# Fanconi anemia: Diagnostic methods and the applicability of laboratory genetics

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## ABSTRACT

Fanconi Anemia (FA) is an autosomal recessive or X-linked hereditary disease that manifests itself in the first years of life, being responsible for congenital anomalies and progressive bone marrow failure, with aplastic anemia (AA) as its main characteristic. This review article aimed to identify the diagnostic methodologies used to detect AF involving genetic, clinical and laboratory applicability. For this study, electronic scientific databases were consulted, such as the Portal States National Library of Medicine National Institutes of Health (Medline/PubMed), Web Of Science, and Scientific Electronic Library Online (SciELO). A total of 1563 scientific articles were found in English, Spanish and Portuguese, 6 of which were included for analysis. It was demonstrated that there are two chromosomal fragility tests, a molecular biology method and a DNA sequencing method, and that the degree of specificity found in the Western blot and mitomycin C (MMC) methods complement the diagnosis of AF. Diepoxybutane (DEB) differs from Western blot and MMC by being more specific in chromosomal breaks, with next generation sequencing (NGS) being the most specific and most informative as it leads to a personalized approach due to ease of access to somatic variations in tumors and changes in gene expression. Therefore, it is concluded that these methods provide greater diagnostic specificity by identifying the genetic association of SCA and somatic mosaicism, understanding the levels of severity, bringing greater agility in taking therapeutic measures.

**Keywords:** Fanconi Anemia, Aplastic Anemia, Genetic variation, Mosaicism, Research.

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## INTRODUCTION

Fanconi Anemia (FA) is an autosomal recessive hereditary disease that appears in the first years of life causing hematological abnormalities, capable of triggering aplastic anemia (AA) and can lead to progressive bone marrow failure in addition to congenital anomalies which lead to malformation or even the absence of bones in the individual (Shimamura & Alter, 2010; Crossan & Patel, 2012). SCA is described as a rare disease with heterogeneous characteristics, which can be found in several ethnic groups with a predominance of 1 in every 360 thousand births. The cause of its appearance lies in several genes responsible for triggering it, related to inheritance linked to the X chromosome, which controls the repair mechanisms of deoxyribonucleic acid (DNA) (Zen, Moraes, Rosa, Graziadio, & Paskulin, 2011).

FA is known for its specificity in causing the hematological system to collapse, due to widespread and progressive bone marrow failure with a high risk of cancer, in addition to being an invariably fatal disease with onset of clinical manifestations in the first years of life, resulting in an expected of life between 16-25 years of age or, depending on the level of severity, this expectancy can vary between 0-50 years with an average of 23 years of life (Nowzari, Jorgensen, Ta, Contreras, & Slots, 2001; Joenje & Patel, 2001). The reduction in lifespan is associated with a highly variable phenotype, which makes the diagnosis of the disease difficult, especially regarding clinical aspects (Auerbach, 2009).

Bone marrow failure can range from asymptomatic cytopenia to severe bone marrow aplasia, which is associated with complications in the hematological system resulting from a deficit in the production of blood cells (Alter, 2003). The increased risk of neoplasms in addition to hematological changes that appear throughout life leads to successive complications such as thrombocytopenia with granulocytic changes, anisocytosis, macrocytosis, thrombocytopenia, neutropenia, poikilocytosis, elevated fetal hemoglobin, elevated alpha protein and clinical manifestations that vary due to abnormal hematopoiesis (Alter, 2003; Kutler et al., 2003). Furthermore, the appearance of hyper or hypochromic skin spots, hypoplasia in the bones of the upper limbs such as the radius and thumbs, changes in the male gonads, abnormalities in the endocrine system with prevalence of hypothyroidism, changes in metabolism, dyslipidemia and syndrome are associated. metabolic (Auerbach, 2009).

Among patients, it is common for some to present a reserved phenotype, with normal progress of the skeletal system, hematological changes initially without apparent signs and little longer survival that can reach more than 30 years of age. Others, due to developing more expressive phenotypic manifestations, will present more severe abnormalities, with early appearance of bone marrow dysfunctions with a strong chance of triggering cancer, which in most cases lead to death

within the first decade of life (Crossan & Patel , 2012; Kee & D'andrea, 2012; Garaycoechea & Patel, 2014).

The clinical manifestations of SCA are grouped into four large groups: physical anomalies existing at birth; endocrinopathies and growth retardation; emergence of solid tumors and hematological abnormalities (Sagaseta de Ilurdoz, Molina, Lezáun, Valiente, & Durán, 2003). Among other abnormalities found at the beginning of their lives, skin pigmentation stands out, with café-au-lait spots and short stature found in more than 50% of cases (Alter, 2002). These spots are the result of melanin deposition (De Kerviler, Guermazi, Zagdanski, Gluckman, & Frija, 2000). In 10 to 40% of cases, one can also find the absence of the first metacarpal, hypoplastic fingers and ulna, congenital dislocation of the hip, absent or horseshoe-shaped kidneys revealed on imaging exams, malformation in the female reproductive system, hypoplasia or absence of ovary, bicornuate uterus, changes in the digestive system with imperfect folds, obstruction in the intestine and narrowing of the esophagus, duodenum and anus that make the digestive process difficult, in addition to tracheo-esophageal fistula, lip hypoplasia, microcephaly, insufficient hormone production (GH ) responsible for growth, visual defects, cardiac structural abnormalities, hearing problems, developmental delay, low birth weight, changes in insulin and glucose metabolism, in addition to a series of events that can trigger other pathologies resulting from the type of genetic inheritance (Alter, 2002; De Kerviler, Guermazi, Zagdanski, Gluckman, & Frija, 2000; Sagaseta de Ilurdoz, Molina, Lezáun, Valiente, & Durán, 2003; Auerbach, 2009; Shimamura & Alter, 2010).

The most common cause, which leads to the emergence of AF, is directly associated with gene mutations that occur through DNA repair and genomic stability (Nowzari, Jorgensen, Ta, Contreras, & Slots, 2001). Currently, the genetic characterization of the disease includes the identification of at least 19 genes that are groups that have several different mutations each. In some countries, such as Brazil, the frequency in certain ethical groups varies due to their greater occurrence (Gille et al. al., 2012). Associated with genetic characterization is pancytopenia, which can appear, in a less serious way, through somatic mosaic. This condition is characterized by genetically different somatic cells, that is, normal and altered, probably triggered by the appearance of new mutations or by spontaneous reversions arising from groups of complementations, as established in table 1 (Gregory et al., 2001).

Table 1- Complementation groups (genetic subtypes) of Fanconi Anemia.

| *SUBTYPES* | *GENES* | *FUNCTION OF PROTEINS* | *CHROMOSOME LOCATION* |
|---|---|---|---|
| *FA-A* | FANCA | It constitutes the main FA complex and performs ubiquitination of the FA-ID complex | 16q24.3 |
| *FA-B* | FANCB (FAAP95) | It constitutes the main FA complex and performs ubiquitination of the FA-ID complex | XP22.31 |
| *FA-C* | FANCC | It constitutes the main FA complex and acts on cytoplasmic functions with ubiquitination of the FA-ID complex | 9q22.3 |
| *FA-D1* | FANCD1 (BRCA2) | Doubles BRC – recruitment of RAD51 in addition to acting as a mediator of HR, activated by the FA-ID complex | 13q12-13 13q13.1 |
| *FA-D2* | FANCD2 | Monoubiquitinated FANCD2 – association with BRCA2, BRCA1 and MRE11 complex ubiquitination occurs after DNA damage | 3p25.3 |
| *FA-E* | FANCE | Formation of the AF main complex recruitment of FANCD2 direct binding to ubiquitinated FANCD2 | 6p21-22 |
| *FA-F* | FANCF | Formation of the main complex Homology with ROM - binding to nucleic acids Ubiquitination of the FA-ID complex | 11p15 |
| *FA-G* | FANCG (XRCC9) | Formation of the AF main complex Removal of the AF complex after replication Ubiquitination of the FA-ID complex | 9p13 |
| *FA-I* | FANCI (KIAA1794) | monoubiquitinated FANCI – associated with FANCD2, forms Complex downstream on the AF/BRCA pathway Ubiquitinated after DNA damage | 15q26.1 |
| *FA-J* | FANCJ (BRIP1/BACH1) | Helicase – uncoils DNA in the 5'→3' direction BRCA1 membership Helicase, activated by the FA-ID complex | 17q23.2 |
| *FA-L* | FANCL (PHF9) | WD40 repeats – stabilization of the AF complex PHD – ubiquitin ligase Associated with E3 ligase in the ubiquitination of the FA-ID complex | 2p16.1 |
| *FA-M* | FANCM (Hef/FAAP250) | Formation of the AF main complex Helicase, DNA translocase Helicase, localizes the main complex to DNA | 14q21.3 |
| *FA-N* | FANCN (PALB2) | BRCA2 association/stabilization Activated by the FA-ID complex | 16p12 |
| *DO IT* | RAD51c | Activated by the FA-ID complex | 17q23 |
| *FA-P* | SLX4, MUS312 | Activated by the FA-ID complex | 16p13.3 |
| *FA-Q* | XPF, ERCC4 | Activated not elucidated, only that it interacts with FANCP | 16p13.12 |
| *FA-R* | RAD51 | Responsible for protecting the new DNA strand | 15q15.1 |
| *FA-S* | BRCAI, BRCC1 | Cancer susceptibility protein | 17q21.31 |
| *FA-T* | UBE2T, E2 | Associated with E2 ubiquitinase, involved in the ubiquitination of the FA-ID complex | 1q32.1 |

Source: Adapted from Gurtan & D'Andrea (2006); Taniguchi & D'Andrea (2006).

Mosaicism is a genetic phenomenon of FA that results in the reversion of harmful hereditary mutations, involved in two cell populations when it is discovered that there is one within the normal range and another outside it, in the same individual (Hirschhorn, 2003). There are several types of mosaicism, in SCA the most common is the presence of a mutation in which the allele presents with emphasis on normal functioning in a range that varies from 15 to 25% of patients affected by the

syndrome and who demonstrate some type of spontaneous mosaicism. (Hirschhorn, 2003; Gregory et al., 2001).

Mosaicism is characterized through the chromosomal break test, in this test the presence of two subpopulations of lymphocytes is verified, confirmation is made when sensitivity to DNA cross-link inducing agents occurs in one of them, the other will present a condition of normality that satisfies more than 50% of the cells analyzed (Gregory et al., 2001). With the progress of the abnormalities found in the hematopoietic system, the individual will present a condition of AA responsible for bone marrow failure associated with an increase in malignant predisposition, which reduces life span, especially when clinical manifestations begin in the first years of life, making this expectation around 16 to 25 years of age. Each of the groups of genes involved carries several different mutations, which allows controlling DNA repair mechanisms (Nowzari, Jorgensen, Ta, Contreras, & Slots, 2001; Porto et al., 2011).

The most impactful clinical complications triggered by AF occur in the hematological system and massively affect patients during their fight against the disease. It is believed that at least 90% of them develop progressive medullary insufficiency that can lead to severe AA. severe in which the bone marrow stops producing quantities of blood cells, resulting from a congenital condition of AF leading to a decrease in erythrocytes, granulocytes and megakaryocytic cells found in the bone marrow. Generally, in laboratory tests carried out at birth, normal peripheral blood counts are found, and over time they tend to change and worsen, leading to pancytopenia around seven years of age due to the progressiveness of the disease. (Tulpule et al., 2010).

There is also a high percentage of patients with the potential to develop acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) that appear in the middle of their lifespan, that is, between 10 and 15 years of age, which are due to complications due to the evolution of the patient's clinical condition. MDS is a clonal hematopoietic disease that induces cytopenias, imbalance in hematopoietic cell differentiation and a high risk of progressing to AML (Hasle, 2016). AML is a typical SCA neoplasm that is characterized by an 800 times greater risk of progression when compared to the general population. This disease that occurs in the bone marrow is defined by the disordered growth in the number of immature leukocytes and low production of platelets (Alter, 2003; Rosenberg, Huang, & Alter, 2004). Typically, progressive bone marrow failure occurs when a reduction in hematimetric values in peripheral blood is noticed, which involves thrombocytopenia, leukopenia and anemia. Severity begins with excessive chromosomal breaks responsible for damaging DNA repair, apoptosis and mutations that selectively benefit the growth of progenitor cells (Mathew, 2006). With regard to AA, AML and MDS developed in patients in the first years of life, it is important to investigate the probable occurrence of AF, even if clinical manifestations resulting from the disease are not apparent (Alter, 2003).

With the advances that have occurred in recent years in technologies involving the health field, mainly diagnostic techniques and treatment of abnormalities found in the hematological system associated with hereditary factors, new methods that deal with the diagnosis of rare diseases have been widely debated. in addition to the fact that there are already methodologies capable of replacing or complementing existing ones due to the need to avoid difficulties, rework and diagnostic confusion, and that several of them are already used in different parts of the world, especially when it comes to their discoveries. Thus, the objective of this article is to analyze, through an integrative review, the diagnostic methodologies used to detect Fanconi anemia involving genetic, clinical and laboratory applicability.

## METHODOLOGY

The development of this integrative review took place through qualitative bibliographic research, which consists of a type of research capable of answering the study question in a broad way, and which includes structured qualitative techniques that cover studies related to the research topic (Prodanov, 2013). This includes the interpretation of events defined in other studies, mainly those of investigative origins that attribute important meanings in the process of detailing the results as well as being a main characteristic of a qualitative study (Pereira, Shitsuka, Parreira, & Shitsuka, 2018 ). Therefore, in order to research and interpret the results obtained by studies related to the topic, as well as the objective of this review, a search and selection of scientific articles published in electronic scientific databases such as Portal *States National Library of Medicine National Institutes of Health* ( *Medline/PubMed* ) , *Web Of Science, and Scientific Electronic Library Online* ( *SciELO* ).

As this is a topic that seeks to evaluate the main FA diagnostic methodologies of genetic characterization, studies published between 2000 and 2019 were considered eligible, as there is a scarcity of work carried out on the chosen theme. To select these studies involving the evolution of diagnostic methods for SCA, as well as medullary aplasia, the following Health Sciences Descriptors (DeCS/MeSH) in Portuguese, English and Spanish were used: erythrocyte aplasia, fanconi anemia, leukemia acute myeloid, myelodysplastic syndrome, diagnosis, genetic diversity, mosaicism, hematopoietic stem cells, diagnosis of fanconi anemia and bone marrow/ *erythrocyte aplasia, fanconi anemia, acute myeloid leukemia, myelodysplastic syndrome, diagnosis, genetic diversity, mosaicism, hematopoietic stem cells , diagnosis of fanconi anemia and bone marrow* / erythrocyte aplasia, fanconi anemia, acute myeloid leukemia, myelodysplastic syndrome, diagnosis, genetic diversity, mosaicism, hematopoietic mother cells, diagnosis of fanconi anemia and bone marrow. As exclusion criteria, theses, thesis chapters, books, book chapters, congress or conference proceedings, technical and scientific reports, as well as other study designs and gray literature were not analyzed.

Table 1 - Search strategy and results in databases.

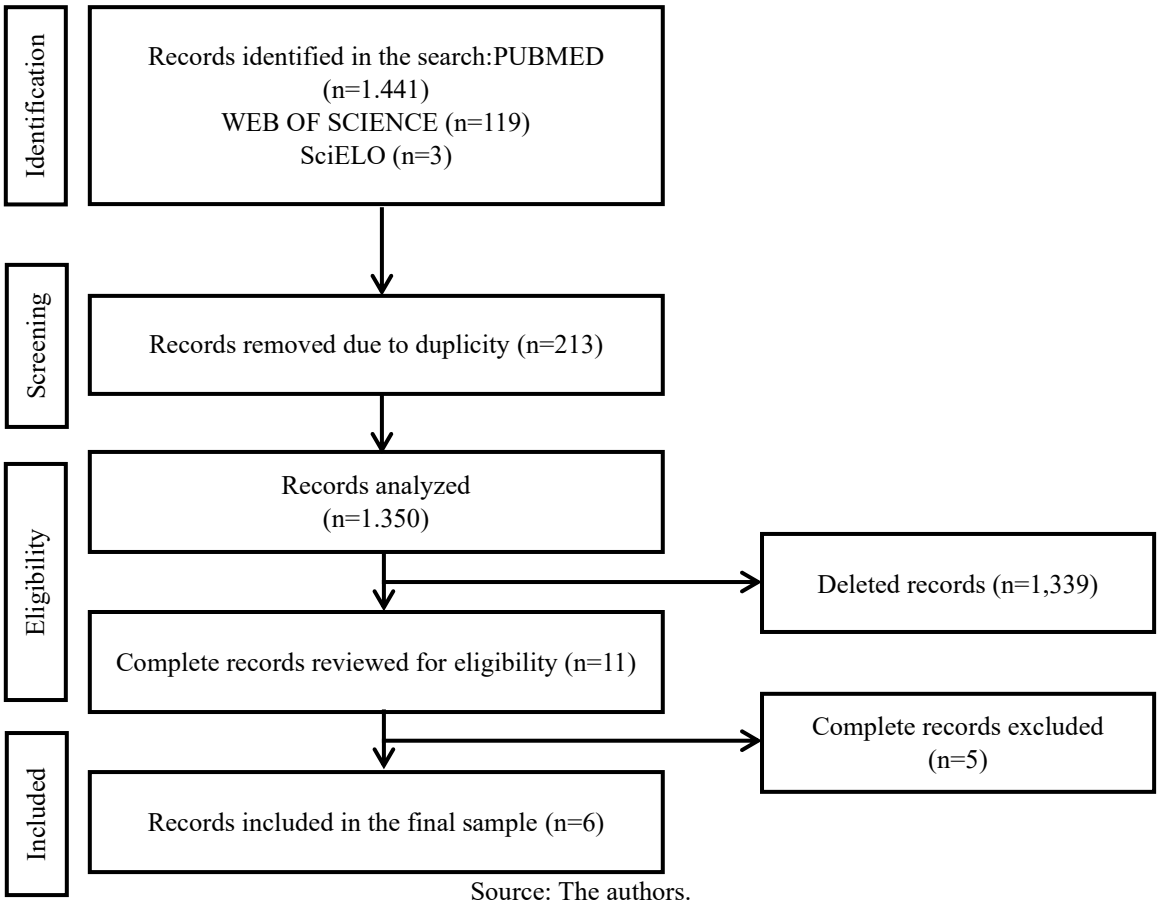
| Search date | Search strategy | | Results |
|---|---|---|---|
| **PubMED** | | | |
| 06/11/2020 | #1 | Fanconi anemia OR Diagnostic | 127,989 |
| | #two | (("*fanconi anemia* " OR " *erythrocyte aplasia* " OR " *acute myeloid leukemia* " OR " *myelodysplastic syndrome* " OR " *diagnosis* " OR " *genetic diversity* " OR " *mosaicism* " OR " *hematopoietic stem cells and bone marrow* ") AND ( *genetic* OR tic *diagnoses of fanconi anemia* )) | 1,629 |
| | #3 | #1 AND #2 Filters: text availability (Free full text and Full text); article type (Clinical Trial); article language (English, Portuguese and Spanish) and publication date (20 years). | 1,441 |
| **Web Of Science** | | | |
| 06/15/2020 | (((((((((ALL=( *fanconi anemia* )) AND ALL=( *erythrocyte aplasia* )) OR ALL=( *acute myeloid leukemia* )) OR ALL=( *myelodysplastic syndrome* )) OR ALL=( *diagnosis* )) OR ALL =( *genetic diversity* )) OR ALL=( *mosaicism* )) OR ALL=( *hematopoietic stem cells and bone marrow* )) OR ALL=( *genetic* )) AND ALL=( *diagnosis of fanconi anemia* ) | | 119 |
| | Filters applied: Years of publication (2000-2019); Meso creation topics (all); Researcher profiles (All); Document types ( *Article* ); *Web of Science* Categories (All); Publication titles (All); Editor (All). | | |
| **SciELO** | | | |
| 06/15/2020 | Fanconi anemia AND Diagnosis | | 3 |
| | Filters applied: All Open Access (Open Access and Gold), Year (2000-2019), Author Name (All), Subject Area (All), Document Type (Article), Publication stage (Final), Language ( Spanish ). | | |

Source: The authors.

At the end of the search stage, a total of 1,563 articles were found in the respective databases. These studies were analyzed by two reviewers and, before that, 213 duplicate studies were removed, leaving 1,350 articles for analysis and selection based on reading titles and abstracts.

After applying the eligibility criteria, 1,339 articles were excluded as they were not relevant to the study question, leaving 11 of them to be read in full. After reading, 6 studies were included in the final sample as shown in figure 1.

Figure 1 - Identification of studies through databases and records.




Source: The authors.

## RESULTS AND DISCUSSION

In the end, with 6 studies included, relevant information was extracted and organized in a table to answer the study question. The selected studies are investigative, descriptive and observational, described and ordered according to table 2.

Table 2 – Characterization of the studies.

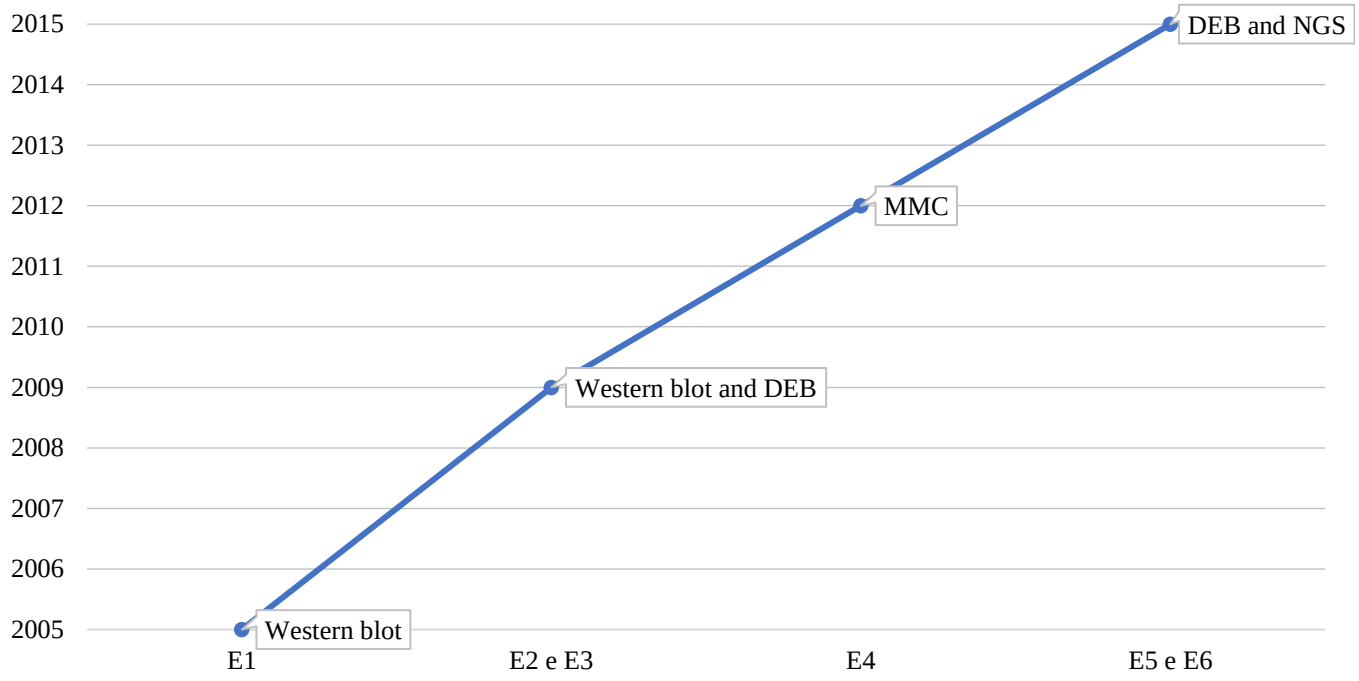
| ID* | Authors | Method | goal | Key Takeaways |
|---|---|---|---|---|
| E1 | Soulier et al., 2005 | Use of the Western blot technique. | To analyze the AF/BRCA pathway in 53 patients with AF by FANCD2 immunoblotting in chromosomal breakage tests. | A highly recurrent pattern of somatic abnormalities related to chromosomal translocations and FANCD2 mono-ubiquitination detected in peripheral blood lymphocytes was evidenced. |
| E2 | Pilonetto et al., 2009 | Use of the Western blot technique in the diagnosis of AF. | To determine the effectiveness of the method in diagnosing 84 Brazilian patients with AF, and with positive results in the diepoxybutane test, and 98 healthy controls. | All 98 healthy controls were FANCD2S+/FANCD2L + and negative in the DEB test. The 84 DEB+ patients were classified into three phenotypic classes. The majority of patients (77/84; 91.7%), including 68 probands and their respective 9 siblings, were FANCD2S+/FANCD2L-. |
| E3 | Auerbach, 2009 | Laboratory study of chromosomal breakage induced by diepoxybutane (DEB). | Identify pre-anemic cases, as well as patients with aplastic anemia or leukemia who do not present physical findings characteristic of clinical diagnosis. | The availability of such tests has revealed an increasing number of individuals affected with AF who, by clinical criteria, appear to have idiopathic aplastic anemia and appear phenotypically normal. |
| E4 | Oostra, Nieuwint, Joenje, & de Winter, 2012 | Cytogenetic analysis of chromosome breakage induced by Mitomycin C (MMC) in whole blood cultures. | Provide a detailed laboratory protocol for accurate assessment of the diagnosis of AF. | The test reliably differentiates between AF and non-AF blood samples, including non-AF patients with aplastic anemia, Nijmegen break syndrome, Roberts syndrome, Baller-Gerold syndrome, VACTERL, and other thrombus and erythropenia syndromes, as these conditions do not have elevated cell fractions in the G2 phase. |
| E5 | Aslan, Ameziane, & De Winter, 2015 | Next generation sequencing (NGS) analysis . | To analyze, using NGS, a case of AF with inconclusive signs in a negative chromosomal breakage test. | Confirmation of AF diagnosis through NGS, this being the first molecularly diagnosed using this method. |
| E6 | Auerbach, 2015 | Application of chromosomal breakage test induced by diepoxybutane (DEB). | Describe the application through a protocol in order to rule out the prenatal diagnosis of AF based on a peripheral blood sample. | DEB is considered the most recommended in the prenatal diagnosis of SCA as it has greater sensitivity and specificity, resulting in lower false-positive and false-negative rates. |

*ID = Identification.
Source: The authors.

The studies presented in table 2 are characterized by articles that explore genetic methods as a relevant factor in the diagnosis of SCA by representing, through analyzes and gnomic tests involving chromosomes, the main discoveries of genes as well as the specificity and high sensitivity of each one. regarding its association with the signs and symptoms of the syndrome found in studies that serve to confirm the diagnosis of SCA in the first years of life. With the analysis of the studies and

their characterization, described in table 2, the methods covered by the studies (E1, E2, E3, E4, E5 and E6) are represented in graph 1, as well as the evolution and adoption of the more specific ones in the diagnosis of AF over time.

Graph 1 – Characterization of methods identified by studies




Source: The authors.

From the analysis of graph 1, it is possible to infer that the studies mentioned used four methods in the diagnostic evaluation in order to define the genomic clinical decision-making of AF, such as Western blot techniques, chromosomal instability induced by diepoxybutane ( DEB) , mitomycin C (MMC) and next generation sequencing ( NGS). All of these tools are used in hypersensitivity with chromosomal breaks under the clastogenic effect of blood cells and in gene sequencing, being identified as genetic advances that strengthen the genetic investigation of the syndrome in addition to other factors involving chromosomal instability and acquired aplastic anemia.

According to Auerbach (2015), the diagnosis of AF cannot be based solely on clinical manifestations due to the considerable overlap of the phenotype and the genetic variability that means that the available genetic methods have specificities in decision-making when diagnosing AF. This even implies disposal during the diagnostic investigation period. Because Williams et al. (2014), state that currently the cross-link sensitivity test to rule out FA and the telomere length test to rule out other syndromes such as Dyskeratosis Congenita (CD) should be part of the investigation before treating acquired aplastic anemia as they are considered standard tests that assist in diagnosis. Oostra, Nieuwint, Joenje, & de Winter (2012), warn that, as it is a cancer-prone chromosomal instability

disorder, the typical cellular phenotype of AF can also be determined using whole blood cultures (T lymphocytes) stimulated by phytohemagglutinin. However, for the diagnosis of AF, the test is not 100% specific, which requires other, more specific methodologies in its determination.

Soulier et al. (2005), state that the use of Western blot allows the overexposure of almost imperceptible bands capable of highlighting residual levels of the FA-D2 protein, and that it is possible to classify patients with AF included in this protein group. And who also analyzed patients with AF through clinical data, chromosomal breakage tests and immunoblots of the FANCD2 protein, finding that the majority of them demonstrate abnormal patterns of the FANCD2 protein in peripheral blood lymphocytes, confirming the specific defect of the AF/ BRCA. Specifically, a single small isoform of FANCD2 (FANCD2-S) but no large isoform (FANCD2-L) was detected in primary fibroblasts that showed normal FANCD2 patterns but positive chromosomal breakage tests, suggesting somatic mosaicism. Furthermore, FANCD2 protein immunoblot patterns allow determination of the level at which the AF/BRCA pathway is disrupted by detecting a single short, non-ubiquitinated FANCD2 isoform classifying patients as “AF core”.

Based on the information provided by E1 and E2 and analyzed in graph 1, it is possible to see that the Western blot technique appears as one of the most adopted methods in the period from 2005 to 2009 and that, according to Pilonetto et al. (2009), the results using this method involving molecular biology corroborate global data in which core complex complementation groups present great genetic variety for FA, including mutations in any of the genes that encode the central complex of proteins (FANCA , B, C, E, F, G, L and M). And they may also belong to other complementation groups to be identified and may be associated with the central complex of the FANCD2 protein. Soulier et al. (2005), highlight that the investigation by immunoblotting of the FANCD2 protein in fibroblasts due to the specificity of the Western blot, allowing the separation of antigens by electrophoresis, facilitates the monoubiquitination of this protein and may indicate situations of AF reversal. Therefore, when the FANCD2 protein in fibroblasts is monobiquitinated to normal levels, chromosomal breakage tests in fibroblasts can be performed if necessary to confirm AF and abnormalities in the AF/BRCA2 pathway, or should be investigated by immunoblotting and/or molecular analysis in order to detect functional reversal of AF in addition to classifying carriers as “unidentified downstream group”. This allows the confirmation of the diagnosis, detection of potential and classification of patients with SCA, facilitating the determination of the complementation group and identification of the mutations that constitute the syndrome through molecular analysis and the presence of somatic mosaicism.

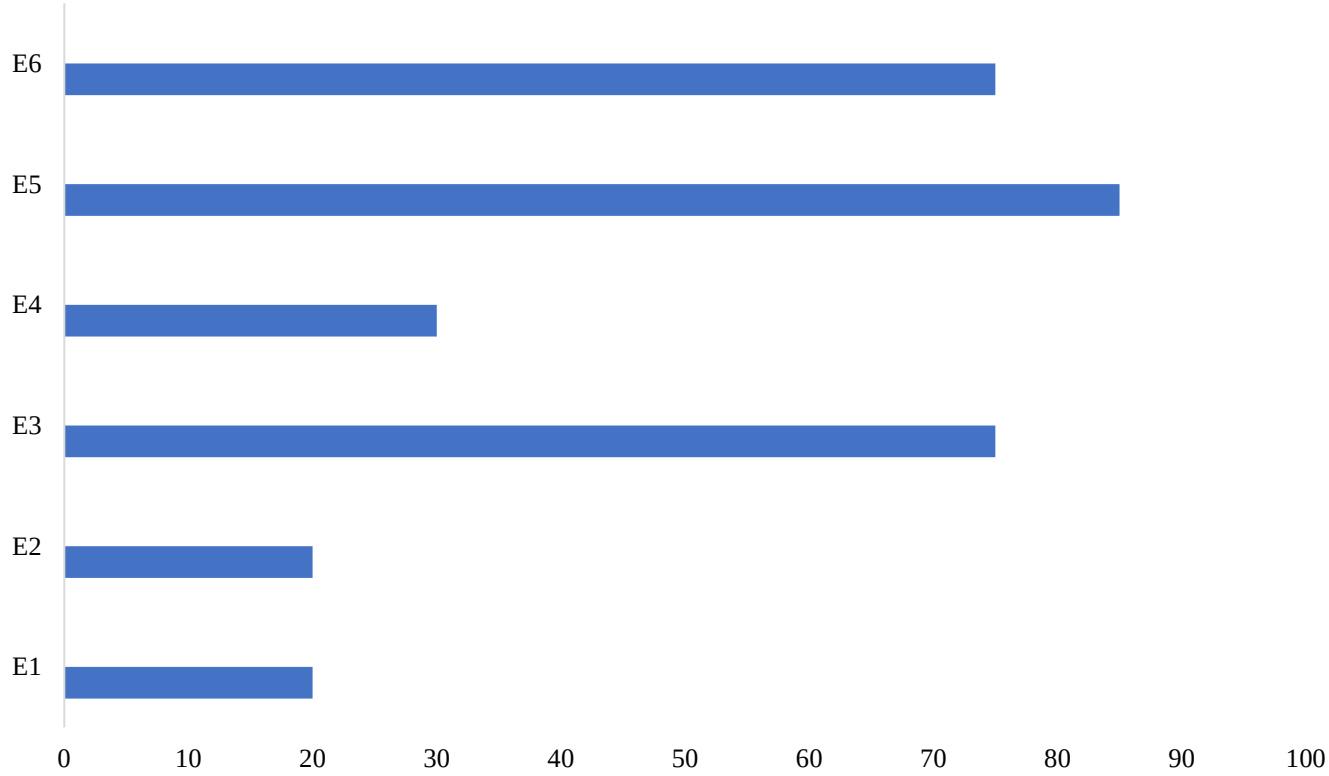
The information described by E3 and E4 highlights the adoption of DEB and MMC as new methods in the period from 2009 to 2012, which, according to Auerbach (2009), the hypersensitivity of the cells comprising AF to the clastogenic effect (chromosome breakdown) of the agents Cross-

linking provides a reliable cellular marker for diagnosis. Because DEB as well as MMC are the most used agents for the diagnosis of AF, since the great experience with MMC and DEB tests demonstrates the sensitivity, specificity and reproducibility of the results in patients who present congenital malformations associated with AF, and who the tests, mainly carried out with DEB, indicate that there is a great genetic variability when it involves chromosomal breakage, even though there is little overlap in patients with SCA in the number of breaks per cell or in the number of breaks per aberrant cell, somatic mosaicism can be configured and that in Tests performed with DEB will reveal two populations of peripheral blood lymphocytes stimulated by phytohemagglutinin where one demonstrated an AF phenotype with chromatid breaks and exchanges and the other normal. And even though there is currently no generally accepted level of aberrant cells at which an individual would be considered mosaic, about 10% of SCA patients have 50% or less aberrant cells.

According to Oostra, Nieuwint, Joenje, & de Winter (2012), although the MMC is an extremely sensitive test for FA cells due to the chromosome breaking effect of cross-linking agents, it can be omitted if a proband belongs to a ethnic population with a high frequency of carriers for a specific mutation of the AF gene. Therefore, demonstration of this mutation in the proband would be diagnostic of FA. However, the result may not provide information about possible mosaicism , which means that DEB is routinely used to diagnose AF.

As seen in graph 1, it can be seen that since 2009, DEB continues to be the most indicated in the diagnosis of SCA because according to Auerbach (2015), its specificity in identifying the patient's complementation group, as well as the pathogenic variants involved, make this method the best option in diagnosis due to the extensive phenotypic heterogeneity of FA, including the heterogeneity in the degree of sensitivity to cross-linking agents in some rare cases, and the phenotypic overlap with this large number of other genes that, with the adoption of other methods such as NGS addressed by E5, can further favor the diagnosis of fanconi syndrome, especially in postnatal diagnosis. Aslan, Ameziane, & De Winter, (2015), state that the use of NGS in the study of DNA, isolated by standard protocols, allows the identification of almost all genetic subtypes of FA (including the FANCA subtype) since in some conditions , the chromosomal breakage test can be inconclusive and confirmation of the diagnosis of FA at the DNA level is crucial by NGS as seen in graph 2, which points out in E5 the NGS as being the most specific compared to the others.

Graph 2 – Specificities of the methods identified in the studies.




Source: The authors.

The specificity of each method was highlighted in order to find in the studies included for analysis the potential of each one based on the results indicated, and according to what is presented in graph 2, DEB thus appears in second place with greater qualitative predominance in the studies, but different from what is presented by studies E1 and E2, DEB appears as the most updated when analyzing its characterization and period of use according to table 2, mainly in what involves E1 and E2. DEB has a different specificity in relation to Western blot and MMC, capable of providing results with higher levels of confidence. According to Auerbach (2015), hypersensitivity to the clastogenic effect caused by DEB in DNA cross-linking agents provides a unique marker for the diagnosis of AF, with a cellular characteristic capable of identifying whether the patient is considered pre-anemic, as well as if it has signs of aplastic anemia and leukemias that may or may not result in classic physical stigmata associated with SCA , which is not seen when using the Western blot method in studies E1 and E2 and the MMC in E4 since it has the ability to demonstrate residual levels of AF proteins. This is due to the variety of chemical agents used to test the sensitivity of DNA cross-links, being the preferred test for diagnosing Fanconi syndrome as it has the highest sensitivity and specificity, while other agents have higher rates of test results. false positives and false negatives.

According to Aslan, Ameziane, & De Winter, (2015), given the clinical characteristics of AF, methods such as NGS can replace studies of chromosomal breakage induced by DEB, as there is evidence that in the existence of a negative chromosomal breakage test, NGS would be the best

method used. Well, according to Bettoni et al. (2017), this occurs because NGS is an informative tool to guide cancer treatment and conduct a personalized approach in oncology due to the ease of access to somatic variations in tumors and changes in gene expression, which make it possible to refine diagnosis and predict the immune suppression response to medications, something that cannot be predicted in studies of DEB-induced chromosome breakage. Furthermore, NGS-based diagnosis is improved by developing a simple DNA integrity assessment assay that can be used to estimate levels of fragmentation and modification of DNA extracted from samples and establish integrity parameters to optimize preparation and demonstrate the implications of sequencing samples considered to be of low quality, which makes their specificity closer to the highest possible.

Thus, regarding the types of diagnostic methodologies used in the detection of AF involving genetic, clinical and laboratory applicability, NGS and DEB stand out as being the most relevant in the diagnosis of AF. Furthermore, the analysis of the studies (E1, E2, E3, E4, E5 and E6) allows us to conclude that the advancement of tools in genetic and molecular diagnosis over this time allowed specificity in finding and classifying related genes as well as complementation groups and genetic subtypes directly associated with Fanconi syndrome and aplastic anemia, which is one of the most common symptoms found in patients with SCA.

## FINAL CONSIDERATIONS

The description of FA, in the past, established the diagnosis based on family history and, later, it was characterized as a rare form of aplastic anemia, which has always been considered the most common cause of progressive bone marrow failure in patients with the syndrome. Despite the low incidence of the disease in different parts of the world, over the years, efforts have always been made to improve the diagnosis, with the main focus being the search for the quality and specificity of the methods used for this purpose. In this context, this work addressed the importance of methodologies with genetic applicability used in the diagnosis and monitoring of SCA with the purpose of verifying the available methods not only currently used to detect the disease, but also with regard to the use of sequencing technology. of DNA to better characterize it.

Over the years, there has been an intense technological evolution followed by investments in research that have led to the development of new methods and techniques with the aim of increasing the level of specificity of the diagnostic methodologies addressed in this review, capable of delivering good results and thus reducing the chances uncertainty in the diagnosis of AF. As a result, four types of diagnostic methods were found, such as Western blot, DEB, MMC and NGS, belonging to the genetic techniques routinely used in the discovery of genes associated with syndromes like this. It was evident that the degree of specificity found in each method satisfactorily complements the diagnosis of SCA, increasing the understanding of the disease, allowing us to discover the types of

genes and complementation groups involved in the symptoms of the disease, which makes it possible to characterize it more clearly. the functional abnormalities of organs, tissues and the hematopoietic system, being able to verify the progression of bone marrow failure that substantially interfere with the patient's life expectancy.

It was identified that the DEB diagnostic method has an extremely important difference capable of differentiating itself in relation to Western blot and MMC as it is more specific in chromosomal breaks in AF, in addition to being considered the gold standard in the discovery of the disease. However, even with the specificity that DEB has in reducing false-positive and false-negative test results as much as possible, it has become clear that NGS is more specific than DEB as it is capable of performing parallel sequencing of fragments of DNA without confirmation of the existence of false-positive and false-negative tests. Thus, although molecular tests have their specificities, they take into account an important difference, which is to identify related genes resulting from chromosomal instability triggered by SCA, as well as to differentiate somatic variations through ease of access, making it possible to refine the diagnosis and predict adverse responses to genetic counseling.

Although the research carried out in this review highlights the genetic methodologies used for this purpose, the absence of more studies involving genomic and statistical analyzes related to AF in terms of the discovery of new genes through other techniques in molecular biology in different parts of the world limits the results of this study as the scarcity of further research involving the genes involved in fanconi syndrome in the last two decades tends to hide relevant information associated with the prevalence of AF in various parts of the world, mainly with the discovery of new genetic technologies used in other pathologies that could also be used in further studies involving AF.

Therefore, it is essential that more studies associated with hematological disorders involving AF and AA using cytogenetics are widely developed, as this is the most valuable method due to its greater specificity in diagnosing the disease when compared to other methods. It is important to highlight the importance of all other diagnostic methods mentioned throughout this review aiming to assess and monitor the level of severity, providing clinical information as it is an autosomal recessive and invariably fatal hereditary disease. Because as DNA sequencing technology advances, the goal will be faster, more accurate sequencing with lower error rates.

Considering the topic in question, especially regarding its complexity in discovering new genes, it is essential to carry out studies that enable the discovery of new diagnostic methodologies with a focus on genetic applicability that aim to investigate with more specificity the heredity patterns between the different members of the family as well as the mechanisms of action that the types of genes associated with AF carry out to manifest the disease in order to provide greater clarity when associating the types of genes that are precursors to AF.

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