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Personalized Medicine of Long-
Acting Aripiprazole:
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and Pharmacogenetics

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Personalized Medicine of Long-Acting Aripiprazole: Therapeutic Drug Monitoring and Pharmacogenetics

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Conflicto de interés

El doctorando, Francisco José Toja Camba, declara
no tener ningún conflicto de interés en relación con esta Tesis Doctoral.

Fdo. Francisco José Toja Camba

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Good, Better, Best.*

ACRONYMS AND ABBREVIATIONS

AGNP: German working group for Neuropsychopharmacology and Pharmacopsychiatry.

AM: Active Moiety

AOM: Aripiprazole Once-Monthly

ARI: Aripiprazole

BBB: Blood–Brain Barrier

BMI: Body Mass Index

C/D: Concentration/Dose

CL/F: Clearance relative to bioavailability

CL: Clearance.

DHA: Dehydroaripiprazole

IM: Intermediate Metabolizer

INJS: Injection Site

IQ: Interquartile range

IVOL: Injection Volume

KA: First-order Absorption rate constant

K_{max}: Maximum Absorption rate

LAI: Long-Acting Injectable

NDLL: Needle Length

NM: Normal Metabolizer

P-gp: P-glycoprotein

PM: Poor Metabolizer

popPK: Population Pharmacokinetics

PP1M: Once-Monthly Paliperidone Palmitate

PP3M: Once every 3 Months Paliperidone Palmitate

t_{1/2}: Apparent half-life of elimination

TDM: Therapeutic Drug Monitoring

T_{max}: Time to maximum concentration after administration

T_{ss}: Time to reach steady state

UHPLC-MS/MS: Ultra-High Performance Liquid Chromatography-tandem Mass Spectrometry

UM: Ultra Metabolizer

VD/F: Apparent Volume of distribution relative to bioavailability

VD: Apparent Volume of Distribution

9-OH-R: 9-Hydroxy-Risperidone

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RESUMEN/ RESUMO/ SUMMARY

RESUMEN

La esquizofrenia es una enfermedad mental grave que afecta al 1% de la población a lo largo de su vida. La enfermedad modifica la forma en la que los pacientes piensan, sienten o se comportan. Esto dificulta su participación en actividades de la vida cotidiana, la incorporación al mercado laboral y las relaciones sociales. Los síntomas se pueden dividir en cuatro categorías: síntomas positivos (alucinaciones, delirios, etc.), negativos (abulia, anhedonia, etc.), cognitivos (dificultad de concentración, atención, etc.) y afectivos (ansiedad, depresión, etc.).

Actualmente, el pilar fundamental del tratamiento farmacológico de la esquizofrenia son los fármacos antipsicóticos. Estos se dividen clásicamente en antipsicóticos típicos o de primera generación (haloperidol, clorpromazina, etc.) o atípicos o de segunda generación (risperidona, paliperidona, aripiprazol, etc.). Ambos grupos presentan afinidad por los receptores D2, siendo este efecto responsable de la reducción de los síntomas positivos de la enfermedad. Además, los antipsicóticos atípicos poseen diferentes afinidades sobre otros receptores como 5HT-2A ó 5HT-1A, lo que le confiere un mayor perfil de seguridad. La comercialización de los antipsicóticos inyectables de acción prolongada mejoró significativamente la adherencia a estos tratamientos con respecto a las formas farmacéuticas orales, siendo de especial importancia en este grupo de pacientes con baja conciencia de enfermedad. A pesar de esto, sigue existiendo un amplio margen de mejora en los que respecta a la eficacia y seguridad de estas terapias y es en este punto donde la medicina personalizada cobra una especial importancia.

En las últimas dos décadas, la farmacocinética y la farmacogenética se han utilizado cada vez más en la práctica clínica en psiquiatría debido a la alta variabilidad en cuanto a la respuesta y los efectos adversos de los fármacos antipsicóticos. En concreto, los antipsicóticos inyectables de acción prolongada tienen un perfil farmacocinético diferente al de las formulaciones orales debido a sus características de liberación sostenida. Además, la mayoría de estos fármacos son metabolizados por CYP2D6 y CYP3A4, cuya variabilidad genética interindividual da lugar a diferentes estados metabolizadores y, en consecuencia, a diferentes concentraciones plasmáticas de los fármacos. En este contexto, existen pruebas consistentes que apoyan el uso de la monitorización de las

concentraciones plasmáticas de fármacos junto con pruebas farmacogenéticas para mejorar el balance beneficio-riesgo de la farmacoterapia antipsicótica.

En la **primera sección** de esta tesis doctoral se evalúan dos métodos analíticos diferentes con el fin de facilitar la traslación a la práctica clínica diaria de la determinación de concentraciones plasmáticas de aripiprazol. La Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas (UHPLC-MS/MS) se considera el *gold-standard* para la determinación de concentraciones plasmáticas de fármacos. Esta técnica ofrece una alta sensibilidad y especificidad para la detección y cuantificación de compuestos en muestras biológicas. En el caso concreto de aripiprazol, el método permite su determinación, así como de su metabolito activo, dehidroaripiprazol, con alta especificidad y precisión. Por ello, esta técnica se posiciona como un método útil para la monitorización terapéutica de pacientes psiquiátricos a tratamiento con este fármaco. Sin embargo, a pesar de la capacidad para distinguir entre compuestos muy similares en matrices biológicas complejas, presenta grandes inconvenientes a la hora de su introducción en la práctica clínica diaria debido a los procesos manuales de procesamiento de muestra que requiere, causantes de una alta variabilidad, potenciales errores y un alto consumo de tiempo.

Por otro lado, el Sistema Alinity C (Laboratorios Abbot), es una alternativa prometedora debido a las ventajas que aporta en cuanto a su automatización, versatilidad y facilidad de uso. Todas estas ventajas lo convierten en un método eficiente con gran potencial para ser implementado en un entorno clínico real, donde tanto los tiempos de procesamiento como los de respuesta deben ser bajos. Alinity C utiliza técnicas fotométricas y de potenciometría para la cuantificación de analitos. Comprender las ventajas y limitaciones comparativas de estos métodos es obligatorio para avanzar en las prácticas de seguimiento terapéutico.

Para ello, se ha realizado el primer estudio comparativo hasta la fecha en la literatura disponible de UHPLC-MS/MS frente a Alinity C en la medición de las concentraciones plasmáticas de aripiprazol y dehidroaripiprazol. Para ello, se analizaron un total de 86 muestras de plasma. La fracción activa de aripiprazol se midió en 60 muestras utilizando ambos sistemas y 26 muestras se analizaron dos veces utilizando Alinity C con un periodo intermedio de 6 meses para evaluar su reproducibilidad y la estabilidad de las muestras después de un periodo de congelación. La correlación de Spearman reveló una buena asociación entre los dos ensayos ($r_s = 0,96$) y no se encontraron diferencias significativas mediante test de McNemar al clasificar las muestras entre rangos infra, supra y

terapéuticos. La regresión Passing-Bablok mostró una buena correlación entre los métodos ($r_s = 0,93$) y una pendiente de 1,12, lo que indica una ligera tendencia de Alinity C a medir valores más altos que UHPLC-MS/MS. Además, se obtuvo una buena correlación intramétodo en los dos análisis secuenciales con Alinity C ($r_s = 0,99$). No obstante, las decisiones clínicas podrían ser diferentes en el 15% de los casos en función del método elegido. No se encontraron diferencias en la determinación del *active moiety* (suma de aripiprazol y dehidroaripiprazol) mediante Alinity C en función de la concentración de aripiprazol y dehidroaripiprazol de las muestras.

La formulación inyectable de aripiprazol de administración mensual presenta una importante variabilidad farmacocinética interindividual con implicaciones significativas para su uso clínico. CYP2D6 y CYP3A4 contribuyen en gran medida a esta variabilidad, ya que metabolizan el aripiprazol en su metabolito activo, el dehidroaripiprazol y éste en metabolitos inactivos. Por ello, en la **segunda sección** de esta tesis doctoral se ha evaluado el efecto de los polimorfismos CYP2D6 y CYP3A4 en combinación y la presencia de inductores e inhibidores concomitantes de estos citocromos sobre las concentraciones plasmáticas de aripiprazol y dehidroaripiprazol en un entorno clínico real. Para ello, se realizó un estudio observacional de una cohorte de 74 pacientes caucásicos a tratamiento con formulación inyectable de aripiprazol de administración mensual.

En cuanto al CYP2D6, se observaron concentraciones más elevadas de *active moiety* (67%), aripiprazol (67%) y relación aripiprazol/dehidroaripiprazol (77%) en los metabolizadores pobres (PM) que en los metabolizadores normales (NM). No se encontraron diferencias para el dehidroaripiprazol. Los PM para CYP2D6 y CYP3A4 mostraron un 58% más de *active moiety* y un 66% más de concentración plasmática de aripiprazol en comparación con los PM para CYP2D6 y los NM para CYP3A4. Además, los PMs para CYP2D6 y CYP3A4 mostraron concentraciones de dehidroaripiprazol un 45% más altas que los NMs para ambos citocromos y un 41% más de dehidroaripiprazol que los PMs para CYP2D6 y los NMs para CYP3A4, lo que sugiere un papel significativo del CYP3A4 en la eliminación del dehidroaripiprazol.

 De este modo, se objetiva que la evaluación del efecto del estado metabolizador de CYP2D6 y CYP3A4 en combinación sobre las concentraciones plasmáticas de aripiprazol, dehidroaripiprazol y la relación

aripiprazol/dehidroaripiprazol, teniendo en cuenta los tratamientos concomitantes con inductores e inhibidores, podría optimizar la terapia de los pacientes en tratamiento con formulación inyectable de aripiprazol de administración mensual.

Además del papel relevante que tienen los citocromos CYP2D6 y CYP3A4 en la farmacocinética de aripiprazol, otra tercera proteína cobra especial importancia a la hora de comprender la variabilidad interindividual de las concentraciones plasmáticas del fármaco y su metabolito. La glicoproteína P (P-gp), una glicoproteína transmembrana que desempeña un papel crucial en la eliminación de compuestos potencialmente nocivos para el organismo. Los polimorfismos genéticos en *ABCB1* pueden provocar cambios en la función de la P-gp, dando lugar a diferencias individuales en la disposición del fármaco.

La tercera sección de esta tesis doctoral pretende determinar, en esta misma cohorte de pacientes, cómo afectan las distintas variantes de los tres SNP más prevalentes del gen *ABCB1* (C1236T, G2677T C3435T) a las concentraciones plasmáticas de aripiprazol, de su metabolito activo dehidroaripiprazol y a la relación aripiprazol/dehidroaripiprazol en pacientes en tratamiento con formulación inyectable de aripiprazol de administración mensual. Se tuvo en cuenta el estado metabólico de las dos principales enzimas metabolizadoras del aripiprazol (CYP2D6 y CYP3A4) para estudiar específicamente el efecto de la P-gp en las concentraciones plasmáticas del compuesto original y de su metabolito activo, evitando así los posibles sesgos e interferencias.

El estudio halló una clara relación entre los genotipos encontrados para los distintos SNPs de *ABCB1* y el ratio aripiprazol/dehidroaripiprazol. En concreto, los pacientes con genotipo TT en G2677T presentan casi el doble de ratio en comparación con el genotipo CC. Del mismo modo, este aumento también se encuentra en C3435T con 1,4 veces más y en C1236T con 1,6 veces más para los mismos genotipos. En cuanto a los haplotipos, se obtuvieron diferencias significativas entre los pacientes CC-GG-CC y TT-TT-TT, con una proporción un 87,9% mayor en los pacientes con el haplotipo CC-GG-CC. Se observó una clara tendencia hacia concentraciones más bajas de aripiprazol y más altas de dehidroaripiprazol cuando aumenta la presencia de alelos T mutados. El gen *ABCB1* podría ser un buen aliado junto con el genotipado de CYP2D6 y CYP3A4 a la hora de monitorizar las concentraciones plasmáticas de aripiprazol.

RESUMO

A esquizofrenia é unha enfermidade mental grave que afecta ao 1% da poboación ao longo da súa vida. A enfermidade cambia a forma de pensar, sentir ou comportarse dos pacientes. Isto dificulta a súa participación nas actividades da vida cotiá, a súa incorporación ao mercado laboral e as relacións sociais. Os síntomas pódense dividir en catro categorías: síntomas positivos (alucinacións, delirios, etc.), síntomas negativos (abulia, anhedonia, etc.), síntomas cognitivos (dificultade de concentración, atención, etc.) e síntomas afectivos (ansiedade, depresión, etc.).

Actualmente, o pilar fundamental do tratamento farmacolóxico da esquizofrenia son os antipsicóticos. Estes divídense clásicamente en antipsicóticos típicos ou de primeira xeración (haloperidol, clorpromazina, etc.) ou antipsicóticos atípicos ou de segunda xeración (risperidona, paliperidona, aripiprazol, etc.). Ambos grupos teñen afinidade polos receptores D2, sendo este efecto o responsable da redución dos síntomas positivos da enfermidade. Ademais, os antipsicóticos atípicos teñen diferentes afinidades sobre outros receptores como 5HT-2A ou 5HT-1A, o que lles confire un maior perfil de seguridade. A comercialización de antipsicóticos inxectables de longa acción mellorou significativamente a adherencia a estes tratamentos con respecto ás formas farmacéuticas orais, sendo de especial importancia neste grupo de pacientes con baixa conciencia de enfermidade. A pesar disto, aínda hai unha gran marxe de mellora en canto á eficacia e seguridade destas terapias e é neste punto onde a medicina personalizada cobra especial importancia.

Nas últimas dúas décadas, a farmacocinética e a farmacoxenética foron cada vez máis utilizadas na práctica clínica en psiquiatría debido á alta variabilidade na resposta e aos efectos adversos dos antipsicóticos. En concreto, os antipsicóticos inxectables de acción prolongada teñen un perfil farmacocinético diferente ao das formulacións orais debido ás súas características de liberación sostida. Ademais, a maioría destes fármacos son metabolizados por CYP2D6 e CYP3A4, cuxa variabilidade xenética interindividual orixina diferentes estados de metabolización e, en consecuencia, diferentes concentracións plasmáticas dos fármacos. Neste contexto, hai probas consistentes que apoian o uso da monitorización das concentracións plasmáticas de fármacos xunto con probas

farmacoxenéticas para mellorar o balance beneficio-risco da farmacoterapia antipsicótica.

Na **primeira sección** desta tese de doutoramento abórdase a traslación da determinación das concentracións plasmáticas de aripiprazol á práctica clínica diaria. A Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas (UHPLC-MS/MS) considérase o "gold-standard" para a determinación das concentracións plasmáticas de fármacos. Esta técnica ofrece unha alta sensibilidade e especificidade para a detección e cuantificación de compostos en mostras biolóxicas. No caso específico do aripiprazol, o método permite a súa determinación, así como o seu metabolito activo, o dehidroaripiprazol, con alta especificidade e precisión. Polo tanto, esta técnica sitúase como un método útil para o seguimento terapéutico dos pacientes psiquiátricos tratados con este fármaco. Non obstante, a pesar da capacidade de distinguir entre compostos moi similares en matrices biolóxicas complexas, presenta grandes inconvenientes á hora da súa introdución na práctica clínica diaria debido aos procesos de procesamento manual das mostras que require, provocando unha gran variabilidade, erros potenciais e tempos elevados.

Por outra banda, o Sistema Alinity C (Abbot Laboratories) é unha alternativa prometedora polas vantaxes que aporta en termos de automatización, versatilidade e facilidade de uso. Todas estas vantaxes convérteno nun método eficiente con gran potencial para ser implantado nun entorno clínico real, onde os tempos de procesamento e resposta deben ser baixos. Alinity C utiliza técnicas fotométricas e de potenciometría para a cuantificación de analitos. Comprender as vantaxes e limitacións comparativas destes métodos é obrigatorio para avanzar nas prácticas de seguimento terapéutico.

Para iso, preséntase o primeiro estudo comparativo ata a data na literatura dispoñible de UHPLC-MS/MS fronte a Alinity C na medida das concentracións plasmáticas de aripiprazol e dehidroaripiprazol. Para iso analizáronse un total de 86 mostras de plasma. A fracción activa de aripiprazol foi medida en 60 mostras utilizando ambos sistemas e 26 mostras foron analizadas dúas veces mediante Alinity C cun período intermedio de 6 meses para avaliar a súa reproducibilidade e a estabilidade das mostras despois dun período de conxelación. A correlación de Spearman revelou unha boa asociación entre as dúas probas ($r_s = 0,96$) e non se atoparon diferenzas significativas coa proba de McNemar ao clasificar as mostras entre rangos infra, supra e terapéuticos. A regresión Passing-Bablok mostrou unha boa correlación entre os métodos ($r_s =$

0,93) e unha pendente de 1,12, o que indica unha lixeira tendencia de Alinity C a medir valores máis altos que UHPLC-MS/MS. Ademais, obtívose unha boa correlación intramétodo nas dúas análises secuenciais con Alinity C ($r_s = 0,99$). Non obstante, as decisións clínicas poden ser diferentes nun 15% dos casos dependendo do método elixido. Non se atoparon diferenzas na determinación da fracción activa (suma de aripiprazol e dehidroaripiprazol) utilizando Alinity C dependendo da concentración de aripiprazol e dehidroaripiprazol nas mostras.

A formulación inxectable mensual de aripiprazol presenta unha variabilidade farmacocinética interindividual significativa con implicacións significativas para o seu uso clínico. CYP2D6 e CYP3A4 contribúen en gran medida a esta variabilidade, xa que metabolizan o aripiprazol no seu metabolito activo, o dehidroaripiprazol e o dehidroaripiprazol en metabolitos inactivos. Por iso, na **segunda sección** desta tese doutoral avalíase o efecto dos polimorfismos CYP2D6 e CYP3A4 en combinación e a presenza de indutores e inhibidores concomitantes destes citocromos sobre as concentracións plasmáticas de aripiprazol e dehidroaripiprazol nun contexto clínico real. Para iso, realizouse un estudo observacional nunha cohorte de 74 pacientes caucásicos tratados con formulación inxectable mensual de aripiprazol.

Respecto do CYP2D6, observáronse concentracións máis altas de fracción activa (67%), aripiprazol (67%) e relación aripiprazol/dehidroaripiprazol (77%) en metabolizadores pobres (PM) que en metabolizadores normais (NM). Non se atoparon diferenzas para o dehidroaripiprazol. Os PM para CYP2D6 e CYP3A4 mostraron un 58% máis de fracción activa e un 66% máis de concentración plasmática de aripiprazol en comparación cos PM para CYP2D6 e os NM para CYP3A4. Ademais, os PM para CYP2D6 e CYP3A4 mostraron concentracións de dehidroaripiprazol un 45% máis altas que os NM para ambos os citocromos e concentracións de dehidroaripiprazol un 41% máis altas que as PM para CYP2D6 e NM para CYP3A4, o que suxire un papel importante para o CYP3A4 na eliminación de dehidroaripiprazol.

Neste apartado da presente tese doutoral obxetívase que avaliar o efecto do estado metabolizador de CYP2D6 e CYP3A4 en combinación coas concentracións plasmáticas de aripiprazol, dehidroaripiprazol e a relación aripiprazol/dehidroaripiprazol, tendo en conta os tratamentos concomitantes con indutores e inhibidores, podería optimizar a terapia dos pacientes tratados con formulación inxectable mensual de aripiprazol.

Ademais do papel relevante que teñen os citocromos CYP2D6 e CYP3A4 na farmacocinética do aripiprazol, outra terceira proteína é de especial importancia cando se trata de comprender a variabilidade interindividual das concentracións plasmáticas do fármaco e do seu metabolito. A glicoproteína P (P-gp), unha glicoproteína transmembrana que xoga un papel crucial na eliminación de compostos potencialmente nocivos do corpo. Os polimorfismos xenéticos en *ABCB1* poden causar cambios na función de P-gp, o que provoca diferenzas individuais na disposición do fármaco.

A **terceira sección** desta tese doutoral pretende determinar, na mesma cohorte de pacientes, como as diferentes variantes dos tres SNPs máis prevalentes do xene *ABCB1* (C1236T, G2677T C3435T) afectan ás concentracións plasmáticas de aripiprazol, o seu metabolito activo dehidroaripiprazol e a relación aripiprazol/dehidroaripiprazol en pacientes tratamento con formulación inxectable mensual de aripiprazol. O estado metabolizador dos dous principais encimas metabolizadores de aripiprazol (CYP2D6 e CYP3A4) tívose en conta para estudar especificamente o efecto da P-gp sobre as concentracións plasmáticas do aripiprazol e do seu metabolito activo, evitando polo tanto posibles sesgos e interferencias.

O estudo atopou unha clara relación entre os xenotipos para os diferentes SNPs de *ABCB1* e o cociente aripiprazol/dehidroaripiprazol. En concreto, os pacientes co xenotipo TT en G2677T teñen case o dobre do cociente en comparación co xenotipo CC. Do mesmo xeito, este aumento tamén se atopa en C3435T con 1,4 veces máis e en C1236T con 1,6 veces máis para os mesmos xenotipos. En canto aos haplotipos, obtivéronse diferenzas significativas entre os pacientes CC-GG-CC e TT-TT-TT, cunha proporción un 87,9% maior nos pacientes co haplotipo CC-GG-CC. Observouse unha clara tendencia cara a concentracións máis baixas de aripiprazol e máis altas de dehidroaripiprazol coa presenza crecente de alelos T mutados. O xene *ABCB1* podería ser un bo aliado xunto co xenotipado de *CYP2D6* e *CYP3A4* á hora de monitorizar as concentracións plasmáticas de aripiprazol.

SUMMARY

Schizophrenia is a serious mental illness that affects *1% of the population* in their lifetime. The disease modifies the way patients think, feel or behave. This makes it difficult for them to participate in activities of daily living, enter the labor market and social relationships. Symptoms can be divided into four categories: positive symptoms (hallucinations, delusions, etc.), negative symptoms (abulia, anhedonia, etc.), cognitive symptoms (difficulty in concentration, attention, etc.) and affective symptoms (anxiety, depression, etc.).

Currently, the mainstay of pharmacological treatment of schizophrenia are antipsychotic drugs. These are classically divided into typical or first-generation antipsychotics (haloperidol, chlorpromazine, etc.) or atypical or second-generation antipsychotics (risperidone, paliperidone, aripiprazole, etc.). Both groups have an affinity for D2 receptors, and this effect is responsible for the reduction of the positive symptoms of the disease. In addition, atypical antipsychotics have different affinities for other receptors such as 5HT-2A or 5HT-1A, which confers a higher safety profile. The commercialization of long-acting injectable antipsychotics significantly improved adherence to these treatments with respect to oral pharmaceutical forms, being of special importance in this group of patients with low disease awareness. Despite this, there is still wide margin for improvement in terms of efficacy and safety of these therapies, and this is where personalized medicine is of particular importance.

In the last two decades, pharmacokinetics and pharmacogenetics have been increasingly used in clinical practice in psychiatry due to the high variability in response and adverse effects of antipsychotic drugs. In particular, long-acting injectable antipsychotics have a different pharmacokinetic profile than oral formulations due to their sustained release characteristics. In addition, most of these drugs are metabolized by CYP2D6 and CYP3A4, whose interindividual genetic variability results in different metabolizing states and, consequently, different plasma concentrations of the drugs. In this context, there is consistent evidence to support the use of therapeutic drug monitoring together with pharmacogenetic testing to improve the benefit-risk balance of antipsychotic pharmacotherapy.

The **first section** of this doctoral thesis focuses on the translation of the determination of plasma concentrations of aripiprazole to daily clinical practice.

Ultra-High performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) is considered the gold-standard for the determination of plasma drug concentrations. This technique offers high sensitivity and specificity for the detection and quantification of compounds in biological samples. In the specific case of aripiprazole, the method allows its determination, as well as that of its active metabolite, dehydroaripiprazole, with high specificity and precision. Therefore, this technique is positioned as a useful method for the therapeutic monitoring of psychiatric patients under treatment with this drug. However, despite the ability to distinguish between very similar compounds in complex biological matrices, it has major drawbacks when introduced into daily clinical practice due to the manual sample processing operations required, causing high variability, potential errors and high time consumption.

On the other hand, the Alinity C System (Abbot Laboratories), is a promising alternative due to its advantages in terms of automation, versatility and ease of use. All these advantages make it an efficient method with great potential to be implemented in a real clinical environment, where both processing and turnaround times must be low. Alinity C uses photometric and potentiometric techniques for analyte quantification. Understanding the comparative advantages and limitations of these methods is mandatory to advance therapeutic monitoring practices.

For this purpose, the first comparative study to date in the available literature of UHPLC-MS/MS/MS versus Alinity C in the measurement of plasma concentrations of aripiprazole and dehydroaripiprazole is presented. For this purpose, a total of 86 plasma samples were analyzed. The active fraction of aripiprazole was measured in 60 samples using both systems and 26 samples were analyzed twice using Alinity C with an intervening period of 6 months to assess their reproducibility and the stability of the samples after a period of freezing. Spearman's correlation revealed a good association between the two assays ($r_s = 0.96$) and no significant differences were found by McNemar's test when classifying samples between infra-, supra- and therapeutic ranges. Passing-Bablok regression showed a good correlation between the methods ($r_s = 0.93$) and a slope of 1.12, indicating a slight tendency for Alinity C to measure higher values than UHPLC-MS/MS. In addition, a good intramethod correlation was obtained in the two sequential analyses with Alinity C ($r_s = 0.99$). However, clinical decisions could be different in 15% of cases depending on the chosen method. No differences were found in the determination of active moiety (sum

of aripiprazole and dehydroaripiprazole) by Alinity C as a function of the concentration of aripiprazole and dehydroaripiprazole in the samples.

Aripiprazole once-monthly has significant interindividual pharmacokinetic variability with significant implications for clinical use. Cytochromes CYP2D6 and CYP3A4 are major contributors to this variability, as they metabolize aripiprazole to its active metabolite, dehydroaripiprazole, and the latter to inactive metabolites. Therefore, the **second section** of this doctoral thesis aims to evaluate the effect of CYP2D6 and CYP3A4 polymorphisms in combination and the presence of concomitant inducers and inhibitors of these cytochromes on plasma concentrations of aripiprazole and Dehydroaripiprazole in a real clinical setting. For this purpose, an observational study of a cohort of 74 Caucasian patients treated with aripiprazole once-monthly was performed.

For CYP2D6, higher concentrations of active moiety (67%), aripiprazole (67%) and aripiprazole/dehydroaripiprazole ratio (77%) were observed in poor metabolizers (PM) than in normal metabolizers (NM). No differences were found for dehydroaripiprazole. PMs for CYP2D6 and CYP3A4 showed 58% higher active moiety and 66% higher plasma concentration of aripiprazole compared with PMs for CYP2D6 and NMs for CYP3A4. Furthermore, PMs for CYP2D6 and CYP3A4 showed 45% higher dehydroaripiprazole concentrations than NMs for both cytochromes and 41% more dehydroaripiprazole than PMs for CYP2D6 and NMs for CYP3A4, suggesting a significant role of CYP3A4 in dehydroaripiprazole clearance.

In this section of the present doctoral thesis is observed that the evaluation of the effect of CYP2D6 and CYP3A4 metabolizer status in combination on plasma concentrations of aripiprazole, dehydroaripiprazole and the aripiprazole/dehydroaripiprazole ratio, considering concomitant treatments with inducers and inhibitors, could optimize the therapy of patients on treatment with aripiprazole injectable formulation of aripiprazole once-monthly.

In addition to the relevant role of cytochromes CYP2D6 and CYP3A4 in the pharmacokinetics of aripiprazole, a third protein is of particular importance in understanding the interindividual variability of plasma concentrations of the drug and its metabolite. P-glycoprotein (P-gp), a transmembrane glycoprotein that plays a crucial role in the elimination of potentially harmful compounds from the body. Genetic polymorphisms in *ABCB1* can lead to changes in P-gp function, resulting in individual differences in drug disposition.

The third section of this doctoral thesis aims to determine, in the same cohort of patients, how the different variants of the three most prevalent SNPs of the *ABCB1* gene (C1236T, G2677T and C3435T) affect the plasma concentrations of aripiprazole, its active metabolite dehydroaripiprazole and the aripiprazole/dehydroaripiprazole ratio in patients treated with aripiprazole once-monthly. The metabolizing states of the two main aripiprazole metabolizing enzymes (CYP2D6 and CYP3A4) was taken into account to specifically study the effect of P-gp on plasma concentrations of the parent compound and its active metabolite, thus avoiding potential biases and interferences.

The study found a clear relationship between the genotypes found for the different *ABCB1* SNPs and the aripiprazole/dehydroaripiprazole ratio. Specifically, patients with TT genotype at G2677T have almost double the ratio compared to the CC genotype. Similarly, this increase is also found in C3435T with 1.4 times more and in C1236T with 1.6 times more for the same genotypes. Regarding haplotypes, significant differences were obtained between CC-GG-CC and TT-TT-TT patients, with an 87.9% higher proportion in patients with the CC-GG-CC haplotype. A clear trend toward lower aripiprazole and higher dehydroaripiprazole concentrations was observed when the presence of mutated T alleles increases. The *ABCB1* gene could be a good ally together with *CYP2D6* and *CYP3A4* genotyping when monitoring aripiprazole plasma concentrations.

INTRODUCTION

1. PERSONALIZED MEDICINE IN PSYCHIATRY

Schizophrenia is a severe mental disorder that is presented clinically heterogeneously in patients. It is estimated that approximately 0.7–1% of the world population suffer from this condition, currently affecting 24–25 million people worldwide according to data from the World Health Organization ^{1–3}. Mainly, it causes disorder of behavior, thoughts, perception and emotions. The core features are positive symptoms (delusions, hallucinations and disorganized speech), negative symptoms (such as abulia, anhedonia and social withdrawal), cognitive impairment (mainly in speed of mental processing, working memory and executive functions) and affective symptoms (i.e., anxiety and depression) ⁴. There are well-defined instruments such as the Brief Psychiatric Rating Scale (BPRS), the Scale for the Assessment of Positive Symptoms (SAPS), the Scale for the Assessment of Negative Symptoms (SANS) or the Positive and Negative Symptoms Scale (PANSS) to measure these positive and negative symptoms as well as general psychopathology. In clinical studies, reductions in these scales have been used to define treatment response ⁵.

There are several theories that try to explain the pathophysiology of this complex and highly polygenic disease, including brain structure defects or neurochemical alterations ⁶. Recently Genome Wide Association Studies have supported some of those classic neurochemical hypotheses such as the dopaminergic and glutamatergic from a genetic perspective ⁷. Nowadays, the pharmacological treatment of schizophrenia is based on antipsychotics, a family of drugs which are classified into two separate groups: first generation or typical (i.e., haloperidol, chlorpromazine) and second generation or atypical (i.e., risperidone, paliperidone, aripiprazole). Both of them present affinity toward D2 receptors and reduce positive symptoms. Atypical antipsychotics form a heterogeneous group of drugs with regard to their different receptor affinity. In addition to D2 receptor affinity, they have other pharmacological actions involving different signaling pathways such as serotonergic receptors (5-HT_{2A}, 5-HT_{1A} and 5-HT_{2C}) that could be the main cause of their safer profile compared to typical antipsychotics ⁸.

The effectivity of these drugs is far from expected. Treatment resistance requires dose optimization that is based on a trial-and-error method caused by the current inability to predict response to treatment; this added to severe and frequent adverse events are commonly the reasons that lead the patients to discontinue antipsychotic therapies. The main handicap of oral antipsychotics

formulations is adherence. Many factors have influence on non-adherence such as forgetting doses of medication, adverse events during therapy and poor insight that condition drug intake. According to various studies, which define adherence as taking medication at least 75% of the time, non-adherence is around 50% of treated patients ^{9,10}. This problem has been partially solved since typical long-acting injectable (LAI) antipsychotics were released. Later, arrival of atypical LAI antipsychotics introduced an improvement in the safety profile of these drugs, which are administered at long intervals of time (from 2 weeks to 3 months). At this point, the tandem of pharmacogenetics and pharmacokinetics plays a key role, and personalized prescription of antipsychotics improves the safety and efficacy of pharmacotherapy. Combining pharmacogenetics with therapeutic drug monitoring (TDM) of LAI antipsychotics would be a great tool to predict and anticipate patient response in order to achieve mental wellness and minimize side effects. Furthermore, population pharmacokinetic (popPK) models should be considered as a useful tool to understand the relationship between patient characteristics and drug exposure.

Among all over the genes involved in pharmacokinetics of drugs, the *Cytocrome P450* family is, by far, the most important in terms of interindividual variability. Most of drugs that are commonly used in psychiatry are CYP2D6-dependent ^{11,12}, an enzyme encoded from a highly polymorphic gene. Nowadays, there are already 145 alleles described for *CYP2D6*. These alleles can have different functions, frequency and clinical impact on metabolism across different populations ¹³⁻¹⁵. Depending on allele combination, it is possible to differentiate the following metabolizer status ¹⁶⁻²¹: normal metabolizer (NM): their metabolic pathway is not altered; poor metabolizers (PM): the activity enzyme is significantly reduced or absent; intermediate metabolizer (IM): those who can metabolize substances in lower ratios compared to an EM but better than a PM. Rapid or ultrarapid metabolizer (UM): enzymatic hyperactivity compared to NM. Those attracting more interest are PM, IM and UM due to their risk of developing adverse reactions or lack of efficacy in clinical practice ²²⁻²⁴, so currently development in this area of knowledge holds great promise for reducing time between diagnosis and seeking an effective treatment. Lastly, a mismatch has been observed between the genotype-based prediction of *CYP450*-mediated drug metabolism and the true capacity of an individual to metabolize drugs (phenotype) due to non-genetic factors, which is called phenoconversion

Compared to classical pharmacokinetics, where several samples at different time points are collected from a patient to estimate their individual pharmacokinetic parameters (clearance, distribution volume, half-life, etc.), population pharmacokinetics (popPK) analyzes the pooled data available of several patients in order to estimate pharmacokinetic parameter values and their variance, identify covariates and study the random effects (parameter variance which cannot be explained by covariates). All this information implemented in a mathematical model (popPK models) plus Bayesian computational methods allows us to estimate our patients' individual pK parameters, which can be a very helpful tool to individualize dosing and optimize the treatment efficacy and safety.

The three major prescribed LAI-antipsychotics are risperidone, paliperidone and aripiprazole, while LAI-olanzapine prescription has been decreasing over time ²⁶.

2. LONG-ACTING RISPERIDONE

Risperidone is an atypical antipsychotic derived from benzisoxazole with potent D2 and 5-HT₂ receptor antagonism. LAI risperidone is an aqueous suspension of microspheres formed by a glycolic and lactic acid degradable matrix where risperidone is encapsulated ²⁷. More recently, a new formulation of once-monthly subcutaneous LAI risperidone has been approved by the FDA in which risperidone is suspended in ATRIGEL[®], a polymer that solidifies in contact with tissues ^{28,29}.

2.1. Pharmacokinetics

Considering the physicochemical properties of polymers of risperidone, a reduced release occurs a few days after injection (around 1% of the dose administered) followed by a lag time of 3 weeks and then a significant release is observed in weeks 4–6 ^{27,30}. Due to this lag phase, oral supplementation should be given in the first three weeks of treatment. Metabolism of risperidone goes through hydroxylation and N-dealkylation processes in which CYP2D6 plays a predominant role in the formation of 9-OH-risperidone (9-OH-R), main active and “equipotent” metabolite ³¹. Product monograph states that the half-life ($t_{1/2}$) of risperidone plus 9-OH-R is 3–6 days ³² and the steady-state is reached after the fourth injection and maintained for 4–5 weeks after the last injection ³⁰. As well as $t_{1/2}$, steady-states were determined by inspection of the concentration–time

curve and not by the rule of the five $t_{1/2}$. As Lee et al.³³ point out, this can cause an underestimation of the time when the steady state is reached and can lead to an increase in dosage and an accumulation of the drug.

More recently, a new formulation of once-monthly subcutaneous LAI risperidone has been approved by the FDA, in which risperidone is suspended in ATRIGEL®, a polymer that solidifies in contact with tissues, providing a sustained release. Risperidone ATRIGEL® does not need oral supplementation due to its characteristic pharmacokinetic profile. After subcutaneous injection, a first peak appears 4–6 h post-dose followed by a second one 10–14 days after. Steady state is reached by the end of the second injection and the apparent half-life ranges between 8 and 9 days^{28,29}.

With regard to the therapeutic range of reference, the German working group for neuropsychopharmacology and pharmacopsychiatry (AGNP) Consensus Guidelines for Therapeutic Drug Monitoring established between 20 and 60 ng/mL for risperidone plus 9-OH-R³⁴. This range is derived exclusively from orally given risperidone studies, extrapolating this range to patients treated with long-acting formulations, which has not yet been extensively studied. Two different studies used this AGNP range^{35,36} but the other two studies differ and propose other ranges for LAI risperidone^{37,38}. De Leon et al.³⁹ proposed an updated therapeutic range (20–30 ng/mL) for LAI risperidone as a consequence of the prolonged absorption of LAI formulations and the reduction in the administration frequency, which leads to less oscillations at steady-state conditions. Despite this, until new data about LAI risperidone are available, the AGNP therapeutic reference range is the most recommended⁴⁰.

2.2. Pharmacogenetics

2.2.1. Efficacy

Polymorphisms in the *CYP2D6* gene could affect plasma concentrations of risperidone plus 9-OH-R (active moiety) although the product monograph reflects that concentration of the active moiety is the same in PM individuals as in other phenotypes³². Therefore, adjusting the dose to the patient's phenotype is not necessary but many published studies differ on this point. It is suggested that *CYP2D6* metabolizer status cause differences in active moiety exposure and consequently in efficacy and tolerability, but there are still controversies (Table 1).

Table 1. Studies between CYP2D6 phenotype and relation with active moiety exposure. Classified in relevant (significant association between CYP2D6 phenotype and active moiety exposure) or irrelevant (no significant association between CYP2D6 phenotype and active moiety exposure or incorrect assignment of CYP2D6 genotype due to missing copy number variation analysis). Abbreviations: PM, Poor Metabolizer; IM, Intermediate Metabolizer; NM, Normal Metabolizer; UM, Ultra Metabolizer; NR: Not reported. Those studies that do not analyze the number of copies of the gene have been classified as irrelevant. Among those classified as relevant, it is important to highlight that they should be taken as a guideline, taking into account that the main limitation is the high heterogeneity that exists between the different methods of analysis used, as well as the polymorphisms explored in the determination of the phenotype of CYP2D6.

Study	LAI/Oral	n	Race	Age (Median)	Male/Female	CYP2D6 Phenotypes	Outcome
Vermeulen A et al. ⁴¹	Oral	407	NR	38	267/140	PM/IM/NM	Irrelevant
Scordo MG et al. ⁴²	Oral	37	Caucasians	41	30/7	PM/IM/NM/UM	Irrelevant
Cho HY et al. ⁴³	Oral	24	Asian	24.6	NR	PM/NM	Irrelevant
Hendset, M et al. ³⁵	LAI	90	Caucasians	38	53/37	PM/IM/NM/UM	Relevant (higher plasma exposure for IM and PM)
Llerena A et al. ⁴⁴	Oral	35	Caucasians	43	NR	PM/IM/NM/UM	Irrelevant
Choong, E et al. ⁴⁵	LAI	42	Caucasian (76%)	35	30/12	PM/IM/NM/UM	Relevant (higher plasma exposure for IM and PM and lower for UM)
León, J. D et al. ⁴⁶	Oral	277	Caucasian (78%)	43.7	150/127	PM/IM/NM/UM	Relevant (higher plasma exposure for PM)
Jovanović, N et al. ⁴⁷	Oral	83	Caucasians	30.3	17/66	PM/NM/IM	Irrelevant
Jukic, M et al. ⁴⁸	Oral	725	Caucasians	42.8	355/370	PM/IM/NM/UM	Relevant (higher plasma exposure for IM and PM and lower for UM)
Locatelli, I et al. ⁴⁹	LAI	50	Caucasian	30	39/11	PM/IM/NM/UM	Irrelevant
Vandenbergh, F et al. ⁵⁰	Oral	150	Caucasian (81%)	39	82/68	PM/IM/NM/UM	Relevant (higher plasma exposure for PM)
Gunes, A et al. ⁵¹	Oral	46	Caucasian	45	35/11	PM/NM/PM	Relevant (higher plasma exposure for PM)

A recent meta-analysis of 15 studies involving 2125 patients taking oral risperidone concluded that CYP2D6 activity is associated with increased exposure of both risperidone and active moiety. Risperidone steady-state plasma concentration was 2.35-fold higher in IM and 6.20-fold higher in PM, while active moiety concentration was 1.18-fold higher in IM and 1.44-fold higher in PM ⁵².

Furthermore, some studies suggest that PM are associated with a greater number of adverse events and treatment discontinuation which decreases clinical

efficacy ^{53,54}. In addition, some popPK models based on oral regimens of risperidone showed large differences in CL/F depending on *CYP2D6* phenotype. Mean CL/F values reported by Feng et al. were 65.4, 36 and 12.5 L/h for EM, IM and PM, respectively ⁵⁵. The popPK model developed by Sherwin et al. ⁵⁶ obtained mean values of CL/F for EM, IM and PM patients of 37.4, 29.2 and 9.38 L/h, respectively. Similar differences in CL/F between PM (three-fold lower) and NM individuals were reported by Thyssen et al. ⁵⁷ in a study including pediatric and adult patients. More recently, a popPK model based on oral regimen of risperidone showed that a reduced activity of *CYP2D6* (PM) could increase up to a 106% steady-state plasma concentration and up to a 53% higher C_{max} compared with NM, so dose adjustment may be necessary ⁵⁸.

Nevertheless, LAI antipsychotics exhibit large differences compared to oral formulations in terms of their PK profile due to their slow and sustained absorption. To our knowledge, only a popPK LAI model of the risperidone ATRIGEL[®] formulation has been published, which found body mass index and dose as covariates of absorption rate constants and risperidone and 9-OH-R distribution volumes, respectively. In contrast, *CYP2D6* polymorphisms were not statistically significant to any structural popPK parameter ⁵⁹, which might be explained by the unbalanced and reduced number of patients recruited.

It is possible to obtain approximated information about *CYP2D6* activity using quantitative methods by the following ratios. On the one hand, the R/9-OH-R ratio (relates risperidone and 9-OH-R plasma concentrations) values < 1 are normal plasma ratios that indicate normal *CYP2D6* activity (UM, NM, IM) while a value > 1 indicates a lack of activity (PM) or the presence of a strong inhibitor [39,60,61]. On the other hand, total risperidone C/D ratio is a measure of drug clearance that relates the concentration of the active moiety with the dose of risperidone. The normal value for oral risperidone and for LAI formulation is approximately around 7 ng/mL per mg/day and patients with ratios > 14 ng/mL per mg/day are expected to be *CYP2D6* PM ^{40,60}. Previous procedures could be an alternative for psychiatrists and could be a possibility for clinicians who do not have access to genotype *CYP2D6*, but it is important to take into account that these methods would only be useful when *CYP2D6* constitutes the main pathway of metabolism. It is important to take into account that the *CYP2D6* allele combination in a patient is an invariable condition; once analyzed it will not change. Thus, analyzing polymorphisms in *CYP2D6* by pharmacogenetics can be useful to select the most appropriate drugs for a patient, avoiding adverse effects

or lack of efficacy concerning its *CYP2D6* metabolizer status. As a result, individualized treatment selection would become easier for prescribers.

Therefore, it seems clear that genetic variations in *CYP2D6* have an impact on plasma concentrations of risperidone, but there are controversies when it comes to relating it to the efficacy of the treatment and clinical outcome. A study of 136 patients diagnosed with schizophrenia and evaluating clinical improvement using the Positive and Negative Syndrome Scale (PANSS) did not showed association between *CYP2D6* activity variations and clinical outcome ⁶¹. Along the same line another study of 83 drug-naïve patients experiencing a first episode of psychosis did not match the clear relation between *CYP2D6* genetic variations and clinical improvement ⁴⁷. On the contrary, another study of 76 patients with schizophrenia evaluated changes on total PANSS and *CYP2D6* polymorphism founding correlation between PMs and better response to risperidone treatment. Only three patients were classified as PMs but the power of the study was enough to establish an association with PANSS improvement. ⁶². Likewise, Jukic et al. recently identified a higher incidence of therapeutic failure (discontinuation or switch to another antipsychotic) in PMs ⁴⁸. These pharmacogenetic studies are based on oral risperidone administration and not all of them had been conducted in patients with schizophrenia.

2.2.2. *Safety*

Antipsychotics can cause hyperprolactinemia by blocking dopaminergic receptors at the tuberoinfundibular system ⁶³. Risperidone was reported to have a high prevalence of hyperprolactinemia among all atypical antipsychotics ^{64,65}. Association between *CYP2D6* and hyperprolactinemia was described in a observational study of 47 children and adolescents with autism spectrum disorder in a long-term treatment with risperidone; the number of patients with hyperprolactinemia was 100% (2/2) for PM, 47% (8/17) for IM, 48% (12/25) for NM and 0% (0/2) for UM ⁶⁶. Sex influence in hyperprolactinemia incidence has been studied, observing a higher incidence in female than in males, a study of Schoretsanitis et al. ⁶⁷ involving 111 patients (61 males and 49 females) evaluates association between *CYP2D6* activity, risperidone levels and sex finding a significant association across them. Another cause of reduced patient compliance is weight gain produced by risperidone, relation between genetic polymorphisms and this adverse drug reaction is still under study. Some studies like the one performed by Lane et al. found significant association between

CYP2D6×10 allele (PM) and weight gain but data about this relation up-to-date are limited ⁶⁸.

Extrapyramidal Symptoms (EPS) are the most evaluated adverse effects with CYP2D6 in a large number of studies. Nevertheless, it is not clear the impact of CYP2D6 variations on EPS from risperidone. Several studies showed no significant difference between these two variables and only a few find some correlation. Adverse effects were also examined in a study of 70 healthy volunteers, several genes and respective polymorphisms were associated with adverse effects (CYP2C9, NAT2, DRD2, CYP2C19) but no relation was found between CYP2D6 polymorphisms and EPS ⁶⁹. De Leon et al. ⁵³ showed this association in 73 patients with moderate to severe EPS and 81 patients that discontinued risperidone due to EPS. CYP2D6 PM phenotype was associated with the first group (OR = 3.4; CI = 1.5–8.0, $p = 0.004$) and with the second group. (OR = 6; CI = 1.4–25.4, $p = 0.02$). Another recent study evaluated 22 patients and the Drug-induced Extrapyramidal Symptoms Scale (DIEPSS) score; they observed that DIEPSS score was significantly higher in the IM group (7) than in the NM group (15) ⁷⁰. A summary of LAI-risperidone pharmacokinetic and pharmacogenetic characteristics is available in Table 2.

Table 2. Summary of PK/PG characteristics of LAI-Risperidone. Abbreviations: T_{max} , time to maximum concentration after administration; T_{ss} , time to reach steady state; $t_{1/2}$, apparent half-life of elimination. TDM Levels of recommendation: 1: Strongly recommended; 2: Recommended; 3: Useful; 4: Potentially useful (33). Genetic test; informative: the label contains information stating that a particular gene affects or does not affect drug efficacy, not clinically significant.

Drug	Main Active Metabolite	Therapeutic Reference Range	Metabolism	TDM Recommendation (33)	Mean PK Values	Genetic Test Recommendation (94)
LAI-Risperidone microspheres	9-OH-Risperidone	20–60 ng/mL	CYP2D6/CYP3A4	2	T_{max} : 28 days T_{ss} : 8 weeks $T_{1/2}$: 3–6 days	Informative
LAI-Risperidone ATRIGEL®	9-OH-Risperidone	20–60 ng/mL	CYP2D6/CYP3A4	2	Double T_{max} : 4–6 h and 10–14 days T_{ss} : 8 weeks $T_{1/2}$: 8–9 days	Informative

3. LONG-ACTING PALIPERIDONE

Paliperidone (9-OH-Risperidone) is the primary active metabolite of risperidone. This long-acting drug is formulated as a palmitate ester of paliperidone in an aqueous suspension of nanocrystals resulting in a sustained release profile ^{71,72}. This technology confers an increased solubility, absorption and bioavailability ⁷³. Monthly formulation (PP1M) was approved by the FDA in 2009 and trimestral formulation (PP3M) in 2015, a semi-annual formulation is currently being developed ⁷⁴. For developing PP3M, the manufacturer used a PP1M population-pharmacokinetic model and avoid phase II study ⁷⁵. The main difference between PP1M and PP3M is that this last one has an increased particle size what allows its longer sustained release ⁷⁶.

3.1. Pharmacokinetics

Paliperidone palmitate is slowly dissolved at the injection site after the intramuscular, deltoid or gluteal, administration and then rapidly hydrolyzed. Peak plasma concentration is achieved 13 days after injection while steady-state conditions last nearly 8 months. The apparent half-life ranges between 25 and 49 days. In contrast to risperidone, paliperidone is not extensively metabolized in the liver and nearly half of the dose is excreted unchanged in urine, so dose reduction is recommended in patients with mild renal impairment ⁷¹. LAI Paliperidone treatment is not recommended in patients with moderate to severe renal impairment.

A popPK model of LAI PP1M statistically identified BMI, creatinine clearance, injection site (deltoid vs. gluteal), injection volume and needle length as statistical covariates, demonstrating that administration procedure and the site of injection play a key role on PP1M pharmacokinetic profile ⁷⁷. The summary of product characteristics recommends a first deltoid injection of 234 mg of paliperidone palmitate followed by a second deltoid injection of 156 mg one week later ⁷¹. Different studies evaluated therapeutical equivalence of deltoid versus gluteal injection sites. While some authors stated that differences between deltoid and gluteal injection are not clinically relevant ⁷⁸, Yin et al. reported that it can compromise maintenance treatment ⁷⁹. A case report observed that absorption rate after gluteal injection decreased in obese patients due to subcutaneous fat ⁸⁰. According to this, another study estimates that time to reach

steady-state differs by 4 weeks between deltoid (38w) and gluteal (42w) injection⁸¹. It seems that deltoid injection provides better absorption so it should be chosen instead of the gluteal zone whenever is possible, especially in obese patients.

On the other hand, PP3M is approved for use in patients previously treated with PP1M formulation for at least 4 months with a dose 3.5 times higher than the previous PP1M dosage. PP3M has a larger particle size compared to PP1M, which allows for longer sustained release and administration every 3 months. Peak plasma concentrations are achieved 30–33 days after administration while consecution of steady-state lasts nearly 15 months. Apparent half-life ranges from 84 to 95 days (deltoid administration) versus 118 to 139 days (gluteal administration). This difference in half-life of PP3M depending on site of injection could be due to flip-flop kinetics that occur on these long-acting formulations. According to Schoretsanitis et al., urgent real-world half-life studies are needed in steady-state conditions to clarify dose-dependent half-lives on dose and influence of injection site⁴⁰. The most relevant parameters of the popPK models of PP1M and PP3M are summarized in Table 3.

Table 3. Population pharmacokinetic models of paliperidone palmitate. Abbreviations: LAI: long-acting injectable. PP1M: once-monthly paliperidone palmitate. PP3M: once every 3 months paliperidone palmitate. IVOL: injection volume. INJS: injection site. BMI: body mass index. NDLL: needle length. KA: first-order absorption rate constant. $K_{a\max}$: maximum absorption rate. CL: clearance. VD: apparent volume of distribution. CL/F: clearance relative to bioavailability (in the absence of intravenous administration). VD/F: apparent volume of distribution relative to bioavailability (in the absence of intravenous administration).

Author	Formulation	Model	Covariates	Parameters Values	Equations
Samtani et al. ⁷⁷	LAI-PP1M	- One-compartment model with first-order elimination - Dual and sequential input absorption: rapid zero order followed by first-order absorption after a lag time. - Flip-flop kinetics due to dissolution rate limited absorption.	- SEX, AGE, IVOL and INJS on Ka - CLCR on CL - BMI and SEX on Vd	KA: 0.488 h ⁻¹ CL: 4.95 L/h VD: 391 L	$CL = 4.95 \times \left(\frac{CLCR}{110.6}\right)^{0.376}$ $V = SEX \times 391 \times \left(\frac{BMI}{26.8}\right)^{0.889}$ SEX = 1 for males SEX = 0.726 for females
Magnusson et al. ⁸²	LAI-PP3M	- One-compartment model with first-order elimination - Dual and sequential input absorption processes: rapid zero absorption followed by first-order absorption.	- IVOL on Ka - INJS and SEX on $K_{a\max}$ - CLCR on CL - BMI on Vd	KA: not available. CL/F: 3.84 L/h VD/F: 1960 L	$CL = 3.84 \times \left(\frac{CLCR}{115}\right)^{0.316}$ $V = 1960 \times \left(\frac{BMI}{26.15}\right)^{1.18}$

With regard to the reference range, AGNP consensus guidelines established the paliperidone palmitate reference range between 20 and 60 ng/mL, but this range is again an extrapolation from oral paliperidone studies and discordance exists. A study of non-steady-state patients revealed that 45% of them were under the AGNP range ⁸³; on the other hand, two different studies agreed with the AGNP therapeutic range ^{84,85}.

3.2. Pharmacogenetics

Metabolism and elimination information provided by the manufacturer are based on a study of five healthy male subjects given a 1 mg single dose of ¹⁴C-paliperidone oral solution; 59% of the dose was excreted unchanged in urine. They identified four metabolic pathways but none of them metabolized more than 6.5% of the dose. Three subjects were classified as NM and two were classified as PM for *CYP2D6* using the dextromethorphan metabolic ratio. No differences in the overall plasma pharmacokinetics were observed between NM and PM. A total of 80% of the administered radioactivity was recovered in urine and 11% in the feces ⁸⁶. The small sample size (n = 5) of the study and the fact of oral administration instead of injectable does not make it possible to draw definitive conclusions with a sufficient level of evidence. Another study including 31 patients treated with paliperidone (Oral: 9 LAI: 22) did not find a statistical difference in either for the C/D ratios between different *CYP2D6* phenotypes (4 PM, 3 IM, 22 EM, 2 UM) ⁸⁷. A summary of LAI-paliperidone pharmacokinetic and pharmacogenetic characteristics is available in Table 4.

Table 4. Summary of PK/PG characteristics of LAI-Paliperidone. Abbreviations: T_{max} , time to maximum concentration after administration; T_{ss} , time to reach steady state; $t_{1/2}$, apparent half-life of elimination. TDM Levels of recommendation: 1: Strongly recommended; 2: Recommended; 3: Useful; 4: Potentially useful (33). Genetic test; informative: label contains information stating that a particular gene affects or does not affect drug efficacy, not clinically significant.

Drug	Main Active Metabolite	Therapeutic Reference Range	Metabolism	TDM Recommendation (33)	Mean PK Values	Genetic Test Recommendation (94)
LAI-Paliperidone PP1M	-	20–60 ng/mL	60% excreted unmetabolized/ <i>CYP2D6/CYP3A4</i> In vitro	2	T_{max} : 13 days T_{ss} : 8–9 months $T_{1/2}$: 25–49 days	Informative
LAI-Paliperidone PP3M	-	2–60 ng/mL	60% excreted unmetabolized/ <i>CYP2D6/CYP3A4</i> In vitro	2	T_{max} : 30–33 days T_{ss} : 15 months $T_{1/2}$: 84–95 days (deltoid) 118–139 days (gluteal)	Informative

With these data it is expected that co-treatment with cytochrome inducers does not play any role in metabolism of paliperidone; however, a study performed by Yasui-Furukori et al.⁸⁸ in steady-state conditions observed that paliperidone concentrations decrease nearly 48% when carbamazepine, a potent CYP3A4 inductor, was administered concomitantly. Due to the lack of evidence regarding the influence of CYP2D6 polymorphisms in paliperidone plasma concentrations (Table 5), it is not expected that it could be associated with a higher risk of adverse effects.

Table 5. Studies between CYP2D6 phenotype and the relation with paliperidone plasma exposure. Irrelevant outcome (no significative association between CYP2D6 phenotype and paliperidone plasma exposure or incorrect assignment of CYP2D6 genotype due to missing copy number variation analysis). Abbreviations: PM, Poor Metabolizer; IM, Intermediate Metabolizer; NM, Normal Metabolizer; UM, Ultra Metabolizer; NR: Not reported. * Phenotyped using the dextromethorphan metabolic ratio.

Study	Oral/LAI	n	Race	Age (Median)	Male/Female	CYP2D6 Phenotypes	Outcome
Vermeir et al. ⁸⁶	Oral	5	Caucasian	51	5/0	PM/NM *	Irrelevant
Lisbeth et al. ⁸⁷	Oral/LAI	31	Caucasian	35	22/9	PM/IM/EM/UM	Irrelevant
Berwaerts et al. ⁸⁹	Oral	60	Caucasian (75%)	NR	60/0	NM/UM	Irrelevant (Coadministration with paroxetine)

4. LONG-ACTING ARIPIRAZOLE

Aripiprazole was the first antipsychotic to have partial agonist effects at D2 receptors. This property, plus D3 and 5-HT1A partial agonism and 5-HT2A receptor antagonism translates, into a reduction in negative, positive and cognitive symptoms of schizophrenia, minimizing the risk of some adverse effects compared with other atypical antipsychotics.

4.1. Pharmacokinetics

The aripiprazole once monthly (AOM) long-acting injectable is the monohydrate polymorphic form of aripiprazole⁹⁰; currently, doses of 400 mg and 300 mg are approved for induction (along with 14 days of oral supplementation)

and maintenance therapy of schizophrenia. Recently, an induction start of two injections of 400 mg has been approved, avoiding the need for oral supplementation for 14 days ⁹⁰. Due to low solubility, AOM absorption into the systemic circulation is slow and prolonged after intramuscular injection. Peak plasma concentration is reached after 4 days in deltoid injection versus 5–7 days in gluteal injection, in steady-state conditions. The mean apparent half-life is 29.9 days for 300 mg and 46.5 days for 400 mg; steady-state concentrations are achieved by the fourth injection for both sites of injection according to the manufacturer ^{90,91}.

Systemic metabolism is carried out mainly by liver biotransformation, mediated by *CYP3A4* and *CYP2D6*, yielding to its main active metabolite dehydroaripiprazole ⁹². Dehydroaripiprazole is also a ligand at the D2 receptor and has similar pharmacological properties to the original compound ⁹³. At steady state, this metabolite represents up to 29% of plasma drug concentration ⁹⁴. Therefore, both compounds are thought to contribute to the antipsychotic effects. As well as risperidone and paliperidone, aripiprazole and dehydroaripiprazole are substrates of P-gp ⁹⁵. In this case, to our knowledge no literature is available about binding affinity to P-gp of aripiprazole and dehydroaripiprazole.

Recently, the new lauroxil LAI aripiprazole formulation has been approved. After intramuscular injection, aripiprazole lauroxil slowly dissolves and it is then hydrolyzed to aripiprazole. Peak plasma concentrations are achieved 41 days post-dose, while the steady-state is reached after 4 months. Supplementation with oral aripiprazole is needed 21 days after first administration. A 1-day regimen composed of 30 mg oral aripiprazole plus an intramuscular 675 mg nanocrystalline aripiprazole has also been approved. Aripiprazole lauroxil can be administered every 4, 6 or 8 weeks depending on the dose.

Therapeutic reference range based on oral formulation studies were reported between 100 and 350 ng/mL for aripiprazole and 150 and 500 ng/mL for the active moiety (aripiprazole plus dehydroaripiprazole) ³⁴. AOM 400 mg provided sustained mean plasma concentrations comparable to those achieved by oral aripiprazole, 10–30 mg/day at steady-state. These data were evaluated in a 24-week, open-label, phase I study conducted in 41 patients with schizophrenia, receiving oral supplementation with 10 mg of aripiprazole for the first 14 days following the initial injection ^{91,96}. Pharmacokinetic data about AOM are to date limited; no third-party studies with PK analysis are available.

A study performed by the manufacturer comparing deltoid and gluteal administration verified that exposure was similar between the two injection sites but absorption rate and C_{max} were higher in the deltoid group; it seems that, as in other LAI antipsychotics, deltoid administration is a better injection site ⁹⁶.

Most relevant parameters of the popPK models of aripiprazole lauroxil are summarized in Table 6, which simultaneously modeled aripiprazole and dehydroaripiprazole observations. Surprisingly, factors related with its administration (needle length or injection volume) were not statistically significant covariates in LAI-aripiprazole lauroxil pharmacokinetics, in contrast to PP1M and PP3M findings. CYP2D6 and total weight significantly affected LAI-aripiprazole pharmacokinetics.

Table 6. Population pharmacokinetic model of LAI aripiprazole. Abbreviations: KA: first-order absorption rate constant. CL/F: clearance relative to bioavailability (in the absence of intravenous administration). VD/F: apparent volume of distribution relative to bioavailability (in the absence of intravenous administration).

Author	Formulation	Model	Covariates	Parameters values	Equations
Hard et al. ⁹⁷	Aripiprazole lauroxil	- 2-compartment model with sequential zero-order absorption	- CYP2D6 PM on CL	KA: 0.574 h ⁻¹ CL/F: 0.767 L/H (PM)	CL equation not reported $VD = 268 \times \left(\frac{WT}{70}\right)$
		followed by a first-order process	- Total weight on Vd	vs. 2.02 L/h (non-PM)	
		- Zero-order conversion of aripiprazole lauroxil to aripiprazole		VD/F: 2122 L	

4.2. Pharmacogenetics

The FDA recommends dose adjustment for aripiprazole in patients who are known *CYP2D6* PMs and AOM product monograph recommends an adjusted dose of 300 mg for this group of patients ⁹⁰. According to these data, C/D ratios observed in 62-patient study treated with oral aripiprazole indicated that PMs typically need 30–40% lower doses to achieve similar serum concentrations as NMs ⁹⁸. As aripiprazole and dehydro-aripiprazole are regarded as equipotent ⁹³, Suzuki et al. ⁹⁹ in a study of 89 healthy patients demonstrated that subjects with any or reduced functional alleles ($\times 5$ and $\times 10$) for *CYP2D6* had higher C/D ratios of the active moiety of the two compounds than those without the alleles. In a recent retrospective cohort study including pharmacokinetic data of 890 patients it was found that aripiprazole active moiety exposure increased 1.6 times and 1.4 times for PMs and IMs, respectively ⁴⁸. All these data and those of other studies are available in Table 7, showing the necessary dose adjustment in PMs.

Current FDA recommendation only takes into account dose adjustment in *CYP2D6* PMs, but available studies show that dose reduction should also be carried out in *CYP2D6* IMs. In this sense, Tveito et al. ¹⁰⁰ established that IM phenotype increases aripiprazole plasma concentration by 50% and active moiety concentration by 40%, both compared to NM. Further pharmacogenetic studies must be carried out in order to confirm this effect on plasma exposure, which could entail updating the product information regarding this group of patients. Among all the studies, only a few of them were performed with AOM, in which substantial influence of *CYP2D6* phenotype on serum concentrations was observed too, as in oral formulations ¹⁰⁰.

Aripiprazole is generally well tolerated due to its good safety profile concerning extrapyramidal reactions which are less common than in the other atypical antipsychotics, except for akathisia ^{101,102}. Hendset et al. ⁹⁸ observed that *CYP2D6*-defective alleles patients were associated with more potent adverse effects. In addition, a case-control study with eight patients suggested that IM and PM *CYP2D6* status are more frequently associated with extrapyramidal reactions ¹⁰³. Another study found that PM and IM were associated with higher incidence of nausea/vomiting due to higher plasma concentrations of aripiprazole in these subjects ¹⁰⁴. A summary of LAI-aripiprazole pharmacokinetic and pharmacogenetic characteristics is available in Table 8.

Table 7. Studies between CYP2D6 phenotype and the relation with aripiprazole plasma exposure. Classified in relevant (significant association between CYP2D6 phenotype and active moiety exposure) or irrelevant (no significant association between CYP2D6 phenotype and active moiety exposure or incorrect assignment of CYP2D6 genotype due to missing copy number variation analysis). Abbreviations: PM, Poor Metabolizer; IM, Intermediate Metabolizer; NM, Normal Metabolizer; UM, Ultra Metabolizer; NR: Not reported. * Those studies which do not analyze the number of copies of the gene have been classified as irrelevant. Among those classified as relevant, it is important to highlight that it should be taken as a guideline, taking into account that the main limitation is the high heterogeneity that exists between the different methods of analysis used, as well as the polymorphisms explored in the determination of the phenotype of CYP2D6. ** Single-dose study.

Study	Oral/LAI	n	Race	Age (Median)	Male/Female	CYP2D6 Phenotypes	Outcome
Suzuki et al. ⁹⁹	Oral	89	Asian	38	46/43	IM/NM/UM	Relevant (higher plasma exposure for IM and PM)
Suzuki et al. ¹⁰⁵	Oral	63	Asian	NR	36/33	PM/IM	Relevant (For *10 allele)
Hendset et al. ⁵⁴	Oral	266	Caucasian	33	NR	IM/NM	Irrelevant
Hendset et al. ⁹⁸	Oral	62	Caucasian	31	29/33	PM/NM	Relevant (higher plasma exposure for PM)
Belmonte et al.* ¹⁰⁴	Oral	148	Caucasian	26	85/63	PM/IM/NM/UM	Relevant (higher plasma exposure for PM and IM)
Tveito et al. ¹⁰⁰	Oral/LAI	635(469/166)	Caucasian	40	294/341	PM/IM/NM/UM	Relevant (higher plasma exposure for PM and IM)
Jukic et al. ⁴⁸	Oral	890	Caucasian	37	400/490	PM/IM/NM/UM	Relevant (higher plasma exposure for PM and IM)
Lisbeth et al. ⁸⁷	Oral/LAI	18(17/1)	Caucasian	36	11/7	PM/IM/NM/UM	Relevant (higher plasma exposure for PM)
van der Weide et al. ¹⁰⁶	Oral	130	Caucasian	NR	NR	PM/IM/NM/UM	Irrelevant
Azuma et al.** ¹⁰⁷	Oral	27	Asian	NR	NR	IM/NM	Relevant (Coadministration with CYP2D6 inhibitors)
Kubo et al.** ¹⁰⁸	Oral	20	Asian	24	20/0	IM/NM	Irrelevant

Table 8. Summary of PK/PG characteristics of LAI-Aripiprazole. Abbreviations: T_{max} , time to maximum concentration after administration; T_{ss} , time to reach steady state; $t_{1/2}$, apparent half-life of elimination. TDM Levels of recommendation: 1: Strongly recommended; 2: Recommended; 3: Useful; 4: Potentially useful (33). Genetic test; Informative: this label contains information stating that a particular gene affects or does not affect drug efficacy, not clinically significant; Actionable: this label may contain information about changes in efficacy due to gene variants.

Drug	Main Active Metabolite	Therapeutic Reference Range	Metabolism	TDM Recommendation (33)	Mean PK Values	Genetic test Recommendation (94)
LAI-Aripiprazole Monohydrate	Dehydro-aripiprazole	100–350 ng/mL Active moiety: 150–500 ng/mL	CYP2D6/CYP3A4	2	T_{max} : 4–7 days T_{ss} : 4 months $T_{1/2}$ 400 mg: 46.5 days 300 mg: 29.9 days	Actionable
LAI-Aripiprazole Lauroxil	Dehydro-aripiprazole	100–350 ng/mL Active moiety: 150–500 ng/mL	CYP2D6/CYP3A4	2	T_{max} : 41 days T_{ss} : 4 months $T_{1/2}$: 53.9–57.2 days	Actionable

Only a few of the summarized studies are developed using LAI formulations of antipsychotics; there is an urgent need for independent and real-world LAI antipsychotics TDM studies to clarify pharmacokinetic data such as the half-life and therapeutic reference range.

5. PHARMACOKINETICS/PHARMACOGENETICS IMPLEMENTATION

Pharmacogenetics can also contribute to understand antipsychotics behavior in the organism, and this information can be totally complemented with pharmacokinetics data of LAI. The use of pharmacogenetics on treatment optimization is exemplified in different case reports^{109–111}. Moreover, nowadays there are multiple initiatives that are already translating pharmacogenetics into clinical care around the world. To cite several examples, there is the Electronic Medical Records and Genomics (eMERGE-PGx) and St. Jude Children's Research Hospital that are using pharmacogenetic analysis to guide treatment in patients; both initiatives are from the United States^{112,113}. In Europe, the Ubiquitous

Pharmacogenomics project (U-PGx) can be found, with seven countries taking part, one of them Spain ¹¹⁴. In our country, there are already hospitals incorporating pharmacogenetics into health services ¹¹⁵. There is also a Pharmacogenomic Society, the Spanish Society of Pharmacogenetics and Pharmacogenomics (SEFF), which is actively working on promoting pharmacogenetics knowledge and developing guidelines for healthcare professionals ¹¹⁶.

Viability of pharmacogenetics testing in psychiatry has been observed. Five years ago, two different studies were performed where physicians were asked about pharmacogenetics in psychiatry practice. Around 95% of the physicians believe that this can help them and their patients in decision-making ¹¹⁷ and 80–85% think that pharmacogenetics will become a standard practice in the future ^{117,118}, which is currently a reality as aforementioned ¹¹⁹. Curiously, in 2012 Mas et al. ¹²⁰ studied *CYP2D6* polymorphisms in 151 patients treated with risperidone and realized that PMs received the lowest doses and UM the higher doses, without physicians knowing patients' metabolic status. However, a preventive pharmacogenetics test would resolve the casuistry with better cost-effectiveness for the health care system and the patient versus traditional prescribing methods ¹²¹.

As we have mentioned, schizophrenia implies a great economic cost for health care systems, families and society. A study performed in patients with psychiatric conditions taking drugs not recommended based on genetic information showed that they have 69% more total health care visits, 67% more medical visits and three times more medical absence days compared to patients taking drugs recommended based on their genotypes ¹²². Another study associated *CYP2D6* PMs and UMs to longer hospital stay duration compared to EMs ¹²³. In 2013, Herbild et al. ¹²⁴ demonstrated cost-effectiveness of preemptive *CYP2D6* or *CYP2C19* genotyping in patients with schizophrenia. TDM and preemptive genotyping of patients could reduce the time spent seeking the right antipsychotic drug and dose, minimizing hospital stay and visits, preventing adverse drug reactions and reducing associated costs. To all of these expenses must be added the expensive treatment with LAI, so studies focused on therapies with this group of drugs are needed to assess the cost–benefit ratio of preemptive *CYP2D6* genotyping.

6. UNSOLVED QUESTIONS AND FUTURE DIRECTIONS

According to the LAI risperidone product monograph, 9-OH-R has similar pharmacological activity to risperidone ³²; however, oral paliperidone has been approved with twice the daily dose of risperidone. Accordingly, de Leon et al. ^{60,125} suggest that risperidone is twice as potent as paliperidone. In this sense, brain imaging studies showed that lower doses of risperidone cause a similar percentage of binding to higher doses of paliperidone ^{126,127}. This could be explained by the high affinity of paliperidone to P-gp, minimizing blood–brain barrier (BBB) penetration.

The correlations between plasma and brain concentration for risperidone and 9-OH-R have been studied by Aravagiri et al. ¹²⁸ in rats documenting differences in the distribution of both compounds in the brain, showing plasma/brain ratios of 6.7 for risperidone and 32.9 for 9-OH-R. These differences could be explained by two different factors: first, affinity of risperidone and 9-OH-R for P-glycoprotein (P-gp) in BBB. Wang et al. ¹²⁹ conducted a study in *abcb1a/b* knockout mouse model dysfunctional in P-gp. Data showed that the ratio of plasma to brain of 9-OH-R was 2.4-fold higher than that for risperidone. This could mean that 9-OH-R presents a higher affinity for P-gp than risperidone and it could explain the greater toxicity of risperidone than 9-OH-R ⁶⁰; second, hydroxylation of risperidone to 9-OH-R makes it more hydrophilic, decreasing the ability to cross the BBB.

A preliminary assessment has demonstrated the prediction performance of D2/3 receptor occupancy a priori to optimize the dosing regimen based on risperidone blood concentrations. Accordingly, a 65–80% D2/3 receptor occupancy is associated with maximal effectiveness, minimal risk of adverse events and reduced risk of relapse in patients receiving supra-therapeutic doses ¹³⁰. Similar D2/3 receptor occupancy was also reported by Shin et al. after aripiprazole administration, demonstrating the improvement of the cognitive function in a small sample size of patients with schizophrenia ¹³¹. Therefore, predictive popPK models are encouraged to better characterize the PK properties of LAI antipsychotics and identify the sources of inter-individual variability in order to establish a PK/PD framework able to assess D2/3 receptor occupancy. The mathematical framework would allow the evaluation of dosing strategies in

special sub-groups of patients, which will help to achieve an optimal efficacy/safety balance.

Atypical LAI have resulted in a great increase in safety compared with the previously available typical LAI. However, limited evidence has restricted the proper characterization of their pharmacokinetic properties, specifically regarding LAI of risperidone, paliperidone and aripiprazole. The role of CYP2D6 has been partially demonstrated on the PK of LAI aripiprazole, although it remains uncertain for LAI risperidone and LAI paliperidone. In spite of that fact, the developed popPK models showed common aspects regarding the influence of body weight, administration site and needle characteristics on the pharmacokinetic behavior of these drugs. However, the reduced number of patients enrolled, the prolonged drug half-life and the limited number of clinical studies available may limit the identification of covariates and pharmacogenetic effects that would help to explain the large inter-individual differences concerning drug exposure. Pharmacogenetic analysis should be considered with caution because the CYP2D6 polymorphisms analyzed in each study are different. Assuming that all the polymorphisms that were not analyzed are wild-type allele; consequently, exceedance of NM would be obtained. The CYP2D6 gene is a complex gene, which presents duplications, deletions and even non-functional hybrids and, therefore, requires a more exhaustive approach to be able to determine the relationship between kinetics and genetics. Additional evaluations, including continuous PK endpoints, are encouraged to provide a more comprehensive characterization of the PK/PD properties of LAI in order to guide the individual dose selection.

BACKGROUND AND OBJECTIVES

Mental health disorders, such as schizophrenia and bipolar disorder, pose a significant public health challenge due to their clinical complexity and substantial impact on patients' quality of life ^{1,2}. The effective management of these conditions often involves the use of antipsychotic medications, among which aripiprazole, a second-generation drug, has proven efficacy in treating psychotic and affective symptoms.

Antipsychotic drugs are often far less effective than expected. Treatment resistance requires dose optimization based on a trial-and-error approach because the therapeutic response cannot currently be predicted. Together with serious and frequent adverse events, these are the main reasons leading to treatment discontinuation.

In the clinical setting, changes to the original prescribed dosage shown in the summary of product characteristics (SmPC) are frequently required to achieve an adequate clinical response while minimizing adverse effects. It is a huge challenge for physicians to predict the optimal dose in the absence of objective data such as plasma drug concentrations, due to excessive inter-individual variability. Therefore, this trial-and-error procedure may delay the achievement of clinical goals and even lead to the occurrence of relapse or adverse events, which are closely related to poor prognosis and poor treatment compliance.

Aripiprazole (ARI) is an atypical antipsychotic whose efficacy may be mediated through a combination of partial agonist activity at D2 and 5-HT_{1A} receptors and antagonist activity at 5-HT_{2A} receptors ^{132,133}. Binding affinity and functional selectivity of ARI at dopamine D2 receptors are essential to its mechanism of action. As a partial agonist, it has a moderate intrinsic activity at D2 receptors, which is lower than that of dopamine itself, suggesting a nuanced modulation of dopaminergic neurotransmission ¹³². Moreover, the ability to act as a dopamine system stabilizer is thought to be mediated through its action at presynaptic D2 autoreceptors, decreasing dopamine release, while also serving as an antagonist at postsynaptic D2 receptors at higher doses. This dual activity helps to modulate dopamine levels more finely than typical antipsychotics, which are known for their strong antagonistic effects on dopamine receptors ¹³³.

The pharmacokinetics of ARI, particularly its plasma concentrations, exhibit considerable inter-individual variability, which has significant implications for its clinical use. One of the key factors contributing to ARI variability is the polymorphic nature of the cytochromes P450 family (CYP2D6 and CYP3A4) ,

responsible for metabolizing ARI into its active metabolite, dehydroaripiprazole (DHA) ¹³⁴. According to the Neuropsychopharmacology Therapeutic Drug Monitoring Consensus Guidelines, the therapeutic reference range is 100–350 ng/mL for ARI and 150–500 ng/mL for ARI plus DHA, called active moiety (AM) ³⁴. Recently, new reference ranges were proposed of 120–270 ng/mL and 180–380 ng/mL, respectively, for ARI and its active moiety by Hart et al. ¹³⁵.

The introduction of aripiprazole long-acting injectable once-monthly (AOM) dosing has improved the treatment compliance of this drug. AOM is the polymorphic monohydrate form of ARI; currently, 400 mg and 300 mg formulations are approved for the induction treatment of schizophrenia and maintenance therapy ⁹⁰. Due to low solubility, AOM is slowly absorbed into the systemic circulation after intramuscular injection. Maximum plasma concentrations under steady-state conditions are reached 4 days after deltoid injection and 5–7 days after gluteal injection. The mean apparent half-life is 29.9 days for 300 mg and 46.5 days for 400 mg; according to the manufacturer, steady-state concentrations are achieved with the fourth injection at both injection sites ⁹¹.

As a member of the cytochrome P450 family, CYP2D6 is responsible for catalyzing several metabolic reactions that ARI undergoes, specifically its metabolism into the active metabolite DHA. In addition, this cytochrome also takes part in the conversion of the DHA into inactive metabolites ^{94,136}. Consequently, genetic polymorphisms in the *CYP2D6* gene could significantly influence the plasma concentrations and pharmacokinetics of both ARI and DHA. Today, 163 alleles of CYP2D6 have been described have different functions, frequencies, and clinical metabolic effects in different populations ^{14,15}. Depending on the combination of alleles, the following metabolizer states can be distinguished in different phenotypes: Normal metabolizer (NM), Intermediate Metabolizer (IM), Poor metabolizer (PM) and ultrarapid metabolizers (UM) ¹⁶.

Regarding CYP3A4, it is known that it plays a certain role in the metabolism of ARI to DHA and the latter into inactive metabolites. A recent study has described that the variant *CYP3A4**22 produces reduced enzyme activity and may affect the metabolism of antipsychotics ¹³⁷. On the other hand, *CYP3A4**20 polymorphism produces a non-functional protein that may result in elevated drug concentrations in individuals carrying this allele. However, no studies have examined the effect of *CYP3A4**20 on the pharmacokinetics of ARI. In addition to genetics, concomitant drugs can influence drug metabolism ¹³⁸. In

this sense, phenoconversion is the mismatch between the genotype-based prediction of CYP450-mediated drug metabolism and the true capacity of an individual to metabolize drugs (phenotype) due to nongenetic factors ¹³⁹. Accordingly, Lisbeth et al. found phenoconversion to be the limiting factor for predicting an accurate dose based on CYP2D6 genotype ¹⁴⁰. The genotype-phenotype mismatch of CYP2D6 could have significant consequences in the clinical setting and may result in suboptimal treatment of patients ¹³⁹. The Summary of Product Characteristics (SmPC) approved by the Food and Drug Administration (FDA) and Dutch Pharmacogenetics Working Group recommend dose adjustment of AOM if concomitant treatments with CYP2D6 and CYP3A4 inhibitors or CYP3A4 inducers ¹⁴¹. However, recommendations for dose reductions based on cytochrome phenotype are only established for CYP2D6 PMs, but not for CYP3A4. In addition, FDA SmPC states that DHA represents about 29% of the ARI exposure in plasma without specifying the metabolizer phenotype for *CYP2D6* or *CYP3A4* ⁹⁰.

Considering that there is available a target plasma concentration and the evidence that exists on the influence of the metabolizing state of CYP2D6 and CYP3A4 on these concentrations (dose recommendations included in Summary of product characteristics), the influence on ARI plasma concentrations of another important player in the pharmacokinetics of numerous drugs in the body, P-Glycoprotein (P-gp), needs to be further explored.

P-gp is a transmembrane glycoprotein, first described in 1976, which plays a crucial role in the defense against potentially harmful compounds for the organism, including a variety of drugs ¹⁴². Its location in the cell membrane, as an efflux transporter pump, allows it to actively transport drugs from the intracellular space to the extracellular environment, thereby affecting both plasma and tissue concentrations of pharmaceutical agents ¹⁴³.

The strategic placement of P-gp across various locations such as blood-brain-barrier (BBB), gut lumen, and within the renal and hepatic systems that underscores its integral role in drug metabolism and clearance, ensuring that xenobiotics and other substances are effectively removed from the body ¹⁴⁴.

P-gp is encoded by the human *ABCB1* gene, also known as Multidrug Resistance protein 1 (*MDR-1*), which is located in chromosome 7¹⁴⁵. In this sense, genetic polymorphisms in *ABCB1* may lead to changes in P-gp expression or function, leading to individual differences in drug disposition ¹⁴⁶. Specifically, among the reported single nucleotide polymorphisms (SNPs) in the *MDR1* gene,

there are three variants which have been the most thoroughly studied, C1236T (rs1128503) in exon 12, G2677T (rs2032582) in exon 21 and C3435T (rs1045642), in exon 26. These variants show strong linkage disequilibrium and were found to be functionally significant and ethnically distinct when associated with this region of the gene ^{147–151}. It is suggested that the higher the number of T alleles present, the lower the function or expression of P-gp ¹⁵².

The influence of drug-drug interactions on plasma drug concentrations due to P-gp inhibitory drugs have been extensively studied ¹⁵³. Mostly, these studies evaluate the bioavailability of orally administered drug as a function of P-gp activity in the intestine ¹⁵⁴ or the outcomes in cancer treatment due to P-gp activity in tumor derived tissue ^{155,156}.

There are many drugs which are substrates of P-gp, so that variations in its function or expression can lead to alterations in their plasma concentrations and, consequently, affect their efficacy and safety ¹⁵⁷.

The first study to find differences in plasma drug concentrations depending on the P-gp polymorphisms presented was developed by Hoffmeyer et al., reflecting the impact of polymorphisms in exon 26 (C3435T) on digoxin pharmacokinetics ¹⁵⁸. In addition, the inhibition of p-Gp located in liver and kidney due to concomitant drugs has been evaluated by Taskar et al., who found increases in systemic concentrations of the P-gp substrates tested due to concomitant P-gp inhibitors ¹⁴⁴.

Regarding ARI and DHA, it has been proven that both are substrates of P-gp ¹⁵⁹. Specifically, in patients under oral aripiprazole treatment, differences in plasma concentrations of ARI due to P-gp polymorphisms have been found ¹⁵².

In recent years, an increasing body of literature has emphasized the significance of personalized medicine in psychiatric care, highlighting the importance of tailoring treatments based on individual patient profiles ^{160–162}. The advent of precision psychiatry has underscored the need for robust analytical methods capable of delivering accurate and reliable data for informed decision-making in the clinical setting ^{163,164}. In this regard, achieving optimal therapeutic outcomes of ARI requires the precise monitoring of plasma concentrations. In this sense, accurate quantification of ARI is crucial for tailoring treatment strategies to individual patient needs, minimizing adverse effects and optimizing therapeutic efficacy ^{165,166}. Liquid chromatographic methods for the analysis of

ARI are widely described in the literature: there are LC-MS/MS^{167–170}, HPLC-UV/DAD^{171–173} or GC-MS¹⁷⁴-based methods.

Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) has emerged as a powerful analytical tool, offering a high sensitivity and selectivity for the quantification of compounds in biological samples. Its ability to discriminate between ARI and its metabolite through mass spectrometry enables precise and specific detection, addressing the demand for accurate therapeutic drug monitoring in psychiatric patients. As the field of psychopharmacology advances, the need for analytical methods that can keep pace with the evolving landscape of psychiatric medications increases. The UHPLC-MS/MS methodology is considered the gold standard in pharmacokinetic studies, providing unparalleled sensitivity and specificity¹⁷⁵. Its ability to quantify analytes at lower concentrations, coupled with the ability to distinguish between closely related compounds, positions it as an ideal candidate for the analysis of ARI and its metabolite in complex biological matrices. However, sample processing prior to analysis is completely manual, which introduces potential variability, and it is very time consuming.

Conversely, the Alinity C system (Abbot Laboratories), which uses potentiometry and photometry, is a promising alternative, providing advantages in terms of automation and user-friendliness, potentially facilitating implementation in clinical settings. Automation is a hallmark of the Alinity C system, potentially reducing the analytical variability associated with manual sample handling. This feature aligns with the growing demand for high-throughput analytical methods in clinical laboratories, where efficiency and reproducibility are paramount. Understanding the comparative advantages and limitations of these methods is mandatory in order to advance therapeutic monitoring practices, ultimately optimizing treatment outcomes for individuals grappling with mental health disorders.

In view of the above, the main objective of this doctoral thesis is to elucidate the influence that different *CYP2D6*, *CYP3A4* and *ABCB1* polymorphisms may have on the plasma concentrations of ARI and its active metabolite, as well as to perform a comparison and validation of an automated analytical method against its gold standard in order to implement in clinical practice the pharmacokinetic monitoring of this antipsychotic.

In order to achieve these purposes, the following specific objectives are proposed:

1. Analyze the performance of two analytical methods for the plasma determination of ARI: UHPLC-MS/MS and the Alinity C system, with a focus on sensitivity, precision and accuracy, and assess the impact of sample freezing on the reproducibility of the analysis as well as the stability of plasma samples.
2. Evaluate the effect of *CYP2D6* and *CYP3A4* polymorphisms and the presence of concomitant inducers and inhibitors of these cytochromes on ARI and DHA plasma concentrations in a real clinical setting.
3. Explore the influence of *ABCB1* polymorphisms on plasma concentrations of ARI and DHA in patients under AOM treatment.

WORKING PLAN AND METHODS

1. WORKING PLAN

The organization of this Doctoral Thesis complies with the regulations of the International Doctoral School of the University of Santiago de Compostela (Spain) regarding the structure of the work, language, ethics and intellectual property rights.

In accordance with the proposed objectives, this Doctoral Thesis is presented as a monographic work with partial reproduction of publications of 1 revision article and 1 original article.

According to the objectives, the development of will be divided into three sections.

Firstly, the comparison between an automated analytical method and the gold standard for the determination of plasma concentrations of ARI will be addressed, as well as the study of the stability of the frozen sample. This first point will allow the translation to daily clinical practice of the pharmacokinetic monitoring of this drug.

Secondly, an observational study will be carried out in which the plasma concentrations of ARI and its active metabolite will be determined in real clinical practice, as well as the genotyping and phenotyping of the coding genes for *CYP2D6* and *CYP3A4*. This will allow to determine the influence of the different metabolizing states on the plasma concentrations of the drug.

Finally, another observational study in the same cohort of patients will be carried out in which the plasma concentrations of ARI and DHA will be determined together with the genotyping of the three main *ABCB1* SNPs in order to determine their influence on the plasma concentrations of both compounds.

2. METHODS

The methodology for each of the studies carried out is presented below.

2.1. Comparison between analytical methods

A retrospective study is proposed, where plasma samples of patients treated with aripiprazole monohydrate were selected. The study and data collection strictly adhered to the principles of the Declaration of Helsinki. Ethical approval was given by the local Institutional Review Board and the autonomous region of Galicia (2021/285).

2.1.1. UHPLC-MS/MS

Concentrations of ARI and DHA in plasma were measured by a validated UHPLC-MS/MS method on a Xevo TQD® triple quadrupole mass spectrometer (Waters, Milford, MA, USA). Aripiprazole-d8 (deuterated aripiprazole) was used as the internal standard. ARI, DHA and aripiprazole-d8 detection were monitored at the following transition pairs m/z 448.2 $\rightarrow$ 285.2, m/z 446.04 $\rightarrow$ 285.02 and m/z 456.3 $\rightarrow$ 293.07, respectively.

For this purpose, 850 μ L of plasma with 100 μ L of ARI and DHA at a concentration of 0.25 mg/L and 50 μ L of aripiprazole-d8 at a concentration of 10 mg/L are added in an Eppendorf and shaken by vortex-mixing for 1 min. Four samples were extracted from this solution, each consisting of 200 μ L of the combined solution and 600 μ L of acetonitrile. Subsequently, they were vortex-mixed for 1 min and centrifuged (3500 rpm 10 min 4 °C) and 500 μ L of the resulting supernatant are extracted. Then, the samples were evaporated to dryness and finally dissolved in 500 μ L of a solution composed of water and acetonitrile in a ratio of 80:20.

A linear calibration curve was obtained over a concentration range of 25–1000 ng/mL ($R^2 = 0.998$). The detection and quantification limits for both ARI and DHA were 10 and 25 ng/mL, respectively. An ACQUITY UHPLC BEH C18 column (2.1 $\times$ 50.0 mm, 1.7 μ m) was used at a temperature of 40 °C. The sample manager was set to 4 °C, the flow rate to 0.6 mL/min and the injected volume of sample to 5 μ L. The obtained data were processed using MassLynx® software (Version 4.20.0001).

2.1.2. Alinity C

Alinity C is based on photometric and potentiometric detection technologies for the quantitative determination of analytes in human serum, plasma, urine, cerebrospinal fluid, hemolysate and whole blood. Alinity C assay for determination of the active moiety of aripiprazole is based on photometric detection, using a tungsten lamp, a diffraction grating and a silicon photodiode detector. The test was performed at a wavelength of 604 nm. The MyCare Psychiatry Kit for total aripiprazole (Saladax Biomedical, Bethlehem, PA. USA) was used to determine the concentration of active moiety. It is based on competition between drug and drug-conjugates for binding to drug specific antibodies covalently bound to nanoparticles. The extent of particle aggregation can be followed spectrophotometrically on clinical chemistry analyzers as Alinity C. Cross-reactivity was tested by the kit manufacturer using more than 150 drugs as well as the presence of interfering substances such as rheumatoid factor, lipids and hemolyzed blood. The lower limit of quantification of the kit is 45 ng/mL and the upper limit of quantification is 1000 ng/mL.

Since the determination by Alinity C only provides values of active moiety (ARI plus DHA), the sum of drug and active metabolite found by UHPLC-MS/MS has been used for the comparison between both methods.

A total of 86 plasma samples, corresponding to trough concentrations from 86 patients, were collected. Samples were stored at -80 °C until analysis.

On the one hand, 60 samples were analyzed by both UHPLC-MS/MS and Alinity C. Due to the clinical repercussions of possible variations in the use of one method or another, the samples have been stratified according to the consensus therapeutic range into infra-therapeutic (<150 ng/mL), therapeutic (150–500 ng/mL) and supra-therapeutic (>500 ng/mL). Furthermore, it was studied whether there were differences between the two methods when the aripiprazole/dehydroaripiprazole (ARI/DHA) ratios were different, and thus whether the amount of each analyte individually affected the determination by Alinity C. For this purpose, the proportion between the concentration measured by the two methods (UHPLC-MSMS/Alinity C) was classified into 5 different groups according to the ARI/DHA ratio (1–2, 2–3, 3–4, 4–5, 5–6).

On the other hand, 26 samples previously analyzed by Alinity C and stored at -80 °C were selected and reanalyzed by the same method 6 months later to

determine the reproducibility of the method and the stability of the plasma samples.

2.1.3. Statistics comparison

Normality was tested using the Shapiro–Wilk test, and correlations were assessed using the Spearman rank correlation coefficient (r_s). The Wilcoxon rank sum test was used to determine differences between drug levels. Bland–Altman analysis was performed to assess the agreement between the two quantitative measurements. A Bland–Altman plot shows the difference (y-axis) between these two measurements. The mean (x-axis) represents the average difference between two measurements, and the limits of agreement are expressed as the mean difference plus or minus 2 standard deviations of the difference. Additionally, Passing–Bablok regression was performed to estimate the best-fit line by comparing observed ranks between concentrations measured by UHPLC-MS/MS and Alinity C and measurements from two consecutive Alinity C analyses.

Agreement between classification of UHPLC-MS/MS and Alinity C into three different therapeutic range groups was determined using weighted Cohen kappa (κ), and differences in classification for each group were assessed using McNemar's test. To assess if the presence of higher concentrations of ARI or its active metabolite DHA affects the active moiety determined by Alinity C system, an ANOVA test was performed to determine differences between group means.

2.2. Influence of *CYP2D6* and *CYP3A4* polymorphisms

2.2.1. Study design and procedures

An observational study of a cohort of patients under AOM treatment was conducted to evaluate the effect of *CYP2D6* and *CYP3A4* genetic polymorphisms on plasma concentrations of ARI and DHA. All procedures performed were in accordance with the ethical standards of the relevant national and institutional committees on human experimentation and with the 1975 Declaration of Helsinki (as revised 2008). All procedures involving patients were approved by the Drug Research Ethics Committee of Galicia (2020/486) and written informed consent was obtained from all subjects. The study included patients who received at least four doses of AOM (Abilify Maintena®; Otsuka, Tokyo, Japan). Exclusion criteria

were age <18 years, pregnancy, and cognitive impairment. Three blood samples were obtained from each patient, one for pharmacokinetic analysis, one for genotyping and one for evaluation of biochemical data. Demographic characteristics and biochemical data including gender, age, height, weight, dosage, concomitant medication, liver, and kidney function were recorded.

2.2.2. Pharmacokinetic analysis

Blood samples for pharmacokinetic analysis were collected in EDTA tubes immediately prior to AOM administration (Ctough). All samples were centrifuged at 3500 rpm for 10 min at 4 °C to obtain the plasma. A volume of 180uL of plasma sample was spiked with 20ul of aripiprazole-d8 (internal standard) and 500uL of acetonitrile and centrifuged. The supernatant was evaporated to dryness and the dry residue was reconstituted with 500uL of 10mM Ammonium Formate/Acetonitrile (85:15). Concentrations of ARI and DHA in plasma were measured with an ultra-high performance liquid chromatography-tandem mass spectroscopy (UHPLC-MS/MS) Xevo TQD® triple quadrupole mass spectrometer (Waters, Massachusetts, USA), using a validated method previously published ¹⁷⁶.

2.2.3. Genotype and phenotype

Genetic analysis was performed for single nucleotide polymorphic variants (SNPs) or insertion-deletions (indels) for *CYP2D6* and *CYP3A4* genes. Analyses were performed using Taqman assays on the QuantStudio 12K Flex (Applied Biosystems, Foster City, California). For *CYP2D6*, the following variants were analyzed: rs1065852, rs201377835, rs5030862, rs5030865, rs774671100, rs28371706, rs16947, rs1135840, rs59421388, rs35742686, rs72549356, rs267608319, rs3892097, rs28371725, rs72549346, rs1135822, rs79292917, rs5030655, rs5030867, rs5030865, rs769258, rs5030656. Copy number variations in the *CYP2D6* gene were also determined. For *CYP3A4* the presence or absence of the alleles *22 (rs35599367) and *20 (rs67666821) was evaluated. After genotyping *CYP2D6*, each allele is assigned an activity value corresponding to the activity value described by Caudle et al. Individuals with activity score (AS) 0 are classified as PMs, individuals with AS 0.25-1 are classified as IMs, individuals with AS 1.25-2.25 are classified as NMs, and individuals with AS > 2.25 are classified as UMs ¹⁶.

Regarding *CYP3A4*, patients with presence of heterozygous **1/*22* and homozygous **22/*22* were grouped under *CYP3A4* PM phenotype.

Once phenotyping has been performed, depending on the presence of concomitant *CYP2D6* and *CYP3A4* inhibitors or inducers, the appropriate phenoconversion was performed for each patient.

2.2.4. Data analysis

The main statistics analysis afforded two approaches: drug concentrations and dose-adjusted drug concentrations. Adjustment to a normal distribution was not assumed in doses, concentration levels and ratios variables (Kolmogorov-Smirnov with Lilliefors correction normality test), so nonparametric tests were carried out. For comparison of two independent samples the Mann-Whitney tests were performed and for comparison of more than two independent samples the Kruskal-Wallis test were used to test the significance of differences between the different *CYP3A4* and *CYP2D6* groups. P values of less than or equal to 0.05 were considered to be statistically significant. Data were presented as median and interquartile ranges.

2.3. Influence of *ABCB1* polymorphisms

Another study in the same cohort of patients under AOM treatment was conducted to evaluate the effect of the most prevalent *ABCB1* genetic polymorphisms on plasma concentrations of ARI and DHA. The study included patients who received at least four doses of AOM (Abilify Maintena®; Otsuka, Tokyo, Japan). Exclusion criteria were age <18 years, pregnancy, and cognitive impairment. Three blood samples were obtained from each patient, one for concentration analysis, one for genotyping and one for evaluation of biochemical data. Demographic characteristics and biochemical data including gender, age, height, weight, dosage, concomitant inhibitors and inducers for *CYP2D6*, *CYP3A4* and P-gp, liver, and kidney function were recorded.

All procedures performed were in accordance with the ethical standards of the relevant national and institutional committees on human experimentation and with the 1975 Declaration of Helsinki (as revised 2008). All procedures involving patients were approved by the Drug Research Ethics Committee of Galicia (2020/486) and written informed consent was obtained from all subjects.

2.3.1. Concentration analysis

Blood samples for pharmacokinetic analysis were collected in EDTA tubes immediately prior to AOM administration (Ctough). The same method described in 1.1 and 2.2 was used for the analysis.

2.3.2. Genotype and phenotype

Genetic analysis was performed for SNPs for *ABCB1* gene. Analyses were performed using Taqman assays on the QuantStudio 12K Flex (Applied Biosystems, Foster City, California). For *ABCB1*, the following variants were analyzed: C3435T (rs1045642), C2677 T/A (rs2032582) and C1236T (rs1128503). Also, due to the influence of CYP2D6 and CYP3A4 metabolizer status in ARI and DHA concentrations, the genotypes and phenotypes for these cytochromes were extracted from electronic clinical records. Phenotypes were established by assigning each allele an activity value corresponding to the activity value described by Caudle et al.¹⁷⁷.

To avoid biases in the interpretation of the results, due to the demonstrated influence of the metabolizing phenotype of these cytochromes on plasma ARI concentrations, only patients with normal phenotype metabolizer state for *CYP2D6* and absence of alleles *22 and *20 for *CYP3A4* were ultimately selected. In addition, the presence of concomitant inhibitors or inducers for *CYP2D6*, *CYP3A4* and *P-gp* were taken into account for patient screening.

The relationship between *P-gp* and ARI and DHA concentrations have been evaluated for the three SNPs individually, in pairs and as haplotypes. In addition, patients with mutations in any of the SNPs were grouped as T-carriers and compared with those who were Non-T carriers for each SNP.

2.3.3. Statistical Analysis

Adjustment to a normal distribution was not assumed in concentration for ARI or DHA and ratios variables (Kolmogorov-Smirnov with Lilliefors correction normality test), so nonparametric tests were carried out. For comparison of two independent samples the Mann-Whitney tests were performed and for comparison of more than two independent samples the Kruskal-Wallis test were used to test the significance of differences between the different *ABCB1* groups. P values of less than or equal to 0.05 were considered to

be statistically significant. Data were presented as median and interquartile ranges.

RESULTS

1. ALINITY C VS. UHPLC-MS/MS IN PLASMA ARIPIRAZOLE DETERMINATION

1.1. UHPLC-MS/MS vs. Alinity C

Aripiprazole active moiety concentrations were measured by both methods. The median values were 230.6 ng/mL (IQR: 166.2–418.8) for the UHPLC-MS/MS assay and 239 ng/mL (IQR: 167.8–456.5) for the Alinity C assay. The UHPLC-MS/MS method yielded a mean concentration for active moiety of 275.3 ng/mL, with a standard deviation of 140.2. In comparison, the Alinity C method exhibited a slightly higher mean concentration of 299.9 ng/mL, with a standard deviation of 162.5 (Table 9). The Spearman's rank correlation coefficient showed a good correlation in active moiety values between the two tests ($r_s = 0.96$).

Table 9. Descriptive statistics of both assays.

	UHPLC-MS/MS Dehydroaripiprazole (ng/mL)	UHPLC- MS/MS ARI (ng/mL)	UHPLC- MS/MS Active Moiety (ng/mL)	Alinity C Active Moiety (ng/mL)	Ratio ARI/DHA UHPLC- MS/MS
Samples	60	60	60	60	60
Minimum	21.01	53.40	74.40	45.00	1.32
Maximum	185.30	551.90	714.10	646.00	5.23
25% Percentile	49.81	114.40	166.20	167.80	169.30
Median	66.01	162.50	230.60	239.00	229.00
75% Percentile	99.32	289.20	418.80	456.50	455.50
Mean	77.84	197.40	275.30	299.90	2.54
Std. Deviation	36.43	110.60	140.20	162.50	0.86
Std. Error of Mean	4.70	14.28	18.10	20.97	0.11
Coefficient of variation	46.81%	56.02%	50.93%	54.18%	34.08%

1.2. Bland–Altman and Regression Analysis

In the present study, we conducted a Bland–Altman analysis to assess the agreement between two measurement methods (Figure 1). The mean difference, or bias, between the methods was found to be 5.560. The standard deviation of the bias was 14.28. The 95% limits of agreement, calculated using the Bland–Altman method, were determined to be -22.43 and 33.55, establishing an interval within which it is expected that 95% of the differences between the methods will fall. These findings were supported by the regression that estimated the line of best fit between both methods (Figure 2). Revealing a slope of 1.122, an intercept of -8.931 and a high correlation coefficient (r) of 0.936. The p -value (<0.0001), which is significantly below the conventional threshold of 0.05, underscores the statistical significance of this correlation. Furthermore, the F-test, with an F-statistic of 860.4 and a p -value < 0.0001 , emphasizes the statistical significance of the slope, confirming a significant deviation from zero.

Difference vs. average: Bland-Altman of UHPLC-MS/MS vs ALINITY C

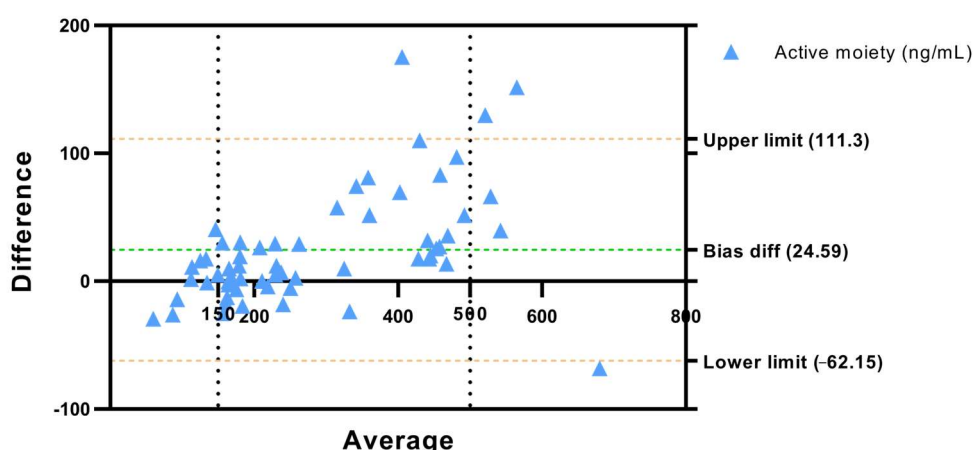


Figure 1. Bland–Altman’s plot. Difference in active moiety concentrations vs. the average (ng/mL) between UHPLC-MS/MS and Alinity C. The dashed green line represents the bias and dashed orange lines represent the limit of agreement.

1.3. Stratification in Ranges

The concentrations determined by both methods were classified according to the sub- (<150 ng/mL), supra (>500 ng/mL) and therapeutic range (>500 ng/mL). Cohen’s weighted kappa showed a substantial agreement between the

measurements of both methods ($K = 0.643 \pm 0.105$). The qualitative agreement between the two analytical methods was up to 84.99% of the measured samples. Considering this percentage in each range, the concordance of results was 8/9 (88.88%) samples for the sub-therapeutic range, 41/44 (93.18%) for the therapeutic range and 2/7 (28.5%) for the supratherapeutic range. The therapeutic strategy decision could have been different in nine samples (15%) (Table 10). To complete the comparison, McNemar's test was performed to determine whether the classification was discordant between the subtherapeutic and therapeutic range and between the therapeutic and supratherapeutic range. No significant differences ($p = 0.625$ and $p = 0.0625$) were found between the classification between these groups by both methods.

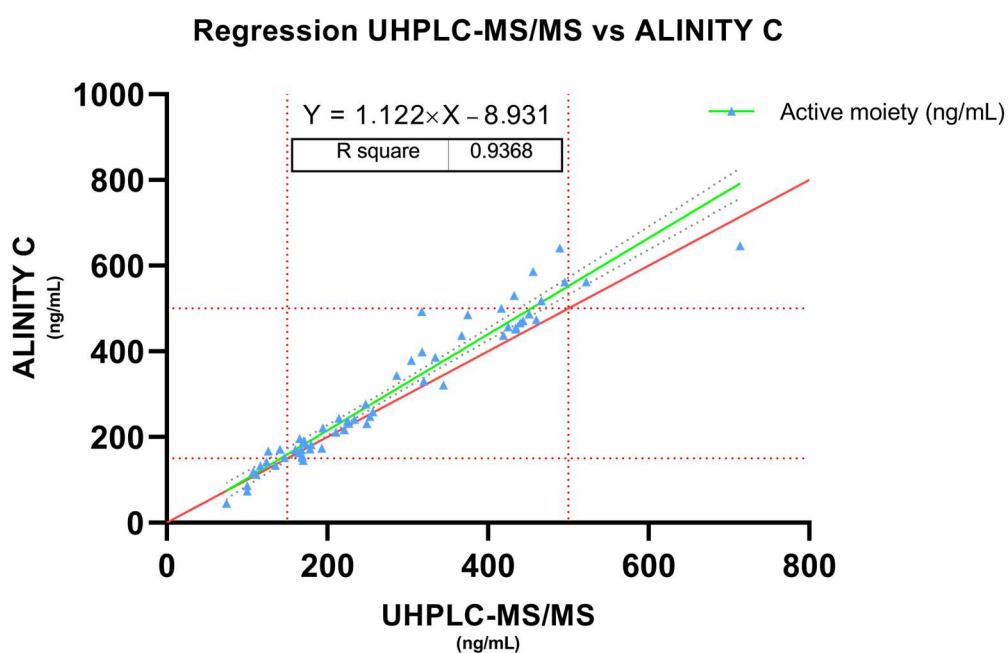


Figure 2. Passing–Bablok. Regression of measured concentrations of the active moiety between the UHPLC-MS/MS and Alinity C system. Red line represents the identity line. Grey dashed lines are the 95% confidence bounds. Dashed red lines represents aripiprazole therapeutic range (active moiety).

Table 10. Stratification of samples between the three possible ranges according to UHPLC-MS/MS and Alinity C active moiety measured concentrations.

Number of Samples (%)						Statistics		
Active Moiety	Alinity C				Total	Cohen's Weighted Kappa		
	<150 ng/mL	150–500 ng/mL	>500 ng/mL			K	SE	CI 95%
<150 ng/mL	8 (13.33)	3 (5)	0		11 (18.33)	0.643	0.105	0.437–0.849
150–500 ng/mL	1 (1.67)	41 (68.33)	5 (8.33)		47 (78.33)	McNemar test (<150 ng/mL)- (150–500 ng/mL) (150–500 ng/mL)- (>500 ng/mL)		
>500 ng/mL	0	0	2 (3.33)		2 (3.33)			
Total	9 (15)	44 (73.33)	7 (11.67)		60 (100)	<i>p</i> -value = 0.625	<i>p</i> -value = 0.0625	

Since the Alinity C system does not provide concentrations of ARI and DHA singularly, but only reports the active moiety, a stratification according to the ARI/DHA ratio obtained by UHPLC-MS/MS was conducted to check the influence of both analytes (ARI and DHA) concentrations over Alinity C performance and to verify that the measurements were not dependent on the concentration of a particular analyte. For this purpose, an ANOVA test was performed which revealed no significant differences ($p = 0.587$) between the means of the differences between UHPLC-MS/MS and Alinity C between the five groups as a function of the ARI/DHA ratio.

1.4. Alinity C Reanalysis

A total of 26 samples were analyzed twice 6 months apart by Alinity C to determine the consistency of the method. During this period, samples were stored at -80°C . Descriptive analyses of the results are available in Table 9.

Linear regression revealed a slope of 1.080, a y-intercept of -0.003400 and an x-intercept of 0.003148. The model demonstrates an exceptional goodness of fit, with an R-square value of 0.9914, The F-test, with an F-statistic of 2759 and a *p*-value less than 0.0001, underscores the statistical significance of the slope, confirming a significant deviation from zero (Figure 3).

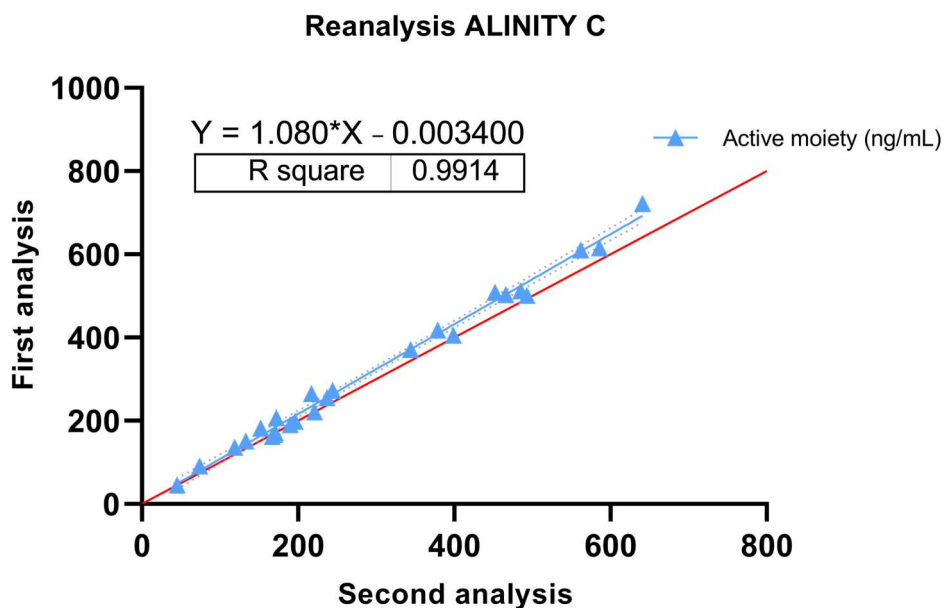


Figure 3. Passing–Bablok. Regression of measured concentrations of the active moiety between the first and second analysis by the Alinity C system. Red line represents the identity line. Grey dashed lines are the 95% confidence bounds.

1.5.Discussion

The landscape of psychiatric pharmacotherapy is continuously evolving, requiring analytical methods that can provide accurate and reliable data for personalized treatment approaches. As far as we know, this study stands out as the initial attempt to compare these two methods in determining ARI, a drug that has accumulated substantial evidence supporting the need for monitoring its plasma concentrations.

In the field of psychiatry research, the choice between analytical methods extends beyond technical considerations to encompass practical aspects, such as cost, ease of use and scalability¹⁷⁸. The Alinity C system, with its potential for automation and simplified workflows, may present advantages in resource-limited clinical settings where short turnaround times and cost-effectiveness are crucial considerations. Conversely, the UHPLC-MS/MS method, while more complex, offers unparalleled sensitivity and specificity, making it indispensable for research settings and situations where the highest level of analytical precision is required.

The Bland–Altman approach provides a comprehensive insight into the agreement between measurements, offering a nuanced understanding of the potential discrepancies between the two methods under consideration. Bland–Altman analysis was conducted to rigorously evaluate the agreement between two distinct measurement methods. The mean difference, indicative of bias, was calculated at 5.56, which indicates a systematic tendency towards higher values in Alinity C analysis. In parallel, the regression analysis confirms these results showing a slope of 1.122, suggesting a systematic tendency for the Alinity C system to yield slightly higher values than the UHPLC-MS/MS, especially at high concentration values.

The high correlation coefficient (r) of 0.936 further underscores the strength and direction of the relationship between the two methods. This implies that a substantial proportion of the variability in one method can be explained by the variability in the other, reinforcing the notion of a robust association.

The stratification of concentrations into sub-therapeutic, therapeutic and supratherapeutic ranges showed substantial agreement between the two methods, with a Cohen's weighted kappa of 0.643 ± 0.105 ¹⁷⁹. The qualitative agreement was high across the ranges, particularly in the therapeutic range (93.18%) and sub-therapeutic range (88.88%). The McNemar test indicated no significant differences in classification between the sub-therapeutic and therapeutic or therapeutic and supratherapeutic ranges, emphasizing the reliability of both methods in guiding ARI dosing towards the achievement of the commonly targeted therapeutic ranges.

A total of 3/11 (27.7%) patients with sub-therapeutic ranges measured by UHPLC-MS/MS were classified as therapeutic by Alinity C, and 5/47 (10.6%) in a therapeutic range by UHPLC-MS/MS were classified as supra-therapeutic by Alinity C. Taking into account all the discrepancies, the therapeutic decision to be taken would be different on nine occasions (15%) according to the method used. This leads us to realize that, in case of doubt and especially at high plasma concentrations, it is always necessary to confirm with a reference method and to be cautious before making clinical decisions.

In addition, to determine whether the presence of a higher concentration of ARI or its active metabolite DHA influences the active moiety determined by Alinity C, we compared the ratio between the result of the analysis by Alinity C and UHPLC-MS/MS and grouped them according to the ARI/DHA ratio, proving that Alinity C is a robust method regardless of the concentration of both

analytes. This is important to know, since the ratio between ARI/DHA does not remain constant over the time of administration or between different patients, especially in those with metabolizing phenotypes for *CYP2D6* different from the normal phenotype ¹⁶⁰.

UHPLC-MS/MS, while considered the gold standard in pharmacokinetic studies, has manual sample processing, introducing potential variability and time-consuming steps ¹⁸⁰. Its high sensitivity and specificity, especially in distinguishing closely related compounds, makes it valuable for research purposes ^{99,105}. The Alinity C system, on the other hand, offers automation, reducing the analytical variability associated with manual sample handling. Its user-friendliness makes it suitable for routine clinical use. However, it is essential to note that it provides values only of the active moiety and not of each separate compound.

When it comes to clinical implementation, the choice between UHPLC-MS/MS and the Alinity C system should consider the specific needs of the clinical setting. UHPLC-MS/MS remains valuable for research and situations requiring the highest sensitivity and specificity. However, the Alinity C system's automation and user-friendliness makes it an attractive option for routine therapeutic drug monitoring, offering efficiency without compromising accuracy ¹⁸¹.

On the other hand, our study also assessed the consistency of the Alinity C method and the stability of plasma samples through the reanalysis of 26 samples within a 6-month interval. Linear regression analysis of the reanalyzed samples demonstrated a slope of 1.080, indicating a minimal increase in concentration over time. This 8% increase at six months is within the $\pm 15\%$ variation allowed ¹⁸². The exceptionally high R-square value of 0.991 suggests a robust fit of the linear regression model, highlighting the consistency of the Alinity C method over the storage period in the aforementioned conservation conditions. The F-test with a significant F-statistic of 2759 and a p-value less than 0.001 further confirmed the statistical significance of the slope, reinforcing the reliability of the reanalysis results. These findings suggest that the Alinity C method maintains consistency and accuracy even after storing samples at $-80\text{ }^{\circ}\text{C}$ for six months. In this sense, Jansen et al. published the only other study which evaluates by this same technique the stability of samples after freezing, with similar results ¹⁸³.

While the study provides evidence into the comparability and consistency of the UHPLC-MS/MS and Alinity C methods, certain limitations should be

acknowledged. The sample size, although sufficient for the intended analysis, might limit the generalizability of the findings to a broader population. Additionally, the discordance observed in the supratherapeutic range warrants further investigation. Future studies could explore factors contributing to this discrepancy, such as the presence of interfering substances or method-specific limitations in accurately quantifying higher concentrations.

The advent of precision psychiatry underscores the importance of analytical methods capable of delivering accurate and reliable data for informed decision-making. As the field of psychopharmacology continues to advance, the ongoing evaluation of analytical methodologies is essential to ensure that clinical practices align with the evolving landscape of psychiatric therapies. This study contributes to this ongoing effort by providing evidence for the suitability of the Alinity C system as a practical and reliable tool for the therapeutic monitoring of ARI in these patients. These findings contribute to the ongoing dialogue in refining analytical methods for therapeutic drug monitoring, ultimately benefiting clinicians and patients in ensuring the accurate and consistent assessment of antipsychotic medication concentrations in plasma.

The findings support the integration of the Alinity C system into clinical laboratories, emphasizing its potential to enhance efficiency and reproducibility in therapeutic monitoring practices. However, it is crucial to acknowledge the limitations of each method and carefully consider the specific requirements of individual clinical scenarios.

This study provides valuable insights into the comparative analysis of UHPLC-MS/MS and the Alinity C system for the plasma determination of ARI and reproducibility after freezing samples. Both methods demonstrated a high precision and substantial agreement, particularly in the therapeutic range. These results affirm the incorporation of the Alinity C system into clinical laboratories, highlighting its capacity to improve effectiveness and consistency in therapeutic drug monitoring. However, it is essential to recognize the constraints of each approach and meticulously assess the unique needs of different clinical situations.

Although systematic upward method drift must be taken into account, the Alinity C system with its automation and ease-of-use features presents a

promising alternative for routine clinical use, addressing the need for high-throughput analytical methods.

2. INFLUENCE OF CYP2D6 AND CYP3A4 POLYMORPHISMS

2.1. Study population

Observational study of a cohort of 74 Caucasian patients under AOM treatment (40 male and 34 female); Median age was 48 years (35-54); Weight 82.15 Kg (68.5-93); BMI 29.82 kg/m²; Cr: 0.8 mg/dL (0.7-0.97); AST: 23 IU/L (19-30.5); ALT: 30 IU/L (18-43); GGT: 26 IU/L (17-42); Median dose administrated of AOM was 400 mg (300-400); Injection site (28 Deltoid; 46 Gluteus); smoking status (51 smokers; 23 non-smokers). CYP2D6 enzyme genotyping was performed for all the variants. Phenotyping was done according to the activity score of each allele and phenoconversion was performed. CYP3A4 genotyping was also performed for all individuals but phenoconversion was not performed due to the absence of inhibitors/inducers for this cytochrome. Obtained frequencies for CYP2D6 and CYP3A4 were depicted in Table 11.

Table 11. Biochemical data and obtained phenotypes frequencies for CYP2D6 and CYP3A4. PM: Poor Metabolizer; IM: Intermediate Metabolizer; NM: Normal Metabolizer; UM: Ultra metabolizer; Cr: Creatinine; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyl transferase. IQ: Interquartile Range. *Presence of heterozygous *1/*22 and homozygous *22/*22 were grouped under CYP3A4 PM phenotype.

	PM n (%)	IM n (%)	NM n (%)	UM n (%)
CYP2D6 Phenotype	2 (2.7)	29 (39.19)	40 (54.05)	3 (4.05)
CYP2D6 after phenoconversion	12 (16.22)	24 (32.43)	35 (47.29)	3 (4.05)
CYP3A4 Phenotype*	5 (6.75) *1/*22 (3) *22/*22 (2)	-	69 (93.24)	-

2.2. Effects of CYP2D6 phenotypes on plasma concentrations

After reviewing the concomitant medication, 11 patients were found to have concomitant treatment with strong CYP2D6 inhibitors and none for CYP3A4. Phenoconversion for CYP2D6 was performed following calculator developed by

the University of Florida Health Precision Medicine Program ¹⁸⁴. To explore the effect of CYP2D6 metabolizer status on plasma concentrations, comparative analyses were performed between the three phenotype groups (PMs, IMs and NMs); since UMs group did not reach a sufficient n, it was discarded from the analysis (Table 12). Significant differences were found between the three groups for AM ($p=0.0468$) (Figure 4A), ARI ($p=0.0302$) (Figure 4B) and ARI/DHA Ratio ($p=0.0174$) (Figure 4C). In addition, group to group comparisons showed a difference in the AM of PMs (327.1 ng/mL) vs NMs (196.2 ng/mL) $p=0.0038$; in the case of ARI, PMs (243.1 ng/mL) showed higher concentrations than IMs (153.3 ng/mL) $p=0.0486$ and also with respect to NMs (145.7 ng/mL) $p=0.0027$; Differences were also found between the above groups in the case of ARI/DHA ratio for PMs (3.63) vs IMs (2.31) $p=0.0055$ and PMs vs NMs (2.04) $p=0.0015$. No differences were found in the case of DHA. To determine whether these differences were not due to AOM doses, the same analysis was performed correcting each value by the administered dose (Figures 4A, 4B, 4C). After dose-adjusted drug concentrations were calculated, the differences remained statistically significant and, in addition, statistical significance was reached in the comparison of AM of PMs (0.85) vs IMs (0.56) $p=0.0317$. No differences were found in the case of DHA either. The results of the dose-adjusted analysis are available in Table 13.

Table 12. Plasma concentrations (ng/mL) and Ratio ARI/DHA among CYP2D6 phenotypes after phenoconversion. Presented as Median (Interquartile Range). AM: Active moiety ARI: Aripiprazole; DHA: Dehydroaripiprazole; PM: Poor Metabolizer; IM: Intermediate Metabolizer; NM: Normal Metabolizer. *: $p<0.05$; **: $p<0.01$.

	PM (12)	IM (23)	NM (36)	P	P PM vs IM	P PM vs NM	P IM vs NM
AM	327.1 (202.9-353.50)	216.8 (171.4-313.60)	196.2 (162.3-274.7)	0.0468*	0.0971	0.0038**	0.2722
ARI	243.1 (144.5-282.9)	153.3 (110.5-228.4)	145.7 (100.00-182.80)	0.0302*	0.0486*	0.0027**	0.279
DHA	63.9 (60.28-88.40)	61.89 (53.8-76.91)	57.65 (41.71-74.42)	0.5752	0.6493	0.3375	0.3695
ARI/DHA Ratio	3.631 (2.29-4.25)	2.31 (1.79-3.03)	2.04 (1.78-2.97)	0.0174*	0.0055**	0.0015**	0.7162

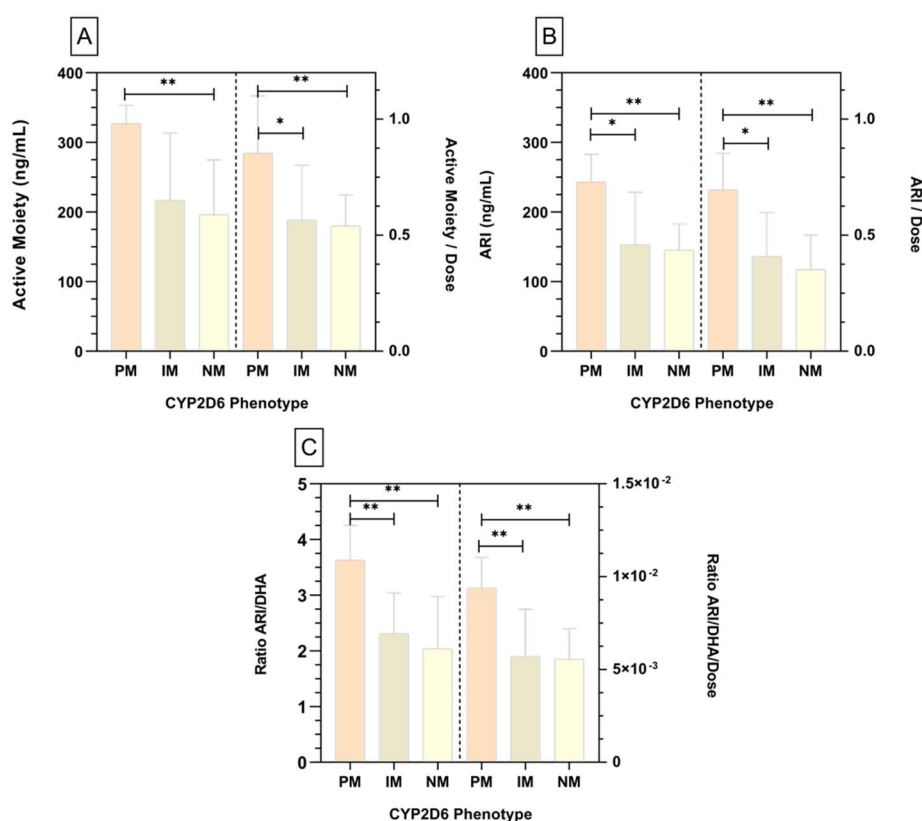


Figure 4. AM, ARI and ARI/DHA Ratio concentrations and dose-adjusted ratios among the different CYP2D6 Phenotype groups after phenoconversion. *: $p < 0.05$; **: $p < 0.01$.

Table 13. Plasma concentrations (ng/mL) and Ratio ARI/DHA corrected by dose among CYP2D6 phenotypes after phenoconversion. Presented as Median (Interquartile Range). AM: Active moiety; ARI: Aripiprazole; DHA: Dehydroaripiprazole; PM: Poor Metabolizer; IM: Intermediate Metabolizer; NM: Normal Metabolizer. *: $p < 0.05$ **: $p < 0.01$.

	PM (12)	IM (23)	NM (36)	P	p-PM vsIM	p-PM vsNM	p-IM vsNM
AM/Dose	0.85 (0.54-1.10)	0.56 (0.43-0.80)	0.54 (0.40-0.67)	0.0181*	0.0317*	0.0045**	0.2655
ARI/Dose	0.69 (0.39-0.85)	0.40 (0.28-0.59)	0.35 (0.26-0.50)	0.0125*	0.0219*	0.0015**	0.259
DHA/Dose	0.18 (0.15-0.24)	0.16 (0.13-0.23)	0.15 (0.11-0.20)	0.3477	0.655	0.2211	0.286
ARI/DHA/Dose Ratio	0.00938 (0.00757- 0.01105)	0.00570 (0.00455- 0.00824)	0.005553 (0.00457- 0.00718)	0.0074**	0.0019**	0.0015**	0.8124

2.3. Effect of CYP2D6 and CYP3A4 combined on plasma concentrations.

Since ARI and DHA are also metabolized via CYP3A4, a stratification of patients was performed considering CYP2D6 and CYP3A4 metabolizer states.

NMs for both cytochromes showed lower AM (196.61 ng/mL) and ARI (148.10 ng/mL) (Figure 5A and 5B) concentrations than PMs for both cytochromes (344.41 ng/mL) and (271.13 ng/mL) respectively, with p-values <0.001. DHA concentrations were higher in PM-PM (86.9 ng/mL) in comparison with PM-NM (61.42 ng/mL) and NM-NM (59.8 ng/mL) with p-values <0.05 (Figure 5C). In the case of the ARI/DHA ratio (Figure 5D), significant differences were found both between NM-NM vs PM-PM (P=0.0304), NM-NM vs PM-NM (P=0.0122) as well as in the case of IM-NM vs PM-NM (p=0.0253) (Table 14). CYP2D6NM-3A4PM (n=1) and CYP2D6UM-3A4NM (n=3) groups were discarded from the analysis in order not to increase the bias due to random effects.

Table 14.. Plasma concentrations and Ratio ARI/DHA corrected among CYP2D6 phenoconverted phenotypes and CYP3A4 phenotypes. AM: Active Moitey; ARI: Aripiprazole DHA: Dehydroaripiprazole; PM: Poor Metabolizer; IM: Intermediate Metabolizer; NM: Normal Metabolizer. *: p<0.05; **: p<0.01; ***:P<0.001.

	2D6NM- 3A4NM (34)	2D6IM- 3A4NM (24)	2D6PM- 3A4NM (8)	2D6PM- 3A4PM (4)	p	P NM- NM vs IM- NM	P NM- NM vs PM- NM	p IM-NM vs PM-NM	P PM- NM vs PM- PM	p NM-NM vs PM-PM
AM	196.61 (160.30-274.80)	216.80 (173.37- 313.57)	253.00 (183.33- 344.12)	344.41 (324.02- 491.60)	0.0574	0.2819	0.13	0.5355	0.1535	0.0003***
ARI	148.10 (99.59-193.92)	153.30 (110.46- 228.40)	191.21 (137.68- 280.35)	271.13 (230.62- 398.51)	0.0485*	0.289	0.0997	0.3132	0.2141	0.0005***
DHA	59.8 (41.52-76.45)	61.89 (53.8-76.9)	61.42 (45.17-64.15)	86.9 (72.97-99.9)	0.2612	0.3925	0.8138	0.5076	0.0162*	0.0344*
ARI/DHA Ratio	2.03 (1.77-3.05)	2.31 (1.79-3.03)	3.63 (2.29-4.23)	3.70 (2.53-4.25)	0.0701	0.7333	0.0122*	0.0253*	0.901	0.0304*

On the other hand, dose adjusted ratio for AM, ARI and ARI/DHA ratio were calculated (Figures 5A, 5B, 5C). Statistical significance was maintained between the same groups. Furthermore, after adjustment, significant differences were obtained between the PM-CYP2D6-NM-CYP3A4 and PM-CYP2D6-PM-CYP3A4 groups for AM and ARI (Figure 5A, 5B, 5C and 5D). Results of Dose-adjusted ratios are available in Table 15.

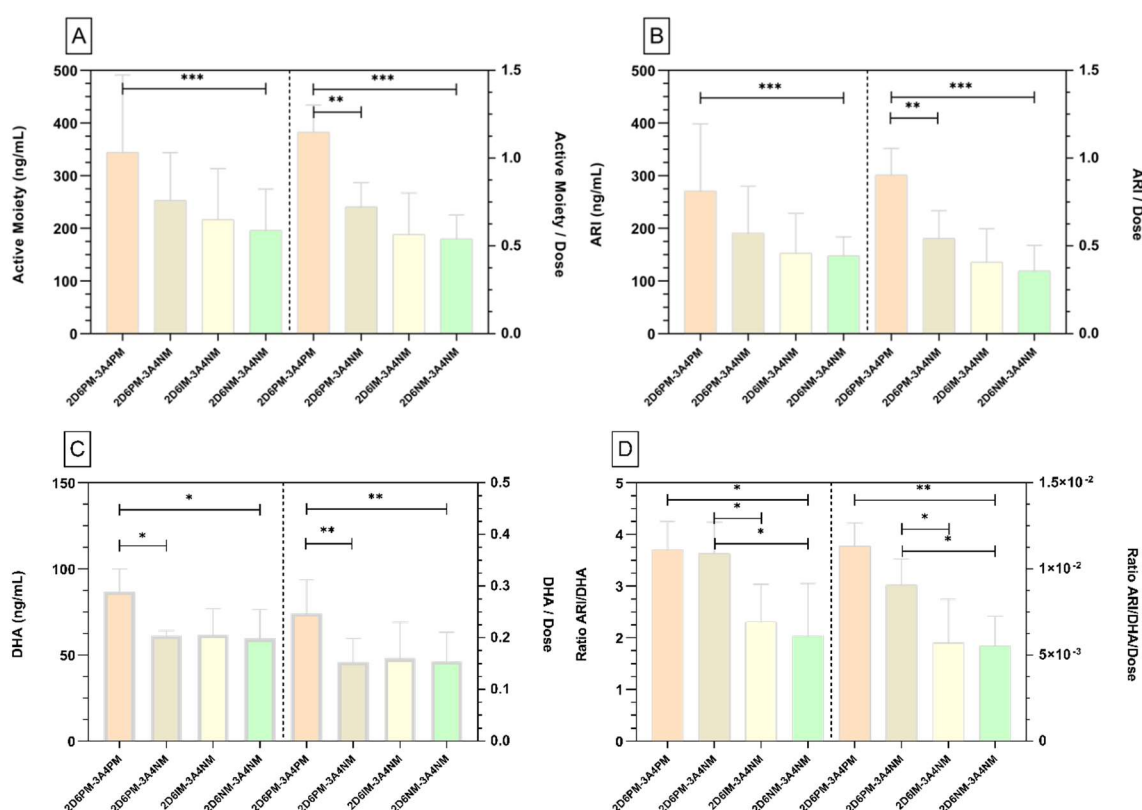


Figure 5. Active moiety, ARI and ARI/DHA Ratio concentrations and dose-adjusted ratios among the different CYP2D6-CYP3A4 combined phenotypes. *: $p < 0.05$; **: $p < 0.01$; ***: $P < 0.001$.

Table 15. Plasma concentrations and Ratio ARI/DHA corrected by dose among CYP2D6 phenoconverted phenotypes and CYP3A4 phenotypes. AM: Active Moitey; ARI: Aripiprazole DHA: Dehydroaripiprazole; PM: Poor Metabolizer; IM: Intermediate Metabolizer; NM: Normal Metabolizer. *: $p<0.05$; **: $p<0.01$; ***: $P<0.001$.

	2D6NM- 3A4NM (34)	2D6IM- 3A4NM (24)	2D6PM- 3A4NM (8)	2D6PM- 3A4PM (4)	p	P NM- NM vs IM-NM	P NM-NM vs PM- NM	P IM- NM vs PM- NM	P PM- NM vs PM-PM	P NM-NM vs PM- PM
AM/Dose	0.54 (0.39-0.67)	0.56 (0.43-0.80)	0.72 (0.45-0.86)	1.14 (1.08-1.30)	0.0114*	0.2819	0.1475	0.5076	0.004**	0.0003***
ARI/Dose	0.35 (0.26-0.50)	0.40 (0.28-0.59)	0.54 (0.34-0.70)	0.90 (0.76-1.05)	0.0109*	0.2749	0.0808	0.3567	0.0081**	0.0001***
DHA/Dose	0.155 (0.1131-0.2109)	0.161 (0.1398-0.23)	0.1535 (0.1129-0.1988)	0.248 (0.2432-0.3122)	0.0693	0.3187	0.8382	0.4037	0.0040**	0.0065**
Ratio ARI/DHA/Dose	0.0055 (0.0045-0.0072)	0.0057 (0.0045-0.0082)	0.0090 (0.0062-0.010)	0.011 (0.0082-0.012)	0.0268*	0.8204	0.0262*	0.0201*	0.2141	0.0097**

2.4.Discussion

To the best of our knowledge, this is the first study that explores how the combination of CYP2D6 and CYP3A4 genotypes and their predicted phenotypes affects plasma concentrations of AM, ARI, DHA and parent-to-metabolite ratio in patients under AOM treatment. Also, it is the first to take in account a strict and most validated up to date CYP2D6 phenoconversion¹⁸⁴. The phenotypic distributions found correspond to the values expected in the Caucasian population^{185,186}.

Our results showed a strong effect between different CYP2D6 metabolizer states on AM, ARI and ratio ARI/DHA. Particularly, this trend is observed in PMs that have 1.7-fold concentration of AM compared with NMs. FDA SmPC of AOM warns of the need to adjust the dose to 300 mg in PMs for CYP2D6 as they found 60% higher AM concentrations in these metabolizers⁹⁰. In real world data, we observed 67% higher concentrations in these metabolizers for both AM and ARI and 77% higher in the case of the ARI/DHA ratio. On the other hand, the SmPC recommends that, with concomitant medication that strongly inhibits CYP2D6, the dose adjustment should also be made to 300 mg, without considering the baseline metabolizer status.

The SmPC does not contemplate recommendations for IMs, nor does the Dutch Pharmacogenetics Working Group¹⁸⁷. In other available works, IMs exhibit higher concentrations of AM and ARI with respect to NMs^{100,188}, but these non-prospective studies lack information about concomitant drugs and they have not been taken into account for the analysis. In our work, significant differences are obtained between PMs and IMs, although an upward trend is observed in IMs vs NMs, but significance was not reached.

The influence of certain concomitant drugs affecting *CYP2D6* on ARI concentrations have been reported by other authors^{189,190}. These findings are in line with our results but, in addition, in our study it has been carried out the stratification of patients according to the activity score associated with each allele. Also, , it was possible to obtain information about the analysis of more than 20 allelic variants, as well as the number of copies of variants, and a strict phenoconversion was done taking into account the drugs included in the FDA list of *CYP2D6* inhibitors, which is one of the most authoritative resources¹⁵⁷. This influence on ARI concentrations is also observed after dose-adjusted concentrations, where this increase continues to be observed. The adjusted dose-concentrations for AM and ARI in the NMs in our study are practically identical to those observed in other works available in the literature^{191,192}.

In our study, no relationship was found between the metabolizing phenotype for *CYP2D6* and plasma DHA concentration, as previously stated by other authors¹⁰⁰. In the present work, we provide the parent-to-metabolite ratio ARI/DHA, where 77% higher ratios were found for PMs compared to NMs. Therefore, the increase in AM observed is at the expense of ARI concentration. NMs ARI/DHA ratios obtained are in accordance with the AOM SmPC, but a certain inter-variability is observed⁹⁰.

With most of the focus remaining on the genetic polymorphisms of *CYP2D6* and their consequent effect on the pharmacokinetics of ARI, nonetheless, the role of *CYP3A4* cannot be entirely dismissed. Furthermore, observing the constant and unchanging concentration of DHA across the different *CYP2D6* metabolizing groups, the role of *CYP3A4* should be studied. To study this possible effect, patients were grouped according to predicted activity for *CYP2D6* and *CYP3A4*. We observed 75% higher AM and 83% higher ARI concentrations in the PM-*CYP2D6*-PM-*CYP3A4* group compared to the NMs group for both cytochromes. Statistical significance was maintained when the comparison was made using dose-adjusted ratios. On the other hand, when

performing this adjustment, PM-CYP2D6-PM-CYP3A4 groups showed a 58% higher AM and 66% higher ARI compared with PM-CYP2D6-NM-CYP3A4. All these differences were also observed in the ARI/DHA ratio between the same groups. On the contrary, other studies have explored the influence of CYP3A4 on ARI metabolism without finding a correlation ^{152,193}, this could be due to the fact that the combined effects of the two cytochromes were not taken into account and that their samples did not include homozygotes for the *22 allele.

Our analysis also reveals that PMs for both cytochromes have 45% higher DHA concentration than NMs. This suggests that CYP3A4 plays a more significant role than expected, in the elimination of DHA. In the possible metabolic pathways that can undergo DHA shown in the molecule's FDA approval documents ¹⁹⁴, CYP3A4 catalyzes up to 3 possible reactions and two of them independently of CYP2D6. Therefore, the reduction in the metabolizing capacity of this pathway could explain the increase in DHA concentrations and the increases observed in AM and ARI for this metabolizer states. Furthermore, our analysis reveals that CYP2D6PM-3A4PM have 41% more DHA than CYP2D6PM-3A4NM, which reinforces this hypothesis.

This study increases the evidence that PMs for CYP2D6 should be treated with lower doses of AOM. On the other hand, a trend towards higher concentrations of AM and ARI in IMs were observed. In addition, the evidence provided by this study shows that considering concomitant drugs is needed to stratify patients when it comes to the clinical implementation of these techniques, and it is probably the cause of the difference in the results found with other studies that do not take this effect into account. On the other hand, CYP3A4 metabolizer status also influences AOM metabolism and affects plasma concentrations of AM, ARI and DHA. Therefore, it would be advisable to be genotyped and combined with CYP2D6 activity.

Genotyping and phenotyping of *CYP2D6* and *CYP3A4* as well as consider concomitant drugs may constitute an important tool to obtain optimal concentrations within the therapeutic reference range and, consequently, maximizing therapeutic efficacy while reducing the potential adverse events. Further studies should be performed to relate our results with clinical outcomes of patients under AOM therapy.

3. INFLUENCE OF *ABCB1* POLYPMORPHISMS

3.1. Study population

A total of 72 patients under AOM treatment were evaluated. Median age was 47 years (35-54); Weight 80.43 Kg (67.3-91); BMI 29.1 kg/m²; Cr: 0.82 mg/dL (0.7-0.97); AST: 23 IU/L (19-30.5); ALT: 30 IU/L (18-42); GGT: 27 IU/L (17-42); Median dose administrated of AOM was 400 mg (300-400); Injection site (28 Deltoid; 44 Gluteus); smoking status (49 smokers; 23 non-smokers). The genotypes and phenotypes of *CYP2D6* and *CYP3A4* are depicted in Table 16. Finally, after considering only patients with normal phenotype metabolizer state for *CYP2D6* and absence of alleles *22 and *20 for *CYP3A4* together with the absence of concomitant inhibitors or inducers, a total of 35 patients were selected. The distribution of *ABCB1* genotypes for these 35 patients is shown in Table 17.

Table 16. *CYP3A4* presence of *22 and *20 alleles detected, *CYP2D6* Phenotypes and *ABCB1* single nucleotide polymorphisms (SNPs) variants and haplotypes.

	SNP	n (%)	Haplotype	n (%)
C3435T	CC	23 (31.9)	CC-GG-CC	10 (13.89)
	CT	34 (47.22)	CC-GA-CC	2 (2.78)
	TT	15 (20.83)	CC-GT-CC	1 (1.39)
G2677T	GG	22 (30.56)	CC-GG-CT	4 (5.56)
	GT	36 (50)	CC-GG-TT	3 (4.17)
	TT	11 (15.28)	CC-GA-TT	1 (1.39)
	GA	3 (4.17)	CC-GT-CT	2 (2.78)
C1236T	CC	19 (26.39)	CT-GG-CC	2 (2.78)
	CT	33 (45.83)	CT-GG-TT	3 (4.17)
	TT	20 (27.78)	CT-GT-CC	1 (1.39)
<i>CYP2D6</i> Phenotype	PM	2 (2.7)	CT-GT-CT	20 (27.78)
	IM	27 (37.5)	CT-GT-TT	5 (6.94)
	NM	40 (55.5)	CT-TT-CC	1 (1.39)
	UM	3 (4.1)	CT-TT-TT	2 (2.78)
<i>CYP3A4</i> Genotype	*1/*22	3 (4.1)	TT-GT-CT	7 (9.72)
	*22/*22	2 (2.7)	TT-TT-CC	2 (2.78)
			TT-TT-TT	6 (8.33)

Table 17. *ABCB1* single nucleotide polymorphisms (SNPs) variants and Haplotypes after selection of normal metabolizing states for CYP2D6 and absence of alleles *22 and *20 for CYP3A4.

	SNP	n (%)	Haplotype	n (%)
C3435T	CC	15 (42.86)	CC-GG-CC	8 (22.86)
	CT	14 (40)	CC-GA-CC	2 (5.71)
	TT	6 (17.14)	CC-GG-CT	1 (2.86)
G2677T	GG	13 (37.14)	CC-GG-TT	2 (5.71)
	GT/GA	18 (51.43)	CC-GA-TT	1 (2.86)
	TT	4 (11.43)	CC-GT-CT	1 (2.86)
C1236T			CT-GG-CC	1 (2.86)
			CT-GG-TT	1 (2.86)
	CC	12 (34.29)	CT-GT-CT	12 (34.29)
	CT	16 (45.71)	TT-GT-CT	2 (5.71)
	TT	7 (20)	TT-TT-CC	1 (2.86)
			TT-TT-TT	3 (8.57)

3.2. Effect of variations in individual *ABCB1* SNPs

A comparative analysis of the concentrations of ARI, DHA as well as the ratio of their plasma concentrations (ARI/DHA ratio) between the different variants found was carried out (Table 18). For ARI concentrations, no significant differences were found between the different variations for the three SNPs analyzed. However, there was a clear trend across the three SNPs to lower ARI concentrations as the presence of mutated T alleles increases.

On the other hand, in the case of DHA concentrations, there was also a trend towards higher DHA concentrations the higher the number of T alleles. Specifically, in the case of C1236T significant differences ($p < 0.05$) were found with 59.9% higher DHA concentrations for CT (68.99 ng/mL) compared to CC (43.13 ng/mL).

Regarding ARI/DHA ratio, significant differences between the different variants were observed in the three SNPs analyzed. Specifically, for C3434T it was obtained an ARI/DHA ratio 37.4% higher for CC (2.35) compared to the TT variant (1.71), for G2677T 93% higher for GG (3.27) vs TT (1.69) and for C1236T 64.2% higher for CC (2.93) vs TT (1.78) (Figure 6A). Comparing Non-T carriers vs. T carriers variants as a whole, yielded 22.3%, 70.07% and 47.43% more respectively, maintaining statistical significance (Figure 6B).

Table 18. Plasma concentrations of aripiprazole (ARI) and dehydroaripiprazole (DHA) and ARI/DHA ratios among the different ABCB1 single nucleotide polymorphisms (SNPs) variants and haplotypes. Results are presented as median and Interquartile Range (IQ); *Significant difference between Non-T carriers and homozygous T carriers ($p < 0.05$); *Significant difference between Non-T carriers and heterozygous T carriers ($p < 0.05$).

SNP	Genotype (n)	ARI (ng/mL) (IQ)	DHA (ng/mL) (IQ)	ARI/DHA RATIO (IQ)
C3435T	CC (15)	152.9 (98.38-187.4)	55.97 (40.82-68.4)	2.35* (1.86-3.44)
	CT (14)	138.9 (105.5-178.9)	62.49 (43.3-79.51)	2.034 (1.71-2.86)
	TT (6)	117.5 (67.52-271)	66.3 (41.52-114)	1.71* (1.56-2.30)
	T Carrier (CT or TT) (20)	133.5 (101.2-181.5)	65.46 (42.38-80.51)	1.929* (1.63-2.67)
G2677T	GG (13)	152.9 (129.3-188.8)	55.97 (40.43-66.66)	3.274* (1.92-3.71)
	GA/GT (18)	127 (97.7-183.9)	62.49 (43.72-85.4)	2.009 (1.79-2.65)
	TT (4)	117.5 (74.17-312.8)	66.3 (46.6-173.5)	1.694* (1.48-2.03)
	T Carrier (GT or TT) (22)	127 (97.7-183.9)	65.46 (43.72-85.4)	1.925* (1.71-2.32)
C1236T	CC (12)	151.1 (95.8-165.5)	43.13* (34.63-64.18)	2.934* (1.90-3.73)
	CT (16)	138.9 (104.9-197.3)	68.99* (54.32-85.21)	2.034 (1.75-2.70)
	TT (7)	134.9 (98.38-187.4)	63.6 (53.53-94)	1.786* (1.57-2.12)
	T Carrier (CT or TT) (23)	134.9 (100-187.4)	67.32* (53.72-86.1)	1.99* (1.73-2.35)

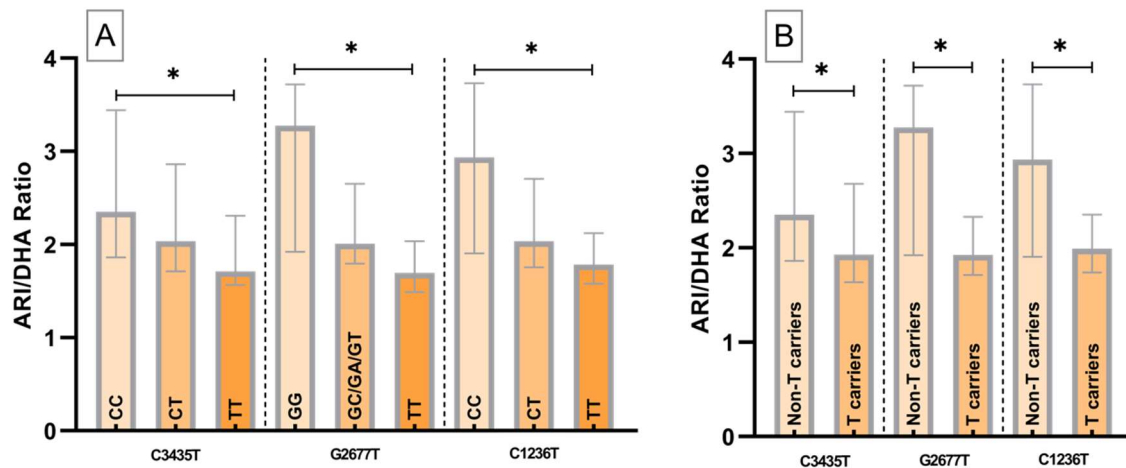


Figure 6. Aripiprazole/dehydroaripiprazole (ARI/DHA) ratio among the different *ABCB1* single nucleotide polymorphisms (SNPs) variants and comparison between Non-T carriers vs T carriers for each SNP; *: ($p < 0.05$).

3.3. Effect of *ABCB1* haplotypes

The different *ABCB1* haplotypes were analyzed together (Table 19). The 3 groups with the highest number of patients were used for the analysis. Regarding ARI and DHA concentrations, no significant differences were found between the three groups, but a slight trend towards higher ARI and lower DHA concentrations was observed with increasing presence of T alleles. Significant differences were obtained between CC-GG-CC and TT-TT-TT patients, with an 87.9% higher ratio in patients with the CC-GG-CC haplotype (Figure 7A). Non-T carriers, heterozygous T carriers and homozygous T carriers patients were also compared. In this case, 67.09% higher ratios were obtained in Non-T carriers compared to heterozygous T carriers ($p < 0.05$) (Figure 7B). This difference was maintained when comparing Non-T carriers to T-carriers (homozygous and heterozygous) (Figure 7C).

Table 19. Plasma concentrations of aripiprazole (ARI) and dehydroaripiprazole (DHA) and ARI/DHA ratios among the different ABCB1 haplotypes. Results are presented as median and Interquartile Range (IQ); *Significant difference between Non-T carriers and homozygous T carriers ($p<0.05$); +Significant difference between Non-T carriers and heterozygous T carriers ($p<0.05$). Heterozygous T carriers: CC-GA-CC; CC-GG-CT; CC-GG-TT; CC-GA-TT; CC-GT-CT; CT-GG-CC; CT-GG-TT; CT-GT-CT; TT-GT-CT; TT-TT-CC

Haplotype	ARI (ng/mL) (IQ)	DHA (ng/mL) (IQ)	ARI/DHA RATIO (IQ)
CC-GG-CC (8)	156.3 (137-201.7)	59.47 (41.95-67.53)	3.357* (2.08-3.73)
CT-GT-CT (12)	138.9 (108.5-181.5)	68.99 (56.44-89.7)	2.034 (1.75-2.67)
TT-TT-TT (3)	134.9 (100-372.1)	69 (63.6-208.3)	1.786* (1.45-2.12)
Non-T carriers (CC-GG-CC) (8)	156.3 (137-201.7)	59.47 (41.95-67.53)	3.357** (2.08-3.73)
Heterozygous T carriers (24)	127 (96.38-181.5)	56.79 (41.13-80.51)	2.009+ (1.74-2.76)
TTT haplotype homozygous carriers (3)	134.9 (100-372.1)	69 (63.6-208.3)	1.786* (1.45-2.12)
Non- T carriers (8)	156.3 (137-201.7)	59.47 (41.95-67.53)	3.357* (2.08-3.73)
T carriers (27)	132.1 (98.36-182.8)	57.65 (41.71-82.54)	1.99* (1.73-2.59)

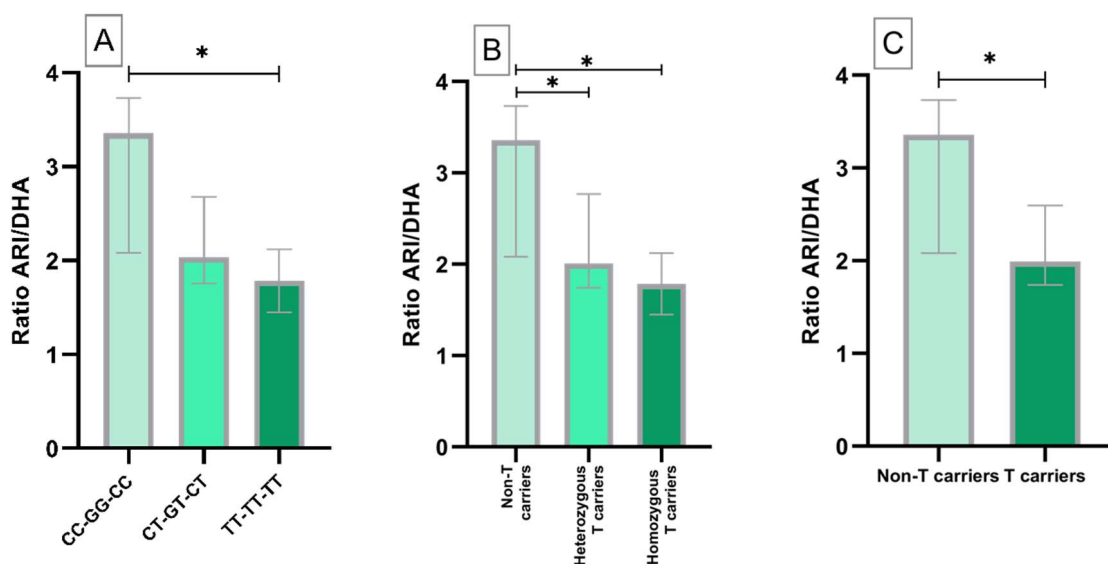


Figure 7. Aripiprazole/dehydroaripiprazole (ARI/DHA) ratio among the three different ABCB1 haplotypes analyzed and comparison between T carriers and Non-T carriers; *:($p<0.05$).

To reinforce the results obtained and to confirm whether the observed differences were maintained when grouping the patients by pairs of variants of the three SNPs, the same comparative analyses were carried out for the pairs C3435T-C1236T, C3435T-G2677T and G2677-C1236T. Identical results were obtained for the rest of the comparisons performed, with even greater statistical significances ($p < 0.01$) (Figure 8A and 8B). The results of this analysis are shown in Table 20.

Table 20. Plasma concentrations of aripiprazole (ARI) and dehydroaripiprazole (DHA) and Ratios ARI/DHA among the different paired ABCB1 single nucleotide polymorphisms (SNPs) variants. Results are presented as median and Interquartile Range (IQ); *Significant difference between Non-T carriers and homozygous T carriers ($p < 0.05$); **Significant difference between Non-T carriers and homozygous T carriers ($p < 0.01$); +Significant difference between Non-T carriers and heterozygous T carriers.

	SNP	ARI (ng/mL) (IQ)	DHA (ng/mL) (IQ)	ARI/DHA RATIO (IQ)
C3435T - C1236T	CC-CC (10)	152.3 (116.3-178.8)	51.16 (35.34-65.8)	2.93* (1.99-3.70)
	CT-CT (12)	138.9 (108.5-181.5)	68.99 (56.44-89.7)	2.03 (1.75-2.67)
	TT-TT (3)	134.9 (100.372.1)	69 (63.6-208.3)	1.78* (1.45-2.12)
	T carriers (25)	134.9 (99.19-185.1)	63.6 (48.95-84.32)	1.99* (1.68-2.58)
C3435T- G2677T	CC-GG (11)	159.7 (132.1-202.2)	57 (45.33-68.4)	3.27* (2.03-3.693)
	CT-GT (12)	138.9 (108.5-181.5)	68.99 (56.44-89.7)	2.03 (1.75-2.67)
	TT-TT (4)	117.5 (74.17-312.8)	66.3 (46.6-173.5)	1.69* (1.48-2.03)
	T carriers (24)	127 (96.38-181.5)	60.63 (41.13-80.51)	1.92* (1.66-2.50)
G2677-C1236T	GG-CC (9)	152.9 (141.3-190.3)	57 (38.47-66.66)	3.44** (2.12-4.07)
	GT-CT (15)	132.1 (104.6-182.8)	67.32 (53.72-82.54)	2.02+ (1.73-2.82)
	TT-TT (3)	134.9 (100-372.1)	69 (63.6-208.3)	1.78* (1.45-2.12)
	T carriers (26)	127 (97.7-183.9)	60.63 (43.72-83.	1.92** (1.71-2.41)

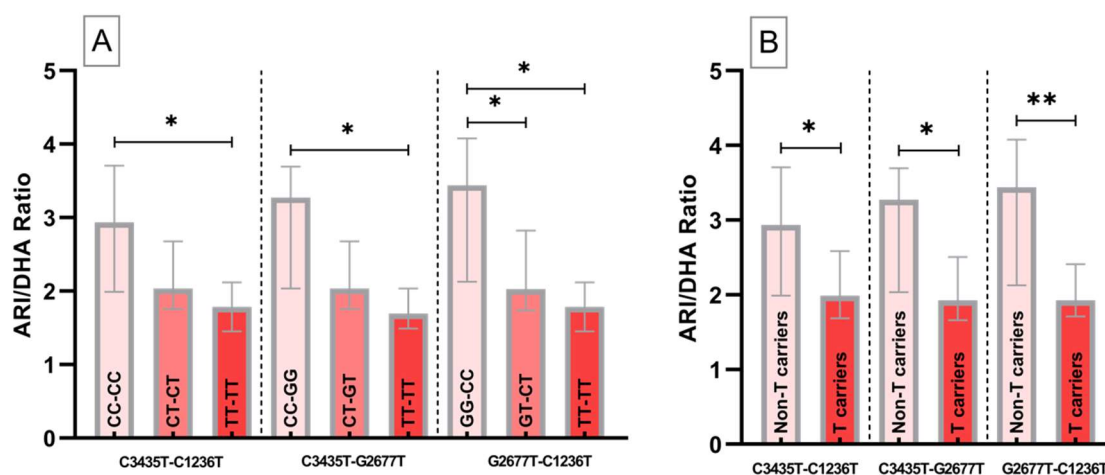


Figure 8. ARI/DHA ratio among the different paired *ABCB1* SNPs variants and comparison between T carriers and Non-T carriers. *: ($P < 0.05$); **: ($P < 0.01$).

3.4. Discussion

This study explores how three of the most studied SNPs of the *ABCB1* gene to date affect plasma ARI concentrations in patients under AOM treatment for the first time. In addition, this study has considered the metabolizing state of the two main ARI metabolizing enzymes (CYP2D6 and CYP3A4), in order to eliminate biases due to metabolizer status of these cytochromes and to specifically study the effect of P-gp on plasma concentrations of the parent compound and its active metabolite. The allele frequencies for the *ABCB1* gene obtained in our study population were similar to those of the reference population^{195,196}.

Our results show a clear relationship between the genotypes found for the different *ABCB1* SNPs and the ARI/DHA ratio. Individually, patients with TT genotype in G2677T have almost twice the ratio compared to CC genotype. Similarly, this increase is also found in C3435T with 1.4-fold and in C1236T with 1.6-fold for the same genotypes. These results are reinforced by comparing the three haplotypes analyzed and obtaining significant differences between Non-T carriers and T carriers.

Other previous studies analyzed some of these SNPs individually, but not haplotypes, nor did they consider CYP2D6 or CYP3A4 genotypes to select patients. Specifically, Suzuki et al. observed trends to lower ARI concentrations for TT-TT genotype of C2677T and C3435TT but no significant differences were

found¹⁹⁷. In contrast, Rafaniello et al. found significant differences in patients with TT genotype for these SNPs, in whom they observed lower ARI concentrations¹⁹⁸. These results are in agreement with those obtained in this work, in which T homozygous carriers patients for the three SNPs individually show a tendency towards lower ARI concentrations. On the other hand, another work developed by Belmonte et al. found higher ARI concentrations in those patients with the presence of T alleles¹⁵². These studies were performed with oral ARI, so part of the observed effect was artifactual due to the influence of intestinal P-gp. In contrast, this work was performed with AOM, so the contribution of intestinal P-gp should be minimal, focusing on the effects caused by the different variants of the polymorphisms in the other tissues where P-gp is present.

Regarding DHA concentrations, higher values were found as the presence of T alleles increases both for the SNPs individually and for the haplotypes studied. In particular, a greater influence is observed in DHA concentrations than in the parent compound, being its greater P-gp affinity comparing to ARI one of the possible causes (21).

The effect of the different P-gp polymorphisms produce significant differences in ARI/DHA ratio. However, regarding plasma concentrations of ARI and DHA, despite there is a clear trend towards lower ARI concentrations and higher DHA concentrations when the presence of mutated T alleles increases, no significant differences were found. This may be due to the different hepatic P-gp activity between patients, as well as to the aforementioned different P-gp affinity for both substrates. Although the pharmacological activity of DHA seems to be similar to that of ARI, it would be necessary to study whether these differences in the ratio, in favour of DHA, produce a decrease in the time in which the therapeutic effect is maintained, due to the shorter half-life of this metabolite compared with the parent compound⁹⁰.

Regardless of whether or not it affects the active moiety of plasma concentrations, both ARI and DHA are obvious substrates of P-gp, and therefore at the BBB these polymorphisms may predispose to lower drug effectiveness or greater adverse effects, this hypothesis is reinforced by differences at this level, found by Kirschbaum et al. and Wang et al., in P-gp knockout animal models^{159,199}. Regarding limitations, this study focuses on the three most studied SNPs of the *ABCB1* gene in the ARI/DHA ratio, neglecting the potential influence of rarer *ABCB1* polymorphisms that have not been explored. These rarer variants might hold significant weight in individual responses to medications, and their absence

from the analysis could lead to an incomplete picture of the impact of *ABCB1* on ARI pharmacogenetics²⁰⁰.

In conclusion, the *ABCB1* gene could be a good partner along with *CYP2D6* and *CYP3A4* genotyping in conjunction with monitoring plasma concentrations of ARI. Further studies should evaluate clinical efficacy and adverse effects, together with analysis of the different *ABCB1* SNPs, as well as *CYP2D6* and *CYP3A4* genotype, to clarify the clinical impact of these three *ABCB1* polymorphisms.

CONCLUSIONS

CONCLUSIONS

This doctoral thesis has provided answers to key points of the clinical translation of personalized medicine in the field of mental health and specifically in the treatment with long-acting aripiprazole. From the comparison of two analytical techniques in order to determine their feasibility in daily clinical practice, to the determination of the influence on plasma concentrations of aripiprazole and dehydroaripiprazole of genetic polymorphisms of cytochromes CYP2D6 and CYP3A4, as well as the *ABCB1* gene coding for P-glycoprotein.

In relation to the proposed objectives, the conclusions of this doctoral thesis are as follows:

1. Alinity C is a suitable analytical method for the determination of plasma concentrations of aripiprazole in daily clinical practice.
 - a. This method is comparable in a wide range of concentrations to the *gold-standard* for this type of determination, Ultra-high performance liquid chromatography-tandem mass spectrometry. It provides a higher speed of response and a lower need for processing compared to Ultra-high performance liquid chromatography-tandem mass spectrometry.
 - b. In concentrations higher than 500 ng/mL there is discordance between both methods, being the concentrations determined in Alinity C higher than those determined by Ultra-high performance liquid chromatography-tandem mass spectrometry.
 - c. Freezing the samples at -80°C for 6 months does not affect the reproducibility of the method or the stability of the sample.
2. Genotyping and phenotyping of CYP2D6 and CYP3A4 together with consideration of concomitant drug treatment is an important tool for obtaining plasma concentrations of aripiprazole within the therapeutic range. Potentially allowing to increase effectiveness and decrease adverse effects.
 - a. Patients with a poor metabolizer phenotype for CYP2D6 have higher plasma concentrations of active moiety, aripiprazole and higher aripiprazole/dehydroaripiprazole ratios.

- b. Patients with intermediate metabolizer phenotype for *CYP2D6* show a trend toward higher plasma concentrations of Active moiety, aripiprazole and aripiprazole/dehydroaripiprazole ratio.
 - c. Adjusted-dose correction does not modify the results obtained, thus reinforcing the influence of phenotypes on plasma concentrations.
 - d. The *CYP3A4* metabolizing state also influences plasma concentrations of active moiety, aripiprazole and aripiprazole/dehydroaripiprazole ratio, so it is advisable to perform its genotyping together with that of *CYP2D6*.
3. *ABCB1* genotyping is a potentially useful tool if combined with *CYP2D6* and *CYP3A4* genotyping, in addition to consideration of concomitant medication:
- a. Individually, all SNPs with mutated T alleles have higher aripiprazole/dehydroaripiprazole ratios.
 - b. With respect to haplotypes, as the presence of T alleles increases the aripiprazole/dehydroaripiprazole ratio increases.
 - c. The determination of plasma concentrations taking into account the *CYP2D6* and *CYP3A4* metabolizing states is essential to avoid biases when interpreting the influence of P-glycoprotein.

CONCLUSIONES

En esta tesis doctoral se ha abordado la traslación clínica de la medicina personalizada en el ámbito de la psiquiatría, concretamente de la terapia con aripiprazol inyectable de acción prolongada. Para ello, por un lado, se han comparado dos técnicas analíticas con el fin de determinar su viabilidad en la práctica clínica diaria y, por otro lado, se ha determinado la influencia sobre las concentraciones plasmáticas de aripiprazol y dehidroaripiprazol de los polimorfismos genéticos de los citocromos CYP2D6 y CYP3A4, así como del gen *ABCB1* codificante para la Glicoproteína P.

En relación con los objetivos propuestos, las conclusiones de esta tesis doctoral son:

1. Alinity C es un método analítico adecuado para la determinación de concentraciones plasmáticas de aripiprazol en la práctica clínica diaria.
 - a. Este método es comparable en un amplio rango de concentraciones al “gold-standard” para este tipo de determinaciones, la Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas. Aportando una mayor velocidad de respuesta y una menor necesidad de procesamiento frente a la Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas.
 - b. En concentraciones superiores a 500 ng/mL existe discordancia entre ambos métodos, siendo las concentraciones determinadas en Alinity C superiores a las determinadas por Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas.
 - c. La congelación de las muestras a -80°C durante 6 meses no afecta a la reproducibilidad del método ni a la estabilidad de la muestra.
2. El genotipado y fenotipado de *CYP2D6* y *CYP3A4* de forma conjunta, junto con la consideración del tratamiento con fármacos concomitantes son una herramienta importante de cara a obtener concentraciones plasmáticas de Aripiprazol dentro del rango terapéutico. Permitiendo, potencialmente, aumentar la efectividad y disminuir los efectos adversos.

- a. Los pacientes con fenotipo metabolizador pobre para *CYP2D6* presentan mayores concentraciones plasmáticas de Active moiety, aripiprazol y mayores ratios aripiprazol/dehidroaripiprazol.
 - b. En los pacientes con fenotipo metabolizador intermedio para *CYP2D6* se observa una tendencia hacia mayores concentraciones plasmáticas de Active moiety, aripiprazol y ratio aripiprazol/dehidroaripiprazol.
 - c. La corrección por dosis no modifica los resultados obtenidos por lo que se refuerza la influencia de los fenotipos en las concentraciones plasmáticas.
 - d. El fenotipo metabolizador de *CYP3A4* también influye en las concentraciones plasmáticas de Active Moiety, aripiprazol y ratio aripiprazol/dehidroaripiprazol, por lo que es recomendable realizar su genotipado en conjunto al de *CYP2D6*.
3. El genotipado de *ABCB1* es una herramienta potencialmente útil si se combina con el genotipado de *CYP2D6* y *CYP3A4*, además de la consideración de la medicación concomitante:
- a. De forma individual, todos los SNPs con alelos T mutados presentan mayores ratios de aripiprazol/dehidroaripiprazol.
 - b. Respecto a los haplotipos, a medida que aumenta la presencia de alelos T aumenta el ratio aripiprazol/dehidroaripiprazol.
 - c. Realizar la determinación de concentraciones plasmáticas, teniendo en cuenta los estados metabolizadores de *CYP2D6* y *CYP3A4*, es esencial para evitar sesgos a la hora de interpretar la influencia de la glicoproteína P.

CONCLUSIÓN

Nesta tese doutoral deuse resposta a puntos clave da translación clínica da medicina personalizada no campo da saúde mental e en concreto no tratamento con Aripiprazol de acción prolongada. Dende a comparación de dúas técnicas analíticas co fin de determinar a súa viabilidade na práctica clínica diaria, ata a determinación da influencia sobre as concentracións plasmáticas de Aripiprazol e Dehidroaripiprazol dos polimorfismos xenéticos dos citocromos CYP2D6 e CYP3A4, así como do xene *ABCB1* codificante para a Glicoproteína P.

En relación cos obxectivos propostos, as conclusións desta tese doutoral son:

1. Alinity C é un método analítico adecuado para a determinación de concentracións plasmáticas de aripiprazol na práctica clínica diaria.
 - a. Este método é comparable nun amplo rango de concentracións ao “gold-standard” para este tipo de determinacións, a Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas. Achegando unha maior velocidade de resposta e unha menor necesidade de procesamento fronte a Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas.
 - b. En concentracións superiores a 500 ng/mL existe discordancia entre ambos métodos, sendo as concentracións determinadas en Alinity C superiores ás determinadas por Cromatografía Líquida de Ultra-Alta Resolución en tándem de Masas.
 - c. A conxelación das mostras a -80°C durante 6 meses non afecta á reproducibilidade do método nin á estabilidade da mostra.
2. O xenotipado e fenotipado de *CYP2D6* e *CYP3A4* de forma conxunta, xunto coa consideración do tratamento con fármacos concomitantes son unha ferramenta importante para obter concentracións plasmáticas de Aripiprazol dentro do rango terapéutico. Permitindo, potencialmente, aumentar a efectividade e diminuír os efectos adversos.
 - a. Os pacientes con fenotipo metabolizador pobre para *CYP2D6* presentan maiores concentracións plasmáticas de Active moiety, aripiprazol e maiores cocientes aripiprazol/dehidroaripiprazol.

- b. Nos pacientes con fenotipo metabolizador intermedio para CYP2D6 obsérvase unha tendencia cara a maiores concentracións plasmáticas de Active moiety, aripiprazol e cociente aripiprazol/dehidroaripiprazol.
 - c. A corrección por dose non modifica os resultados obtidos polo que se reforza a influencia dos fenotipos nas concentracións plasmáticas.
 - d. O fenotipo metabolizador de CYP3A4 tamén inflúe nas concentracións plasmáticas de Active Moiety, aripiprazol e cociente aripiprazol/dehidroaripiprazol, polo que é recomendable realizar o seu xenotipado en conxunto co de CYP2D6.
3. O xenotipado de *ABCB1* é unha ferramenta potencialmente útil se se combina co xenotipado de *CYP2D6* e *CYP3A4*, ademáis da consideración da medicación concomitante:
- a. De forma individual, todos os SNPs con alelos T mutados presentan maiores cocientes de aripiprazol/dehidroaripiprazol.
 - b. Respecto aos haplotipos, a medida que aumenta a presenza de T aumenta o cociente aripiprazol/dehidroaripiprazol.
 - c. Realizar a determinación de concentracións plasmáticas tendo en conta os estados metabolizadores de CYP2D6 e CYP3A4, é esencial para evitar sesgos á hora de interpretar a influencia da glicoproteína P.

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APPENDIX

Appendix

1. CONFLICT OF INTEREST

The doctoral student declares that there is no conflict of interest with respect to the Doctoral Thesis.

2. CLINICAL STUDIES

Three clinical studies were carried out in this Doctoral Thesis. The first of them was a retrospective study in which 60 samples from 60 different patients were collected with the objective of comparing an automated analytical method with the current gold standard, as well as the stability of the sample once frozen. The second observational clinical study included 74 patients treated with Aripiprazole monohydrate in which, on the one hand, the plasma concentrations of Aripiprazole, dehydroaripiprazole and biochemical parameters were determined and, on the other hand, CYP2D6 and CYP3A4 genotyping was performed. In the third and last clinical study developed, 72 patients were recruited in whom the genetic analysis of three SNPs of ABCB1, CYP2D6 and CYP3A4 was performed, as well as the determination of plasma concentrations of Aripiprazole and dehydroaripiprazole.

Studies were conducted in accordance with Good Clinical Practice (GCP) standards and applicable regulatory requirements. The studies were conducted in accordance with the ethical principles based on the Declaration of Helsinki. The collection, processing, storage and transfer of data and samples will be in accordance with the General Data Protection Regulation (EU Regulation 2016-679 of the European Parliament and of the Council of 27 April 2016), the Spanish regulations on personal data protection in force, Law 14/2007 on biomedical research and RD 1716/2011. The study protocols were approved by the Galician Research Ethics Committee (CEIm) with registration codes 2021/285 and 2020/486. The committee complies with the current legal requirements applicable to the Research Ethics Committee, following the Good Clinical Practice Standards.



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CERTIFICA:

Que este Comité evaluó en su reunión del día 28/10/21 la modificación del estudio:

Título: Caracterización farmacocinética de los tratamientos farmacéuticos mediante el desarrollo de modelos poblacionales de tipo no lineal de efectos mixtos

Versión modificación: protocolo Versión: 1.1, data 04/06/2021 y HIP/CI Versión: 2, data 14/10/2021

Tipo de estudio: EPA SP

Promotor: Anxo Fernández Ferreiro

Código del Promotor: AFF-LAM-2020-01

Código de Registro: 2020/486

Y que este Comité acepta de conformidad con sus procedimientos normalizados de trabajo y tomando en cuenta los requisitos éticos, metodológicos y legales exigibles, que dicha enmienda sea incorporada al estudio de investigación.

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Promotor: *Cristina Mondelo García*

Tipo de estudio: EOM Seguimiento prospectivo

Version: v 2.0 de 14 de julio de 2021 y HIP/CI v 2.0 de 14 de julio de 2021

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Y, tomando en consideración las siguientes cuestiones:

- La pertinencia del estudio, teniendo en cuenta el conocimiento disponible, así como los requisitos legales aplicables.
- La idoneidad del protocolo en relación con los objetivos del estudio, justificación de los riesgos y molestias previsibles para el sujeto, así como los beneficios esperados.
- Los principios éticos de la Declaración de Helsinki vigente.
- Los Procedimientos Normalizados de Trabajo del CEIm-G de Galicia

Emite un **INFORME FAVORABLE** para la realización del estudio por el/la investigador/a del centro:

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4. ARTICLES DERIVED FROM THIS DOCTORAL THESIS

In compliance with the regulations for doctoral studies of the University of Santiago de Compostela in the “Reglamento dos estudos de doutoramento na USC, DOG of September 16, 2020”, information is provided about the articles included in this work and the journals where they are published. In particular, the name of the authors, the title of the journals, the year of publication of each article, the publisher, the DOI type link, the Impact Factor of the Journal and the quartile of the Journal Citation Reports, relevant information regarding copyright and use of articles and the author's contribution to publications are provided.

TITLE: Review of Pharmacokinetics and Pharmacogenetics in Atypical Long-Acting Injectable Antipsychotics

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AUTHOR CONTRIBUTION: The doctoral student has participated in conceptualization, methodology, formal analysis, investigation and writing original draft and preparation of the manuscript.

TITLE: Towards Precision Medicine in Clinical Practice: Alinity C vs. UHPLC-MS/MS in Plasma Aripiprazole Determination

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5. FIGURES AUTHORSHIP

The author declares that all unreferenced figures in this Thesis are his own, created with GraphPad Prism version 9.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA.

6. FIGURES AND TABLES

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In this doctoral thesis, the clinical implementation of personalized medicine in the field of psychiatry has been addressed, specifically regarding therapy with long-acting injectable aripiprazole. To this end, the plasma concentrations of this drug and its main active metabolite, dehydroaripiprazole, have been determined. In addition, the pharmacogenetic characterization of *CYP2D6*, *CYP3A4* and *ABCB1*, main agents involved in the metabolism and transport of these molecules, has been carried out. The introduction into clinical practice of pharmacokinetic monitoring of aripiprazole, together with the analysis of genetic polymorphisms involved in its elimination, constitutes a fundamental pillar to implement the therapeutic individualization of this drug, as well as to optimize its benefit-risk balance.