfe R, McNeil JJ.** Rapid clozapine dose titration and concomitant sodium valproate increase the risk of myocarditis with clozapine: A case-control study. Schiz Res 2012; 141: 173-178.
11. **Ifteni P, Nielsen J, Burtea V, Correll CU, Kane JM, Manu P.** Effectiveness and safety of rapid clozapine titration in schizophrenia. Acta Psychiat Scand 2014; 130:

25-29.
12. **Ifteni P, Correll CU, Nielsen J, Burtea V, Kane JM, Manu P.** Rapid clozapine titration in treatment-refractory bipolar disorder. J Affect Disorders 2014; 166:168-72.
13. **American Psychiatric Association.** Diagnostic and Statistical Manual, DSMIV-TR. Washington DC: American Psychiatric Association Press; 2000.
14. **Everitt BS.** Medical statistics from A to Z: a guide for clinicians and medical students. New York: Cambridge University Press; 2003, pp. 154.
15. **Gardner DM, Murphy AL, O'Donnell H, Centorrino F, Baldessarini RJ.** International consensus study of antipsychotic dosing. Am J Psychiat 2010; 167: 686-693.
16. **Baptista T, Rojas N, Dávila D.** Heterogeneity of CLZ-associated myocarditis: an opportunity for novel preventing strategies. Aust NZ J Psychiat 2015; 49 (11):1068.
17. **Novartis Pharmaceutical. Clozaril.com. 2015.** Accessed on June 10, 2016.
18. **Li XB, Tang YL, Wang CY, de Leon J.** Clozapine for treatment-resistant bipolar disorder a systematic review. Bipolar Disord 2015; 17: 235-247.
19. **Sabaawi M, Singh NN, de Leon J.** Guidelines for the use of clozapine in individuals with developmental disabilities. Res Dev Disabil 2006; 27: 309-336.
20. **Guidelines for the use of clozapine.** http://www.clozapinepluswerkgroep.nl/wp-content/uploads/2013/07/ 2013.Pdf, accessed on February 25, 2015).
21. **Spina E, de Leon J.** Clinical applications of CYP genotyping in psychiatry. J Neural Transm 2015; 122: 5-28.
22. **Ronaldson KJ, Taylor AJ, Fitzgerald PB Topliss DJ, Elsik M, McNeil JJ.** Diagnostic characteristics of clozapine induced myocarditis identified by an analysis of 38 cases and 47 controls. J Clin Psychiat 2010; 71: 976-981.
23. **Ronaldson KJ, Fitzgerald P, McNeil JJ.** Evolution of troponin, C-reactive protein and eosinophil count with the onset of clozapine-induced myocarditis. Aust N Z J Psychiat 2015; 49: 486-487.
24. **Fehily SR, Forlano R, Fitzgerald PB.** C-reactive protein: an early critical sign of clozapine-related myocarditis. Australas Psychiatry 2016; 24: 181-184.
25. **Wang JF, Min JY, Hampton TG, Amende I, Yan X, Malek S, Abelmann WH, Green AI, Zeind J, Morgan JP.** Clozapine-induced myocarditis: role of catecholamines in a murine model. Eur J Pharmacol 2008; (1-3): 123-127.
26. **Abdel-Wahab BA, Metwally ME.** Clozapine-induced cardiotoxicity: role of oxidative stress, tumor necrosis factor alpha and NF-kappabeta. Cardiovasc Toxicol 2014; 15: 355-365.
27. **Ronaldson KJ, Taylor AJ, Fitzgerald PB, Topliss DJ, McNeil JJ.** A new monitoring protocol for clozapine induced myocarditis based on an analysis of 75 cases and 94 controls. Aust N Z J Psychiat 2011; 45: 458-465.
28. **Curto M, Comparelli A, Ciavarella GM, Gasperoni C, Lionetto L, Corigliano V, Uccellini A, Mancinelli I, Ferracuti S, Girardi P, Baldessarini RJ.** Impairment of left ventricular function early in treatment with clozapine: a preliminary study. Int Clin Psychopharm 2015; 30: 282-289.
29. **Chow V, Yeoh T, Ng AC C, Pasqualon T, Scott E, Plater J, Whitwell B, Hanzek D, Chung T, Thomas L, Celermajer DS, Kritharides L.** Asymptomatic left ventricular dysfunction with long-term clozapine treatment for schizophrenia: a multicentre cross-sectional cohort study. Open Heart 2014;26 (1):e000030.