# Familial Amyloid Polyneuropathy: A Proposal for an Epidemiological Study Through the Creation of a Virtual Platform

## Polineuropatia Amiloidótica Familiar: Uma proposta de Estudo Epidemiológico por meio da Criação de uma Plataforma Virtual

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**ABSTRACT**

Amyloidosis are characterized by mutations in the gene coding for transthyretin (TTR), located on chromosome 18. TTR is a set of four 127-aminoacid polypeptides structured as homo-tetrameric protein of 56 kDa with a secondary ß sheet structure. It plays the role of thyroxin (T4) carrier, and has a binding domain for retinol (vitamin A). It is synthesized in the liver, although a small quantity is also produced by the choroid plexus, and retinal cells. Mutations of this gene result in loss of tetramer stability. Insoluble amyloid fibrils (AF) are formed and deposited in tissues and organs. The abnormal aggregation of TTR protein trigger several syndromes, such as familial amyloid polyneuropathy (FAP-TTR), cardiomyopathies (CMP), and senile systemic amyloidosis (SSA). It is estimated there are 5,000 to 10,000 cases of FAP-TTR globally.

**Objective:** The study intends to develop an online platform for the diagnosis of FAP-TTR. The aim is to facilitate the diagnosis process and promote a tool for epidemiological study.

**Methods:** The project was based on a literature review featuring clinical and epidemiological evidence for the development of a practical platform (applied research).

**Results:** It was elaborated a platform containing a questionnaire to allow a more dynamic, cheaper, and efficient operation, mediated by a better characterization of the disease to enable its early diagnosis.

**Conclusion:** The platform might become a valuable resource for the characterization, diagnosis, and future epidemiological study of FAP-TTR.

**Keywords:** Amyloidosis. Mutation. Transthyretin. Polyneuropathy. Epidemiology

**RESUMO**

As amiloidoses se caracterizam por mutações no gene codificante da transtirretina (TTR) no cromossomo 18. A proteína TTR compõe-se de uma corrente de polipeptídios de 127 resíduos, que constituem uma proteína homotetramérica de 56kDa com estrutura secundária de folha ß, que serve como proteína de deslocamento para a tiroxina (T4), e uma proteína de ligação ao retinol (vitamina A). O principal local de produção dessa proteína é o fígado, embora uma pequena quantidade seja produzida pelo plexo coroide e pelas células retinianas. O gene codificante da TTR (18q11.2-12) é pequeno (7 kb) e contém quatro éxons. As mutações convertem-se em perda do equilíbrio do tetrâmero proteico. Surgem assim, fibrilas amiloides (FA) em cadeias não ramificadas de 10 a 12 nm de diâmetro e fibrilas indissolúveis, que se condensam nos tecidos e órgãos. As síndromes concernentes ao acúmulo da proteína TTR são: polineuropatia amiloidótica familiar (PAF-TTR), miocardiopatias (MCP) e amiloidose sistêmica senil (ASS). Estimativa recente relatou a existência de 5.000 a 10.000 casos de PAF-TTR no mundo.

**Objetivo:** O estudo objetiva elaborar uma plataforma de diagnóstico PAF-TTR *on-line* para auxiliar como ferramenta de contribuição para o estudo da epidemiologia e facilitar o diagnóstico.

**Métodos:** O projeto baseou-se em uma pesquisa bibliográfica capaz de levantar evidências clínicas e epidemiológicas na elaboração de uma plataforma facilitadora (pesquisa aplicada).

**Resultados:** O resultado alcançado foi a elaboração da plataforma contendo um questionário, que tornará o trabalho dos profissionais mais dinâmico, barato e eficiente, caracterizando melhor a doença e promovendo um diagnóstico precoce.

**Conclusão:** A plataforma poderá tornar-se recurso valioso para caracterização, diagnóstico e futuro estudo epidemiológico da PAF-TTR.

**Palavras-chave:** Amiloidoses. Mutação. Transtirretina. Polineuropatia. Epidemiologia

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## INTRODUCTION

Familial Amyloid Polyneuropathy (FAP) due to transthyretin (TTR) gene mutation is the most common presentation among hereditary amyloidosis and is characterized by high morbidity and mortality rates. It is a rare and progressive autosomal dominant disease caused by transthyretin (TTR) instability, leading to fibrillar protein aggregates and consequent deposits of amyloid fibrils. Once accumulated in the peripheral nervous system, it results in sensory-motor polyneuropathy with dysautonomia[11].

FAP was first reported in the north of Portugal, where the missense variant was most prevalent. Later, the disease was observed in large groups of individuals in Japan and Sweden. It was also reported in Brazil. However, epidemiological data in this country are still very scarce[8, 9]. The diagnosis of FAP-TTR is confirmed by biopsy/genetic test, which allows the identification/visualization of amyloid deposits or amyloidogenic mutation, respectively[2] . The assumed difficulty to access research centers and the need for preliminary treatment reinforce the importance of a facilitating tool for the FAP-TTR diagnosis.

Therefore, a questionnaire was developed based on the literature review, considering the disease's primary clinical and epidemiological aspects. The objective of the questionnaire is to aid in screening FAP-TTR patients. The tool is easy and quick to use. Free access was made available at an online diagnostic platform for FAP-TTR at www.neuropatia.com.br.

## METHODOLOGY

This study used two specific techniques: bibliographic and applied research.

Bibliographic research was used to fundament the essential knowledge for the comprehension, analysis, and discussion of the topic in question. To achieve this goal, academically recognized research instruments such as PUBMED and Academic Google platforms were used. The expression familial amyloid polyneuropathy (polineuropatia amiloidótica familiar) was used as the main descriptor.

An exploratory applied research, also called Pilot Study, was necessary at a second moment to achieve the main specific goal: to create an online platform directed to help professionals based on a quick, precise, and low-cost diagnosis of FAP-TTR.

It is important to emphasize that this study aims only at creating a tool that may help in the diagnosis of FAP-TTR. Furthermore, its applicability, sensitivity, and accuracy can be evaluated along with major research reference centers such as CEPRAM (Centro de Pesquisas Antonio Rodrigues de Melo) located in Rio de Janeiro.

A questionnaire was organized to establish the main criteria for the identification of the clinical condition in question. The questionnaire (Attachment 1) contains 11 questions – the first one being only additional information (if the patient has a diagnosis of diabetes mellitus type 2 — DM2, and if they are on a glycemic control).

For the following ten questions, different scores were established, totaling the maximum result of 13 points. The higher the score, the greater the possibility of a positive diagnosis for FAP-TTR. The questions were based on the most relevant symptoms mentioned in the leading scientific studies, also considering the hereditary character of the comorbidity. The weight of each question does not depend on the patient's particular characteristic since the objective is to find similarities in the cases based on standardized data.

### Inclusion criteria

- For neurologists who will use the platform in the future:

a) Active neurologists;
b) Neurologists with patients suspected of FAP-TTR diagnosis;

- For participating patients:

a) Patients with suspected diagnosis of FAP: small-fiber neuropathy, symmetric and progressive axonal sensory-motor polyneuropathy, autonomic symptoms, positive family history, bilateral carpal tunnel syndrome which may be associated with non-ischemic cardiomyopathy, ophthalmopathy, and kidney injury of undetermined cause.
b) No age restriction;
c) Both genders

### Exclusion criteria

- For neurologists:

a) If the patient's signed consent form (ICF-Informed Consent Form) was not presented by the neurologist;
b) If the acceptance form from the patient was not presented (patient ICF);

- For participating patients:

Patients with a suspected diagnosis of FAP-TTR who do not show written evidence of acceptance (ICF).

An exclusive virtual environment was developed by a programmer linked to a private company so that the Familial Amyloid Neuropathy Platform was made available to any interested professional at www.neuropatia.com.br.

Every time a questionnaire is answered, the results will be sent directly to the Researcher, and will be possible to be analyzed in real-time. A collaborative and networked approach to FAP-TTR management, associated with further epidemiological knowledge, will allow the consistent sharing and the use of new data to improve the prognosis and quality of life of the patients and knowledge about the epidemiology of this disease in Brazil.

This study is in line with resolution number 196/96 and the 1988 Medical Code of Ethics. Since the research is based purely on developing a virtual diagnostic aid platform for FAP-TTR, there is no need for contact or evaluation of the patients by the physicians of the research team. This project was approved by the Antonio Pedro Hospital's Institutional Review Board under number 07284818.0.0000.5243.

## RESULTS

The result of this study was the creation of a free, online tool of easy access that physicians can use whenever they suspect the diagnosis of FAP-TTR for a patient.

The platform will provide a structure capable of synthesizing symptoms and generating data that will promote low-cost diagnosis. This resource will also allow the identification of groups that are more likely to present familial amyloid polyneuropathy, and the endemic areas. Hopefully, it will contribute to timely treatment, thereby reducing expenses for patients, government or health insurance.

Plans of a future partnership with the Brazilian reference center CEPRAM and other institutions for applying this platform will allow statistically quantifying its sensitivity and accuracy.

The data collected may be insufficient to predict the risks of developing the disease. However, in the future, the platform will aid the physicians to quantify the minimum chance of the patient being a carrier. This will work as a screening tool to highlight patients at higher risk of developing the disease and who need closer monitoring. The platform as a screening and surveillance tool will also foster the generation of objective features to estimate the probability of disease development.

## DISCUSSION

Familial amyloid polyneuropathy (FAP) is a hereditary, rare, progressive condition with high morbidity and mortality. It originates from the instability of the transthyretin protein, generating fibrilar protein aggregates, culminating in a widespread accumulation of amyloid.

Amyloidoses are classified according to the type of amyloid protein that causes the disease: TTR, apolipoprotein A1 (APLA1), or gelsolin (GL)[8].

The PNS and the heart are frequently affected, and most patients show sensory-motor polyneuropathy and dysautonomia. It can also affect leptomeninges and eyes and, less frequently, the kidneys.

Amyloidosis is relatively rare and can be systemic or punctual, spontaneous or hereditary.

TTR is the most common form of hereditary amyloidosis. Pathogenic substitution frequently occurs by the substitution of valine for methionine in position 50 of the gene TTR (Val50Met), nevertheless, other pathogenic substitutions have already been described. Currently, 119 punctual mutations have been identified, including 113 pathogenic variants in TTR gene 20 .

TTR is a plasmatic tetrameric transport protein; each monomer consists of a single polypeptide chain of 127 amino acid residues of about 14,000Da. The entire structure is approximately 56,000Da. The tetrameric structure has surface receptors for retinol-binding proteins (vitamin A), and binding sites for thyroxine (T4). The protein breakdown leads to inadequate aggregation and deposition of amyloid in various systems and organs. Most of the plasma TTR protein is synthesized by the liver, but some are produced in the pigment epithelium of the retina and in the choroid plexus of the brain.

Recently studies have reported TTR synthesis in Schwann cells of the peripheral nervous system (PNS). In humans, the TTR gene has been found in chromosome 18 in *locus* 18p11.1-q12.3. Its size is approximately 7kb, and it has four exons[2] . Most mutations are the product of a single substitution of nucleotides in the TTR gene. The abnormal amyloid fibrils (AF) were found to result from a replacement of valine by methionine at position 50 of the TTR gene (Val50Met). Since then, several other missense variants in the TTR gene have been described, but Val50Met remains the most common mutation.

FAP-TTR was first described by Andrade (1952)[1] in northern Portugal and was later observed in Japan and Sweden. Initially, its occurrence was believed to be restricted to endemic areas, but with the advanced techniques developed by immunohistochemistry and molecular biology, the diagnosis became less underestimated and could be observed even in sparsely isolated cases[2] . In the Brazilian population, the most common mutation is the TTRVal50Met, which is due to the Portuguese colonization[3, 4]. To date, four other missense variants have been reported: TTRVal71Ala[5] , TTRIle107Val[3], TTRAla19Asp[6] , and TTRAsp38Tyr[7] . The age at onset of symptoms varies from the second to the ninth decade of life.

Sensory-motor polyneuropathy occurs in patients with familial amyloid polyneuropathy due to mutation of the transthyretin gene (FAP-TTR) associated with autonomic disorders and non-neurological manifestations. Patients may have different patterns of neuropathy such as focal neuropathies, sensory-motor polyneuropathy, autonomic neuropathy, or any combination of all these types. The most common is length-dependent polyneuropathy. However, the disease may manifest as focal deficits resulting from local amyloid deposits. The medium nerve of the wrist is a place affected by FAP. Some patients present an early deterioration due to autonomic disfunction[9] .

The initial symptoms are burning of the feet, numbness, and loss of thermal sensibility. Symptoms begin in the lower limbs and may extend to the upper limbs.

Motor alterations are related to loss of strength and muscular atrophy in the feet and legs, occurring after sensitive symptoms and usually associated with suppression of deep tendon reflexes[1°].

Cardiovascular, genitourinary and gastrointestinal symptoms are also frequent and there may be orthostatic hypotension, dizziness, fatigue, and blurred vision. Gastrointestinal symptoms include postprandial diarrhea alternating with constipation[19].

The diagnosis of FAP-TTR should be considered in the presence of progressive sensory-motor polyneuropathy associated with at least one of the following: family history of small-fiber neuropathy, autonomic dysfunction, cardiac involvement, diarrhea or constipation, weight loss, bilateral carpal tunnel syndrome, renal involvement, or vitreous opacities[11].

The accepted tools for the definitive diagnosis are tissue biopsy and genetic tests. However, the diagnosis should be guided by clinical findings, followed by investigation with additional tests such as electroneuromyography (ENMG), electrocardiogram (ECG), Holter, and two-dimensional echocardiographic scan should be performed during the follow-up to screen for cardiac involvement.

Genetic tests detect specific amyloidogenic TTR mutations. When patients have suspected FAP, some differential diagnoses should be considered: light chain amyloidosis, chronic inflammatory demyelinating polyneuropathy, and other neuropathies (Fabry disease, leprosy neuropathy, among others). The diagnosis by tissue biopsy/genetic test is based on the identification/visualization of amyloid deposits or amyloidogenic mutation[2]. The FAP mutant gene is detected by DNA sequence analysis in exons 2, 3, and 4 of the TTR gene. It can determine whether the sequences found show mutations compared to the original template deposited in the NCBI (National Center for Biotechnology Information) – Reference Sequence: NG_009490.1. This analysis is performed by PCR (polymerase chain reaction), using the donated biological material as the substrate to confront the template[12]. Genetic testing and tissue biopsy of affected organs are performed in few places in Brazil and are usually expensive, often forcing patients to travel long distances to obtain a definitive diagnosis[13]. There are specific drugs for FAP-TTR that minimize or block amyloid deposits, aiming to better offer quality of life and reduce morbidity and mortality. Gene therapy comes up as promising[2, 9].

Previously, the only treatment was orthotopic liver transplantation (OLT). However, candidates for this therapy were patients with an early diagnosis (before age 50), confirmation of the TTRVal50Met mutation, minimum duration of the disease and greater modified body mass index (mBMI). Innovative procedures have gained prominence in recent years, such as tetramer stabilizers and gene therapies[14].

The classification of the FAP-TTR stages is based on different scores 2.

**1. Clinical staging of FAP-TTR:**
Stage 0: Asymptomatic;
Stage 1: Light; able to walk; symptoms limited to lower limbs;
Stage 2: Moderate; able to walk but needs assistance;
Stage 3: Serious; confined to a wheelchair or bed with diffuse weakness

**2. Polyneuropathy Disability score (PND score):**
I: Sensory alterations in the extremities, but able to walk (stage 1)
II: Difficulty walking, but still does not need assistance (stage 1)
IIIa: Walks with unilateral support (stage 2)
IIIb: Walks with bilateral support (stage 2)
IV: Confined to a wheelchair or bed (stage 3).

Considering the mentioned data and the importance of a tool that may help in the diagnosis of patients presenting FAP-TTR, the project focused on elaborating a questionnaire which takes into account the main clinical and epidemiological aspects of the illness as well as its hereditary and an easy access to the virtual environment.

The selection of the questions was based on a previous bibliographic review done before the elaboration of the questionnaire. It was based on the most relevant signs and symptoms of the disease —mentioned in the leading scientific studies— besides valuing the hereditary characteristic of the comorbidity.

The initial question is just an additional information and does not interfere with the final score of the questionnaire. It questions whether the patient presents a diagnosis of *diabetes mellitus* type 2 (DM2), and if there is glycemic control since this disease promotes a small-fiber neuropathy which also figures as a characteristic for FAP diagnosis, therefore being of great importance the previous knowledge whether or not the patient has DM2 in order to exclude factors that might bias in the final result.

**Question 01.** "Is FAP family history confirmed? —First- or second-degree kinship"— was rated 2. It was considered relevant due to the hereditary characteristic of FAP already identified by Corino de Andrade in 1952, and later having its autosomal dominant inheritance genetically elucidated.

**Question 02.** "Neuropathic pain? Ex: burning pain, stabbing or shock" —was also rated 2. Initial manifestations are related to sensitive symptoms (small-fiber neuropathy), which mainly affect lower members with numbness or neuropathic pain (burning). Initially, the types of sensitivity committed are pain and temperature, highlighting the importance of valuing such an nonspecific symptom regarding such a crippling disease. Initially, it appears to be a small-fiber neuropathy[17].

**Question 03.** "Dysautonomia? —Orthostatic hypotension, thermal alterations at extremities—hyperemia/cyanosis, constipation/diarrhea, erectile dysfunction" —was also rated 2.0 as, according to the analyzed literature, a significant amount of patients present dysautonomia. The characterization of these symptoms is described in between brackets in the question, highlighting the fact that the presence of dysautonomia as an initial symptom of the disease tends to make us think of a phenotype of a faster

and more severe progression of the disease, thus justifying the importance of a high score for this question. "Autonomic neuropathy is followed by the installation of sensitive neuropathy and less frequently can present itself as the initial symptom of the disease".[19]

The following questions were valued at 1.0 point each, being considered less significant for the diagnosis although necessary for a deeper view of the disease. When question 04 was elaborated—"Nonischemic cardiopathy? Do not include patients with hypokinesia, segmental akinesia"—it was considering cardiac amyloid deposit, which is observed in patients with ATTR.

**Question 05.** "Bilateral carpal tunnel syndrome?" — Was a must since it refers to a frequently described symptom of FAP-TTR patients due to the component of amyloid deposit on the median nerve, which generates a bilateral compressive syndrome. The amyloid deposits can accumulate in specific areas generating focal lesions such as bilateral carpal tunnel syndrome—more severe and less responsive to decompression surgery than idiopathic forms, described as an early symptom of ATTR[19].

**Question 06.** "Progressive symmetric sensorimotor polyneuropathy?"—The objective is to identify the main routine and clinical aspect of the disease, a neuropathy that initially affects the lower limbs with sensitive symptoms, evolving to motor findings and later affecting the upper limbs. It is an axonal length-dependent sensory-motor neuropathy associated with autonomic symptoms with initial impairment of small fibers.

**Question 08.** "Probable ophthalmopathy?—Abnormal vessels in the conjunctiva, alteration of the shape of the iris, amyloid deposition in the retina/vitreous/crystalline/conjunctiva, not including isolated ischemic or hypertensive retinopathy, or glaucoma"—seemed relevant since not too rarely amyloid deposit occurs in the vitreous body justifying the ophthalmological symptoms in the general context of such entity.

**Question 09.** "Doubtful family history? Family of Portuguese origin, first degree relative with a history of severe restrictive heart failure at a young age/affected with an inability to walk by severe sensory-motor polyneuropathy"—is justified in the same way as Question 01; however, in this case, the family diagnosis is suspected, not established.

**Question 10.** "Weight loss of undetermined cause?"—the choice of this question is imperative since weight loss of undetermined cause is highly related to this disease. Dysautonomia contributes to the alternation between diarrhea and constipation, leading patients to a significant weight loss. Besides the dysautonomic symptoms, throughout the disease's progression, the patient presents sustained weight loss that is not justified solely by their gastrointestinal symptoms [9].

The questionnaire was prepared in a structured way to elucidate the ethnic aspects of the patient, hence trying to identify his/her origin and the clinical aspects of the disease.

Regarding the evaluation criteria, the score chosen for each question does not rely on the patient's particular characteristics, since the aim is finding whatever there is in common in the analysis, allowing thus to build a platform that will present the data in a standardized manner.

The FAP-TTR diagnosis platform can be accessed easily through a link available online containing assertive questions about the condition, scoring the answers in different scales, and generating a data bank for future characterization of the clinical/epidemiological aspects of the disease.

The description of the processes used is relevant. Although there are no other similar instruments for comparison, it reveals knowledgeable strategies based on scientific evidence on the methodological easiness and challenges found in different events and studies. It is often impossible to obtain a satisfactory result through traditional data collection approaches, such as by presential interviews, by telephone or by written questionnaires. These methods do not generate results quickly and efficiently, and also may be expensive In addition, there is the magnitude of the technological resources available nowadays and the fact that the participation rates in epidemiological studies drop when they are not implemented through modern means and methods[21].

With the expansion of internet access throughout the world, studies using virtual environment have shown a fruitful trend in data collection, being the preferred method by most research participants[22]. Even though the use of the internet in research groups is done mainly by young people, the use of this tool has grown lately. Furthermore, it is a resource that allows those involved in the study to establish agile and rigorous contact with the predictions, answers, data, probabilities, and other theories raised.

In Europe, about 85% of homes have access to the internet. In Brazil, the proportion reached 120.7 million people in 2017. According to the study, in 2008, users were no more than 34%, and in 2017 they were already 74.9%. Considering these data, virtual questionnaires are an alternative method for obtaining answers in scientific research. The use of a virtual tool also disseminates the necessary information, enabling the development of research in the area of epidemiology of FAP-TTR. Thus, the objective of this study was to develop the FAP-TTR diagnostic platform through an online questionnaire and

disseminate it in a virtual environment, to systematize data collection related to the disease.

## CONCLUSION

Knowledge about Portuguese heritage, the difficulty of access to large diagnostic centers, the high cost of the confirmatory tests, and the high morbidity and mortality rates of this condition support that this tool can contribute to an early diagnosis and an adequate and timely treatment. Moreover, it is worth highlighting the advent of new therapeutic approaches based on genetic replacement. Therefore, all hypotheses of the need for the applicability of this tool in the near future are justified.

Concluding, this platform will serve as an analysis tool that considers the disease itself and the diseased patient. Due to its low cost and great benefit, it can be used constantly in the daily routine of specialists. Hopefully, the platform will contribute to the improvement and agility of the diagnosis definition, facilitating both early treatment onset and the budget estimation for the patient, the government/the health insurance. In addition, the platform might enable the monitoring of the therapeutic response after the diagnosis determination.

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**Attachment 1**

**STRUCTURED QUESTIONNAIRE**
**Title of the project: Familial Amyloid Polyneuropathy: A Proposal for Epidemiological Study Through the Creation of a Virtual Platform**

Researcher: Osvaldo José M. do Nascimento

IDENTIFICATION:
Referred by Dr.: _____
Physician's phone: (___)_____
Patient's name: _____
Gender: (__) F (__) M
Patient's phone: (___)_____
Date of birth: ___/___/___ (dd/mm/yyy or mm/dd/yyy)
Address: _____

**ADDITIONAL INFORMATION:**
1) Diagnosed for Type 2 Diabetes mellitus? If YES, Classify by:
WELL CONTROLLED: fasting blood glucose <140 mg/dL and/or Hb <7;
PARTIALLY CONTROLLED: fasting blood glucose between 140-200mg/dL and/or Hb between 7-8;
UNCONTROLLED: fasting blood glucose >200mg/dL and/or Hb>8;
NOT POSSIBLE TO CLASSIFY

| Questions | | Points |
|---|---|---|
| 1 | Family history of PAF confirmed?<br>(first or second degree relationship) | 2 |
| 2 | Neuropathic pain?<br>(ex.: burning pain, pang pain or shock pain) | 2 |
| 3 | Dysautonomia?<br>(orthostatic hypotension, thermal alterations in extremities—hyperemia/cyanosis, constipation/diarrhea, erectile dysfunction) | 2 |
| 4 | Non-ischemic cardiopathy?<br>(do not include patients with hypokinesia, segmental akinesia) | 1 |
| 5 | Bilateral carpal tunnel syndrome? | 1 |
| 6 | Progressive symmetrical sensory-motor polyneuropathy? | 1 |
| 7 | Kidney injury?<br>(microalbuminuria of undetermined cause, exclude diabetic patients) | 1 |
| 8 | Suspected ophthalmopathy?<br>(abnormal conjunctiva vessels, iris shape alteration, amyloid deposition in retina/vitreous/iris/crystalline/conjunctiva; do not include isolated ischemic or hypertensive retinopathy or glaucoma, familial carpal tunnel syndrome? ) | 1 |
| 9 | Suspicious family history?<br>(Family of Portuguese origin, first degree relative with history of serious illness with severe restrictive cardiac insufficiency at young age and/or inability to walk due to severe sensory-motor polyneuropathy) | 1 |
| 10 | Weight loss of undetermined cause? | 1 |
| **TOTAL** | | ( _____ ) |