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Laboratory diagnosis for human chimerism: systematic review

Diagnóstico laboratorial para o quimerismo humano: revisão sistemática

Diagnóstico de laboratorio del quimerismo humano: revisión sistemática

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Abstract
The objective of the article was to perform a systematic review of the literature on laboratory diagnosis for human chimerism. The Medical Literature Analysis and Retrieval System Online (Medline), Public Medline or Publisher Medline (PubMed), Scientific Electronic Library (Scielo), Latin American and Caribbean Literature on Health Sciences (LILACS) databases were searched according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology. Articles were included that had as an endpoint evidencing the diagnosis for chimerism in humans, published in the period from 2005 to 2018. Articles whose endpoint was the occurrence of other types of chimerism were excluded. To compose the study, 36 articles were pre-selected, of these only 6 met the inclusion criteria given by the PRISMA method. Regarding the diagnostics used in the articles to detect chimerism, in 100% of the articles there is reference to the Polymerase Chain Reaction (PCR) combined with other techniques as the main diagnostic for detection of chimerism, such as Fluorescent in situ hybridization (FISH), Short Tandem Repeats (STR), Single Nucleotide Polymorphisms (SNPs), Small Insertions and Deletions (INDELs), Single Molecular Inversion Molecular Probes (smMIP) and TaqMan type molecular probes. These combined techniques have many advantages and are decisive for the scientific community in revealing this genetic condition. Thus, it is concluded that there are few techniques that allow a specific diagnosis for chimerism, however, the existing techniques are effective in identifying this genetic condition.
Keywords: Chimerism; Polymerase chain reaction; Systematic Review.

Resumo
O objetivo do artigo foi realizar uma revisão sistemática da literatura sobre o diagnóstico laboratorial para o quimerismo humano. Foram utilizadas buscas bibliográficas nas bases de dados Medical Literature Analysis and Retrieval System Online (Medline), Public Medline or Publisher Medline (PubMed), Scientific Electronic Library (Scielo), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS), conforme a metodologia Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Foram incluídos artigos que tinham como desfecho evidenciar o diagnóstico para o quimerismo em humanos, publicados no período de 2005 a

2018. Foram excluídos artigos cujo desfecho era a ocorrência de outros tipos de quimeras. Para compor o estudo, foram pré-selecionados 36 artigos, desses apenas 6 preencheram os critérios de inclusão dados pelo método PRISMA. Referente aos diagnósticos utilizados nos artigos para detecção do quimerismo, em 100% dos artigos há referência da Reação em Cadeia da Polimerase (PCR) combinada com outras técnicas como diagnóstico principal para detecção do quimerismo, tais como, Hibridação in situ Fluorescente (FISH), Repetição Curta in tandem (STR), Polimorfismos de Nucleotídeo Único (SNPs), Pequenas Inserções e Deleções (INDELs), Sondas de Inversão Molecular de Molécula Única (smMIP) e sondas moleculares do tipo TaqMan. Essas técnicas combinadas possuem muitas vantagens, sendo decisivas para a comunidade científica na revelação dessa condição genética. Dessa forma, conclui-se que existem poucas técnicas que possibilitam um diagnóstico específico para o quimerismo, no entanto, as técnicas existentes são eficazes para a identificação dessa condição genética.
Palavras-chave: Quimerismo; Reação em cadeia da polimerase; Revisão Sistemática.

Resumen
El objetivo del artículo fue realizar una revisión sistemática de la literatura sobre el diagnóstico de laboratorio para el quimerismo humano. Se realizaron búsquedas en las bases de datos Medical Literature Analysis and Retrieval System Online (Medline), Public Medline o Publisher Medline (PubMed), Scientific Electronic Library (Scielo), Latin American and Caribbean Literature on Health Sciences (LILACS) según la metodología Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Se incluyeron los artículos que tenían como criterio de valoración evidenciar el diagnóstico para el quimerismo en humanos, publicados en el periodo de 2005 a 2018. Se excluyeron los artículos cuyo resultado era la aparición de otros tipos de quimerismo. Para componer el estudio, se preseleccionaron 36 artículos, de los cuales sólo 6 cumplían los criterios de inclusión dados por el método PRISMA. En cuanto a los diagnósticos utilizados en los artículos para detectar el quimerismo, en el 100% de los artículos se hace referencia a la Reacción en Cadena de la Polimerasa (PCR) combinada con otras técnicas como diagnóstico principal para detectar el quimerismo, tales como Hibridación fluorescente in situ (FISH), repeticiones cortas en tándem (STR), polimorfismos de un solo nucleótido (SNP), pequeñas inserciones y deleciones (INDEL), sondas moleculares de inversión única (smMIP) y sondas moleculares de tipo TaqMan. Estas técnicas combinadas tienen muchas ventajas y son decisivas para la comunidad científica a la hora de revelar esta condición genética. Por lo tanto, concluimos que hay pocas técnicas que permitan un diagnóstico específico para el quimerismo, sin embargo, las técnicas existentes son eficaces para identificar esta condición genética.
Palabras clave: Quimerismo; Reacción en cadena de la polimerasa; Revisión Sistemática.

1. Introduction

Human chimerism is the presence of more than one population of genetically differentiated cells in an individual. This genetic condition can be acquired through transfusion or transplantation of cells from a donor to the patient, it can also be congenital, resulting from fusion of individual dizygotic embryos or by double-embryonic embryo transfer (Tozzo et al., 2021; Johnson et al., 2020).

In relation to the types of chimerisms mentioned above, the first two occur through surgical interventions, where donor cells start to circulate in the patient's body. The other two conditions occur during pregnancy, the first occurring when one embryo merges with the other in the first days of gestation, and the second occurs when one embryo receives blood from the other embryo (Koehne et al., 2018; Santurtún et al., 2017; Loriaux et al., 2011).

The report of the first case of duplicity of genetic material in humans was discovered in the 1950s. The discovery has astonished the scientific community for many years in the face of the rarity of human chimeras, whose proven incidence reached only 40 individuals worldwide until the beginning of this century (Uysal et al., 2018).

Among these 40 cases that were proven, one of great repercussion was that of Karen Keegan (Sheets et al., 2017; Ramos; Cunha, 2016; Jiménez et al., 2015; Granzen, 2014; Norton & Zehner, 2008). In 1998, in the United States, Karen suffered from kidney failure and needed a transplant. For this, molecular tests were carried out with his three children, in order to verify if one of them could be his donor. After the examinations, it was discovered that two of her three children were not hers because they had a haplotype of a different origin from her (Ramos & Cunha, 2016; Granzen, 2014).

After an extensive study, it was found that Karen's blood was composed of a cell line, while other tissues showed mixed lines, such as oral epithelium, skin and hairy roots (Sheets et al., 2017). The physicians then concluded that the different

deoxyribonucleic acids (DNA's) of the patient originated from tetragametic chimerism, that is, during pregnancy there was fusion of her with an unknown embryo, so she has traces of DNA from both embryos (Granzen, 2014; Oura et al., 2015; Yunis et al., 2007).

Thus, the purpose of the diagnosis for human chimerism is the identification of two different cell types in a single individual and requires DNA analysis. Since 1980, techniques such as Polymerase Chain Reaction (PCR) have been used which allows for the amplification of regions, such as, In Tandem Short Repetitions (STR) (Krick et al., 2018). These are repeated sequences of 3 to 7 base pairs which designate differentiated and named alleles by the number of copies of repeated sequences contained in the regions of the DNA under study (Ahci et al., 2017).

STRs are the most commonly used class of genetic polymorphisms due to rapid and rapid PCR amplification, the possibility of analyzing multiple STRs in a single reaction, high discrimination power, and relatively high mutation rates that give them high variability. These markers are used for genotyping in kinship and trait analyzes, both for individual identification and for population studies (Kristt et al., 2005; Caetano, 2009).

In view of the above, this work aims to perform a systematic review of the literature on laboratory diagnosis for human chimerism, since it is usually quite peculiar and difficult to reveal because it does not present, in the majority, phenotypic signs. Thus, this research will provide information about the laboratory diagnosis for chimerism in humans, thus contributing to a wider knowledge about the subject for the scientific community.

2. Methodology

The present study is a systematic review, conducted according to the methodology Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) as previously described by Pagotto, Bachion & Silveira (2013). To identify the articles about the subject, bibliographic searches were carried out in the databases Medical Literature Analysis and Retrieval System Online (Medline), Public Medline or Publisher Medline (PubMed), Scientific Electronic Library (Scielo) and Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS), January to June 2018, with the following search terms: human chimerism, chimerism diagnosis, PCR, chimerism, tetragametic chimerism, chimera syndrome, chimera DNA tests, chimera tests, chimera, paternity test in chimera. Terms were used in Portuguese, English, French and Spanish, in addition, manual searches were made in the bibliographical references of the articles found (Pagotto et al., 2013).

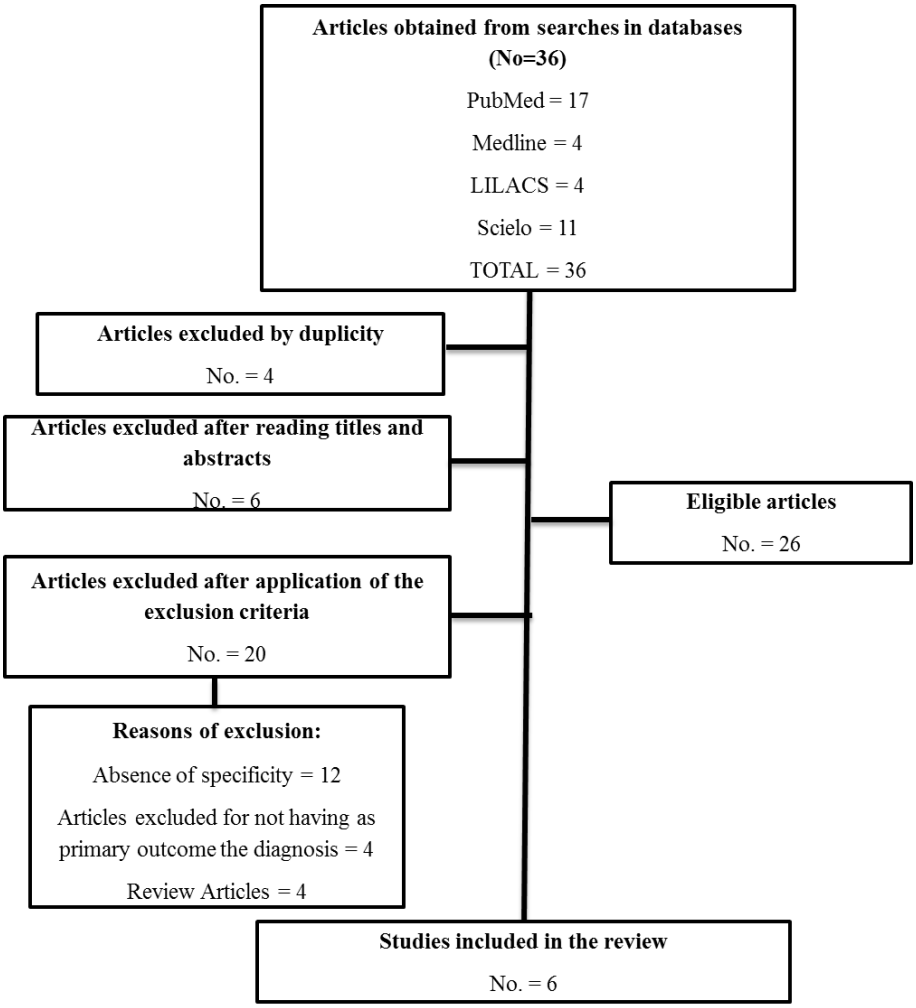
After consulting the databases and applying the search strategies, we excluded studies that showed duplication between the databases. Afterwards, all the resulting abstracts were read. In cases where the summary reading was not sufficient to establish whether the article should be included, considering the defined inclusion criteria, the article was read in its entirety to determine its eligibility. When the summary was sufficient, the articles were selected and then obtained the full version for confirmation of eligibility and inclusion in the study (Pagotto et al.,2013).

For the accuracy of the data of the articles, a table containing the following information was elaborated: title, authors, year of publication, type of study and objective. The data obtained from the articles were tabulated in Excel software (Microsoft Office, 2019). The statistical analysis was descriptive using absolute distributions.

3. Results

In total, they were found in the databases 36 publications on the topic. Duplicates were then retrieved. After reading the abstracts, 26 articles were selected for reading in full and those who did not attend to the guiding question of the research were excluded, and only those who met the inclusion criteria given by the PRISMA method were classified, obtaining a total number of 6 articles, as shown in Figure 1.

Figure 1. Flowchart for identification and selection of articles for the systematic review of human chimerism considering publications from 2005 to 2018. Data presented in absolute number. No = Number.



Source: Survey data.

In Table 1 it is possible to observe the number of articles found in the databases according to the established theme. The PubMed database contained the largest number of articles selected (66.7%) for the systematic review in question.

Table 1. Results of searches in databases according to the established theme for this systematic review on human chimerism (2018).

Databases	Publications found	Abstracts read	Articles initially included	Articles included
PubMed	17	15	13	4
Medline	4	4	2	0
LILACS	4	3	2	0
Scielo	11	10	9	2
Total	36	32	26	6
Duplicate articles in databases				4

Data presented in absolute number. Source: survey data.

The six selected articles that outlined the development of this study are arranged in Table 2, classified according to the title, author, year of publication, type of study and its objective.

Table 2. Methodological classification of articles selected for this systematic review on human chimerism (2018).

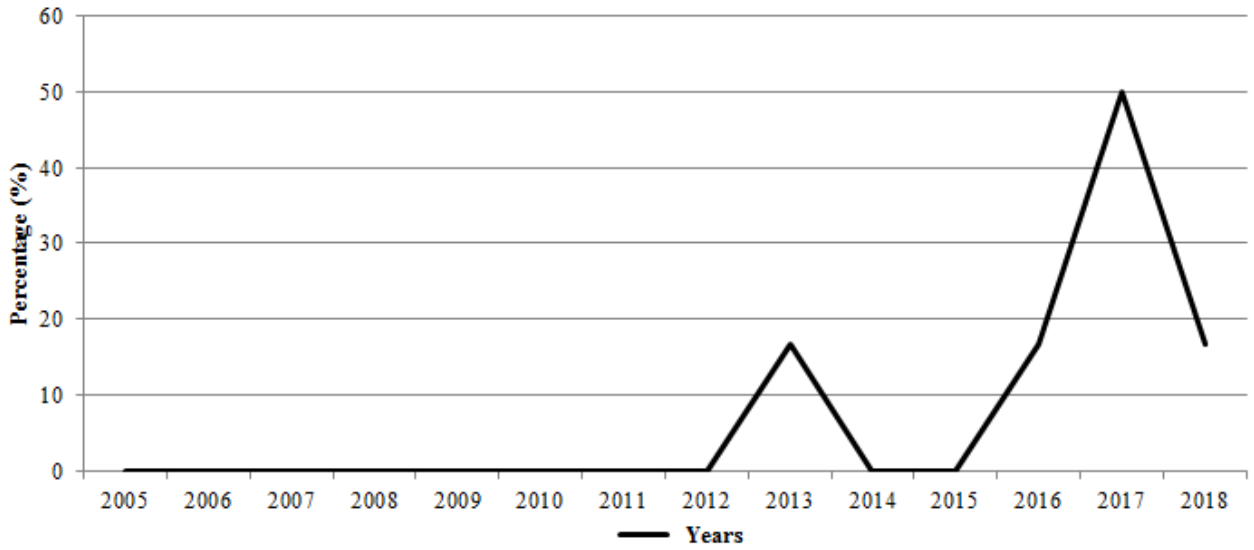
Title	Authors	Year of publication	Type of study	Objective
Ultrasensitive Detection of Chimerism by Single-Molecule Molecular Inversion Probe Capture and High-Throughput Sequencing of Copy Number Deletion Polymorphisms	Wu et al.	2018	Observational study	Demonstrate single-molecule molecular inversion probe (smMIP) capture coupled with high-throughput sequencing to identify and quantify deletion copy number variants for the purposes of chimerism analysis.
Chimerism analysis after hematopoietic cell transplantation: Guidelines from the Francophone Society of bone marrow transplantation and cellular therapy (SFGM-TC)	Dubois et al.	2017	Observational study	Propose some recommendations on methods, analysis of results and therapeutic decision in transplantation of hematopoietic stem cells for malignant or non-malignant diseases.
Relevance of Chimerism Analysis After Allogeneic Stem Cell Transplantation.	Clemente et al.	2017	Observational study	Report the experience in chimerism detection in patients following hematopoietic stem cell transplantation using a multiplex PCR system.
A semi-nested real-time PCR method to detect low chimerism percentage in small quantity of HSC transplant DNA samples	Aloisio et al.	2017	Observational study	The aim of this work was to set up a new reliable protocol useful to define low chimerism percentage when small amount of DNA input is available, especially in the case of rare blood cellular subsets.
Long-term follow-up of patients receiving allogeneic stem cell transplant for chronic lymphocytic leukaemia: mixed T-cell chimerism is associated with high relapse risk and inferior survival	Thompson et al.	2016	Observational study	Determine whether mixed T-cell chimerism post-transplant is associated with higher relapse rates and inferior survival in a large cohort of CLL patients.
The differential diagnosis of inflammatory joint disease in maternal-fetal microchimerism	Reda & Martins.	2013	Case report	This study aimed at making the differential diagnosis of joint disease in a case of genetic chimerism in a female multiparous donor from the Regional Blood Bank of Guarapuava-PR (Hemocentro Regional de Guarapuava-PR), who had had three pregnancies of male fetuses.

Abbreviations used: CLL - chronic lymphocytic leukaemia; DNA - Deoxyribonucleic Acid; HSC -Hematopoietic Stem Cell; PCR - Polymerase Chain Reaction; PR – Paraná. Source: survey data.

In relation to language, 83.3% (n = 5) are in English (Wu et al., 2018; Aloisio et al., 2017; Clemente et al.,2017; Thompson et al., 2017; Reda; Martins, 2013) and 16.6% (n = 1) in French (Dubois et al., 2017). The type of study 83.3% (n = 5) are observational studies and 16.6% (n = 1) refer to case reports.

In relation to the year of publication of the articles, it was observed that the year 2017 presented the highest number of publications, totaling 50% (n = 3) and the remaining years had at most 1 article referring to the topic discussed as illustrated in Figure 2.

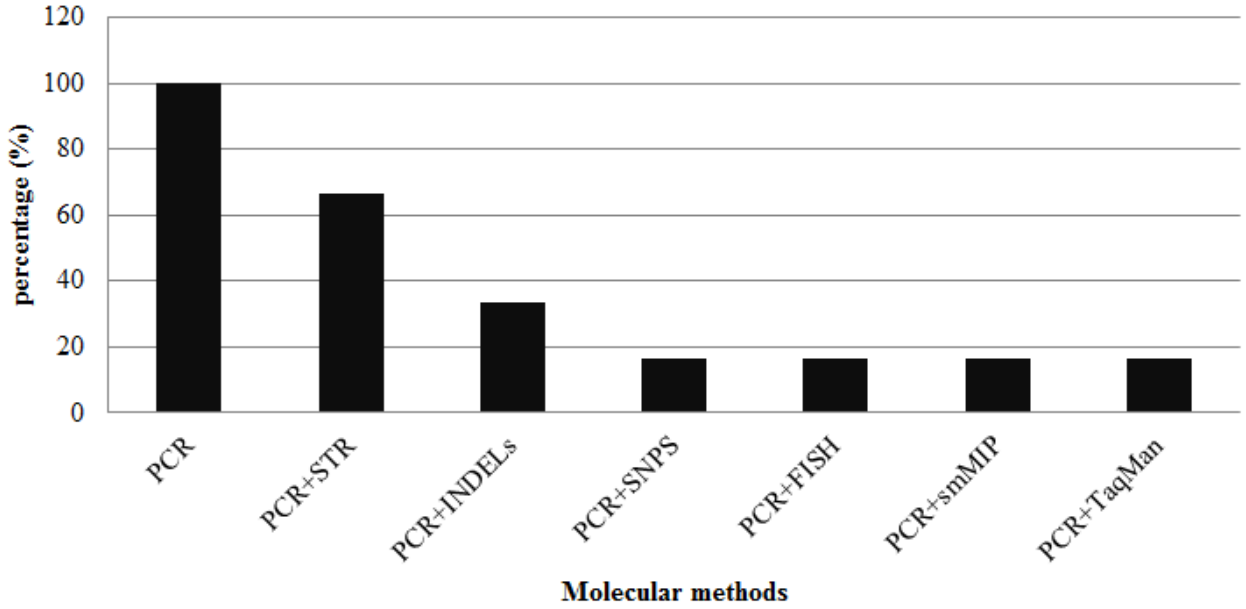
Figure 2. Distribution of the increasing percentage of published scientific articles on human chimerism according to the year of publication (2005 - 2018). Data presented as a percentage.



Source: Survey data.

Regarding the techniques for the diagnosis of human chimerism cited in the articles, in 100% there is the use of the PCR method as the main one that combined with other methods presents great efficacy for the identification of chimeric individuals. According to the articles selected, the most commonly used method in association with PCR is STR (66.6%), followed by the molecular methods Small Insertions and Deletions (INDELs) (33.3%), Single-Nucleotide Polymorphism (SNPs) (16.6%), Fluorescent In Situ Hybridization (FISH) (16.6%), Single-molecule Molecular Inversion Probes (smMIP) (16.6%) and TaqMan molecular probes (16.6%) (Figure 3).

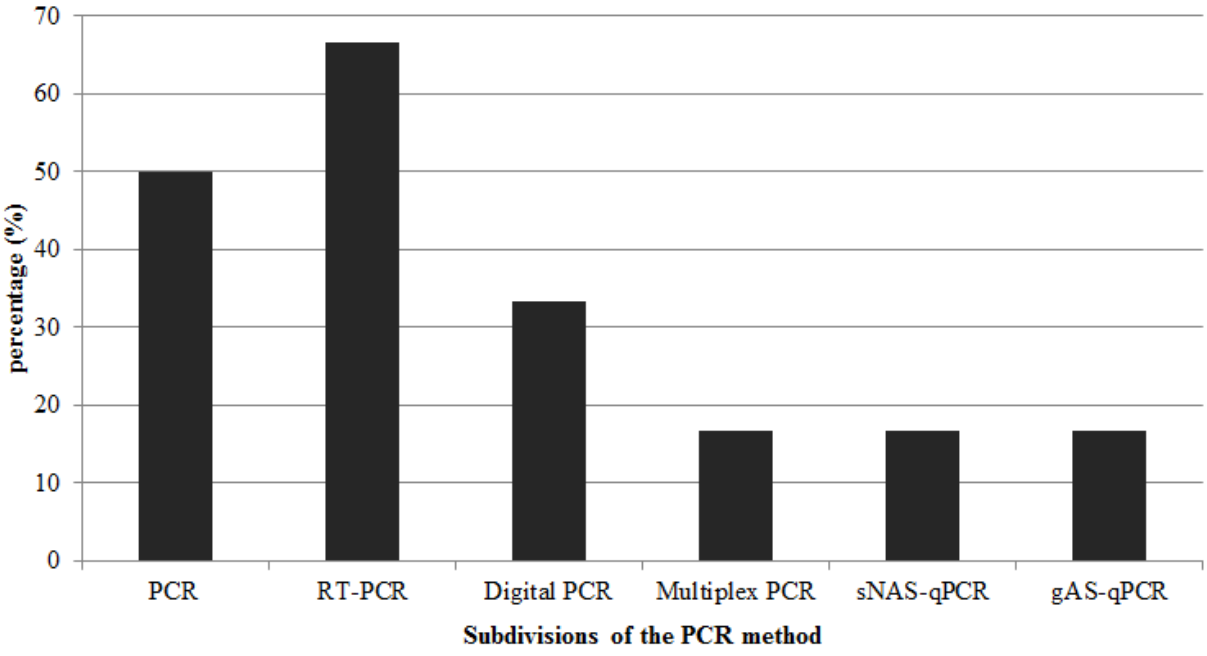
Figure 3. Percentage of molecular methods for the diagnosis of human chimerism present in the articles selected for the present systematic review on human chimerism (2018).



Data presented as a percentage. PCR - Polymerase Chain Reaction; STR - Short Tandem Repetition; INDELs - Small Insertions and Deletions; SNPs - Single-Nucleotide Polymorphism; FISH - Fluorescent In Situ Hybridization; smMIP - Single-molecule Molecular Inversion Probes; TaqMan - TaqMan molecular probes. Source: Survey data.

Some of the articles selected present technological advances in PCR methodology. In total, 66.6% reported the use of (RT-PCR), 33.3% used digital PCR, 16.6% had multiplex PCR, 16.6% had semi-nested allele-specific real-time PCR (sNAS-qPCR) method and 16.6% standard allele-specific real-time PCR (gAS-qPCR) protocol (Figure 4).

Figure 4. Percentage of the subdivisions of the PCR method present in the articles selected for the systematic review on human chimerism (2018).



Data presented as a percentage. PCR - Polymerase Chain Reaction; RT-PCR - real-time PCR; sNAS-qPCR - semi-nested allele-specific real-time PCR; gAS-qPCR - standard allele-specific real-time PCR. Source: Survey data.

4. Discussion

The present study presents the most used methods for the diagnosis of human chimerism. The results demonstrated that the most common methods for the diagnosis of chimerism are combined PCR with other protocols, such as STR, INDELs, SNPs, FISH, smMIP and TaqMan. These are not specific methods, however, they are effective for the diagnosis of chimerism.

According to the literature review, human chimerism is the presence of more than one population of genetically differentiated cells in an individual (Yu et al., 2011). Articles that met all the inclusion and exclusion criteria are presented in greater number in the English language (Aloisio et al., 2017; Clemente et al., 2017; Reda & Martins, 2013; Thompson et al., 2017; Wu et al., 2018), since the best journals and the best impact factors are in that language, so the preference for publications in that language prevails. In addition, English is considered a universal and widely accessible language.

Most articles selected for this review are observational studies, and only one case report was found because of the difficult diagnosis of this genetic condition. In addition, the volume of publications on the subject is not vast if one considers that a total number of 06 articles were obtained and the majority published in the year 2017. However, for the year 2018 the number of articles on the subject is reduced compared to the previous one, since the cut limit of the research was established until June 2018, thus making it impossible to fully collect these data in the year in question.

All works selected for systematic review emphasize PCR as the most accurate diagnosis for chimerism, as through this method a DNA sequence can be amplified millions of times, producing sufficient copies to be analyzed by other techniques. The PCR has brought enormous benefits and scientific developments such as genome sequencing, gene expression in recombinant systems, molecular genetic study, rapid paternity determination and rapid diagnosis of infectious diseases. This method is widely used in biological research and diagnostics because of its high sensitivity and specificity (Clemente et al., 2017).

In the study by Aloisio et al. (2017) we used thirty random DNA samples that were sequenced to determine the genotype through the markers SNPs and INDEL, which are used for human identification and forensic genetics. The authors report that several panels of SNPs and INDELs were selected to evaluate chimerism after the Hematopoietic Stem Cell Transplantation (HSCT) with the RT-PCR method (Aloisio et al., 2017).

According to the authors, the RT-PCR method to date is one of the most sensitive and common used to evaluate chimerism. Since chimerism evaluation represents an association between donor and recipient DNA, RT-PCR allows amplifying the amount of sample DNA to increase the precision of the test, especially during the quantification of the small percentage of chimerism. The lowest percentages of chimerism achieved with these panels range from 0.001% to 0.1% (Aloisio et al., 2017).

In addition to the above methods, the research also made a comparative sensitivity between the genetic markers sNAS-qPCR and gAS-qPCR. Significant differences were detected between these two methods in terms of sensitivity. The sNAS-qPCR has higher sensitivity, however, it is a more expensive and time-consuming procedure compared to the standard, and its use is suggested only when there is a small amount of DNA, for example, in samples of subgroups of blood cells. On the other hand, the gAS-qPCR method demonstrates a significant variability when considering the amount of chimerism obtained in a small percentage (Aloisio et al., 2017).

The study by Wu et al. (2018) did not reveal the sample size, but reports that they were obtained from bone marrow, in which all selected patients were diagnosed with chimerism through molecular or flow cytometry. The authors report that the initial methods for distinguishing chimeric cells involve the use of fluorescence probes labeling the chromosomes by the FISH method or by polymorphism markers by STR which have a sensitivity limit of about 1% (Wu et al., 2018).

The article also presents as diagnostic for chimerism the RT-PCR and digital PCR which is the third generation of PCR. Advances in digital PCR over traditional PCR are ideal for applications requiring the detection of minimal amounts of

nucleic acid samples, such as analysis of copy number variation, rare gene expression analysis, detection of a rare sequence and very sensitive absolute quantification (Wu et al., 2018).

Wu et al. (2018) also demonstrate capture of smMIP coupled with high throughput sequencing to identify and quantitate deletion copy number variants for chimerism analysis purposes. The application of smMIPs in the detection of chimerism offers significant advantages over the conventional and advanced diagnostic methods reported above. First, smMIP capture and error correction allows for the ultrasensitive detection of chimeric populations, exceeding the reported sensitivity of digital PCR based assays. Another advantage is that this technique is highly multiplexable, allowing the simultaneous integration of hundreds or even thousands of different copy number deletion locus, much more than can be examined by multiplex PCR (Wu et al., 2018).

SmMIPs still allow computational inference of genotypes from major or multiple cell populations without the need for donor-independent genotyping, as proved to be necessary by both STR and PCR. Another benefit related to this technique is that it has a favorable and promptly gradual cost benefit for several specimens of patients, facilitating high performance tests in clinical practice (Wu et al., 2018).

The article by Reda and Martins (2013) reports the case of a 49-year-old female patient with three gestations, all of them male, whose last pregnancy occurred 20 years ago. The patient was a regular donor at the Guarapuava Regional Blood Center and after 14 intermittent and continuous donations she began to complain of joint pain, especially in the lower back, right wrist and neck (Reda & Martins, 2013).

After extensive studies, she was diagnosed with maternal-fetal microchemistry once fetal DNA was identified in the patient's current. For this diagnosis, the genotyping of DNA submitted to RT-PCR was carried out using a fluorescence kit to detect chimerism, which allows a RT-PCR machine to monitor the entire reaction (Reda & Martins, 2013).

RT-PCR-based methodologies have improved the sensitivity and objectivity of the interpretation of the results. These benefits apply in different areas, such as in forensics due to the importance of quantification of human DNA and male DNA, detection of qualitative parameters such as PCR inhibitors and levels of DNA degradation, as well as analysis of the results through genotyping, all resolved through RT-PCR (Reda & Martins, 2013).

In the paper by Dubois et al. (2017) monitoring of total blood graft chimerism was performed. The authors report that several methods of molecular biology can be used to determine and quantify chimerism, among them the most commonly used markers are STRs and INDEL-type polymorphisms, by methodology and level of sensitivity. The advantages of these methodologies are represented by the great diversity of markers, the number of alleles available and the small amount of DNA needed per test. The disadvantages are the two-step analysis and the moderate sensitivity of the test (Dubois et al., 2017).

Another widely used methodology cited by the authors as being a very common method for determining and quantifying chimerism is RT-PCR. The advantages of this technique are represented by the low levels of contamination, the time of completion, the number of markers used and the sensitivity very high. However, the disadvantages are based on the large amount of DNA required, on the technical complexity of the manipulations and on the multiple steps required for its execution In addition, the authors also report that digital PCR is an effective method in the molecular diangnóstico for chimerism, with sensitivity of 0.5%. (Dubois et al., 2017).

The study by Clemente et al. (2017) reports the experience in detecting chimerism in patients after hematopoietic stem cell transplantation using the multiplex PCR system. This technique is capable of amplifying in a single reaction more than one genomic segment with specific primers. This can simplify some experiments and also be useful for the introduction of a reaction control. In their study the techniques of PCR and STR are still mentioned to monitor chimerism (Clemente et al., 2017).

All these techniques are used to detect genetic differences between receptor and donor. The two techniques cited above are the most common for the analysis of chimerism, due to its viability and high discrimination between donor and recipient. STRs are highly polymorphic genetic markers and have great ability to detect differences between individuals with variable number of replication units, which can be assessed after a PCR (Clemente et al., 2017).

Similar to the study by Clemente et al. (2017), have the article by Thompson et al. (2017). The authors analyzed the DNA of 143 patients with consecutive refractory relapses of chronic lymphocytic leukemia (CLL), transplanted between 2000 and 2012, to determine the prognostic relevance of mixed chimerism after alogenic stem cell transplantation (TACT) and the capacity of post-transplant immuno-modulation to treat relapse. The methods used to determine post-transplant chimerism were PCR and STR. There are polymorphisms in the number of consecutive repetitions of these sequences at a given locus. This polymorphism is determined in the recipient and donor before transplantation and can be used as a marker in the evaluation of post-transplant chimerism (Thompson et al., 2017).

5. Conclusion

In summary, there are few methods that allow a specific diagnosis for chimerism, however, the existing ones are effective for the identification of such genetic condition. In current research the authors report the combined PCR with other methods such as FISH, STR, SNPs, INDELs, smMIP and TaqMan as the main technique for the diagnosis of chimerism. It is important to emphasize that CRP has evolved over the years, thus facilitating the diagnosis for chimerism. These combined methodologies have many advantages and are decisive for the scientific community in revealing this genetic condition.

It was observed that the scientific production is still scarce and does not seem to demonstrate a continuous growth on the subject addressed in this systematic review. Research on this theme is accepted, as it will enable a wider knowledge about the subject. Thus, it is expected that future research will address these points, enabling the development of further studies on the subject.

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