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REVIEW

DIAGNOSIS OF CHROMOBLASTOMYCOSIS: AN HISTORICAL REVIEW

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ABSTRACT

Chromoblastomycosis is a skin infection caused by dematiaceous fungi, characterized by a verrucous plaque on the limbs. It mainly affects rural workers in tropical countries. The purpose of this review is to identify how the diagnostic methods used in the propaedeutic of chromoblastomycosis emerged and were developed. The MeSH terms "chromoblastomycosis" or "chromomycosis" or "verrucous dermatitis" and "diagnosis" were used to search articles indexed in MEDLINE and LILACS databases. The description of a first-time-used method in diagnosing chromoblastomycosis or modifications and innovations in an existing technique was the criteria used to deem the article eligible. The first methods described in diagnosing chromoblastomycosis were histopathological examination and culture, which characterizes and defines the disease in the early 20th century. Subsequently, they were described as direct microscopic examination, fine needle aspiration for cytology, electron microscopy, serology, molecular tests, scintigraphy, nuclear magnetic resonance and dermoscopy. Tests based on the direct identification of the fungus through biopsy, culture, or direct microscopy are the oldest and more employed methods for diagnosing chromoblastomycosis. The polymerase chain reaction was introduced in the last few decades and is a promising technique. Dermoscopy of chromoblastomycosis shows blackish red dots and white and pink areas along with scaling. Other techniques, such as serology and skin testing for delayed-type hypersensitivity, have not been incorporated into clinical practice.

KEY WORDS: Chromoblastomycosis; diagnosis; review.

INTRODUCTION

Chromoblastomycosis is a disease that causes verrucous lesions on the skin of individuals who work with soil and plants, it is caused by fungi that appear brown when viewed under a microscope. For this reason, they are called dematiaceous or melanized fungi. Chromoblastomycosis mainly affects rural workers in tropical countries (Brito & Bittencourt, 2018).

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The disease manifests clinically as nodular, verrucous, plaque, tumoral, cicatricial or atrophic lesions (Queiroz-Telles et al., 2017) (Figure 1).

Chromoblastomycosis is an infection of the skin and subcutaneous tissue that contains muriform cells (McGinnis, 1983). The term chromoblastomycosis was first applied in 1922 by Terra et al. to distinguish this mycosis from other diseases that clinically presented as verrucous infections (Terra et al., 1922). In 1935, Moore and Almeida proposed replacing the term chromoblastomycosis with chromomycosis to prevent muriform cells from being confused with yeasts (Moore & Almeida, 1935). However, several authors have expanded the use of the term to characterize various infections caused by dematiaceous fungi that do not have muriform cells. To correct this problem Ajello et al. coined the term phaeohyphomycosis in 1974 (Ajello et al., 1974). And in 1983, McGinnis defined chromoblastomycosis as a chronic infection of the skin and subcutaneous tissue which contains muriform cells (also known as sclerotic bodies) and histopathologically shows pseudoepitheliomatous hyperplasia with the formation of microabscesses. The author also suggests to abandon the term chromomycosis in order to avoid confusion with cases of phaeohyphomycosis (McGinnis, 1983).

Currently, there are three types of tests to diagnose the disease: direct microscopy, culture, and skin biopsy. And, in recently published clinical cases researchers have used molecular tests. In this article, we build a timeline of when these tests started to be used, then the discoveries about them were combined to the prior knowledge in medical mycology. The purpose of this review is to build an historical outline and evaluate the evolution of the diagnosis of chromoblastomycosis through articles which described for the first time a diagnostic method in patients with chromoblastomycosis, or that addressed innovations in the technique.

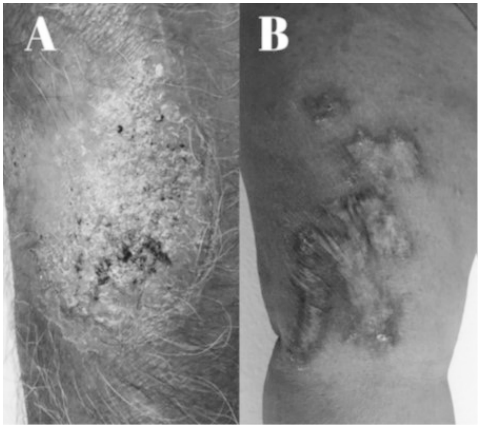

Figure 1. Lesions of chromoblastomycosis. A - Verrucous plaque on the forearm. B – Atrophic lesion on the arm.

MATERIAL AND METHODS

The research questions on this review were: what tests are used to diagnose chromoblastomycosis, when they appeared, and how they have evolved. Articles were located using the MeSH terms "chromoblastomycosis" or "chromomycosis" or "dermatitis verrucosa" and diagnosis" in MEDLINE and LILACS databases by two reviewers. Additional literature was consulted from the references of the articles found in the initial search. Two independent reviewers performed the data analysis and, the inclusion or exclusion of records was done by consensus. Studies describing the first-time-used method in diagnosing chromoblastomycosis or modifications and innovations in existing techniques, written in any language, published up to the date of the survey (March 4, 2022) were also included. Articles in which the complete text were unavailable and that did not address the diagnosis of chromoblastomycosis were excluded, as well as studies exclusively on animals. The search displayed 654 publications, and 22 duplicate articles were excluded. In total, 52 articles were included.

Figure 2 shows the flow diagram with the search strategy of this integrative review.

Figure 2. Flow diagrama with the search strategy of the review

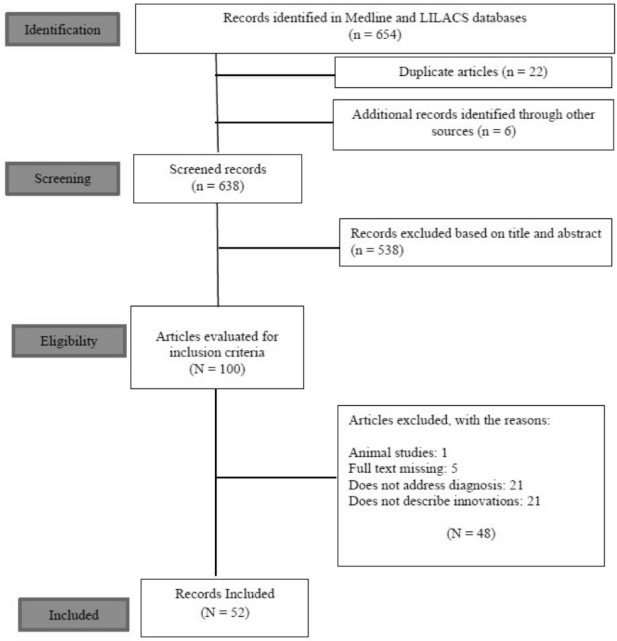

Figure 2. Flow diagram with the search strategy of the review

RESULTS

The first methods described when diagnosing chromoblastomycosis were histopathological examination and culture, which characterized and defined the disease in the early 20th century. Subsequently, direct microscopy, fine needle aspiration, electron microscopy, serology, molecular testing, scintigraphy, nuclear magnetic resonance, and dermoscopy were delineated.

Histopathology

The diagnosis of chromoblastomycosis was initially clinical, with different names including dermatitis verrucosa. The identification of melanized fungi as the cause of the disease occurred in the early twentieth century, through skin biopsy. The first method used in diagnosing chromoblastomycosis was the histopathological examination in 1911, when Pedroso and Gomes found brown spherical cells, which corresponds to current muriform cells, in the skin biopsy from four Brazilian patients with verrucous dermatitis. However, these findings were only published in 1920 (Pedroso & Gomes, 1920).

The first description of the histopathological findings of chromoblastomycosis was done in 1915 by Medlar and Lane in a patient from Boston: granulomatous inflammatory process in the dermis containing brown fungal elements (muriform cells or sclerotic bodies, in the form of "copper coins") (Medlar, 1915; Lane, 1915). In 1930, Fonseca and Leão depicted marked epidermal hyperplasia (pseudoepitheliomatosa hyperplasia) in Brazilian patients (Fonseca & Leão, 1930). In 1933, Wilson et al. published a report of verrucous lesion on the foot´s dorsum of a Texas patient and the clinical and histopathological characteristics of the Texan case was compared with the Brazilian and Bostonian cases. Abscesses or granulomas that may contain giant cells in the dermis, and epidermal hyperplasia, which can be exuberant were found. In addition to fungi with rounded shapes colored dark brown, septated, and with no budding, with different morphology from *Blastomyces* sp, differentiating chromoblastomycosis from blastomycosis. The researchers also pointed out that there was no involvement of internal organs (Wilson et al., 1933).

A histochemical test for glycogen and mucin, based on the liberation of aldehyde groups and subsequent demonstration of the latter by a reduced silver method, has been described by Gomori in 1946 (Gomori, 1946). In this first report, the Gomori technique was not applied to visualize fungi in human tissue. In 1955, Grocott modified Gomori' s technique to demonstrate fungi in tissues (Grocott, 1955). Fungi are sharply delineated in black with the methenamine-silver nitrate procedure, as shown in figure 3.

A survey of many different types of fungal infections revealed the general usefulness of the periodic acid-Schiff (PAS) reaction in mycologic histopathology in 1950. For diagnostic purposes, the organisms are stained with various shades of red (Kligman & Mescon, 1950).

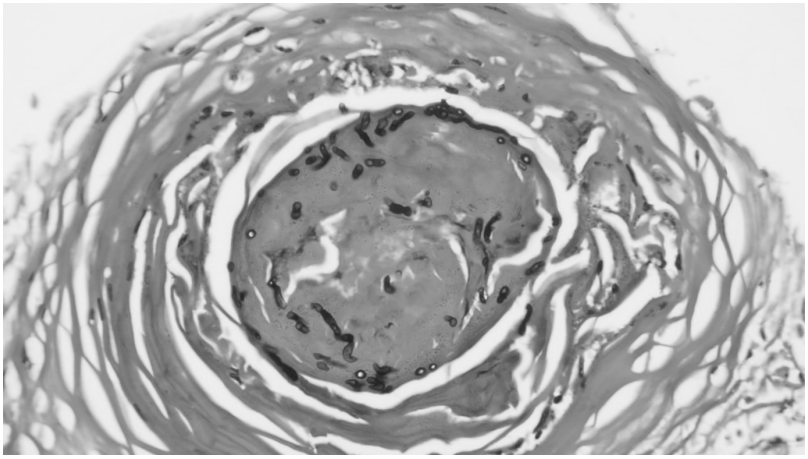

Figure 3. The Grocott-Gomori methenamine-silver nitrate stain shows darkly pigmented fungal elements (horizontal section of skin, 400x magnification).

Culture

In 1914, Rudolph isolated fungi from six Brazilian patients with warty lesions on lower limbs, inoculating them in animals. The macroscopic aspect of the culture was black and velvety. And the fungal morphology was similar to the ones currently described for the species *Fonsecaea pedrosoi*. The disease was known as "fig tree". This was the first publication on chromoblastomycosis which has defined it as a specific entity, with identification of an etiological agent (Rudolph, 1914).

In 1933, Wilson et al. described the culture as black and lumpy in a patient from Texas. They described the formation of conidia chains and the phialides aspect of the fungus, identified as *Phialophora verrucosa*, as well as in the case of Boston, reported in 1915 by Medlar and Lane. The authors noted that the characteristics of micromorphology were different from Brazilian patients' culture, whose agent at the time was called *Acrotheca pedrosoi* (Wilson et al., 1933).

Havyatt suggested in 1957 to curette the lesion until there was bleeding for a quickly chromoblastomycosis diagnosis. He did not use any culture medium containing carbohydrates but instead he used distilled water. According to the author, after three to five days, blackish steel-gray nodular fungal growth was detected (Havyatt, 1957).

In 1972, Borelli described a technique to increase the sensitivity of the culture which was using several tubes, discarding old scales-crusts, and keeping the temperature below 35°C. Because thermotherapy was one form of treatment for the disease and the growth of the fungus would be sensitive to heat (Borelli, 1972).

Direct microscopy

In 1957, Borelli described a rapid diagnostic method that consisted in using direct microscopy of the cutaneous lesion in chromoblastomycosis. The scales were treated with xylol and Canadian balm and then analyzed under the x10 objective, followed by the x40. The author considered that if these tests were negative for the fungus it could exclude the diagnosis of chromoblastomycosis (Borelli, 1957).

In a study published in 1973 by Zaias and Rebell, muriform cells were found under direct microscopy in 100% of cases when the scales are collected from black dots present in the cutaneous lesions of chromoblastomycosis. When present, these dots represent the best place to collect the sample to tests (Zaias & Rebell, 1973).

Miranda and Silva collected scales from deep mycosis lesions using vinyl tape. They were successful in visualizing muriform cells in 11 of 12 cases of chromoblastomycosis. In this research, the authors compared two different methods in which obtained material for direct microscopy analysis. The collection with adhesive tape showed a sensitivity of 91.6% and a specificity of 100%, whereas the conventional technique showed a sensitivity and specificity of 100% (Miranda & Silva, 2005).

In 2015, a case of chromoblastomycosis was reported in a patient who had been treated with an intralesional injection of corticosteroid (triamcinolone) due to the initial clinical hypothesis of hypertrophic lichen planus. Under direct microscopy, large quantities of septate and branched hyphae were observed together with muriform cells. Generally, no large numbers of fungi are seen, and it is even more uncommon to find hyphae and muriform cells in the same field. The authors hypothesized that intralesional injection of triamcinolone could have suppressed the local immune response and allowed for greater reproduction of the agent (Saxena et al., 2015).

Additional methods and stains

Electron microscopy was used on punch biopsy specimen obtained from a lesion of chromoblastomycosis for the first time in 1980. Abundant and easily identifiable muriform cells were found with ovoid shape, a diameter of 10μm, multilamellar and electron-dense cell wall, and typical transverse septation. Organisms were observed both free in tissue and the cytoplasm of histiocytes and giant cells. Cells with pigmented electron-dense material are more resistant to degenerative processes (Rosen et al., 1980).

Immunohistochemically, heat shock protein (HSP) 27 was positively identified in *F. pedrosoi* in a case from chromoblastomycosis (Bayerl et al., 1998).

Through an immunohistochemical study in 2004, *F. pedrosoi* antigens were identified in macrophages, Langerhans cells and dermal dendrocytes in the skin of patients with chromoblastomycosis (Sotto et al., 2004).

Galectin-3 expression was shown by immunohistochemical staining and decreased significantly in diseases with epidermal hyperplasia including seborrheic keratosis, keratoacanthoma, verruca vulgaris, condyloma acuminatum, and chromoblastomycosis (Jiang et al., 2017).

Zielh-Neelsen and Wade-Fite stains proved to be useful in identifying muriform cells when not seen with hematoxylin and eosin, in a study with 4 patients published in 2007 (Lokuhetty et al., 2007).

Fluorescein-labeled chitinase staining exhibited poor sensitivity (16.7%) in detecting muriform cells of chromoblastomycosis compared to periodic acid-Schiff, Gomori's methenamine silver and hematoxilin-eosin stains (Shao et al., 2020).

The cytology of the material aspirated with a fine needle (FNAC) in patients with chromoblastomycosis allowed the identification of muriform cells, erythrocytes and leukocytes, according to data published in 1998 (Sauer & Jebsen, 1998).

Serology

For the first time in 1966, diagnosing chromoblastomycosis by serology was evaluated using the precipitation test. The precipitin reaction was positive in 12 out of 13 patients with chromoblastomycosis. Moreover, it was negative in 22 healthy control patients (Buckley & Murray, 1966).

In 1988, the results of two serology studies in patients with chromoblastomycosis caused by *C. carrionii* were published. The double immunodiffusion technique was reactive in 8 of 13 patients (Villalba & Yegres, 1988). And counterimmunoelectrophoresis was positive in 13 of 15 participants, and it was negative in 3 healthy control (Villalba, 1988).

Five of the 25 patients (20%) with chromoblastomycosis had detectable antineutrophil cytoplasmic antibodies (c-ANCA), but this test is not specific to the infection (Galperin et al., 1996).

In 2003 and 2004, Vidal et al. evaluated the diagnostic accuracy of several serological techniques in patients with positive cultures for *F. pedrosoi*. The control group consisted of participants with sporotrichosis, cutaneous leishmaniasis, and blood donors. Double immunodiffusion (DID) showed a sensitivity of 84% and specificity of 80%. Counterimmunoelectrophoresis (CIE) had a sensitivity of 77% and a specificity of 85%. And the immunoenzymatic test (ELISA) resulted in 68% sensitivity and 87% specificity (Vidal et al.,

2003). The accuracy of immunoblotting was evaluated with two fractions of the *F. pedrosoi* antigen. Immunoblotting with a 54 kDa antigen showed a sensitivity of 99% and a specificity of 98%. Immunoblotting with an antigen of 66 kDa had a sensitivity of 70% and a specificity of 85% (Vidal et al., 2004).

Skin testing for delayed-type hypersensitivity (intradermal test)

The first study using *Fonsecaea pedrosoi* metabolic antigen preparations (chromomycin) for diagnosing chromoblastomycosis due to cutaneous hyperreactivity were carried out in 1969 (Oliveira, 1969). The technique was reproduced in 2014. After three days of growth on Sabouraud dextrose agar, 1g of the culture was removed, grounded, and inoculated into 100mL of Smith's medium, only filtering with a sterile filter after 15 days. Using the Elisa technique, an association was also observed between the diameter of the skin test induration and the levels of IgG antibodies, and the severity of the lesions caused by *F. pedrosoi* (Azevedo et al., 2014).

An evaluation of the specificity of the skin testing for delayed-type hypersensitivity (intradermal test) for the diagnosis of chromoblastomycosis was published before, in 1979. Testing with chromomycin produced from the culture filtrate of *F. pedrosoi* was positive in 8 patients with chromoblastomycosis. It was negative in healthy controls as well as in patients with other mycoses (sporotrichosis and tinea barbae) (Iwatsu et. al, 1979).

In 1982, the intradermal test was found to be species (or perhaps genus) specific. Testing prepared with the culture filtrate of *F. pedrosoi* was positive in 5 out of 6 patients with *F. pedrosoi* infection. It was negative in healthy people, as well as in patients with *Phialophora verrucosa, Exophiala jeanselmi* and *Exophiala dermatitidis* (at the time, *Wangiella dermatitidis*) (Iwatsu et al., 1982).

In 2008, a study was published about evaluating the accuracy of the intradermal test to diagnose chromoblastomycosis. The sample consisted of 194 individuals; 20 patients with chromoblastomycosis, a control group with 86 healthy individuals, and a second control group with 88 patients with other infectious diseases. Intradermal injection of chromomycin was performed on the anterior aspect of the participants' right forearm. After 48 hours, the hardening area was analyzed, and a diameter greater than 5 mm was considered a positive test. When comparing the chromoblastomycosis patients´ group and the healthy individuals' group, the test had a sensitivity of 90% and a specificity of 98.8%. When compared to the control group of patients with other infectious diseases, the test had a sensitivity of 90% and specificity of 100% (Marques et al., 2008).

Diagnostic imaging exams

Gallium-67 scintigraphy was described in a patient with subcutaneous nodules and disseminated verrucous lesions in 1989. Direct microscopy of the subcutaneous nodules showed muriform cells. Gallium citrate accumulated in all subcutaneous nodules and in an enlarged lymph node proved to be useful in assessing the extent of the disease (Sato et al., 1989).

In 2009, a case of chromoblastomycosis was reported that presented itself as a mass on the back of the hand. Its nuclear magnetic resonance showed an unspecific image suggestive of granuloma composed of larger nodules and smaller child nodules (Bahk et al., 2009).

Dermoscopy of a chromoblastomycosis lesion was first described in 2017. Red-black spots were found, which correspond to hemorrhage and fungal elements in the stratum corneum. Yellowish areas that correlate with granulomas in the dermis were also seen (Subhadarshani & Yadav, 2017).

In nodular chromoblastomycosis, white scales and yellow crusts were observed on the periphery of the lesion. In the center, there were polymorphic vessels and a white net. White, pink and yellow amorphous areas were also found (Jayasree et al., 2019).

In reflectance confocal microscopy (RCM), bright white spherical bodies inside round cells were found, likely to correspond to the melanosomes inside muriform cells. The muriform cells were better identified by the special 488-nm filter on RCM (Borges et al., 2021).

Giraldelli et al found small rounded hyperreflective bodies in the epidermis on *in vivo* reflectance confocal microscopy examination suggested the transepidermal elimination of muriform cells. High-frequency ultrasound with Doppler shows epidermal thickening, hyperechogenic round structures, dermal fibrosis, and increased vascularization on the same lesion (Giraldelli et al., 2022).

Figure 4 illustrates muriform cells seen by *in vivo* reflectance confocal microscopy.

Molecular tests

In 1992, a symposium article was published discussing the sequencing of the 18S ribosomal DNA subunit of dematiaceous fungi, including *Exophiala* sp and *Phialophora* sp (McGinnis et al., 1992). In 1995 the sequencing of the ribosomal DNA of *Cladosporium* sp and *Rhinocladiella* sp species was published. (Masclaux et al., 1995; Spatafora et al., 1995).

The mitochondrial DNA sequencing of *Phialophora verrucosa* was analyzed in 1997 and it was compared to other chromoblastomycosis agents, such as *C. carrionii* and *F. pedrosoi* (Yamagishi et al., 1997).

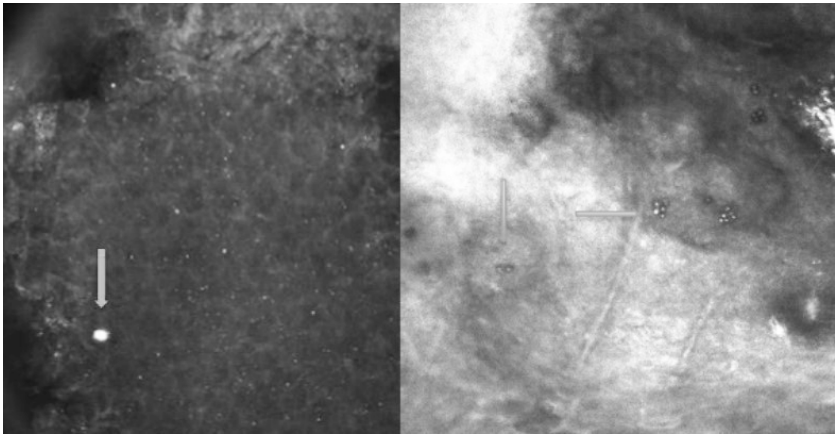

Figure 4. Reflectance confocal microscopy (500x500µm) at the level of epidermis small round hyperreflective bodies on the left, by the 785nm filter (left arrow) and bright white spherical bodies inside round cells by the 785nm filter (right arrows), on the right. VivaScope 1500.

In 1998 the DNA restriction fragment length polymorphism (RFLP) technique, obtained by treating DNA with a restriction enzyme, was used for sequencing ribosomal DNA and the spacer region or internal transcribed space (ITS) of *Fonsecaea* spp. It found a significant variation between the species. In addition, the species defined by morphology did not always coincide with the molecular testing one (Attili et al., 1998).

In 1999, the RAPD (Random Amplified Polymorphic DNA) technique was used to analyze the genetic diversity of pathogenic dematiaceous fungi. A high degree of polymorphism was found among different species and a low degree among strains of the same species. The high similarity between *F. pedrosoi* and *F. compacta* led the authors to suggest that it was not appropriate to consider them distinct species (Caligiorne et al., 1999).

A test for molecular identification and rapid detection of *F. pedrosoi* was developed in 2007 to assist in the chromoblastomycosis laboratory diagnosing, using PCR-duplex with specific primers for *F. pedrosoi,* and universal primers for fungi. This analysis identified 62 of the 64 samples of *F. pedrosoi,* including those that could not be identified in the morphological studies. The two unidentified samples can be considered different species of *F. pedrosoi* or even they may belong to another genus. Genotyping was also performed with the RAPD and RFLP techniques. Also, the authors found great genetic variability among the strains of *F. pedrosoi*, even when they belonged to the same patient (Andrade et al., 2007).

Loop-mediated isothermal amplification (LAMP) is a method capable of amplifying DNA with high specificity. Its use in chromoblastomycosis was described in 2010 with the identification of *F. pedrosoi, F. monophora,* and *F. nubica.* Amplification products can be detected by visual assessment of turbidity, electrophoresis, or with naked eye, which facilitates the interpretation of the results (Sun et al., 2010).

In a 2011 publication rolling circle amplification (RCA) distinguished *Fonsecaea* species from each other and from other members of the ITS sequence of the Chaetothyriales order (internal spacers transcribed from ribosomal DNA). 38 *Fonsecaea* isolates were studied, including 17 *F. pedrosoi*, 13 *F. monophora,* and 8 *F. nubica*. Thirty isolates were obtained from the patients with chromoblastomycosis, two from brain infection, one from an animal ear, and five from the environment (Najafzadeh et al., 2011).

Singh et al examined using molecular methods and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), the diversity of melanized fungi (MF) isolated from patients from India, including two cases of chromoblastomycosis caused by *F. monophora* (Singh et al., 2017).

High resolution melting analysis was able to distinguish *F. pedrosoi, F. monophora,* and *F. nubica* from each other, in clinical isolates from patients with chromoblastomycosis (Shi et al., 2018).

Reverse line blot (RLB) is a gene amplification technique. PCR-RLB hybridization assay was developed to identify the four agents of chromoblastomycosis (*F. pedrosoi, F. monophora, F. nubica* and *Phialophora verrucosa*) it showed 100% specificity with no cross hybridization and it was able to identify all tested strains (Najafzadeh et al., 2018).

Novel specific primers using variations in the centromere microtubule binding gene (CBF5) discriminate pathogenic members of the genus *Fonsecaea* in clinical samples (Schneider et al., 2019).

Fourier Transform Infrared Spectroscopy (FTIR) proved to be a fast and inexpensive alternative for the identification of chromoblastomycosis agents at genera level (Heidrich et al., 2021)

In figure 5, the results are presented in chronological order with their respective references, through a snake-shaped timeline.

DISCUSSION

Diagnosing chromoblastomycosis was previously based on the clinical appearance of the skin lesions and it received various names, including verrucous dermatitis. The identification of melanized fungi as the cause of the disease occurred in the beginning of the 20th century, through skin biopsy in 1911 and then through culture in 1914 (Queiroz-Telles et al., 2017).

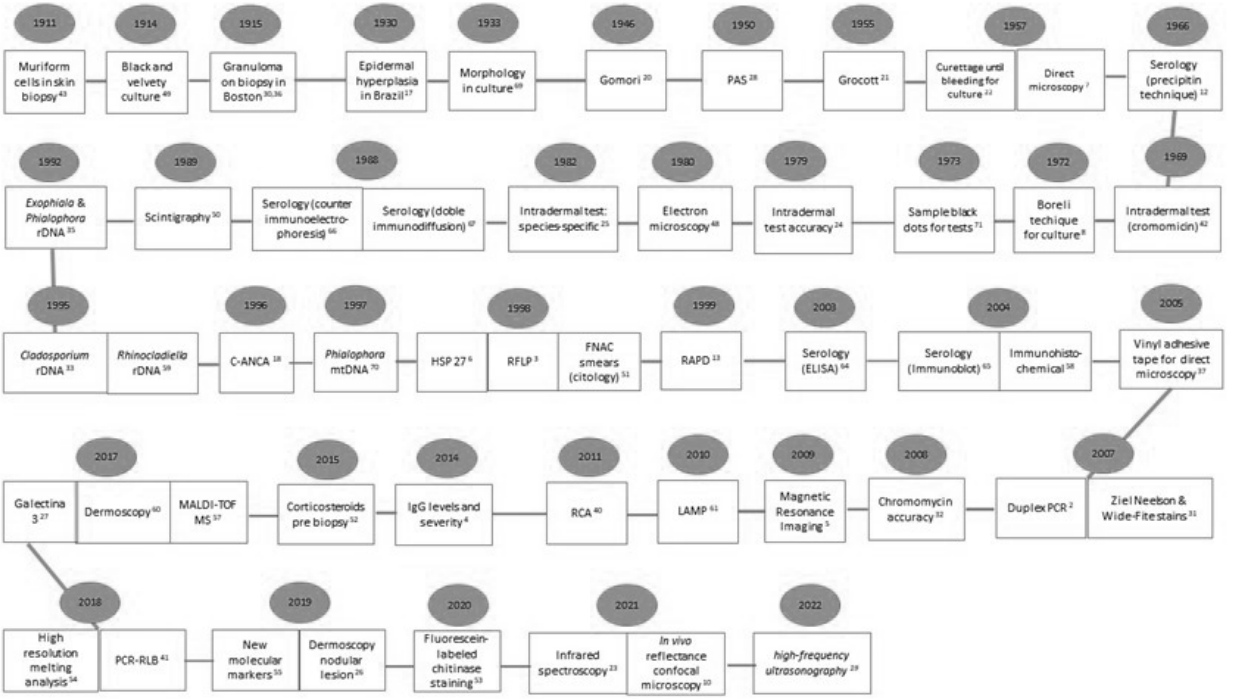

Figure 5. Snake-shaped timeline showing the emergence and the development of tests for chromoblastomycosis diagnosis, with superscript references.

During a histopathological examination, rounded brown fungal elements (muriform cells) should be found, stained with hematoxylin-eosin, periodic acid Schiff, Gomori-Grocott, and also using dyes to identify the mycobacteria (Lokuhetty et al., 2007). There is pseudoepitheliomatous hyperplasia in the epidermis, and inflammatory process in the dermis with granulomas or microabscesses (Pradhan et al., 2007).

The first published cases of chromoblastomycosis in patients from Brazil and from the United States were compared in 1933. The authors found epidermal hyperplasia in all cases (called pseudoepitheliomatous in the Brazilian cases due to its similarity to neoplasia), an inflammatory process in the dermis (granuloma with giant cells or microabscesses), and muriform cells. Thus, for the first time the classic histopathological findings of this disease were defined (Wilson et al., 1933).

The transepidermal elimination of fungi is described in pathological examinations through studies published in the recent decades (Pires et al., 2013; Weedon et al., 2013). However, the presence of fungal elements in the epidermis was presumed much earlier, when the direct mycological examination was described at the first time (Borelli, 1957), and when black spots on the surface were indicated as the best place for collection of material for microscopic examination in the 70's (Zaias & Rebell, 1973).

Electron microscopy of cultured chromoblastomycosis-causing fungi was performed for the first time in 1973, showing the intercellular septa in the species *F. pedrosoi, P. verrucosa,* and *C. carrionii* (Cooper et al., 1973). However, the use of electron microscopy on a skin biopsy sample which was obtained by the punch technique was done seven years after (Rosen et al.,1980). Studies with electron microscopy published later on, have emphasized aspects of the pathogenesis of the disease. Mainly it discusses about the ultrastructural characterization of melanosomes and their role in the resistance of melanized fungi to oxidative stress factors (Silva et al., 2002; Sun et al., 2011; Zhang et al., 2013).

Through fine needle aspiration puncture, it is possible to perform a cytological examination in search of muriform cells. It was used for the first time in 1998 and it was reproduced in 2013. The number of fungal elements is small in chronic verrucous lesions. The technique is most useful on recent injuries (Chavan & Reddy, 2013; Sauer & Jebsen, 1998).

Publications have demonstrated the potential of serological tests for diagnosing chromoblastomycosis. However, they are not commercially available and they are used for research purposes only. According to the technique used in the literature, sensitivity and specificity varied with values above 70% for ELISA and immunoblot and lower values for counterimmunoelectrophoresis (Azevedo et al., 2014; Buckley & Murray, 1966; Villalba, 1988; Villalba & Yegres, 1988; Vidal et al., 2003; Vidal et al., 2004).

Dermoscopy of a chromoblastomycosis lesion was first described in 2017. Red-black spots and yellowish areas were found. These images were observed again in a Brazilian patient in 2018. Dermoscopy is a useful test in the differential diagnosis of squamous skin lesions. The finding of red-black spots suggests the presence of fungal elements in the epidermis. Thus, it can guide the best sample collection site for direct examination, culture and histopathology which increases the chances of finding muriform cells (Borges et al., 2018; Subhadarshani & Yadav, 2017).

Nuclear magnetic resonance imaging was used in a case of chromoblastomycosis which presented as a mass on the back of the hand. The images suggested granuloma and the conditions that causes subcutaneous nodular-cystic lesions, which can suggest hemangioma, lymphangioma, chondroma, fibroma, and neurofibroma as a differential diagnosis (Bahk et al., 2009).

The first genetic study of chromoblastomycosis agents was published in 1992. Ribosomal DNA sequencing of chromoblastomycosis agents occurred a few years later (McGinnis et al., 1992; Spatafora et al., 1995; Yamagishi et al., 1997).

The identification of fungi of the genus *Fonsecaea* in isolates from patients with chromoblastomycosis by the polymerase chain reaction (PCR) technique took place in 2007 (Andrade et al., 2007). Subsequently, other molecular techniques began being used to identify chromoblastomycosis agents: isothermal amplification, AFLP (amplified fragment length polymorphism), and rolling circle amplification (RCA) (Najafzadeh et al., 2011; Sun et al., 2010).

Initially, the morphological aspect of the fungus in microculture was used to identify species. *Fonsecaea compacta* was first described in 1935 (under the name *Hormodendrum compactum*) and *Cladophialophora carrionii* in 1954 (under the name *Cladosporium carrionii*). Over the years, new etiological agents were discovered. From the 2000s onwards, the species were reclassified with the study of the genetic sequence of fungi, mainly ribosomal DNA (Brito & Bittencourt, 2018; Krzyściak et al., 2014; Querioz-Telles et al., 2017).

Currently, the identification of fungi at the genus and species level occurs through the identification of highly variable sequences in the ribosomal DNA known as ITS (internal transcribed space), which are more specific than the micromorphological aspect of the fungi. Several species of the *Fonsecaea genus* (*F. pedrosoi* and *F. nubica*), for example, have similar morphology in microculture but can be differentiated by molecular studies (Attili et al., 1998; Najafzadeh et al., 2010).

Initially employed in phylogenetic studies, the molecular tests have been increasingly used in case series throughout recent years. Although no studies are evaluating the accuracy of PCR for the diagnosis of chromoblastomycosis, the identification of the species may indicate differences in prognosis according to the agents of the disease (Coelho et al., 2018; Rojas et al., 2015; Shi et al., 2015).

Identifying dematiaceous fungi through culture, direct microscopy, or histopathological examination has defined chromoblastomycosis as a nosological entity in the early 20th century and it remains a widely well used diagnostic method. The identification of the etiologic agent at the species level, through molecular tests, has become frequent in case series and it can contribute to predict the disease's prognosis. Serology and intradermal tests showed adequate sensitivity and specificity however these tests did not leave the research laboratories to be applied in daily practices. Electron microscopy, with potential application in chromoblastomycosis diagnosis through the identification of muriform cells in the tissue, has also been used only in research centers for a better understanding of the pathogenesis of the disease. Confocal reflectance microscopy is a potential non-invasive tool for the identification of muriform cells. The findings of nuclear magnetic resonance and scintigraphy were described in isolated clinical cases, but they did not revealed specific findings. Further studies are needed to define a dermoscopic pattern of chromoblastomycosis. We recommend more frequent use of direct microscopy in daily practices as it is a sensitive and specific exam, easy to perform, with low cost and with quick result for the diagnosis of chromoblastomycosis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

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