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Treatment-Resistant Schizophrenia: Clinical Outcomes & Predictors of Response to Clozapine

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Treatment-Resistant Schizophrenia: Clinical Outcomes & Predictors of Response to Clozapine

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Abstract

Treatment-Resistant Schizophrenia (TRS) is diagnosed in one third of patients suffering from Schizophrenia.[1–3] Predictors of TRS have been explored in previous studies but clinical practice is devoid of strategies for early detection of the disease.[4,5] Moreover, Clozapine is the only treatment with proved efficacy in TRS, but it is associated with significant side-effects, requiring specific monitoring of serum levels and weekly-blood analysis.[2,5] All these factors compel TRS patients to experience non-Clozapine antipsychotics high-dosage polypharmacy and numerous Years of Uncontrolled Disease (YUD).[6,7] Finally, 30% of TRS patients properly treated develop Clozapine-Resistant Schizophrenia (CRS), and no predictors have been associated with this prognosis.[4,8–10]

Clinical outcomes of TRS treatment and predictors of response to Clozapine were investigated in a sample of 25 in-patients receiving Clozapine in a specialized-TRS unit during a 6-month-period. All patients underwent standardized clinical assessment of symptoms severity, global functioning and side-effects at baseline and 6 months after, using the *PANSS*, the *GAF* and the *SMARTS* respectively. Laboratory tests for evaluation of Clozapine Serum levels, metabolic function and complete hemograms were also performed during the course of treatment. Sociodemographic information was collected in an initial interview and the history of the disease was assessed according to the patients' consent.

A preliminary analysis confirmed the effectiveness of Clozapine treatment, since the majority of patients improved in all symptom domains and functioning. Positive symptoms were the domain with the highest rate of change, not being influenced by any specific patient characteristic. However, according to *Treatment Response and Resistance In Psychosis* (TRRIP) Working Group criteria, only 64% of patients were considered adequate responders. We found that non-responders presented a distinct initial profile, with greater symptom severity and higher impairment in functioning; and generally experienced more YUD when compared to responders. By testing different variables against the course of disease, we concluded that the severity of negative symptoms was an important prognostic factor, influencing negatively the improvement of this symptom domain and of functioning. A subgroup of patients presented a rather interesting pattern, since they suffered from high negative symptoms severity and received Clozapine relatively early but still did not achieve 20% of negative symptoms change. Patients with many YUD improved regularly around 20% on their negative symptoms' score independently of the initial severity. In addition, we verified that some of the TRS predictors previously studied were detectable in our sample. Hence, some of those patients could have benefited from a TRS early detection algorithm, applied at their First-Episode. Surprisingly, temperature was the most significant predictor of total symptom change. This finding could be related to a hypothesised dysregulation of the glutamate system which has already been associated to TRS.

We concluded that the economic and human resources invested in specialized-TRS units are well compensated by the clinical outcomes achieved, and therefore, more care should be dedicated to TRS patients worldwide. Moreover, there is an utmost need for further investigation on the neurobiology underlying TRS and CRS, in order to allow tailored treatments to be performed.

KEYWORDS: SCHIZOPHRENIA; TREATMENT-RESISTANT; CLOZAPINE; PREDICTORS; TREATMENT OUTCOME.

Resumo

A Esquizofrenia Resistente ao Tratamento (TRS) é diagnosticada num terço dos doentes com Esquizofrenia.[1–3] Alguns preditores da TRS foram anteriormente explorados, mas a prática clínica mostra graves falhas na detecção precoce desta doença.[4,5] Além disso, a Clozapina é o único tratamento com eficácia comprovada na TRS, mas está associada a efeitos adversos significativos, exigindo monitoramento específico dos níveis plasmáticos e análises sanguíneas semanais.[2,5] Todos estes fatores levam os doentes com TRS a receberem polimedicação de antipsicóticos em altas doses durante numerosos Anos de Doença Não Controlada (YUD).[6,7] Por fim, 30% dos doentes com TRS adequadamente tratados desenvolvem Esquizofrenia Resistente à Clozapina (CRS), e nenhum fator preditor tem sido associado a este prognóstico.[4,8–10]

Os outcomes clínicos do tratamento da TRS e os preditores de resposta à Clozapina foram investigados numa amostra de 25 doentes internados numa unidade especializada no tratamento da TRS, que receberam Clozapina durante um período de 6 meses. Todos os doentes foram submetidos à avaliação clínica da gravidade dos sintomas, funcionamento global e efeitos adversos no início do tratamento e 6 meses depois, usando a *PANSS*, a *GAF* e a *SMARTS*, respectivamente; assim como testes laboratoriais para a avaliação dos níveis séricos de Clozapina, função metabólica e hemograma completo. As informações sociodemográficas foram reunidas numa entrevista inicial e a história da doença foi recolhida através da consulta dos processos clínicos, de acordo com o consentimento dos doentes.

Um teste preliminar confirmou a eficácia do tratamento com Clozapina, uma vez que a maioria dos doentes melhoraram em todos os domínios de sintomas e no funcionamento. Os sintomas positivos foram o domínio com maior taxa de mudança, não sendo influenciados por nenhuma característica específica. No entanto, de acordo com os critérios do *Treatment Response and Resistance In Psychosis* (TRRIP) Working Group, apenas 64% dos doentes foram considerados respondedores adequados. Verificamos que os não respondedores apresentaram um perfil inicial distinto, com maior gravidade dos sintomas e maior comprometimento da funcionalidade; e geralmente experimentaram mais YUD quando comparados aos respondedores. Ao testar diferentes variáveis com o curso da doença, concluímos que a gravidade dos sintomas negativos representou um importante fator de prognóstico, influenciando negativamente a melhoria deste domínio e da funcionalidade. Um subgrupo de doentes apresentou um padrão interessante, uma vez que sofrendo sintomas negativos severos e recebendo Clozapina relativamente cedo, não conseguiu atingir 20% de evolução no domínio respectivo. Doentes com muitos YUD diminuiram homogeneamente cerca de 20% dos seus sintomas negativos iniciais, independentemente de sua gravidade. Para além disso, verificamos que alguns dos preditores da TRS previamente estudados se encontravam presentes na nossa amostra. Assim, alguns destes doentes poderiam ter beneficiado de um algoritmo de detecção precoce de TRS, aplicado no seu Primeiro Episódio da doença. Surpreendentemente, a temperatura foi o preditor mais significativo da alteração total dos sintomas, o que pode ser justificado por uma possível desregulação dos níveis de glutamato, previamente associada à neurobiologia subjacente à TRS.

Desta maneira, concluímos que os recursos económicos e humanos investidos em unidades especializadas no tratamento da TRS são compensados com os resultados clínicos alcançados e, portanto, mais cuidados devem ser dedicados à TRS a nível mundial. Paralelamente, há uma necessidade extrema de investigação sobre a neurobiologia subjacente à TRS e à CRS, com o intuito de se proceder a uma adequada adaptação do tratamento a estes doentes.

PALAVRAS-CHAVE: ESQUIZOFRENIA; RESISTÊNCIA; CLOZAPINA; PREDITORES; *OUTCOME* CLÍNICO.

Introduction

Schizophrenia is a severe mental disease affecting around one percent of the worldwide population; one third of those patients end up fulfilling criteria for Treatment-Resistant Schizophrenia (TRS).[1–3] According to the *UK's National Institute of Clinical Excellence* (NICE) Guidelines, TRS is defined as a lack of adequate response to treatment with at least 2 Antipsychotic (AP) drugs, at least one of them being a non-Clozapine Second-Generation Antipsychotic (SGA) in an adequate dose (≥ 600 mg/day of *Chlorpromazine equivalents*) with an adequate adherence and duration of treatment (≥ 6 weeks for each trial).[2,11] TRS has a deep impact in the well-being of patients, leading to diminished quality of life and increased comorbidity.[12] Several demographic and clinical variables, such as an early age of onset, Paranoid Schizophrenia subtype, living in a non-urban area, having family history of Schizophrenia, more schooling, and a previous suicide attempt, have been associated with a higher incidence of TRS.[4,7,10,13] Unexpectedly, being a male and living in an urban area were not proven to be associated with higher incidence of TRS, a fact that supports its classification as a different subtype rather than a severe form of Schizophrenia.[4,9,10,14]

According to international guidelines, Clozapine represents the gold standard treatment for TRS, being efficacious in reducing symptoms and decreasing the risk of psychosis relapse.[2,15–18] This SGA acts as a serotonin and dopamine antagonist, presenting higher affinity to dopamine D4, unlike other SGA; a partial 5-HT1A agonist; and a muscarinic M1, M2, M3, M5, histamine, and alpha-1 adrenergic-receptor antagonist. It is metabolized in the liver by the *cytochrome P450 enzymes* (CYP1A2) into *Norclozapine* (NOR); 50% of the drug is eliminated as NOR by renal clearance and other 30% in the stools.[17,18] Compared to other AP drugs, Clozapine demonstrates an important improvement of positive symptoms, a better compliance, an anti-craving effect for drug abuse and a reduced suicide rate.[8,16]

Nevertheless, it is important to highlight its possible serious side effects (e.g. lethal agranulocytosis, myocarditis, dyslipidemia).[15,17–19] This implies the development of complex protocols of treatment monitoring, which often discourage clinicians to prescribe Clozapine.[5] In addition, there is evident lack of clinical recognition of TRS in early stages, namely because it is extremely difficult to distinguish it from medication nonadherence.[8] As a result, these patients experience high-dosage of AP polypharmacy before getting proper treatment and endure prolonged periods of untreated disease, despite the lack of evidence of polypharmacy clinical benefits.[5,20] According to a recent study, patients with TRS who go without adequate treatment for more than 2.8 years show a decreased response to Clozapine, resulting in prescription of alternative treatments, such as Electroconvulsive Therapy (ECT).[6,7] Ultimately, recent literature shows that 60% of TRS patients develop ultra-resistance, which means they will be partial or non-responsive to Clozapine.[1,8]

There is little and controversial insight on the pathology underlying Clozapine-Resistant Schizophrenia (CRS).[1] Acknowledging Clozapine pharmacokinetics is an important first step to improve treatment, since CYP1A2 inducer and inhibitor substances and liver function may modify Clozapine metabolization and therefore its serum levels.[21,22] However, there is evidence that TRS patients respond with high heterogeneity to Clozapine treatment even when serum levels are properly

controlled.[5] In agreement, recent studies have raised the possibility of classifying TRS as a different subtype of Schizophrenia, with its own neurobiological mechanisms which would explain the lack of response to standard treatment.[4,9,10] Further support to this concept comes from evidence showing that the majority of TRS patients develop treatment resistance from illness onset.[4] Since available neuroimaging and genetic biomarkers do not represent reliable tools to guide the use of Clozapine yet, current practice suggests further investigation to be conducted, in order to identify clinical factors affecting the response of TRS to treatment.[1,5]

This study was conducted at the *Unidade de Cuidados Avançados de Esquizofrenia Resistente ao Tratamento (UCAERe-T)* of the *Department of Psychiatry* at *Centro Hospitalar e Universitário de Coimbra*, the first national unit specialized in the assessment, therapeutic intervention and rehabilitation of patients diagnosed with TRS, established in March 2016. Our goals were to explore the effectiveness and feasibility of Clozapine's treatment in TRS patients, by assessing clinical outcomes and side-effects; to identify the profile of non-responders to Clozapine, when compared to adequate responders; and finally, to study the course of symptoms in order to find predictors and moderators of good clinical and functioning response. A clear knowledge of Clozapine-resistance mechanisms and disease-modifiable variables would have a very important impact in the treatment provided to TRS patients.

Methods

1. Design and Participants

We led an exploratory retrospective cohort study in a sample of TRS patients in treatment at UCAERe-T for a period of 6 months (n=25). Schizophrenia was diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders, 5th edition* (DSM-5) criteria and TRS defined according to NICE criteria. Extrinsic factors such as substance abuse, environmental conditions, and organic causes of psychosis were assessed in order to exclude Pseudo-resistance.[5] (consult Supplementary Table I to guidelines and definitions).

2. Procedure

An initial interview took place in order to collect sociodemographic and clinical data. Medical records were also assessed, including history of diagnosis and treatment and family history of psychiatric diseases. Anthropometric measures, as well as laboratory blood tests were performed both at baseline (T1) and at month 6 (T2). Simultaneously, several assessment instruments were applied at T1 and T2: symptom severity was assessed with the *Positive and Negative Syndrome Scale* (PANSS); functioning was evaluated with the *Global Assessment of Functioning* (GAF); and AP side-effects were self-evaluated with the *Systemic Monitoring of Adverse Events Related to TreatmentS* (SMARTS).

In order to classify TRS according to symptom domains and clinical change of symptoms, we applied the *Treatment Response and Resistance In Psychosis* (TRRIP) Working Group criteria.[11] Therefore, TRS domain was defined as the presence of more than one moderate symptom or one severe symptom in the PANSS score of the respective domain at T1. Symptom change was obtained as the proportion of the PANSS score improved from T1 to T2, adjusting the scale to a baseline of zero (e.g. PANSS T1=90, T2=60, symptoms course of 50%). An improvement equal or higher than 20% in the total symptom ranking and in the specific domain of interest was considered as an indicator of good symptoms course. We considered the following four criteria to define CRS patients: A) an absolute threshold of symptoms with at least one moderate symptom or one severe symptom in the domain of interest at T2; B) a decrease of less than 20% both in the total rating and the specific domain of interest at T2; C) moderate or more severe functional impairment measured by the GAF at T2; D) a minimum treatment duration of 12 weeks.[11] Change in functioning was calculated from the difference of the GAF score between T1 and T2.

We selected candidate predictors based on a literature search of risk factors for treatment-resistant Schizophrenia and/or Clozapine-Resistant Schizophrenia. To that end, we included patient-related factors (sex, age, residence, birthday season, marital status, housing conditions, education level, employment, comorbidity) and disease-related factors (family-history, subtype of Schizophrenia, age of *First-Episode Schizophrenia* (FES), Treatment Resistant from Onset (TRO), number of acute episodes, number of treatment trials, AP polypharmacy and high-dosage prescription, period of

uncontrolled disease since diagnostic, use of psychotropic drugs, history of suicide ideation, adherence to treatment, cigarette consume and history of a previous Clozapine trial).[9] In addition, we included metabolic function factors associated to Clozapine pharmacokinetics and general inflammation levels (Creatinine, INR, LDL and CPR) and vital signs (Mean Arterial Pressure, Heart Rhythm, Temperature, Weight, and Waist Circumference), in order to understand the impact of physical condition in the final outcomes. The number of *Years of Uncontrolled Disease* (YUD) was considered to be the period from the first acute episode without a period of remission to the moment of admission in the UCAERe-T. Variables regarding vital signs and biometric measures corresponded to the mean of the three previous days before clinical evaluation at T2. Variables regarding metabolic function were obtained as the mean of three different laboratory results during hospitalization.

The Clozapine protocol treatment was based on the "*Treatment Review and Assessment Team" of the South London and Maudsley Trust, National Health Service of United Kingdom*.[23] It included, when feasible, the withdrawal of any non-Clozapine AP drug administered at the moment of admission, pursued within one to a three-week-period. Simultaneously, there was progressive titration of Clozapine, initiating with an oral dose of 25 mg twice a day, adding 25 mg/day every 3 days until reaching 100 mg/day. Plus, a supplemental 50 mg would be added every 3 days if necessary, usually until achieving a dose of 400 mg twice a day. Oral dose was adjusted according to clinical response and Clozapine serum levels regularly measured. The recommended therapeutic window of Clozapine was considered between 350 and 600 ng/ml. A slow titration was rather important in reducing side effects.[17] In this investigation, we considered the evaluation of Clozapine and NOR serum levels at a first moment, if Clozapine was already being administrated before admission, and at month 6 (T2). Clozapine serum levels indicated the efficiency of the oral dose, avoiding toxicity levels (superior to 1000 ng/mL); NOR serum levels helped to understand the influence of liver metabolization in the final outcomes; and Ratio Clozapine/NOR was useful to control efficiency (between 1 and 2) and toxicity (ratio ≥ 3) of Clozapine levels in plasma; as well as adherence to treatment.[15] The method performed to analyse Clozapine and NOR serum levels was an *Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry*. Long-Acting Injectable (LAI) Paliperidone Palmitate was administered once-monthly/3-monthly in the majority of patients, in order to guarantee clinical adherence to treatment after hospitalization.[24]

Every participant was provided with a fact sheet explaining the details and goals of this investigation and their written-informed consent was obtained after proper discussion with patients and close relatives. All procedures contributing to this investigation comply with the ethical standards of the institutional committee on research and with the Helsinki Declaration.

3. Statistical Analysis

Data analysis was performed using SPSS Statistics (Version 25; SPSS, Inc:, IBM Company) and PROCESS (Version 3.3)[25] software.

Descriptive data is reported as frequencies and percentages or means and standard deviations, as appropriate. The effectiveness of the Clozapine protocol was assessed with the Wilcoxon Signed Rank test of the PANSS and GAF scores at T1 and T2. Responder and Non-responder groups were classified according to the 4 criteria preconized by the TRRIP Working Group criteria. Both groups were compared with each other according to clinical and pharmacological variables, with an independent-samples Mann-Whitney test and Pearson Chi Square Test, as appropriate. A *p* value of less than 0.05 was considered significant.

Spearman's/Pearson's correlation coefficients were applied to test association between independent variables and each symptom domain and functioning course during the 6 months of treatment, as appropriate (normality tested with the Shapiro-Wilk Test because the n<50). Variables showing the strongest associations with symptoms and functioning course were analysed using Multiple Linear Regression models according to a stepwise method, in order to find causality in their association and eliminate variables that would not improve significantly the model of prediction.[26]

Finally, a linear regression was tested with a Simple Moderation Model for a third interaction variable, chosen for theorical reasons. In this case, we considered as moderators the number of YUD; and the fact that some patients were medicated with Clozapine in a non-oral-dosage-adjusting regime before hospitalization. Figure 1 shows the mathematical model applied in the moderation analysis.[25]

Further detailed information is exposed in the beginning of each chapter of the Results section, when considered necessary for a better understanding of the analysis performed.

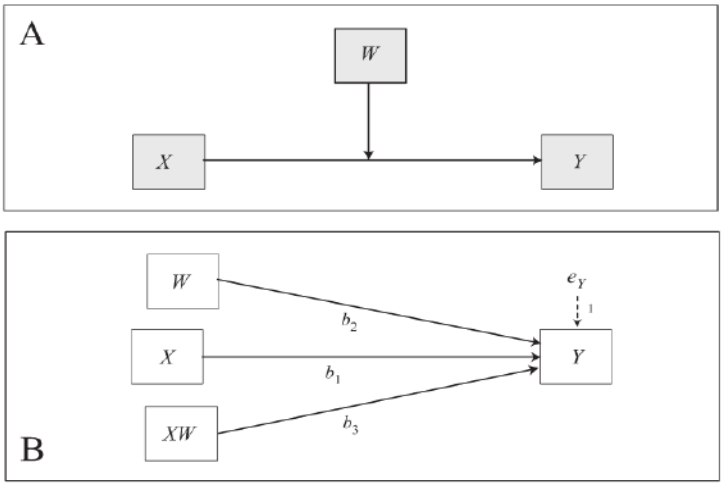

Fig. 1: Moderation Model. A: conceptual diagram. B: statistical diagram.

Results

1. Sample Characteristics

25 patients fulfilled the criteria for inclusion (Appendix – supplementary Graph I). Table 1 presents the socio-demographic characteristics as well as information regarding the history of the disease. All patients were men, and the majority presented Paranoid Schizophrenia subtype (96%). Figure 2 presents the TRS symptom domain(s) at admission.[11] 72% of the patients were already medicated with Clozapine at admission, receiving an average dose of 241.67 (SD 154.59) mg/day (range 50-500mg/day), corresponding to an average Clozapine serum level of 295.57 (SD 284.26) ng/mL (range 32-1102 ng/mL). Before admission, 9 patients (36%) were under treatment with a First-Generation AP (FGA) ($\bar{x}$= 0.56, SD= 0.82), and 20 (80%) with a SGA ($\bar{x}$= 1.28, SD= 0.94). 7 patients (28%) were medicated with both. From those medicated with SGA, 15 were receiving LAI Paliperidone Palmitate (range 100-150mg/monthly). Regarding comorbidities, 4 patients suffered of Obesity; 3 were treated for a Diabetes Mellitus type 2; 1 was followed for an Auto-Immune Hypothyroidism disease; 1 required special care for an Obstructive Sleep Apnea; and finally, 1 presented an antecedent of Myocardial Infarction. None of those were considered as an absolute contraindication for Clozapine. Habitual Medication was evaluated in order to identify the co-administration of CYP1A2 inhibitor and/or inducer substances: except for cigarette smoking, we found no such case.[22]

Table I Characteristics of TRS patients (n=25)

CHARACTERISTIC	N (%)	$\bar{X}$ (SD)
Male Sex	25 (100)	-
Residence: Not urban	16 (64)	-
Birthday Season: Spring & Summer	16 (64)	-
Marital Status: single	24 (96)	-
Housing: living with parents or similar	13 (52)	-
Education Level: under 9th grade	19 (76)	-
Currently Unemployed or Retired	17 (68)	-
Family History of Psychiatric Disease	6 (25)	-
Paranoid Schizophrenia	24 (96)	-
Previously medicated with Clozapine	18 (72)	-
TRO	18 (72)	
History of AP Polypharmacy	24 (96)	-
History of Cannabinoids Consume	14 (56)	-
History of Suicide Ideation	3 (12)	-
History of Electroconvulsive therapy	0 (0)	-
Age (years)	-	39.04 (12.94)
Age of First Episode (years)	-	21 (6.40)
Number of Clinical Acute Episodes	-	10.04 (7.49)
Number of Treatment Trials	-	4.08 (2.73)
Number of FGAs	-	1.44 (1.23)
Number of SGAs	-	2.79 (1.25)
Years of Uncontrolled Disease	-	13.82 (10.41)
Tobacco (cigarettes/day)	-	14.05 (10.00)
PANSS Total score T1	-	90.92 (16.68)
PANSS Positive score T1	-	22.48 (6.38)
PANSS Negative score T1	-	24.12 (5.91)
PANSS General score T1		44.32 (8.24)
Global Assessment of Functioning	-	37.32 (9.80)

AP: Antipsychotic; FGA: First-Generation Antipsychotic; PANSS: Positive and Negative Syndrome Scale; SGA: Second-Generation Antipsychotic; SD: Standard Deviation; TRO: Treatment Resistant from Onset; $\bar{x}$: mean.

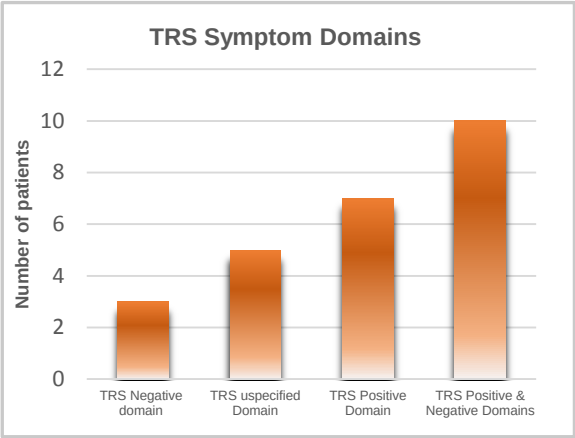

Fig. 2: Classification of TRS Symptom Domains, according to TRIPP Working Group (2017).

2. Preliminary Study of Clozapine Protocol Effectiveness

The effectiveness of the treatment protocol was tested with a Wilcoxon Signed Rank Test (Table II), by analyzing symptom severity level and functioning at T1 and T2. We observed that one patient maintained the same intensity of negative symptomatology during treatment. 2 patients were not assessed with the GAF scale at T1, and consequently we could not calculate their functioning course during treatment.

Table II Symptoms and Functioning course

		n	Mean Rank	Z
PANSS Total	T1>T2	25	13.00	-4.374*[a]
	T1<T2	0	0.00	
PANSS Positive	T1>T2	24	13.50	-4.351*[a]
	T1<T2	1	1.00	
PANSS Negative	T1>T2	22	13.41	-4.152*[a]
	T1<T2	2	2.50	
PANSS General	T1>T2	24	13.44	-4.307*[a]
	T1<T2	1	2.50	
GAF	T1>T2	1	3.50	-4.007*[b]
	T1<T2	21	11.88	

Wilcoxon Signed Rank Test. *If p<0.001; [a] Based on Positive Ranks (T1<T2); [b] Based on Negative Ranks (T1>T2). GAF: Global Assessment of Functioning; PANSS: Positive and Negative Syndrome Scale.

Clozapine's side-effects were monitored during hospitalization (consult Supplementary Table II for complete SMARTS). Most common side-effects were memory and concentration difficulties and affective alterations (n=10); sedation (n=9) and postural hypertension (n=7). No life-threatening events were registered during hospitalization. 18 patients received LAI Paliperidone Palmitate as adjuvant treatment because they were considered resistant in-between the period of 6-months. Other adjuvant treatment was added to 6 patients: 1 an extra FGA; 4 an extra SGA; and 2 performed 12 ECT sessions.

Figure 3 presents Clozapine Serum Levels of patients already medicated at admission (T1) compared to all patients at T2, when the average oral dose was 289 (SD 174.49) mg/day and serum level was 312.72 (SD 132.83) ng/mL. We failed to find significant effect of Clozapine Oral Dose on Serum Levels at T2 (R^2= -0.031, F= 0.268, B= -0.085, SE= 0.165, *p*= 0.610).

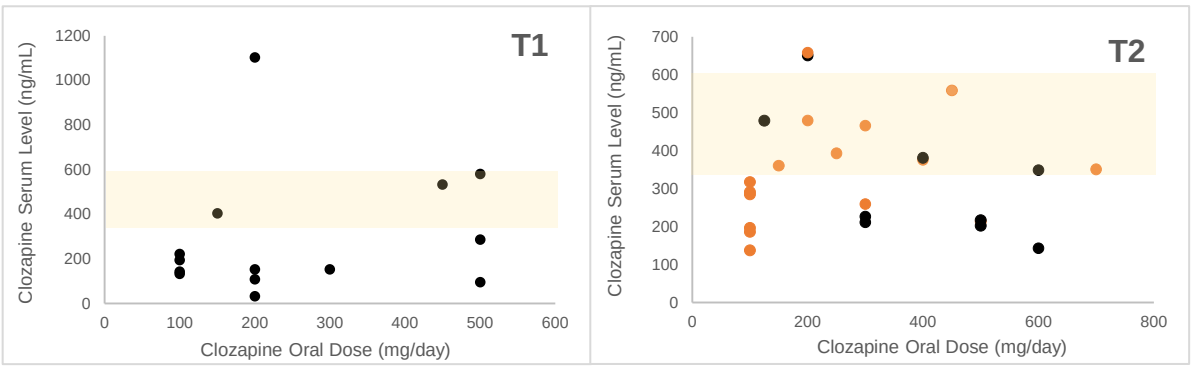

Fig. 3: Clozapine Serum Levels at T1 and at T2 according to Oral Dose. Black Marker: non-responders; Orange Marker: responders. Therapeutic window defined between 350 and 600 ng/mL by the yellow area.

3. Clozapine-Resistant Schizophrenia Profile

According to criteria of resistance suggested by the TRIPP Working Group, we classified 9 patients (36%) as non-responders (CRS) and 16 (64%) as responders (ARC) (verify Appendix - Supplementary Table III for complete assessment). From the group of non-responders, one patient was receiving concomitant LAI Paliperidone Palmitate, another SGA and 12 ECT sessions; one was under LAI Paliperidone Palmitate and another SGA; one under LAI Paliperidone Palmitate and a FGA; another with only an SGA; and the other five were only receiving aduvant LAI Paliperidone Palmitate.

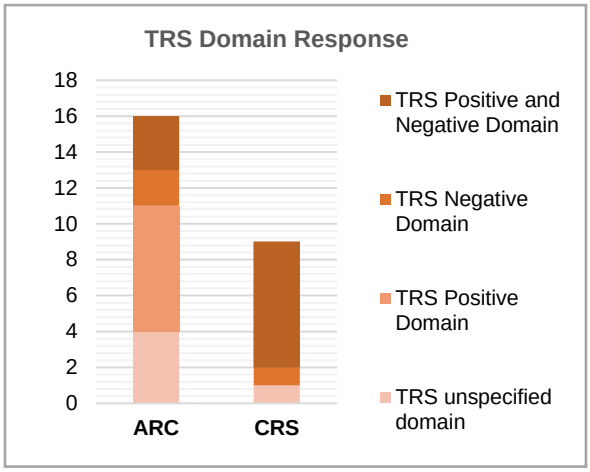

Fig. 4: Response to treatment grouped in TRS Domain Symptoms. ARC: Adequate Response to Clozapine; CRS: Clozapine-Resistant Schizophrenia; TRS: Treatment-Resistant Schizophrenia.

We verified that response in the different TRS Symptom Domains was not equivalent (Pearson Chi Square χ^2= 9.520; df= 3; ***p*= 0.019**), as it is display in figure 4. We emphasize the fact that all 7 TRS Positive Symptom Domain patients responded successfully to treatment. On the other hand, Treatment Resistance from Onset (TRO) seemed to be equivalent between responders and non-responders (Pearson Chi Square χ^2= 2.625; df= 1; *p*= 0.229).

Table III Differences between Responders and Non-Responders

	ARC (n=19)	CRS (n=6)	U Test	*p*
YUD	7	15	30	.057
PANSS Positive T1	21.5	27	24.5	**.007**
P1 Delusions	4	6	37	**.043**
PANSS Negative T1	22	28	20.5	**.003**
N2 Emotional Withdrawal	3	4	42	.077
N3 Poor Rapport	3	4	39	.051
PANSS General T1	40	48	20	**.003**
G9 Unusual Thought Content	4	6	35	**.033**
G12 Lack of Judgement and Insight	4.5	7	39	.056
PANSS Total T1	81.5	105	12	**.001**
GAF T1	41	30	19	**.002**
Oral Dose of Clozapine	200	400	36.5	**.043**
Clozapine Serum Level T2	334.3	226.7	62	.598
Ratio Clozapine/NOR Serum Level T2	1.8	2.2	61.5	.559

Mann-Whitney U Test. Values represent medians of each group of patients.

ARC: Adequate Response to Clozapine; CRS: Clozapine-Resistant Schizophrenia; GAF: Global Assessment of Functioning; NOR: Norclozapine; PANSS: Positive and Negative Syndrome Scale; YUD: Years of Uncontrolled Disease.

We tested different variables in order to find a specific profile for CRS patients (Table III). Symptoms that were close to be proven significantly different in the two groups were also displayed in the table, because the small size of the sample was probably responsible for these results.

4. Variables associated with Clinical Outcomes

In this section we tested correlation of different variables with the course of symptoms and functioning, in order to understand which characteristics were associated with the clinical outcomes of our subjects (Table IV).

Table *IV* ***Correlation Coefficients of Symptoms and Functioning course with different variables (N=25)***

	Total symptoms (N=25)		*Positive symptoms (N=25)*		*Negative symptoms (N=25)*		*General symptoms (N=25)*		*Functioning (N=22)*	
Variables:	**C.Coef.**	**p**	**C.Coef.**	**P**	**C.Coef.**	**p**	**C.Coef.**	**p**	**C.Coef.**	**p**
Age	-.203	.330	-.047	.823	-.201	.334	-.213	.307	.318	.139
Age of Onset	-.316	.142	-.186	.395	-.098	.657	-.233	.285	.077	.741
YUD	-.319	.148	-.708	.730	**-.484**	**.022**	-.358	.102	.402	.079
PANSS Total T1	-.218	.296	-.073	.728	-.359	.078	-.053	.802	-.309	.152
PANSS Positive T1	.078	.713	.218	.295	-.177	.577	.069	.742	-.035	.873
PANSS Negative T1	**-.409**	**.042**	-.142	.498	**-.448**	**.025**	-.221	.289	**-.485**	**.019**
PANSS General T1	-.207	.320	-.215	.302	-.314	.126	-.002	.992	-.241	.269
GAF T1	.209	.316	-.215	.307	.320	.118	.135	.521	-.170	.439
Clozapine Serum Level T2	.125	.553	.296	.151	.048	.821	.093	.658	-.338	.114
NOR Serum Level T2	.313	.128	**.416**	**.039**	-.109	.603	.183	.381	.317	.140
LAI Paliperidone Palmitate	-.477	.045	-.460	.055	.307	.215	-.409	.092	-.208	.440
Creatinine	.039	.855	.086	.683	.193	.356	-.031	.882	-.036	.871
INR	.187	.418	.131	.570	-.078	.737	.242	.290	-.187	.443
LDL	.182	.384	-.252	.224	.092	.660	.390	.054	**.434**	**.039**
CRP	-.010	.961	-.050	.812	.069	.743	.078	.711	.010	.966
Cigarettes per day	-.103	.648	-.170	.449	.075	.741	-.038	.866	.263	.262
Mean Arterial Pressure	-.176	.400	.168	.423	-.274	.184	-.129	.538	-.104	.638
Heart Rhythm	-.006	.977	-.126	.547	-.120	.567	.129	.538	.350	.102
Temperature	**.483**	**.014**	.262	.206	**.482**	**.015**	.370	.069	-.388	.067
Weight	.155	.470	-.059	.783	.055	.798	.232	.275	-.272	.210
Waist Circumference	.104	.627	.218	.305	-.016	.940	.154	.474	-.227	.298

Confidence Interval of 95%. C. Coef.: Spearman's and Pearson's Correlation Coefficient; CRP: C-Reactive Protein; GAF: Global Assessment Functioning; INR: International Normalized Ratio; LDL: Low Density Lipoprotein; NOR: Norclozapine; PANSS: Positive and Negative Syndrome Scale; YUD: Years of Uncontrolled Disease.

5. Predictors of Clinical Outcomes

We tested all the associations found to be significant in the previous section with multiple linear regression models using the stepwise method, in order to find real causality between them and to reject variables sharing similar variance. In the parameters with no more than one predictor to be tested, we used a simple linear regression to justify causality.

Total symptoms course was tested for severity of negative symptoms at T1 and temperature. The severity of negative symptoms was automatically excluded since it did not contribute significantly to the model; ergo temperature was left as the most significant predictor, explaining 17.4% of the total symptoms course.

Table V Model Explaining Total Symptoms course (n=25)

Independent Variable	Adjusted R^2	F (f.d.)	B	SE	*p*
Temperature	**.174**	6.042 (1,23)	**29.409**	11.964	**.022**

Dependent Variable: Total Symptoms course.

Positive symptoms course seemed to be associated to higher NOR serum levels. Notwithstanding, we failed to find a linear relationship of NOR serum levels on positive symptoms course (B= 0.010; SE= 0.007; t= 1.388; *p*= 0.178). This could have happened because correlation of these variables was tested with the Spearman's rho, and consequently it does not represent a linear correlation, but rather an association of any kind.

Regarding the negative symptoms change, we applied again a stepwise method for the multiple regression model. YUD and temperature were automatically excluded from the model. Instead, severity of negative symptoms did explain significantly 16.1% of the course of this domain (Table VI).

Table VI Model Explaining Negative Symptoms course (n=25)

Independent Variable	Adjusted R^2	F (f.d.)	B	SE	*p*
PANSS Negative T1	**.161**	5.023 (1,20)	**-1.937**	.864	**.037**

Dependent Variable: Negative Symptoms course. PANSS: Positive and Negative Syndrome Scale.

At last, functioning course during treatment was significantly explained in 23.6% by the severity of negative symptoms at baseline (table VII). Again, LDL level in blood did not contribute as a good predictor and for this reason it was automatically excluded from the model.

Table VII Model Explaining Functioning course (n=22)

Independent Variable	Adjusted R^2	F(f.d.)	B	SE	*p*
PANSS Negative T1	**.236**	6.470 (1,21)	**-1.050**	.413	**.019**

Dependent Variable: Functioning course. PANSS: Positive and Negative Syndrome Scale.

6. Moderation Model on Negative Symptoms Domain

Since Negative Symptoms were the domain suffering the less significant change under Clozapine, we decided to study the relationship of this symptoms' course and their predictors, interacting with an extrinsic variable. To that end, a Simple Moderation Model was tested for a third variable on the linear regression of severity of initial negative symptoms on course of these symptoms during hospitalization. As explained before, this moderator variable was elected in theorical grounds: in this case our goal was to understand if the number of YUD; and a previous Clozapine trial as an out-patient, in a non-oral-dosage-adjusting regime, influenced the negative symptoms outcomes during hospitalization.

We found that the YUD seemed to contribute significantly to the model (χ^2= 0.1501; F(1,18)= 4.4372; ***p*= 0.0495**). According to Figure 5.A, patients with many YUD improved homogenously around 20% of their initial negative symptomatology, independently of the severity. Patients with a little YUD reached better outcomes when negative symptoms were less severe at T1 (60-80%) than when they presented highly severe at baseline (0-20%). In this last subgroup of patients, we verified that they would improve even less than patients with many YUD and the same PANSS score for negative symptoms at baseline.

Similarly, we observed that previous Clozapine administration presented significant interaction in the model as well (χ^2= 0.1498; F(1,21)= 4.8843; ***p*= 0.038**). According to Figure 5.B, patients submitted to a previous non-monitored Clozapine trial before admission in the UCAERe-T presented greater change of negative symptoms in case these had a low severity at T1 (40-50%), when compared to patients medicated with Clozapine for the first time (<10%). On the other hand, when presenting a high severity of negative symptoms at T1 and not receiving Clozapine for the first time, patients improved less (<20%) than those taking Clozapine for the first time (30-40%).

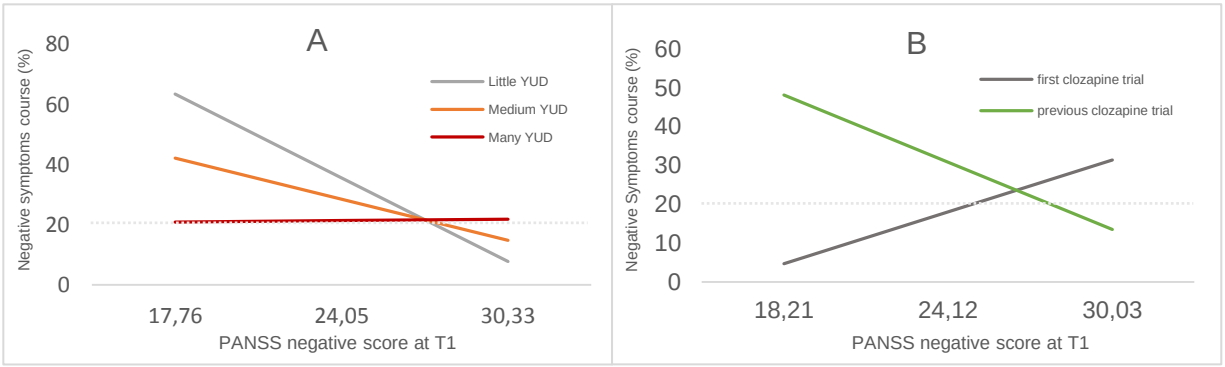

Fig. 5: Simple Moderation Models.

Regression of PANSS Negative Domain score at T1 on course of Negative Symptoms during the 6 months of hospitalization, moderated by A. YUD: Little YUD= 3.41 years ($\bar{x}$ - SD); Medium YUD= 13.82 years ($\bar{x}$); Many YUD= 24.23 years ($\bar{x}$ + SD); B. Previous Clozapine trial before admission in the UCAERe-T.

Relative cut-off of 20% change of symptoms is delimited by a dashed line in both graphs. PANSS: Positive and Negative Syndrome Scale; YUD: Years of Uncontrolled Disease.

Discussion

In this study we sought to **confirm the effectiveness of Clozapine in TRS patients**. In line with recent literature on TRS response to Clozapine, we verified that 64% of our patients developed an adequate response to Clozapine treatment; and that positive symptoms were the clinical domain showing the greatest change during the 6 months of hospitalization, followed by general and negative symptoms.[1]

Measurement of **plasma Clozapine levels** was an important tool for properly adjusting the oral dose of the drug. For instance, one of the subjects receiving Clozapine as an out-patient presented toxic serum levels, which could have led to the development of serious adverse effects. Similarly, patients presenting low serum levels at admission did safely increase Clozapine oral dose, improving their clinical outcome, as a result of close surveillance. However, some patients not showing adequate response did not achieve therapeutic levels of Clozapine in plasma, since they were manifesting several side-effects or presenting a high ratio Clozapine/NOR. Another possible explanation is that, in the clinical setting, some patients were considered to be responders because they improved significantly in the Total and/or Positive score of symptoms, although they did not in the negative Symptom Domain. Nevertheless, in our study, TRS Negative Domain patients were considered non-responders in such cases, since they presented insufficient criteria for an adequate response to Clozapine. Finally, we verified no life-threatening events subjacent to the use of Clozapine. We understand the costs that such specific units and procedures represent to the health care system; but covering the recurrent relapses and the AP high-dosage polypharmacy administrated through all the YUD, can easily over-come this investment.[27,28] In fact, we verified a significant improvement in **functioning levels** in a sample where the majority of patients were single, unemployed or retired and still living with their parents or other non-conjugal relatives in the beginning of the treatment; living out of society since the first clinical manifestations, and representing a burden both for themselves, for their families and caregivers. We consider that the recovery of autonomy can represent the most important trade-off for the financial and human resources invested in the UCAERe-T during the last three years.

For patients who did not achieve an adequate response to treatment, guidelines for subsequent therapies are not well-established. They may include polypharmacy of Clozapine with other AP (a non-evidence-based practice); ECT sessions isolated or associated with Clozapine, which have shown great improvement but has been also associated to important adverse effects; Repetitive Transcranial Magnetic Stimulation;[1] and *Pimavanserin* in case of refractory hallucinations and delusions.[29] During the 6 months of follow-up, some of our patients were not responding significantly to Clozapine; and since it was feasible to monitor possible adverse effects of adjuvant therapy in the unit, they received an add-on treatment with a non-Clozapine AP and/or ECT sessions. Among those patients, four succeeded to respond and three did not, and we considered their contribution equally in the final classification of responders and non-responders. However, we would not follow the same procedure if the size of our sample would have been bigger, excluding them from our statistical analysis.

When looking at the **patients' profile at admission**, we verify that non-responders presented initial greater global impairment, both in symptoms and functioning. Delusions, emotional withdrawal, poor rapport, unusual thought content and lack of judgement and insight were symptoms particularly more severe at baseline in non-responders than in responders. These findings allow us to recognize that TRS is not a homogeneous pathology, since we identified a specific profile for those patients who did not succeed to respond to Clozapine. Concluding, CRS patients were denoted for their higher impairment in terms of symptoms and functioning at baseline. Reasonably, the same group of patients was receiving higher oral doses of Clozapine and presented a higher median ratio Clozapine/NOR. Nevertheless, they did not present significantly increased Clozapine serum levels, which confirms the important heterogeneity of response in TRS patients. The YUD was also higher in non-responders than in responders: however, this may represent a confounding variable as we are going to explore later in this section, meaning that the YUD may not be directly associated with the development of CRS, but rather be a TRS aggravating factor.[14] Equally important, we found a significant difference in response according to the TRS Symptom Domain. This finding reinforces that it is crucial to tailor treatment with regard to symptom profile and disease course.[11]

In agreement, we emphasize the importance of **Clozapine therapy adjustment** in at least two different phases: firstly, TRS patients should be defined as poor metabolizers, extensive metabolizers (normal CYP activity) or ultra-rapid metabolizers, since we did not find any linear relationship between the oral dose and the serum levels of Clozapine. Probably, precisely because each patient's liver profile function define the variance of Clozapine levels in plasma and therefore, the effects and toxicity of the drug.[30] This fact can be properly controlled using laboratory analysis as guidance, and avoiding the administration of CYP1A2 inducer or inhibitor substances concomitantly with Clozapine.[22] Accordingly, we can understand why metabolic variables just as INR, Creatinine and LDL; and cigarette smoking (a well-studied CYP1A2 inducer) showed no causality with symptoms course, since Clozapine Serum Levels were already adjusted to the metabolic function of the patients. Concerning cigarette smoking, we consider that nicotine withdrawal may represent a next step to increase levels of success in our unit, since it would allow us to more efficiently achieve higher concentrations of Clozapine in plasma. Secondly, we believe that Clozapine may influence differently the symptomology of each patient, because its effect will depend on the neurobiology of the disease. This fact complicates the prediction of treatment prognosis, not only because this mechanism is still not well enlightened; but also, because there seems to exist high heterogeneity among TRS patients.[1,8]

There are currently two main theories trying to explain the possible **neurobiological mechanisms** underlying TRS: one is the Dopamine Supersensitive Type-Schizophrenia, which argues that both an increase in the number of the receptors or an increase in their affinity to dopamine DRD2 can justify the lack of effect of non-Clozapine AP after an adequate period of response to first-line AP medication; on the other hand, there is the Normodopaminergic Type-Schizophrenia theory, which states that higher levels of dopamine are not the cause of the symptoms in TRS, and patients are resistant to AP since the FES, showing no response at all.[8] Unfortunately, the size of our sample and the amount of missing data in regards to the initial history of the disease, did not allow us to

exclude an association between CRS and initial response to first-line AP, even if the majority of our subjects were resistant from the illness onset.

Nevertheless, we made some other interesting findings. For instance, it was clear that symptom change did not follow a single pattern in our patients, except for positive and general symptoms. Previous studies found that the heterogeneity of symptom severity is mostly explained by negative symptoms in TRS patients, while non-TRS patients are better defined by the PANSS General Domain.[31] Accordingly, positive and general domains improved significantly with no association with the patients' disease profile, while negative symptoms course was less impacted by Clozapine, being predicted by the severity of the domain at baseline. This means that the mechanism underlying positive and general symptoms is strongly and homogenously affected by Clozapine; while negative symptoms are not so clearly influenced.

We verified that the relationship between the severity of negative symptoms at baseline and negative symptoms course was moderated by the number of **YUD**: unexpectedly, a subgroup of patients that were diagnosed with TRS relatively early and presented an initial high severity of negative symptoms, improved very little during hospitalization, even less than patients with the same level of severity and more YUD. This leads us to think that this subgroup of patients may suffer from a different biologic alteration which is insufficiently affected by Clozapine mechanisms of action: probably a Normodopaminergic Type-Schizophrenia. On the other hand, all patients with less severe negative symptoms at baseline, developed a better course of negative symptoms during hospitalization: we may hypothesize that this subgroup of patients suffered from a Dopamine Supersensitive Type Schizophrenia, since the switch from a DRD2 to a DRD4 receptor antagonist was able to induce an important effect in symptoms. However, since all patients with many YUD did not improve much more than 20% of their initial severity, meaning they all responded equally to Clozapine, we cannot distinguish any subgroup of Schizophrenia-Type. Notwithstanding, this is the group of patients who would have most benefit from an early detection and management of TRS, since some of them could clearly had reached better outcomes with Clozapine, depending on the type of TRS underlying the pathology. This is where the number of YUD may not be responsible for the pathology underlying TRS, but rather correspond to an aggravating and modifiable factor affecting mostly patients suffering from a Dopamine Supersensitive Type-Schizophrenia.

We verified that some of the **TRS predictors** previously studied were present in our subjects, namely an early age of Schizophrenia onset, Paranoid Schizophrenia subtype, living in a non-urban dwelling, family history of psychiatric disease (presented by a quarter of patients) and some with at least one previous suicide attempt. In light of our previous conclusions, we consider of utmost importance the development of a validated algorithm of evaluation applied at the FES in order to screen patients with higher chances of developing TRS. This would allow a closer follow-up to be performed after Schizophrenia diagnosis, reducing the period endured without adequate treatment; and most probably leading the subgroup of patients suffering from a Dopamine Supersensitive Type-Schizophrenia to achieve better rates of response with Clozapine.

On the other hand, we also observed that a **previous non-monitored Clozapine trial** showed to interact with the relationship described above. Patients previously medicated with Clozapine and with a high severity profile of negative symptoms presented an insufficient response. We conceived three different hypothesis to justify this finding: either Clozapine should have been administrated only in a controlled environment as an in-patient, in order to achieve the right serum level as fast as possible and avoid the development of super-sensitivity of dopamine DRD4 receptors; or this subgroup of patients suffered from a Normodopaminergic Type-Schizophrenia and therefore would not improve with Clozapine, independently of the conditions of administration; or finally, Clozapine's effect observed was only the residual course of the disease not yet changed by the drug in ambulatory, and the greatest improvement happened before hospitalization and therefore, we could not register it. In the same line, patients with less severe negative symptoms and receiving Clozapine for the first time, did not achieve successful results during treatment. However, we attribute this result to a single patient unequally contributing to the regression line, who despite having received two extra SGA and 12 sessions of ECT, obtained a worse negative score at T2 than at T1.

Finally, we verified that **temperature** was the most significant predictor of total symptoms course. Recent neuroimaging research has shown that TRS patients present a reduced level of striatal dopamine synthesis compared to non-TRS, but similar to health-controls [32–34]; and higher levels of Glutamate in the Anterior Cingulate Cortex (ACC).[34] Another study proved that TRS patients that present adequate response to Clozapine have higher concentration of glutamate and glutamine in the putamen and decreased levels in the dorsolateral prefrontal cortex compared to non-responders and to first-line responders.[35,36] The thermoregulatory control is carried by the Central Nervous System (CNS) and it is located in the Dorsomedial Hypothalamus, responding to a GABAergic inhibition and glutamatergic excitatory system.[37] In our investigation, we verified that higher body temperature caused higher levels of change in the total symptom score during hospitalization. This could indirectly mean that higher levels of glutamate in the hypothalamus represent lower levels of the neurotransmitter in some other structure of the CNS responsible for the TRS symptoms. Even if several glutaminergic agents have shown not efficient enough in the TRS management,[38] previous pharmacogenomics studies have proved that Clozapine may have some influence in the glutamate distribution, moving it out of the CNS.[5] In line with this, we could predict that the bigger the change in the total symptom score during hospitalization, the bigger the mobilization of glutamate through Clozapine action (from the CCA to the hypothalamus) and the higher the body temperature of the patient. However, this is a hypothetical theory that allows us to explain only 17.4% of the course of total symptoms; and does not sufficiently explain the limiting outcomes of negative symptoms change with Clozapine. Therefore, there is a gap left for other variables, not assessed by us, that would influence more importantly the total symptoms change. To that end, we conclude that further neuroimaging and pharmacogenetics investigation should be done in order to evaluate glutamate role in the neurobiology of TRS patients.

The **strengths** of this study included structured assessment of clinical variables, counting not only with evaluations performed by specialized health care providers, but also with a standardized,

validated symptom rating score and functioning scale; results providing real-world evidence of the feasibility of implementation of units dedicated to the TRS management, which can incentive the worldwide spread of this practice; all results followed the *Treatment Response and Resistance In Psychosis* (TRRIP) Working Group criteria of 2017, allowing the standardization of investigations regarding TRS; and treatment adherence guaranteed by the close monitoring and the ratio Clozapine/NOR frequently evaluated during treatment. The **limitations** of this investigation were the small size of the sample, and the missing data from the initial history of the disease, which did not allow us to perform a more robust statistical analysis. However, it is important to emphasize that we dedicated our study to a very sub-specific pathology and collected our patients in a recent unit that has been active for 3 years only, and therefore the number of patients with a complete follow-up is still restricted. A second limitation to be considered in the interpretation of our results is the multidisciplinary approach of the UCAERe-T, which includes not only pharmacological treatment but also psychotherapy and rehabilitation interventions that contributed to the patients' clinical condition. Ultimately, final outcomes are not only the result of Clozapine high efficiency, but also of the psychosocial care provided during hospitalization. At last, literature shows that response to Clozapine can be achieved up to a year after its initiation[8] and we studied a period of 6-months; so again, we were limited by the number of subjects with a complete follow-up. The optimum study design will require more patients accumulated during the next years of medical practice in the UCAERe-T, enabling the performance of a logistic regression analysis in order to understand clinical course with Clozapine in each TRS Symptom Domains; and adding to this type of investigation more variables, such as genetic and neuroimaging data, to the extent of controlling the maximum possible number of variables associated with TRS and CRS. Future studies should try to gather prediction based on genetic and neuroimaging variables with clinical and environmental predictors.

The literature and our results suggest the following clinical recommendations:

(1) We think that a new approach to TRS patients should be placed. The development of a valid prediction algorithm applicable at the FES could be the key for early identification of TRS patients (maximum 12 months after Schizophrenia diagnosis, 6 months for each trial).

(2) It is of utmost importance to not let TRS patients endure long periods of uncontrolled disease, since this can lead to a worse response to Clozapine therapy. Current practice should envisage the training of physicians in order to identify TRS patients and propose adequate treatment in an early phase.

(3) Units specialized in TRS treatment are rather important in the management of the disease, since they assure the correct administration of Clozapine and eliminate the fear of its ambulatory prescription. As verified in our investigation, we achieved good clinical results, without life-threatening side-effects thanks to close monitoring and rehabilitation programs.

(4) It is essential to continue investigation dedicated to neuroimaging, neurobiology and pharmacogenetics on TRS patients, since recent studies point that TRS may represent a new subtype of Schizophrenia with different underlying mechanisms and to that end, different targets of treatment. We may have found results indirectly supporting that glutamate level dysregulation in the SNC could be involved in the TRS physiopathology.

(5) Understanding the neurobiological profile of each patient would be absolutely mandatory for a trustful interindividual adaptation of the treatment, in order to avoid the development of ultra-resistance to Clozapine and further periods of uncontrolled disease.

Conclusion

We established real-world evidence that units as the UCAERe-T, specialized in the management and rehabilitation of TRS patients, are important in order to limit the impact of the disease both in patients and society. The feasibility and applicability of this project is proven not only by the good clinical outcomes, but also by the functioning levels achieved by the majority of the patients. Further work in this area is required in two different fields: systematic screening of TRS patients after FES is essential because early detection and adequate treatment may minimize TRS clinical burden; and investigation on the neurobiology underlying TRS is demanded in order to properly adjust therapeutics to these patients and avoid AP high-dosage polypharmacy and accumulation of YUD. We think it is highly probable that TRS may correspond to a new subtype of Schizophrenia.

Conflicts of interest

There are no conflicts of interest.

Acknowledgments

“Cuando el jiguero no puede cantar.

Cuando el poeta es un peregrino,

Cuando de nada nos sirve rezar.

“Caminante no hay camino,

Se hace camino al andar...”

Golpe a golpe, verso a verso”

Antonio Machado , de Proverbios y Cantares

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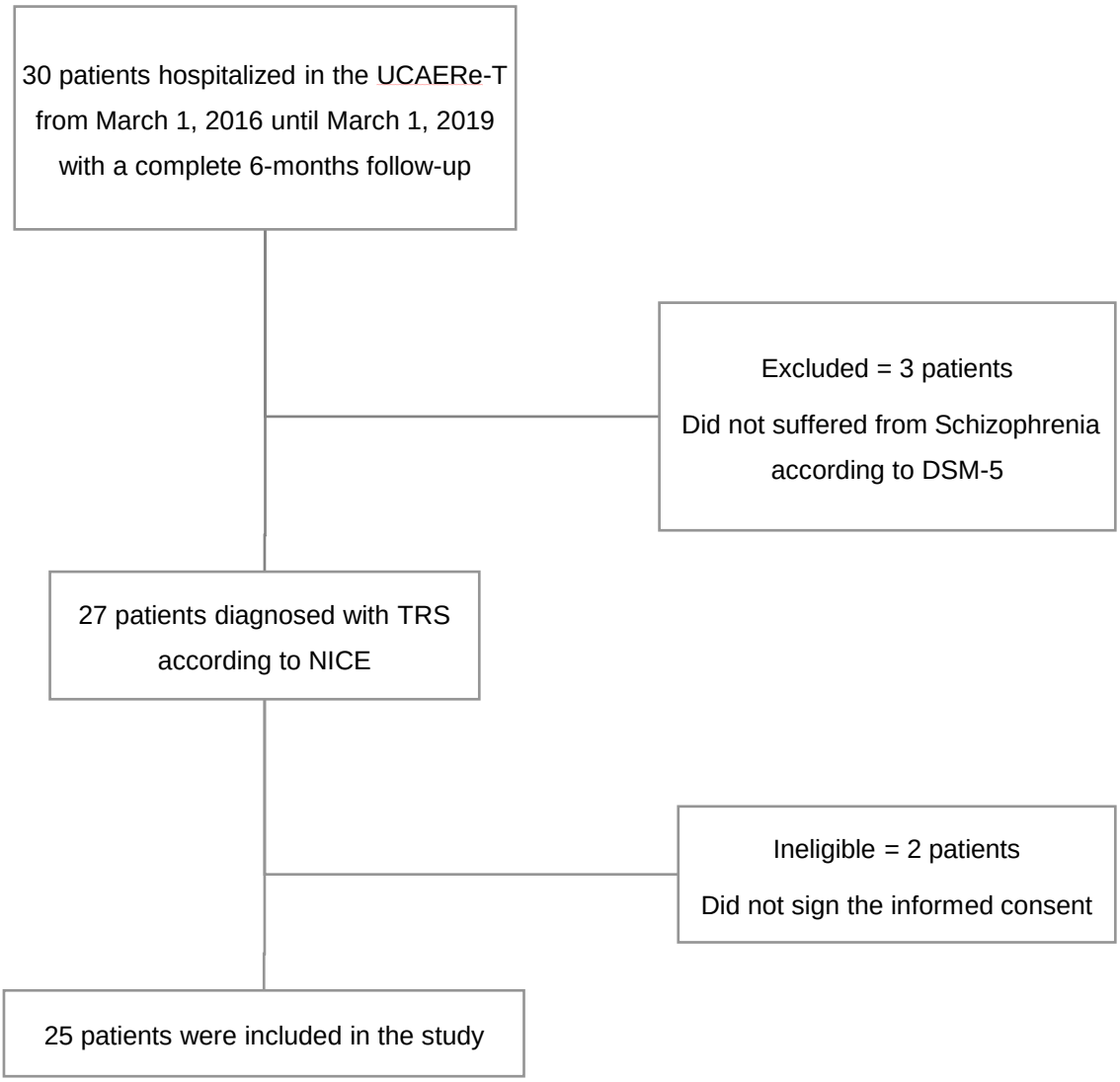
Appendix

Supplementary Table I – Definitions and guidelines		
	Guidelines	Definition
Schizophrenia	DSM-5 (2013)	Presence of ≥ 2 during a 1-month period (or less if successfully treated), with at least 1 of them being (1), (2), or (3): (1) delusions, (2) hallucinations, (3) disorganized speech, (4) grossly disorganized or catatonic behaviour, and (5) negative symptoms.
Treatment-Resistant Schizophrenia (TRS)	NICE (2014)	Lack of adequate response to treatment with at least 2 antipsychotic drugs, being at least one of them a non-Clozapine atypical antipsychotic in an adequate dose (≥ 600 mg/day of *Chlorpromazine equivalents*) with an adequate adherence and duration of treatment (≥ 6 weeks for each).
TRS Symptom Domain	TRIPP Working Group (2017)	More than one moderate symptom or at least one severe symptom in the given domain.
Symptoms Change	TRIPP Working Group (2017)	Change of PANSS score from T1 to T2, adjusting the scale to a baseline of zero (e.g. PANSS T1=90, T2=60, symptoms course of 50%).
Clozapine-Resistant Schizophrenia (CRS)	TRIPP Working Group (2017)	At least one of the following: A) an absolute threshold of symptoms with at least one moderate symptom or one severe symptom in the domain of interest; B) a decrease of less than 20% both for the total rating and for the specific domain of interest at T2; C) moderate or more severe functional impairment at T2; D) a minimum treatment duration of 12 weeks.
Years of Uncontrolled Disease (YUD)		Period from the first acute episode without a period of remission, until the moment of admission in the UCAERe-T.
Functioning Course		Difference of the GAF score between T1 and T2.

Based on recommendations for optimum requirements

CRS: Clozapine-Resistant Schizophrenia; DSM-5: Diagnostic and Statistical Mental Disorders, 5th edition; GAF: Global Assessment of Functioning; NICE: National Institute for Health and Care Excellence; PANSS: Positive and Negative Syndrome Scale; TRRIP Working Group: Treatment Response and Resistance in Psychosis Working Group; TRS: Treatment-Resistant Schizophrenia.

Supplementary Graph I – STROBE Flow-Chart of Participants



DSM-5: Diagnostic and Statistical Mental Disorders, 5th edition; NICE: National Institute for Health and Care Excellence; TRS: Treatment-Resistant Schizophrenia; UCAERe-T: Unidade de Cuidados Avançados de Esquizofrenia Resistente ao Tratamento.

Supplementary Table II - SMARTS evaluation at T2 (N=24)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	**TOTAL**
1. Parkinsonism, tremor	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	**5**
2. Weight and appetite change	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**3**
3. Sexual dysfunction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	**1**
4. Hyperprolactinemia	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
5. Postural Hyperthension	0	0	0	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	**7**
6. Sedation (tiredness and sleepiness)	1	0	0	1	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1	**9**
7. Akathisia	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	**5**
8. Gastrointestinal side-effects	0	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	**6**
9. Urinary symptoms	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	**1**
10. Sedation (Memory and concentration)	1	1	0	1	0	0	1	0	0	0	0	1	1	1	1	0	1	0	0	1	0	0	0	0	**10**
11. Affective side-effects	1	1	0	1	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	1	**10**
12. Miscellaneous side-effects	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	**1**

Self-Evaluation of Side-Effects: 1 if present; 0 if absent.

Supplementary Table III – Assessment of Clozapine-Resistant Treatment

	DOMAIN	Criteria A	Criteria B	Criteria C	Answer
1	Pos&Neg	Ø	Ø	Ø	Responder
2	Pos&Neg	Ø	Ø	Ø	Responder
3	Unspecified	Ø	Ø	Ø	Responder
4	Unspecified	Ø	Ø	Ø	Responder
5	Positive	Ø	Ø	Ø	Responder
6	Negative	Ø	Ø	Ø	Responder
7	Pos&Neg	N_5=6	Ø	GAF=45	Non-Responder
8	Positive	Ø	Ø	Ø	Responder
9	Unspecified	Ø	Ø	Ø	Responder
10	Unspecified	$N_{5,6}$=5	Ø	GAF=21	Non-Responder
11	Pos&Neg	Ø	Ø	GAF=40	Non-Responder
12	Pos&Neg	$P_{1,6}$=5	Ø	GAF=41	Non-Responder
13	Pos&Neg	$P_{1,2}$=6; N_5=7	%N=-4.76; %T=12.20	GAF=30	Non-Responder
14	Pos&Neg	N_5=6	Ø	Ø	Non-Responder
15	Pos&Neg	Ø	Ø	Ø	Responder
16	Positive	Ø	Ø	Ø	Responder
17	Positive	Ø	Ø	Ø	Responder
18	Negative	$N_{1,3}$=7; $N_{2,4,6}$=5; N_5=6	%N=3.23; %T=14.29	GAF=31	Non-Responder
19	Positive	Ø	Ø	Ø	Responder
20	Positive	Ø	Ø	Ø	Responder
21	Positive	Ø	Ø	Ø	Responder
22	Unspecified	Ø	Ø	Ø	Responder
23	Pos&Neg	P_3=6; $N_{1,2}$=5	%N=19.05	-------	Non-Responder
24	Pos&Neg	Ø	%N=18.75	Ø	Non-Responder
25	Negative	Ø	Ø	Ø	Responder

Criteria for CRS:

A) an absolute threshold of symptoms with at least one moderate symptom or one severe symptom in the domain of interest; (symptoms identified by the PANSS score numeration)

B) a decrease of less than 20% both for the total rating and for the specific domain of interest at T2; (%N= change of negative symptoms; %T= change of total symptoms)

C) moderate or more severe functional impairment at T2;

D) a minimum treatment duration of 12 weeks – fulfilled by all patients