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Protocol

DPYD genotyping and 5-fluoropyrimidine toxicity: An overview of systematic reviews protocol

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ABSTRACT

Introduction: The increased risk of severe and life-threatening toxicity in patients with dihydropyridine dehydrogenase (DPD) deficiency, under treatment with fluoropyrimidines, has been widely studied. An up-to-date overview of systematic reviews summarizing existing literature can add value by highlighting most relevant information and supports decision-making regarding treatment in DPD deficient patients. The main objective of this overview of systematic reviews is to identify published systematic reviews on the association between germline variations in the *DPYD* gene and fluoropyrimidine toxicity.

Methods and analysis: This protocol was developed following the Preferred Reported Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) checklist, and the overview of systematic reviews will be reported in accordance with the PRISMA statement. PubMed, Embase, Scopus, and the Cochrane Library will be searched from inception to 2023. Systematic reviews irrespective of study designs that analyze the association between germline variations in the *DPYD* and fluoropyrimidine toxicity will be considered. Methodological quality will be assessed using AMSTAR2 checklist (Measurement Tool to Assess Systematic Reviews 2). Two independent investigators will perform the study selection, quality assessment, and data collection. Discrepancies will be solved by a third investigator.

Registration details: Registration number in PROSPERO: CRD42023401226.

Genotipado del gen DPYD y toxicidad de 5-fluoropirimidinas: Protocolo de una revisión de revisiones sistemáticas

Palabras clave:
Revisión sistemática
5-fluoropirimidina
Genotipado *DPYD*
Toxicidad
Oncología clínica

RESUMEN

Introducción: El incremento del riesgo de toxicidad grave y potencialmente mortal en pacientes con deficiencia de dihidropiridina deshidrogenasa (DPD) en tratamiento con fluoropirimidinas ha sido ampliamente estudiado. Una revisión actualizada de las revisiones sistemáticas publicadas, que agrupe la literatura existente, puede añadir valor al resaltar la información más relevante y respaldar la toma de decisiones con respecto al tratamiento en pacientes con deficiencia de DPD. El objetivo principal de esta revisión de revisiones sistemáticas es identificar revisiones sistemáticas publicadas sobre la asociación entre variaciones en el linaje germinal del gen *DPYD* y la toxicidad de las fluoropirimidinas.

Métodos y análisis: Este protocolo se ha desarrollado siguiendo la lista de verificación de los Protocolos para Revisiones Sistemáticas y Metaanálisis Preferidos (PRISMA-P), y la revisión de las revisiones sistemáticas se comunicará de acuerdo con la declaración PRISMA. Se realizará una búsqueda en PubMed, Embase, Scopus y la Biblioteca Cochrane desde su inicio hasta 2023. Se considerarán aquellas revisiones sistemáticas, independientemente de los diseños de estudio, que analicen la asociación entre variaciones en el linaje germinal del gen *DPYD* y la toxicidad de las fluoropirimidinas. La calidad metodológica se evaluará utilizando la lista de verificación AMSTAR2 (Herramienta de Medición para Evaluar Revisiones Sistemáticas 2). Dos investigadores independientes realizarán la selección de estudios, la evaluación de la calidad y la recopilación de datos. Las discrepancias se resolverán mediante un tercer investigador.

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Introduction

Fluoropyrimidine (FU)-based chemotherapies are widely used as chemotherapeutic drugs for the treatment of different types of cancer.

The *DPYD* gene encodes the dihydropyrimidine dehydrogenase (DPD) enzyme, which plays a vital role in the metabolic catabolism of fluoropyrimidines. Many genetic variants in the *DPYD* gene are known to modify the protein sequence or mRNA splicing, some of them do not clinically affect DPD activity, whereas other variants result in a reduced enzyme function. The increased risk of severe and life-threatening toxicities in patients carrying DPD deficiency treated with fluoropyrimidines has been widely studied. On account of it, patients who are heterozygous for *DPYD* decreased/no function variants demonstrate partial DPD deficiency. Thus, dose FU-based treatment adjustments should be performed.

Many primary studies and systematic reviews have evaluated the associations between *DPYD* polymorphisms and fluoropyrimidine toxicity. Most of the published studies have been summarized in an overview of systematic reviews published in 2016[1] aiming to analyze the associations between germline DPYD variations and fluoropyrimidine and platinum toxicity. This overview of systematic reviews comprehended systematic reviews published between 2009 and 2014, including 2 systematic reviews[2,3] investigating the association of *DPYD* variants with FU-induced toxicity.

Rosmarin et al[2] showed that few genetic variants had convincing evidence of an association with fluoropyrimidine toxicity. Only 4 of the 36 polymorphisms analyzed—*TYMS* 5VNTR 2R/3R, *TYMS* 3UTR 6bpins-del, *DPYD* 2846TA, and *DPYD* *2A—were formally associated with global grade 3 toxicity.

Meta-analysis by Terrazzino et al[3] investigated the impact of the *DPYD* variants IVS14 + 1G>A and 2846A>T on the risk of FU-related toxicities in cancer patients treated with FU. *DPYD* IVS14 + 1G>A and 2846A>T variants were identified as risk factors for the development of severe toxicities following fluoropyrimidine treatment.

Nowadays, 4 genetic polymorphisms in the *DPYD* have demonstrated clinically relevant effects on the DPD activity.[4] Consequently in 2017, the Clinical Pharmacogenetics Implementation Consortium (CPIC) published an updated *Guideline for Dihydropyridine Dehydrogenase Genotype and Fluoropyrimidine Dosing,* including key information regarding the interpretation of clinical *DPYD* genotype tests in order to guide clinicians on the dosing of fluoropyrimidines.[4]

However, several systematic reviews and meta-analyses have been published in the last 5 years, highlighting the importance of other variants and the possibility of various mutations coexistence at different locus of the gene.

The main objective of this overview of systematic reviews is to identify published systematic reviews on the association between germline variations in the *DPYD* gene and fluoropyrimidine toxicity. The secondary objective is to assess the association by subgroups, stratified by cancer type and fluoropyrimidine dosage.

Methods

Eligibility criteria

This protocol was developed following the Preferred Reported Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) checklist,[5] and the overview of systematic reviews will be reported in accordance with the PRISMA statement.[6]

The inclusion criteria for the systematic review according to PICOS (Population, Intervention, Comparison, Outcome, and Study design) design will be the following:

P: Oncologic patients with genetic study of *DPYD* gene variants and under treatment with fluoropyrimidines.

I: Registry of severe adverse events (grades 3–5) related to fluoropyrimidine treatment in patients with *DPYD* gene variants.

C: Patients without *DPYD* gene variants or without comparator.

O: Variables related to toxicity and treatment: severe adverse events, *DPYD* gene variants detected, fluoropyrimidine dosage, and treatment regimen.

S: Systematic review with/without meta-analysis.

The following will be established as exclusion criteria: reviews not following systematic review methodology; in vitro or animal studies; unable to extract genotype; or unable to provide appropriate data.

In addition, there will be no date or language restriction, but the research will have to be accessible in full text.

Information sources and search strategy

The search strategy was carefully designed by the authors in order to identify the largest range of relevant evidence. A comprehensive search will be performed by 2 authors (SOT and RRM) including all available articles from inception until February 2023 in databases of peer-reviewed articles and sources of gray literature.

Sources of peer-reviewed literature to be searched include PubMed, Embase, Scopus (Elsevier Science), and Cochrane Library. A combination of Medical Subject Headings (MeSH) and free terms will be used. An example of our search strategy for PubMed is reported in **appendix 1**.

Gray literature will be included using Google Scholar, as well as the reference lists of identified relevant articles. Common registry databases as ClinicalTrials.gov or the International Prospective Register of Systematic Reviews (PROSPERO) will be searched as well.

Data management, selection process, and data collection process

A peer-review of the literature will be performed by 2 independent investigators (SOT and RRM) and the results will be imported to a reference manager. Once the first search results are obtained, duplicate articles will be discarded. Both, title and abstract of the selected articles will be reviewed taking into account the previously defined inclusion and exclusion criteria, discarding those that do not meet them. In case of discrepancies or uncertainties regarding any review, the full text will be checked to discuss them to reach agreement or to proceed to consultation with a third reviewer (OMP).

With the remaining articles, an exhaustive reading of the full text will be performed, making a table where the excluded articles will be exposed and the reasons for this non-selection will be explained.

One reviewer will independently extract data and a second one will examine the extraction sheets in order to reach consensus and to ensure their accuracy. If there is missing data, the investigators will contact the authors of the systematic reviews, and if it is still not available, this will be stated. For each systematic review, the following variables will be registered:

- **General variables:**
  - Author and year of publication.
  - Aim of systematic review.
  - Number of primary studies.
  - Design of primary studies.
  - Number of participants.
  - Funding statement.
  - Competing interest statement.

- **Specific variables:**
  - Severe adverse events.
  - DPYD gene variants detected.
  - Fluoropyrimidine dosage.
  - Treatment regimen.

Quality of the systematic reviews

Two independent reviewers (SOT and RRM) will carry out the assessment of quality of the systematic reviews using a critical appraisal tool designed for this purpose, *A Measurement Tool to Assess Systematic Reviews 2* (AMSTAR 2).[7] In case of discrepancies in the quality ratings, a common consensus will be reached, and a third reviewer (OMP) will participate, if necessary.

AMSTAR2 quality-assessment tool consists of 16 items whose answers can be "yes", "no", or "partial yes". The overall quality is rated as high, moderate, low, and critically low.

Data synthesis

The data synthesis phase will involve summarizing the results in a table showing the descriptive characteristics of the systematic reviews included: Author and publication year, aim of the systematic review, number and design of the studies, number of participants, polymorphism, treatment, and intervention.

Both narrative findings and meta-analysis of primary study data included in the systematic reviews will be synthesized. For data appropriate for quantitative synthesis, measures of association between DPYD gene variants and toxicity will be expressed as the risk ratio (RR) and difference in means (MD), with consistency (I^2) reported by individual reviews and meta-analyses.

The following subgroup analyses will be performed if feasible: High/moderate quality systematic reviews, systematic reviews with low heterogeneity (I^2<25%) among its primary studies, cancer type, and dosage of the fluoropyrimidine.

Registration details

Systematic review registration number in PROSPERO: CRD42023401226.

Ethics and dissemination

Ethical approval was not sought for this study because the data to be collected are not linked to individuals. Findings will be presented at international conferences and published in peer reviewed journals.

Discussion

The increased risk of severe and life-threatening toxicity in patients with DPD deficiency under treatment with fluoropyrimidines has been widely studied. The majority of the studies that have been published have been summarized in an overview of systematic reviews, which was published in 2016.[1] In contrast, within the past 5 years, there has been a proliferation of systematic reviews and meta-analyses that emphasize the significance of additional variants and the potential for the coexistence of multiple mutations at various gene loci.

An overview of systematic reviews plays a role in summarizing the evidence from existing systematic reviews, providing valuable information to support evidence-based decision-making for prescribers, policy makers, and developers of clinical guidelines. The Cochrane Collaboration recommends an overview of systematic reviews to summarize the evidence of existing systematic reviews that address different outcomes for a single intervention.[8]

Therefore, this updated overview of systematic reviews aims to evaluate published systematic reviews regarding the safety of fluoropyrimidine treatment in relation to germline variations in the DPYD gene. An overview of systematic reviews design provides robust evidence[9]; however, they may also have limitations determined by the limitations of the included systematic reviews. Moderate heterogeneity is expected due to the existence of multiple genetic polymorphisms in *DPYD*, and not all of them are reported in every study. Additionally, they are likely to be studied in different types of tumors. Another limitation could be the quality of the included reviews; however, to minimize bias, a subgroup analysis will be conducted using high and moderate-quality reviews.

In conclusion, this overview of systematic reviews will contribute to the development of an improved approach to fluoropyrimidine treatment by providing reliable evidence for its wide-ranging application.

Author's contributions

SOT, OMP and RRM conceived the study, led the development of the protocol, and coordinated and integrated comments from co-authors. SFM, EFC, ACE contributed to the development of the search strategy. All authors critically revised successive drafts of the manuscript, provided important intellectual input, and approved the final version of the publication.

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Competing interests statement

None declared.

Ethics and dissemination

Ethical approval was not sought for this study since collected data is not linked to individuals. Findings will be presented at international conferences and published in a peer reviewed journal.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.farma.2023.08.009.

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