Vitamin D impact in affecting clozapine plasma exposure: A potential contribution of seasonality

Alessandra Manca [a,1], Jacopo Mula [a,b,1], Alice Palermiti [a,*], Flavio Vischia [c], David De Cori [c], Sara Venturello [d], Guido Emanuelli [e], Domenico Maiese [a], Miriam Antonucci [f], Amedeo De Nicolò [a], Elisa Delia De Vivo [a], Jessica Cusato [a,2], Antonio D'Avolio [a,2]

[a] Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin, Italy
[b] CoQua Lab s.r.l, Italy
[c] Department of Mental Health–Psychiatric Unit West, 10149 Turin, Italy
[d] Department of Mental Health–Psychiatric Unit East, Day Service S.G. Bosco, 10144 Turin, Italy
[e] Department of Mental Health–Psychiatric Unit East, S.G. Bosco, 10144 Turin, Italy
[f] SCDU Infectious Diseases, Amedeo di Savoia Hospital, ASL Città di Torino 10149 Italy

ARTICLE INFO

ABSTRACT

Schizophrenia affects approximately 24 million people worldwide and clozapine is the most effective antipsychotic drug. Nevertheless, its use in therapy is limited due to adverse effects. Therapeutic drug monitoring is a clinical tool useful to reduce the clozapine toxicity. In the literature, papers showed how psychiatric disorders could be associated with low vitamin D levels, but a few studies focusing on its role in affecting clozapine exposure are available. A TDM repository was analyzed: clozapine and vitamin D levels measured with liquid chromatography were considered. 1261 samples obtained from 228 individuals were evaluated: 624 patients (49.5%) showed clozapine plasma levels in therapeutic range (350–600 ng/mL). Clozapine toxic plasma levels (>1000 ng/mL) were more present in winter (p = 0.025), compared to other seasons. Concerning vitamin D, a sub-analysis of 859 samples was performed: 326 (37.81%) were deficient (ng/mL), 490 (57.12%) had insufficient concentrations (10–30 ng/mL), while 43 (5.02%) had sufficient (>30 ng/mL) levels. A correlation between vitamin D and clozapine plasma levels (p = 0.007, Pearson coefficient=0.093) was observed. The role of seasonal variation in clozapine plasma exposure in psychiatric patients treated with clozapine was suggested. Further studies in larger cohorts are needed in order to clarify these aspects.

1. Introduction

Schizophrenia is a severe and heterogeneous mental disorder with poor investigated etiology. It affects approximately 24 million people worldwide [1,2], causing suffering and discomfort in patients and negatively affecting the quality of life. Moreover, it is associated with notable disability, impairing several areas of life such as personal, family, social, educational, and occupational fields [2]. Schizophrenia affected patients are commonly treated with antipsychotic drugs, most with many side effects, such as extra pyramidal symptoms (EPS) and metabolic syndrome [3]. In this context, clozapine (CLZ) is the most effective antipsychotic in drug-resistant patients [1,4,5]. It influences both the positive and negative symptoms of schizophrenia and it is responsible of reduced EPS, it presents poor effect on prolactin, not causing tardive dyskinesia [4]. Nevertheless, CLZ use in therapy is limited due to some severe adverse effects such as agranulocytosis, cardiovascular arrest and seizures, which lead to poor compliance [6].

In the literature, many studies showed how psychiatric disorders

could be associated with low vitamin D (VD) levels [7–10].

VD is a fat-soluble vitamin playing a role as hormone; it is involved in gene expression, calcium and phosphorous homeostasis, bone metabolism, immune modulation and neuromuscular functions [7,8].

It also affects signaling cascades and neurobiological pathways, associated with mental health. In fact, VD plays a role in neurotrophic factors regulation and in brain antioxidative defense [9]. Furthermore, it seems able to modulate the differentiation and maturation of dopaminergic neurons and to affect serotonin levels [8].

Antipsychotic drugs are well known to interact with cholesterol metabolism, increasing the propensity to obesity and hypertriglyceridemia. In particular, the first drug generation could have an important impact on weight gain, increased glucose tolerance, insulin resistance and cardiovascular risks.

In this context, Humble et al. highlighted a link between low VD levels and metabolic syndrome [9].

In addition, Korade et al. reported how different antipsychotics may influence VD precursor level inhibiting 7-dehydrocholesterol reductase, suggesting that further investigations of VD and psychotropic medicaments are needed [3].

Finally, a study considering CLZ as a variable in relation to VD status in mental disorders affected patients, suggested that nearly all CLZ-treated patients showed VD deficiency (< 12 ng/mL) [11].

CLZ showed important hepatic and extrahepatic metabolic variability, due to the differences in the expression of genes encoding enzymes involved in its biotransformation (e.g. CYP2D6, CYP3A4 and CYP2C19): these gene expression is modulated by VD [12]. Furthermore, drug-drug interactions between two antipsychotics or with other co-administered drugs (particularly with antidepressants and antiepileptics), changes in protein binding, enzyme inhibitors and inducers, substance abuse and associated diseases are some of the variables that influencing CLZ pharmacokinetics.

All this considered, the therapeutic drug monitoring (TDM) is a clinical tool to reduce toxicity and to increase compliance in patients.

The aim of this study was to evaluate both CLZ and VD plasma levels trend during the year in order to better understand the possible role of VD in CLZ treatment, through a TDM repository of 1261 samples (obtained from 228 patients).

2. Materials and methods

2.1. Characteristics of enrolled patients

A number of 1261 samples of 228 patients treated with CLZ at the "Amedeo di Savoia" and "Giovanni Bosco" hospitals (Turin, Italy), were analyzed in a retrospective study.

Inclusion criteria was CLZ assumption. All patients received 250 mg of CLZ/die.

The study was performed in compliance with the Declaration of Helsinki and local review board regulations; all patients gave written informed consent, according to the local ethics committee standards ("Appropriatezza farmacologica della terapia anti-infettiva", approved by Ethical Committee "A.O.U. CITTA' DELLA SALUTE E DELLA SCIENZA DI TORINO - A.O. ORDINE MAURIZIANO DI TORINO – A.S.L. CITTÀ DI TORINO", n° 456/2022).

2.2. Pharmacokinetic analyses

Pharmacokinetic analysis was conducted before the new dose assumption (Ctrough). Plasma samples were isolated after whole blood centrifugation at 1400 x g for 10 min at 4 °C.

CLZ plasma concentrations in patients were obtained using the ClinMass® Add-On Set for Neuroleptics kit (RECIPE) in HPLC-MS/MS.

The extracts were injected on a Acquity® UPLC (Waters, Milan, Italy), consisting of a binary pump, a refrigerated sample manager coupled with a triple quadrupole detector (TQD, MS/MS). The chromatographic separation was achieved through a gradient run on a ClinMass® (RECIPE) HPLC column.

2.3. 25-OH-vitamin D analyses

VD plasma determination (25-OH-vitamin D) was performed with the MSMS Vitamin D kit (Perkin Elmer, Wallac Oy, Finland).

Samples analysis was carried out with a LX50 UHPLC (Perkin Elmer). The chromatographic separation was obtained through a gradient run on a Acquity UPLC® BEH C18 1.7 μm 2.1 × 50 mm column.

2.4. Statistical analyses

In order to test normality Shapiro-Wilk test was used. Non-normal variables were resumed as median values and interquartile range (IQR); dichotomic variables were summarized as numbers and percentages.

All genetic variants were evaluated for Hardy-Weinberg equilibrium by the $\chi2$ test for determining the observed genotype frequencies. Kruskal-Wallis and Mann-Whitney tests have been used to test differences in continuous variables between genetic groups, considering the level of statistical significance (p-value) < 0.05. Correlations among drug concentrations at different days were evaluated through Pearson test. In conclusion, the predictive capability of the investigated variables was assessed through univariate (p < 0.2) and multivariate (p < 0.05) linear regression analysis. IBM SPSS Statistics software 27.0 for Windows (Chicago, Illinois, USA) was used.

3. Results

One thousand two hundred sixty-one samples obtained from two hundred twenty-eight psychotic individuals treated with CLZ were analyzed: one hundred forty-sevens were male and eighty-one were female.

Characteristics of subjects regarding age, Body Mass Index (BMI), VD levels and CLZ plasma exposure were resumed in Table 1: patient median age was 48 years old, median BMI was 27.1 Kg/m², median VD exposures were 11.45 ng/mL while median CLZ exposures were 419 ng/mL.

Concerning CLZ exposure, 624 patients (49.5%) showed plasma levels within therapeutic range (350–600 ng/mL). Particularly, 346 (27,4%) patients had CLZ levels between 0 and 350 ng/mL, 241 (19,1%) subjects between 600 and 1000 ng/mL and 50 patients (4,0%) showed CLZ toxic plasma levels (>1000 ng/mL), as illustrated in Fig. 1A.

Concerning VD, a sub-analysis of 859 samples was performed: a suboptimal presence of VD was observed (<30 ng/mL): as reported in Figs. 1B, 57.2% (n = 490) of analyzed patients presented insufficient VD levels (10–30 ng/mL), while 37.81% (n = 326) was deficient (<10 ng/mL) and just 5.02% (n = 43) was sufficient (>30 ng/mL).

VD concentrations showed a seasonal and a monthly trend (Fig. 2A and Fig. 2B respectively). Particularly, regarding seasonal VD plasma exposure, maximum concentrations were observed in summer (17.00 ng/mL), while minimum concentrations were observed in winter (9.07 ng/mL); in spring and autumn patients showed concentrations of 10.03 ng/mL and 12.44 ng/mL, respectively. In details, median concentrations and interquartile ranges are reported in Table 2.

Considering monthly variation, maximum concentrations were

Table1

Characteristics of enrolled subjects.

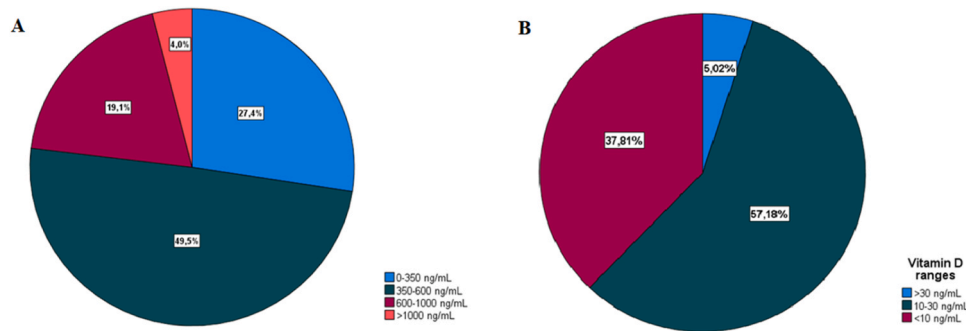| Characteristic	Median	Interquartile Range IQR
Age (years)	48	(40–54)
BMI (Kg/m²)	27.1	(24.4–31.4)
Vitamin D levels (ng/mL)	11.45	(7.59–17.84)
Clozapine plasma Levels (ng/mL)	419	(258.5–601.5)

Fig. 1. A Percentage of patients with different clozapine plasma concentrations. B: Vitamin D concentrations in enrolled subject plasma.
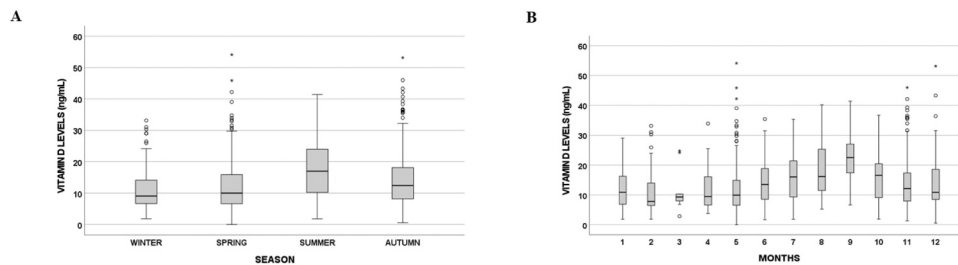

Fig. 2. A Seasonal vitamin D levels variation in patients (Winter N = 94; Spring N = 297; Summer N = 97; Autumn N = 371). B: Monthly vitamin D levels variation in patients (January N = 31; February N = 54; March N = 11; April N = 25; May N = 247; June N = 36; July N = 28; August N = 32; September N = 26; October N = 44; November N = 292; December N = 33).

Table 2
Vitamin D Median and Interquartile ranges in patients.

	Season	Median	Range Interquartile IQR
Vitamin D levels (ng/mL)	*Winter*	9.07	(6.67–14.31)
	Spring	10.03	(6.61–15.88)
	Summer	17.00	(10.21–23.99)
	Autumn	12.44	(8.18–18.18)

showed in September.

Regarding the role of gender in influencing VD and CLZ concentrations, no differences were observed between male and female regarding VD levels and CLZ plasma exposure.

A high number of samples with CLZ> 1000 ng/mL (toxicity cut-off value) was suggested in winter compared to other seasons, as reported in Fig. 3.

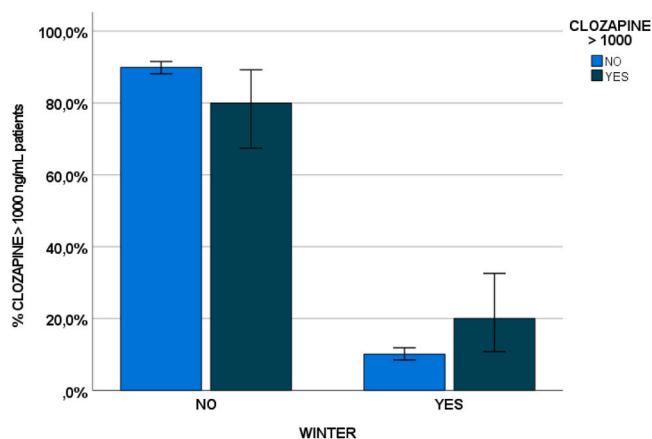A correlation between VD and CLZ plasma levels was observed (p = 0.007), as illustrated in Supplementary Fig. S1.

Fig. 3. Toxic concentrations of clozapine in winter vs other seasons.

Moreover, BMI was correlated with CLZ plasma levels and with VD plasma levels (p < 0.001; p = 0.005), as reported in Supplementary Fig. S2 and Supplementary Fig. S3, respectively.

4. Discussion

Aim of the present study was CLZ and VD plasma levels trend description during the year in order to clarify the possible role of VD in CLZ treatment.

In this study, we described 624 samples (49.5%) showing CLZ plasma levels in therapeutic range (350–600 ng/mL), while other samples showed suboptimal concentrations. According to the literature, as reported [13] by Krivoy et al. in a group of 98 individuals: 21% had concentrations lower than 350 ng/mL, 44.4% had concentrations in therapeutic range, 28.4% had concentrations between 600 and 800 ng/mL and 6.2% had concentrations above 800 ng/mL [13].

In this work, a suboptimal VD level was observed in the analyzed samples: more than 50% of subject was insufficient (10–30 ng/mL) and just 5.02% was sufficient (>30 ng/mL). The current guidelines for VD ranges suggested sufficient levels > 30 ng/mL [14].

As mentioned above, VD is also correlated with neurobiological pathways, which may affect the mental health.

Indeed, despite neuropsychiatric disorders are complex and influenced by different factors, nutritional deficiency could affect pathways fundamental for brain development and functions.

In this context, Rhonda et al. [15] reported how VD regulates serotonin synthesis through tryptophan hydroxylase 2: serotonin pathway-related gene polymorphisms could impact on the dysregulation in the serotonin synthesis and metabolism. Consequently, inadequate VD levels could cause an additional decrease in serotonin levels, exacerbating failures in brain functions.

It is important to highlight that low VD concentrations have been associated with an elevated risk for bipolar disorder, schizophrenia, antisocial behavior, etc.

In the literature, a VD plasma level seasonal trend was reported, with high concentrations in spring and summer and deficiency in autumn and

winter [16]. Considering this, we investigated VD concentrations during each month of the year and seasons as well: a significant increase of VD concentrations in summer and particularly in September was observed. In addition, seasonality seems to have a role in affecting antiretroviral drug concentrations, as reported in the literature [17]. Lindh et al. [18] highlighted a variation in immunosuppressive agent (tacrolimus and sirolimus) concentrations during the year, decreasing in concomitance with an increase in VD levels; other studies reported VD influence on antinfective and anti-neoplastic drug concentrations [19].

CLZ undergoes extensive hepatic metabolism: in vitro studies showed CYP3A4 accounts for around 70% of its clearance, CYP1A2 15%, and 5% or less for CYP2C19, CYP2C8. CYP2D6 plays a very minor role. In vivo CYP1A2 is the major enzyme. CYP3A5 and CYP3A43 may also affect CLZ metabolism [20].

Regarding transport, ABCB1, ABCG2, ABCC1, SLC22A1, SLC22A2, SLC22A3 are involved.

Concerning pharmacogenetics, *ABCB1* variants are associated with increased plasma CLZ levels and with agranulocytosis and neutropenia side effects.

As well known, VD is a modulator of gene expression such as those encoding cytochromes (eg. CYP3A5) and transporters (eg. ABCB1), involved in drugs metabolism and transport.

As reported by Gaebler et al. VD could negatively affect CLZ plasma exposure by enhancing CYP3A4 activity [12].

For these reasons, further studies have to be performed in order to understand if VD-associated genetic variants could affect the expression of genes involved in CLZ metabolism and elimination, thus its exposure and clinical outcome.

The higher percentage of samples with CLZ > 1000 ng/mL in winter compared to other seasons could confirm the link between VD and seasonality. This suggests that inadequate sun exposure in winter leads to lower VD concentrations, as confirmed in this work, probably consequently CYP expression decrease, thus reduced CLZ metabolism and its higher plasma concentrations.

Moreover, a correlation between VD and CLZ plasma levels was observed ($p = 0.007$). It seems to have an opposite trend, but data are related to all the seasons and they are very dispersive.

A correlation between VD and CLZ plasma levels in winter showed not statistically significant results: further studies in larger cohorts are needed in order to clarify these aspects.

In addition, a correlation between BMI and CLZ plasma exposure ($p < 0.001$) was found.

Obesity and metabolic syndrome are the most prevalent side effects for patients treated with CLZ: 28–45% of patients develop metabolic syndrome over a 4 month or longer period of treatment. These affect physical and mental health, compliance, and quality of life [21,22]. Plasma clozapine levels variability may be due to several factors influencing clozapine metabolism: gender, cigarette smoking, inflammation, genetics and obesity leading to higher clozapine levels [23].

5. Conclusions

In conclusion the role of seasonal variation in CLZ plasma exposure in psychiatric patients treated with this drug was suggested: our work confirmed the possible role of VD in affecting antipsychotics blood concentrations, as observed by Gaebler et al. [12].

Our investigation on 228 schizophrenic patients confirmed this trend, showing how low VD levels could affect CLZ efficacy, pointing out a possible relation between low VD exposure and CLZ toxicity.

This suggest a possible role of VD in influencing CYP expression involved in drug metabolism.

For this reason, a future objective in our work will be to perform genetic analysis in a larger cohort of patients, considering cytochrome P450 subfamilies SNPs.

Declaration of Competing Interest

The authors declare no conflict of interest.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and according to the local ethics committee standards "Appropriatezza farmacologica della terapia anti-infettiva" approved by Ethics Committee of "A.O.U. CITTA' DELLA SALUTE E DELLA SCIENZA DI TORINO - A.O. ORDINE MAURIZIANO DI TORINO – A.S.L. CITTÀ DI TORINO", n° 456/2022).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115103.

References

[1] A. Krivoy, D. Joyce, D. Tracy, F. Gaughran, J. MacCabe, J. Lally, E. Whiskey, S. N. Sarkar, S.S. Shergill, Real-World outcomes in the management of refractory psychosis, J. Clin. Psychiatry 80 (5) (2019).
[2] WHO, Schizophrenia. https://www.who.int/news-room/fact-sheets/detail/ schizophrenia.
[3] Z. Korade, W. Liu, E.B. Warren, K. Armstrong, N.A. Porter, C. Konradi, Effect of psychotropic drug treatment on sterol metabolism, Schizophr. Res 187 (2017) 74–81.
[4] D.D. Miller, Review and management of clozapine side effects, J. Clin. Psychiatry 61 (Suppl 8) (2000) 14–17, discussion 18-9.
[5] A.E. Urban, W.J. Cubala, Therapeutic drug monitoring of atypical antipsychotics, Psychiatr. Pol. 51 (6) (2017) 1059–1077.
[6] F.C. Nucifora Jr., M. Mihaljevic, B.J. Lee, A. Sawa, Clozapine as a Model for Antipsychotic Development, Neurotherapeutics 14 (3) (2017) 750–761.
[7] H. Jamilian, E. Amirani, A. Milajerdi, F. Kolahdooz, H. Mirzaei, M. Zaroudi, A. Ghaderi, Z. Asemi, The effects of vitamin D supplementation on mental health, and biomarkers of inflammation and oxidative stress in patients with psychiatric disorders: a systematic review and meta-analysis of randomized controlled trials, Prog. Neuropsychopharmacol. Biol. Psychiatry 94 (2019), 109651.
[8] M. Focker, J. Antel, C. Grasemann, D. Fuhrer, N. Timmesfeld, D. Ozturk, T. Peters, A. Hinney, J. Hebebrand, L. Libuda, Effect of an vitamin D deficiency on depressive symptoms in child and adolescent psychiatric patients - a randomized controlled trial: study protocol, BMC Psychiatry 18 (1) (2018) 57.
[9] M.B. Humble, Vitamin D, light and mental health, J. Photochem. Photobio. B 101 (2) (2010) 142–149.
[10] S. Kapoor, Influence of vitamin D consumption and levels on the development of psychiatric disorders, J. Clin. Psychopharmacol. 34 (4) (2014) 538–539.

[11] S. Ristic, S. Zivanovic, D.R. Milovanovic, V. Janjic, D. Djokovic, A. Jovicevic, M. S. Pirkovic, S. Kocic, Vitamin D deficiency and associated factors in patients with mental disorders treated in routine practice, J. Nutr. Sci. Vitaminol. 63 (2) (2017) 85–95.

[12] A.J. Gaebler, M. Finner-Prevel, S. Lammertz, S. Schaffrath, P. Eisner, F. Stohr, E. Rocher, L. Winkler, P. Kaleta, L. Lenzen, M. Augustin, J. Hovancakova, L. Schwemmer, E. Stormanns, F. Keskin, F. Hendricks, M. Paulzen, G. Grunder, F. Schneider, K. Mathiak, The negative impact of vitamin D on antipsychotic drug exposure may counteract its potential benefits in schizophrenia, Br. J. Clin. Pharm. 88 (7) (2022) 3193–3200.

[13] A. Krivoy, E. Whiskey, H. Webb-Wilson, D. Joyce, D.K. Tracy, F. Gaughran, J. H. MacCabe, S.S. Shergill, Outcomes in treatment-resistant schizophrenia: symptoms, function and clozapine plasma concentrations, Ther. Adv. Psychopharmacol. 11 (2021), 20451253211037179.

[14] C.T. Sempos, A.C. Heijboer, D.D. Bikle, J. Bollerslev, R. Bouillon, P.M. Brannon, H. F. DeLuca, G. Jones, C.F. Munns, J.P. Bilezikian, A. Giustina, N. Binkley, Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D, Br. J. Clin. Pharm. 84 (10) (2018) 2194–2207.

[15] R.P. Patrick, B.N. Ames, Vitamin D and the omega-3 fatty acids control serotonin synthesis and action, part 2: relevance for ADHD, bipolar disorder, schizophrenia, and impulsive behavior, FASEB J. 29 (6) (2015) 2207–2222.

[16] D.D. Bikle, Vitamin D: production, Metab. Mech. Action (2000).

[17] J. Cusato, J. Mula, A. Palermiti, A. Manca, M. Antonucci, V. Avataneo, E.D. De Vivo, A. Ianniello, A. Calcagno, G. Di Perri, A. De Nicolo, A. D'Avolio, Seasonal variation of antiretroviral drug exposure during the year: the experience of 10 years of therapeutic drug monitoring, Biomedicines 9 (9) (2021).

[18] J.D. Lindh, M.L. Andersson, E. Eliasson, L. Bjorkhem-Bergman, Seasonal variation in blood drug concentrations and a potential relationship to vitamin D, Drug Metab. Dispos. 39 (5) (2011) 933–937.

[19] J. Cusato, C. Genova, C. Tomasello, P. Carrega, S. Ottonello, G. Pietra, M. C. Mingari, I. Cossu, E. Rijavec, A. Leggieri, G. Di Perri, M.G. Dal Bello, S. Coco, S. Boccardo, G. Ferlazzo, F. Grossi, A. D'Avolio, Influence of Vitamin D in advanced non-small cell lung cancer patients treated with nivolumab, Cancers 11 (1) (2019).

[20] C.F. Thorn, D.J. Muller, R.B. Altman, T.E. Klein, PharmGKB summary: clozapine pathway, pharmacokinetics, Pharm. Genom. 28 (9) (2018) 214–222.

[21] M. Garriga, A. Mallorqui, S. Bernad, V. Ruiz-Cortes, C. Oliveira, S. Amoretti, G. Mezquida, M. Bioque, O. Molina, M. Gomez-Ramiro, E. Vieta, M. Bernardo, E. Parellada, C. Garcia-Rizo, Antipsychotic-Associated weight gain and clinical improvement under clozapine treatment, J. Clin. Psychopharmacol. 42 (1) (2021) 75–80.

[22] K.A. Costa-Dookhan, T.K. Rajji, V.N. Tran, S. Bowden, D.J. Mueller, G. J. Remington, S.M. Agarwal, M.K. Hahn, Associations between plasma clozapine/ N-desmethylclozapine ratio, insulin resistance and cognitive performance in patients with co-morbid obesity and ultra-treatment resistant schizophrenia, Sci. Rep. 11 (1) (2021) 2004.

[23] A. Hassab Errasoul, M.A. Alarabi, Factors predicting serum clozapine levels in Middle Eastern patients: an observational study, BMC Psychiatry 22 (1) (2022) 269.