U.PORTO

MESTRADO INTEGRADO EM MEDICINA

Role of salivary gland biopsy for the diagnosis of symptomatic onset in Transthyretin Val30Met Familial Amyloid Polyneuropathy

Rui Pedro de Oliveira Lopes

M

2020

Role of salivary gland biopsy for the diagnosis of symptomatic onset in Transthyretin Val30Met Familial Amyloid Polyneuropathy

Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

Rui Pedro de Oliveira Lopes

Aluno do 6º ano de Mestrado Integrado em Medicina

Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

Rua de Jorge Viterbo Ferreira nº228, 4050-313 Porto

rpedro.es2@gmail.com

Orientador: Doutor Ricardo Jorge Ferreira Taipa

Médico Assistente Hospitalar de Neurologia e Neuropatologia, Departamento de Neurociências, do Centro Hospitalar Universitário do Porto;

Prof. Auxiliar Convidado da Unidade de Anatomia e Fisiologia do Sistema Nervoso do ICBAS-UP

Unidade de Neuropatologia, Centro Hospitalar Universitário do Porto - Hospital Santo António

Largo Prof Abel Salazar, 4099 - 001 Porto

rtaipa.neuropat@chporto.min-saude.pt

Coorientador: Doutora Teresa Coelho

Assistente Graduada Sénior de Neurofisiologia, Departamento de Neurociências, do Centro Hospitalar Universitário do Porto

Porto, junho de 2020

U.PORTO
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

centro hospitalar do Porto

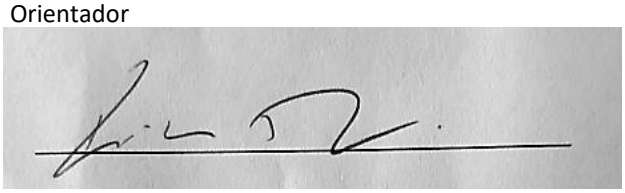
Role of salivary gland biopsy for the diagnosis of symptomatic onset in Transthyretin Val30Met Familial Amyloid Polyneuropathy

Autor

Rui Pedro de Oliveira Lopes

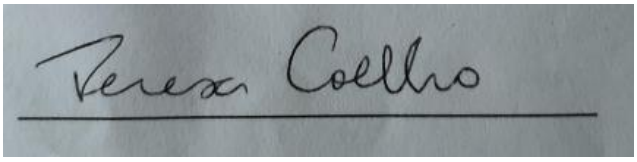
Rui Pedro de Oliveira Lopes

Orientador



Doutor Ricardo Jorge Ferreira Taipa

Coorientador



Doutora Teresa Coelho

Porto, junho de 2020

Agradecimentos

Ao Professor Ricardo Taipa, pela sua disponibilidade, apoio, críticas e ensinamentos indispensáveis no desenvolver deste trabalho. Agradeço ainda pelo impacto que teve no meu interesse por Neurociências desde cedo no meu percurso académico. À Doutora Teresa Coelho pelos conhecimentos transmitidos sobre o mundo da PAF. Ao Professor Pedro Oliveira pelo auxílio importante no tratamento estatístico dos dados.

Aos meus pais, pelo inestimável apoio, carinho e paciência em cada passo deste percurso.

À minha irmã pela inspiração, ensinamentos infindáveis e tempo disponível, e por representar, desde a minha infância, o meu exemplo a seguir, quer enquanto profissional, quer enquanto ser humano.

Aos meus amigos, por me receberem de braços abertos desde o primeiro dia, pelo apoio em qualquer situação, e por todas as histórias e momentos inacreditáveis durante estes seis anos, sem os quais este percurso nunca seria possível.

This page is intentionally left blank.

Resumo

Introdução e objetivos: A amiloidose por transtirretina (ATTR) é a doença mais comum e devastadora no grupo das Polineuropatias Amiloidóticas Familiares (PAF). É uma doença sistémica que se apresenta, predominantemente, sob a forma de neuropatia sensitiva, motora e autonómica. É endémica em Portugal e pode ser fatal após 10 anos se nenhum tratamento for iniciado. Portanto, o reconhecimento e o diagnóstico precoces desta doença são essenciais. Os sintomas iniciais na PAF-TTR podem ser muito subjetivos, sendo geralmente necessário demonstrar deposição de amiloide nos tecidos para estabelecer o início da doença. Com este estudo, procuramos identificar as características clínicas mais associadas ao início da doença e esclarecer o papel da biópsia da glândula salivar labial nessa definição.

Métodos: Foi efetuada uma análise retrospetiva através da consulta dos processos médicos de 357 pacientes com PAF por TTR Val30Met, ou com suspeita da doença, submetidos à biópsia da glândula salivar labial, num período de 5 anos, de janeiro de 2011 a dezembro de 2015.

Resultados: Dos 357 pacientes, 50,1% eram do sexo masculino, com idade mediana de início de sintomas de 36 anos e idade mediana aquando da biópsia de 38 anos. No momento da biópsia, as características clínicas mais comuns eram sintomas autonómicos, particularmente obstipação alternada com diarreia (de novo), sintomas neuropáticos sensitivos, especialmente dor espontânea, e sinais neuropáticos sensitivos, em particular sinais de pequenas fibras. As biópsias foram positivas em 69,5% dos pacientes. O tempo mediano entre o início dos sintomas e a biópsia foi de 14 meses e entre a biópsia e o início do tratamento foi 3 meses. Foi encontrada associação com biópsia com resultado positivo em todos os sintomas autonómicos, com exceção de alteração da função urinária, em todos os sintomas neuropáticos sensitivos, com exceção de sensações não dolorosas, e em todos os sinais neuropáticos sensitivos. Não foi encontrada associação com sintomas oculares ou cardíacos. Seis sintomas/sinais foram associados ao início clínico da doença: dor espontânea, dor evocada, perda sensitiva, obstipação/diarreia, perda de peso involuntária e ausência de reflexos tendinosos profundos. A sensibilidade da biópsia da glândula salivar labial para a deteção do início clínico da doença que encontramos foi de 96,3%, considerando a definição de início clínico aqueles que iniciaram o tratamento até doze meses após a biópsia. A biópsia negativa mostrou-se associada a um início mais tardio do tratamento e, portanto, a um mais tardio início da doença.

Conclusão: A biópsia da glândula salivar labial é um procedimento minimamente invasivo, com muito boa sensibilidade. Deverá ser considerado como um método de escolha para a identificação do início sintomático em pacientes com sintomas iniciais duvidosos.

Palavras-Chave: Amiloidose; Biópsia; Glândula salivar; Polineuropatia amiloidótica familiar; Sensibilidade; Transtirretina.

Abstract

Background and objectives: Transthyretin Amyloidosis (ATTR) is the most common and devastating disease in the group of the Familial Amyloid Polyneuropathies (FAP). It is a systemic disorder presenting predominantly as a sensory, motor, and autonomic neuropathy. It is endemic in Portugal and can be fatal after 10 years if no treatment is provided, so early disease onset recognition and diagnosis is critical. The initial symptoms of ATTR FAP can be very subjective and demonstrating amyloid deposits in tissues may be necessary to establish the disease onset. We sought to identify the clinical characteristics more associated to disease onset and to define the role of labial salivary gland biopsy in this definition.

Methods: We retrospectively analyzed the medical records of 357 patients with Val30Met ATTR FAP or with suspicion of the disorder that underwent labial salivary gland biopsy during a 5 year period from January 2011 to December 2015.

Results: From 357 patients, 50.1% were male with a median age of onset of 36 years and a median age at time of biopsy of 38 years. At the time of the biopsy the most common clinical features were autonomic symptoms, in particular new onset of constipation alternating with diarrhoea; sensory neuropathic symptoms particularly spontaneous pain; and sensory neuropathic signs, in particular small fibre signs. Biopsy results were positive in 69.5% of the patients. The median number of months between the onset of symptoms and biopsy was 14 and between biopsy to start of treatment was 3. Association to a positive amyloid biopsy result was found in all the autonomic symptoms except for impaired urinary function, in all the sensory neuropathic symptoms except for non-painful sensations and in all sensory neuropathic signs. No association was found with ocular or cardiac symptoms. Six symptoms/signs were associated to disease onset: spontaneous pain, evoked pain, sensory loss, constipation/diarrhoea, unintentional weight loss and absent deep tendon reflexes. Our sensitivity found for the labial salivary gland biopsy for the detection of clinical onset of the disease was 96.3%, considering the definition of clinical onset of illness those that started treatment twelve or less months after the biopsy. The negative biopsy was shown to be associated with a later initiation of treatment, and thus, the disease onset.

Conclusion: Labial salivary gland biopsy is a minimal invasive procedure with a very good sensitivity. It should be considered as a method of choice for the identification of symptomatic onset in patients with doubtful initial symptoms.

Keywords: Amyloidosis; Biopsy; Familial amyloid polyneuropathy; Salivary gland; Sensitivity; Transthyretin.

List of abbreviations

ATTR – Hereditary Transthyretin Amyloidosis
CHUP – Centro Hospitalar Universitário do Porto
FAP – Familial Amyloid Polyneuropathy
LSG – Labial Salivary Gland
TTR - Transthyretin

Index

List of tables

List of figures

Background

Transthyretin Amyloidosis is a systemic disorder presenting predominantly as a sensory, motor and autonomic neuropathy[1]. It is also designated as Familial Amyloid Polyneuropathy (FAP), a group of inherited diseases which includes Apolipoprotein A-1 and Gelsolin amyloidosis[2].

Mutations in transthyretin (TTR) gene destabilize TTR protein, a transport protein for Vitamin A and thyroxin, mainly synthetized in the liver, but also in the choroid plexus and retinal epithelium, to misfold from its native tetrameric form to amyloidogenic monomeric form[3]. These monomers have low stability and associate with each other, creating oligomers of increasing size, which precipitate as amyloid fibrils. This results in extracellular deposition of abnormal, insoluble proteins in tissues, leading to ATTR-FAP (transthyretin related familial amyloid polyneuropathy)[2].

The disease was first described in the north of Portugal in 1952 by Corino de Andrade[4], subsequently reported in Japan and Sweden, and we now know that ATTR-FAP is observed worldwide[3] .

More than 150 mutations are described so far in the TTR gene. The Val30Met substitution (alternatively named pVal50Met) is the most frequent pathogenic variant in the endemic countries (Portugal, Sweden, Cyprus)[1,3,5] but also worldwide.

It is transmitted as an autosomal dominant trait[6]. Portugal is the country with the highest number of diagnosed cases, either symptomatic patients or asymptomatic carriers of the mutation[5]. The disease prevalence rate has been estimated as high as 1:1000, in the region of *Póvoa de Varzim* and *Vila do Conde*[7]. Penetrance in Portugal is 87% at 40 years old, a positive familial history is found in 85% of the patients and the mean age at onset is 33 years, remarkably earlier than Sweden[8] and non-endemic regions[3,9,10]. ATTR-FAP is classified based on age at onset. Symptoms before or after the age of 50 distinguish early from late onset form of the disease[11].

Despite the phenotypic variability, ATTR-FAP has an axonal length-dependent sensorimotor polyneuropathy as the main clinical presentation, associated to an autonomic variable dysfunction, or to an infiltrative myocardiopathy[2]. Early-onset disease follows the classical course. In these patients, penetrance is high, and the disease is nearly always associated with a positive family history. Late-onset disease typically presents with peripheral (not autonomic) neuropathy, and the involvement of the upper and lower limbs may appear simultaneously[1,12]. The disease course for late onset is more aggressive and has a shorter survival time than for early onset[11].

Distal small myelinated and unmyelinated nerve fibres carrying pain and temperature sensation become damaged first, resulting in spontaneous and evoked pain or paresthesias in a distal pattern distribution[6,13]. Axonal degeneration then progresses in a distal to proximal pattern, usually reaching the upper limbs 4–5 years after the appearance of the first symptoms[13]. Larger myelinated sensory and motor nerve fibres follow a similar length-dependent progression causing impairing light touch, vibration, and position sensation, with increasing walking difficulty and weakness, leading to muscular atrophy and wheelchair bounding[14]. Life-threatening autonomic dysfunction, along with weight loss and muscle wasting, involves the cardio–circulatory (e.g. orthostatic hypotension), gastrointestinal (e.g. constipation and/or alternating diarrhea, post-prandial vomiting/nausea, early satiety) and genitourinary systems (e.g. erectile dysfunction, urinary retention) and is usually present at this stage, although it can be the first disease manifestation[2,6,15,16].

The natural course of ATTR-FAP can be classified into three stages: I (independent walking ability), II (walking disability, needing uni or bilateral support), and III (wheelchair bound or bedridden)[9,17].

Cardiac involvement, manifested frequently by disturbances of conduction or electrical automaticity, is usually asymptomatic at diagnosis but has been detected in up to 72% of patients when using cardiac imaging and it is the most common extra-neurological manifestation[15,18-20]. Central nervous system (strokelike, focal neurologic deficits) and ophthalmic system (keratoconjunctivitis sicca) may, as well, be involved[14,21].

In terms of treatment, liver transplantation was firstly performed in patients with ATTR-FAP in 1990 in Sweden, and in 1992 in Portugal, and it became the only standard of care until recent years[22,23]. In a 20-year retrospective analysis, the procedure was shown to increase survival especially in early onset patients, with a 15-year survival close to 80%. In contrast, the 10-year survival is below 50% in older Val30Met patients [24,25]. In recent years, treatment of ATTR-FAP has significantly improved with new therapeutic approaches. Treatment with oral Tafamidis was approved in 2011, a TTR stabilizers that prevents the progression of the disease. More recently, new gene silencer therapies: antisense oligonucleotides – Inotersen - and small interfering RNAs - Partisiran have been approved as an alternative to liver transplantation, and new drugs targeting amyloid fibril: anti-serum amyloid P agents or anti-TTR antibodies are being developed and evaluated[16].

If left untreated, the disease can be fatal due to cachexia, infections or cardiac dysfunction after approximately 10 years[1,3,5].

As ATTR-FAP is a progressive disease that can cause largely irreversible tissue damage, timely recognition and diagnosis are critical for appropriate treatment and optimal outcomes[1] .

In the context of genetic counseling, once a mutation has been identified, a predicted date of disease onset (PADO) can be established, taking into account the mutation, the typical age of onset of the associated phenotype and the age of onset in affected family members. Annual follow-up should begin 10 years before this date and should increase in frequency as carriers approach their PADO, particularly for genotypes associated with rapid progression. Based on a Conceição, I., et al. [26], in these patients, it is proposed that a diagnosis of symptomatic ATTR should be made in the presence of the following: at least one quantified or objective symptom or sign definitively related to the onset of symptomatic ATTR; or at least one probably related symptom plus one abnormal definitive or confirmed test result; or two abnormal definitive or confirmed test results in the absence of clinical symptoms [26].

In symptomatic patients, the diagnosis is focused on clinical history and neurological examination. It can be complemented by electrodiagnostic evaluation (EMG, neurophysiologic tests, etc.). The diagnostic confirmation should be done by DNA testing, showing the ATTR mutation [2,3,17].

In the pre-diagnosis era, the only possible way to stablish the diagnosis was through a biopsy showing amyloid deposits. Nowadays, the biopsy value on the diagnosis for ATTR-FAP forms is more limited, and some groups are debating the need to evidence deposition of amyloid substance to establish the clinical diagnosis of ATTR-FAP. However, the initial symptoms on ATTR-FAP can be very subjective and non-specific, and the current neurophysiological markers (small fibre studies) have some limitations, with some patients with autonomic dysfunctions exhibiting normal electromyography tests.

Sensitivity for amyloid detection has been 60-80% via biopsy, considering all tissues [12,27]. Demonstrating amyloid deposits in tissue is important to distinguish carriers from symptomatic patients, as it confirms the beginning of systemic illness[3]. In addition, the mutation is present at birth, and not all patients who are positive for the Val30Met mutation will develop the disease, as the gene penetration is not 100%[2,19,28].

In France and Portugal biopsy of the Labial Salivary Gland (LSG) is preferred over abdominal fat aspiration, which is used in the USA, the UK, and other European countries except Sweden, where fat pad biopsy is used[28]. Since 1947, LSG biopsy has been of considerable value in substantiating the diagnosis of generalized secondary amyloidosis[28-31].

The *Centro Hospitalar Universitário do Porto* (CHUP) is a national reference center for the treatment of the ATTR disease, and the Corino de Andrade Unit preferably uses salivary gland biopsy due to its low

invasive nature, easy access and execution, low morbidity, and high sensitivity when compared to other locations[9,19,21,28,30,32].

Bearing in mind the difficulty in defining disease onset in patients with the TTR Val30Met mutation, as well as the need for an early diagnosis in order to maximize the therapeutic response, this study aims to understand the role of salivary gland biopsy in this definition. This work will also serve to try to understand if there is any clinical-pathological correlation between the characteristics of the biopsies and the clinical-demographic data of the patients.

Methods:

Subject and procedures

This retrospective cross-sectional study was based on the review of clinical files and biopsy results of the population of patients with FAP, or with suspicion of the disorder, undergoing Labial Salivary Gland biopsy, at the Neuropathology Unit of *Centro Hospitalar Universitário do Porto* (CHUP). The analysis included the period of 5 years, from January 2011 to December 2015.

In this population, clinical and demographic data were obtained from the database of the Corino de Andrade Unit and patients clinical records. For each patient, we collected gender, date of birth, date of biopsy, biopsy grade result, date of symptoms onset, date of start of treatment if applicable, neuropathic symptoms and signs, as well as autonomic, cardiac, ophthalmic manifestations present at the time of salivary gland biopsy. The symptoms were classified according to Conceição et al. [26], which separated sensory neuropathic symptoms in positive sensory symptoms, which included spontaneous pain, evoked pain and non-painful sensations; and negative sensory symptoms, which included sensory loss. It also separated the neuropathic signs in small fibre impairment and large fibre impairment which included absence of deep tendon reflexes and muscle waste/weakness. In the category of autonomic symptoms were included sexual dysfunction, impaired bladder function, new onset of constipation alternating with diarrhoea, early satiety, orthostatic dizziness/syncope, and unintentional weight loss (table I).

Additionally, patient follow-up was evaluated, particularly in paucisymptomatic patients or with onset of doubtful symptoms in order to establish the time of clinical onset of the disease.

The global aspect of the LSG and the amount and distribution of the amyloid deposits was graded according to Do Amaral et al.[28] as: G0 – normal morphology without amyloid deposits; G1 – normal morphology of the gland and very discrete amyloid deposits in the interstitial connective tissue stroma or surrounding a few glandular acini; G2 – the global aspect of the gland is preserved with some discrete interstitial fibrosis and more evident amyloid deposits surrounding several glandular acini; G3 – the global aspect of the gland is altered with evident loss of the acinar tissue, pronounced interstitial fibrosis and very abundant amyloid deposits around all glandular tissue

Statistical analysis

Qualitative variables are reported using the absolute and relative frequencies. Quantitative variables are described by medians with interquartile range (25^{th} percentile to 75^{th} percentile). For these variables, normality was assessed using the Kolmogorov-Smirnov test.

To evaluate differences between age or time periods and amyloid presence or gender we performed a Mann-Whitney test. A Pearson's Chi-square was used to evaluate the association between amyloid presence and gender, treatment or presence/absence of symptoms. Kaplan-Meier method was used for the survival comparison between positive and negative biopsies, with time from biopsy to treatment as the index event. Additionally, logistic regression analysis was used to assess the association between the different symptoms or signs and the disease onset, defined as starting treatment.

Statistical analysis was performed using the SPSS® software version 26 for Windows, considering $p<0.05$ for statistical significance.

Ethics Review

This study and the review of clinical information were approved by the Hospital's Ethics Committee, by the coordinator of the department of teaching and had the CHUP Administration Council Authorization. There was no need for an informed consent.

Results

Base Demographic Characterization

From the 480 patients that underwent LSG biopsy, 31 were excluded due to missing data. Ninety-two out of the 449 were patients with FAP acquired after Sequential Hepatic Transplant[33], which were also excluded. The final sample consisted of 357 patients, all with the Val30Met mutation identified by DNA analysis. Flow chart represented in figure 1. Of the 357, 50.1% were male. The median age at the time of the biopsy was 38 years (IQR=31-50). The histogram and box-plot are represented in figure 2.

The median time from the symptoms onset and biopsy was 14 months (IQR=6-26). The symptoms were first recognized by the patients at a median age of 36 years (IQR=19-60). Figure 3 represents the distribution of age of symptom onset. For these two variables we considered 346 of the 357 patients, excluding 11 patients with missing information regarding the time of the symptoms onset.

As of March of 2020, 233 (65.3%) patients have started treatment with Tafamidis. 78 (21.8%) are yet to start treatment, and 46 had contra-indications for treatment, underwent hepatic transplant or were lost in follow-up.

The median time from the biopsy to the start of treatment was 3 months (IQR=1-9). No significant difference for gender with respect to the age at time of biopsy (p=0.181), age of symptoms onset (p=0.237), time from onset of symptoms to biopsy (p=0.691) and time from biopsy to start of treatment (p=0.903) was found (table II).

Regarding the symptoms/signs at the time of the biopsy, the most common group were the autonomic problems, with 305 out of the 357 patients (85.4%) presenting some type of symptoms that could be due to autonomic dysfunction. 83.8% of the patients had sensory neuropathic symptoms, and 75.6% presented sensory neuropathic signs. In our sample, only 5.3% and 5.0% of the patients presented ocular or cardiac symptoms/signs, respectively.

Within the autonomic symptoms, the most common were new onset of constipation alternating with diarrhoea, in 55.2% of patients, followed by unintentional weight loss, in 38.4%, early satiety in 29.7%, orthostatic dizziness in 26.6%, impaired bladder function in 26.6% and sexual dysfunction in 24.4%. Regarding sensory neuropathic symptoms, positive sensory symptoms were present in 73.4% of patients, with spontaneous pain being the most common symptom within this group, and negative

sensory symptoms were present in 40.9%. Within sensory neuropathic signs, 69.7% of patients showed small fibre signs, and 43.4% showed large fibre signs (table III and figure 4).

Amyloid presence and gender

We divided the labial salivary gland biopsies between positive and negative, separated by the presence or absence of amyloid deposition. The positive biopsies were further divided by the amount and distribution of the amyloid deposits. In 69.5% of the patients, LSG biopsies were positive. The most common biopsy result was grade 3, with 35.9% of the total of patients. 21.0% were in grade 2 and 12.6% were in grade 1. 30.5% of the patients did not show amyloid deposition in their biopsy (figure 5).

Out of the 179 men who went through the LSG biopsy, 142 (79.3%) showed positive amyloid deposition. In contrast, women showed positive biopsies in only 106 (59.5%) cases, which is significantly lower than men ($p<0.001$). The most common biopsy result in men was grade 3, in 73 (40.8%) of patients. In women, the most common biopsy was grade 0, negative biopsy, in 72 (40.5%), and after that, grade 3, in 55 (30.9%) patients (table IV).

Amyloid presence and age

The median age at the time of biopsies was higher from patients that had a negative biopsy result (39, IQR=30-55) compared to the patients that had a positive biopsy (37, IQR=31-48). However, no statistically significant difference was found ($p=0.497$). We also found an older age at symptom onset in patient with negative biopsy (38, IQR=28-53) than with positive biopsy (35, IQR=30-50). No significant difference was found ($p=0.237$) as well.

The difference between the time from onset of symptoms and the time of the biopsy was statistically significant ($p=0.041$) with the positive biopsy result group having a longer time (14, IQR=7-28) than the negative biopsy group (12, IQR=5-25).

The average time from the biopsy to the start of the treatment was 24 (IQR=6-40) months in the negative biopsy group, and a significantly shorter time ($p<0.001$) in the positive biopsy group of 3 (IQR=1-7) months.

These results are presented in table V.

Amyloid presence and symptoms

Considering the symptoms categories that we described above, we tried to find an association between them and the presence or absence of amyloid in the LSG biopsy.

We found that 3 out of the 5 groups of symptoms/signs were significantly associated with amyloid presence – autonomic symptoms ($\chi^2(1)=8.699$, $p=0.003$), sensory neuropathic symptoms ($\chi^2(1)=22.439$, $p<0.001$) and sensory neuropathic signs ($\chi^2(1) =23.043$, $p<0.001$).

Within the group of the autonomic symptoms, every clinical presentation was significantly associated to the positive biopsy, except for impaired bladder function ($p=0.113$). The sensory neuropathic symptoms and the sensory neuropathic signs were, as well, correlated to the presence of amyloid, except for non-painful sensation ($p=0.433$).

No association was found between ocular ($p=0.547$) or cardiac ($p=0.427$) symptoms/signs. The results are presented in table VI.

Amyloid presence and treatment

For this statistical test we considered all the patients that have started treatment or that are yet to start treatment. Of the 233 patients that started treatment with Tafamidis, 213 had a positive biopsy result and 20 had a negative biopsy result. Meanwhile, of the 78 patients that hadn't started treatment, 6 had an amyloid deposition, and 72 had a negative biopsy result. As expected, an association was found between amyloid presence and start of treatment $\chi^2(1) =196.643$, $p<0.001$ (table VII).

Diagnostic sensitivity of salivary gland biopsy

Identifying the true beginning of the disease in these patients is exceedingly difficult. There is a wide variety of symptoms related to this disease, some with subjective nature, being hard to relate them to the onset of the disease or not.

Therefore, to define diagnostic sensitivity of salivary gland biopsy in paucisymptomatic TTR Val30Met carriers or with doubtful initial symptoms, we decided to consider all patients who started treatment in the first 12 months after the biopsy as patients with confirmed clinical onset of illness, and not only presence of symptoms like other studies[28]. The decision of starting treatment encloses clinical and para-clinical information, and it is based on follow-up, and consequently was considered for this study

a more robust marker for disease onset. We found 187 patients that respected this condition. Out of the 187, only 7 showed a negative biopsy result, giving us a sensitivity of 96.3%. If we consider the first 6 months after biopsy as patients with ATTR-FAP, we have 157 patients within this assumption, 151 of which show amyloid presence in their biopsy, giving us a remarkably similar 96.2% sensitivity result. Under the first assumption, from the 96.3% of patients with positive biopsy, 54% had a grade 3 result, 27.8% had grade 2 and 14.4% had grade 1 of amyloid deposition. From the population that did not start treatment in the first 12 months, 31.5% had a positive biopsy. (table VII)

Survival Curve

In the Kaplan-Meier plot, we compared the positive biopsy results and negative biopsy result according to the time from the onset of symptoms to the start of treatment. For this statistical test we considered all the patients that have started treatment (233), or that are yet to start treatment (78). For this last case, we used the time from the biopsy results until the last follow up appointment. We did not include patients that were excluded from treatment for reasons mentioned in demographic characterization.

The survival curve showed that a negative biopsy is associated with a later initiation of treatment. Our Log Rank test result was 197.061, with a $p<0.001$ and the Kaplan-Meier curve is represented in figure 6.

Logistic regression analysis – association between symptoms and starting treatment

In the regression analysis, we assessed the association of the different type of symptoms and signs and the decision of starting treatment. Six symptoms/signs were associated to this outcome: spontaneous pain ($p<0.001$), evoked pain ($p=0.049$), sensory loss ($p<0.001$), constipation/diarrhoea ($p=0.026$), unintentional weight loss ($p<0.001$) and absent deep tendon reflexes ($p=0.034$). If the biopsy result (positive vs negative) was added to the model, two symptoms/signs remained associated to shorter time to starting treatment: spontaneous pain ($p=0.016$) and sensory loss ($p=0.005$).

Discussion

Our study was focused on a large ATTR-FAP cohort of patients that underwent labial salivary gland biopsy on a 5-year period. The aim of this study was to characterize the clinical symptoms and signs of this population along with their LSG biopsy result, trying to define the role of LSG biopsy in the management of these patients and identify the clinical characteristics more associated to disease onset.

To define disease onset, patients had to develop consistent and progressive signs and/or symptoms related to the disease[26]. When analyzing patients' age, first symptoms manifested at a median age of 36 years, and, at time of biopsy, we found a median age of 38 years. Compared to other countries, Portugal has an earlier age of onset, generally found within a range of 30 to 35 years, with an average of 33 years[3,9,10,12,34] and 80% of the patients with the Val30Met mutation showing symptoms at 50 years[21,35]. The patients' age in our sample were on the upper limit when compared to the literature for the same geographical area. More recent studies are in this range of age[28,36]. This could reflect an increasing recognition of late onset cases in the Portuguese population. Future epidemiological studies will be important to confirm and explain this potential trend.

There is an equal gender distribution, which goes along with the literature that shows no difference between genders in early onset cases[1,3,5,12].

According to literature, among Portuguese patients with ATTR-FAP, women are known to have a significant later onset than men[10]. In our study women were found to have a later median age of onset (37 vs 36) and median age at biopsy (39 vs 36) than men, but no significant difference was found ($p=0.237$ and $p=0.181$ respectively).

We found a significant difference ($p<0.001$) between men and women concerning biopsy result, with women having significant lower percentage of positive biopsies when compared to man (59.4% vs 79.3%). The same did not happen in Do Amaral et al., where no differences were found[28]. To the best of our knowledge, these were the two only studies that have analyzed this difference. It would be important to confirm if there is an association between gender and amyloid deposition in different populations and other sampling biopsy sites. For instances, it would be important to understand gender differences in reporting (subjective) symptoms that could lead to more frequent referral to biopsy.

The median time from initial symptoms of neuropathy to biopsy was 14 months, a shorter time than the 2- years described by other studies[9,18,20,28,37]. This can be explained by the organization of the ATTR-

FAP clinics, where a significant number of patients have family history and are aware of disease related symptoms. Furthermore, some patients are enrolled in asymptomatic gene carrier's follow-up projects.

This duration was significantly longer (p=0,041) in patients with positive biopsy (14 months vs 12 months). Even though two months do not reflect clinical significance in FAP disease, this result reinforces that the longer the time a patient is symptomatic the more frequent will be to find amyloid deposition in biopsy.

We found a significant longer time between the date of biopsy and the start of treatment when the biopsy result was positive compared with negative biopsy. This was expected due to the importance of the LSG biopsy as a marker for disease onset, along with clinical history and neurophysiological evaluation, leading to earlier start of treatment.

Regarding symptoms/signs at onset, the majority of studies seem to agree sensory neuropathic symptoms, especially spontaneous pain, were the first noticed symptom in the majority of patients[6,12,14,17,20]. This partially goes along with our findings. Spontaneous pain was found to be the most common individual symptom, present in 62.2% of patients, whereas sensory neuropathic symptoms (83.8%) were the second most common group of symptoms. Along with autonomic symptoms, these were the two most common in our sample. However, it must be noted that in our study, a thorough list of symptoms was collected when patients presented, and not only the main symptom, which could increase the likelihood of autonomic symptoms to be included in this group. Autonomic symptoms are generally less specific and, consequently, not referred as the most common first complain at onset [6,9,12,17,20,38] . Regarding symptoms/signs present during the clinical course of Val30Met ATTR-FAP, these two have been the groups of symptoms more associated to early onset, unlike in late onset cases[1,12]. This is consistent with other studies [6,20,21,26]. According to literature, autonomic symptoms were present between 70 to 80%[12,21], a lower value than the one we found (85.4%). It has been described that despite not being the characteristic symptoms when the disease starts, they very often follow and gradually became more apparent[14,20]. These observations underscore the importance of conducting a careful history regarding potential autonomic symptoms at the time of the initial evaluation. In our sample, these were 2 out of the 3 groups of symptoms associated with a positive biopsy result, along with sensory neuropathic signs. Within this group, small fibre signs were the most common signs (69.7%) going along with the literature[21,26].

Within the group of autonomic symptoms, gastrointestinal symptoms – new onset of constipation/diarrhoea and unintentional weight loss complaints - were the most frequent. This is

similar to other studies[6,12,21,26] where they have been described as the main non-neurological complaint. We also found a connection between these symptom and positive biopsy result. Impaired bladder function has been shown on some studies[6,12] as an important manifestation of ATTR-FAP, a finding that was not present on our study, where only 26.6% had complaints regarding this problem. Additionally, we did not find an association between impaired bladder function and positive biopsy (p=0.113). Gender may influence urinary symptoms, as women are far more susceptible to urinary infections. We didn't also find a connection between non-painful sensations and positive biopsy results, and they were only present in 22.7% of our population, what is differently reported in the literature[6,9].

It is also important to mention that sexual dysfunction may be an overlooked symptom when compared with other autonomic manifestations for being essentially a male-only symptom, and a symptom that may not always be revealed on standard examination. In our study, this symptom was present in 43.6% of patients at onset, being the second most present one, if only the male population were considered.

Cardiac symptoms were the least found, only in 5.0% of patients. This is not supported by the literature where Cardiac involvement has been present in 40-50%[11,21] and up to 70% of patients[20]. However, it must be taken in consideration that this cardiac involvement is determined by cardiac imaging/examination, and much less often as a symptom[11,12]. Heart symptoms are not usually present at onset or in the beginning of the course, but later in the course of the disease[1,9,11,21]. They are also more common in late onset cases than early onset[11,12,21] and not as frequently seen in FAP with Val30Met mutation[21,26] as in other mutations, like the Val112Ile[1,14,16,21,39].

As expected, considering the treatment algorithm of the Corino de Andrade Unit, we found positive association between amyloid presence and treatment. There were 20 cases where patients started treatment despite having a negative biopsy. This happened in patients that had a positive biopsy at a later time (11 salivary gland and 3 skin biopsies). There were 5 cases where patients had severe and incapacitating neuropathic or autonomic symptoms at the time of presentation, where the clinicians decided to bypass amyloid deposition demonstration and 1 patient with cardiac amyloidosis symptoms. Interestingly, there were 6 cases were no treatment was started despite of the positive biopsy. This happened in patients that had mild symptoms with improvement between the biopsy and the follow up appointment (4 patients), or had mild and stable symptoms (1 patients), or had symptoms related to other disorder that improved after its resolution(1 patient).

One of our main initial goals was to define the diagnostic sensitivity of salivary gland biopsy in paucisymptomatic TTR Val30Met carriers or with doubtful initial symptoms. In our study, the sensitivity

was 96.3% considering the definition of clinical onset of illness those that started treatment twelve or less months later. According to the literature, LSG biopsy has been shown to be as low as 63%[9] and as high as 100%[32]. Dardiotis et al.[40] and Do Amaral et al.[28] determined the same value of 91%. These findings are consistent with our value of 96.3%. In Do Amaral et al.[28], the study that was conducted with the model most similar to ours, from the 91% of patients with positive biopsy, 63.2% had grade 3 of amyloid deposition, 13.2% grade 2 and 14.5% grade 1. Similar result to what we found: 52.8% grade 3, 28.9% grade 2 and 14.5% grade 1. In our study, only 7 cases of patients within the definition of clinical onset did not show amyloid in their biopsy. This could have happened due to the irregular nature of the amyloid deposition[28]. This value of 31.5% of positive biopsies in patients without the definition of clinical illness, despite being a very lower number when compared with the value found on patients within the definition, it is still a high value.

The Kaplan-Meier curve showed a significant difference between positive and negative biopsy findings concerning time from biopsy to start of treatment, defined by us as clinical onset of the disease. This allows us to infer that a negative biopsy is associated with a much later initiation of treatment, and thus, beginning of the disease.

These findings lead us to conclude that LSG biopsy is a simple procedure with a very good sensitivity and it should be considered, at least, for paucisymptomatic TTR Val30Met carriers or patients with doubtful initial symptoms the diagnosis of TTR amyloidosis.

We were able to identify which symptoms were more associated to amyloid deposition, and perhaps more important, which symptoms were not associated to it. Furthermore, regression analysis identified a core of symptoms which are more associated to the disease onset. Interestingly, all of them are within the group of “Definitively related symptoms” reported by Conceição et al.[26]

Our study presents a series of limitations that should be accounted for. Despite detailed clinical records from the Corino de Andrade Unit, this is a retrospective study. For a better characterization of symptoms, the period between symptom onset and biopsy was considered, according to persistent symptom criteria. Nevertheless, it would be important to address the first symptom expressed by the patients to find what symptoms would be stronger related to the disease onset and that we should be more alert.

We removed patients with FAP after Sequential Hepatic Transplant patients from our study, which could have been interesting to follow its clinical evolution and association with amyloid deposition.

Conclusions

This study analyzed the profile of the population that underwent labial salivary gland biopsy on a 5-year period, in the setting of ATTR-FAP. The most common group of symptoms were autonomic and sensory neuropathic symptoms. Along with sensory neuropathic signs, they were the three groups associated with labial salivary gland positive biopsy result. Spontaneous pain, new onset of constipation alternating with diarrhoea and unintentional weight loss were the three most common symptoms associated to amyloid deposition. Positive biopsy was not related to non-painful sensations and impaired bladder function. Amyloid deposits were more present in men, something that needs to be addressed in future studies.

In this study we found a diagnostic sensitivity value for salivary gland biopsy of 96,3%, considering the definition of clinical onset of illness those that started treatment twelve or less months later. The survival curve showed that amyloid deposition at biopsy is strongly associated to clinical disease onset, defined in this study has starting medical treatment. We also identified a restricted core of symptoms associated to this definition: spontaneous pain, evoked pain, sensory loss, constipation/diarrhoea, unintentional weight loss and absent deep tendon reflexes.

We conclude that LSG biopsy is a simple, minimal invasive procedure with a very good sensitivity. It should be considered in cases with doubtful initial symptoms of ATTR-FAP to confirm diagnosis. This will allow an earlier start of treatment and minimize misdiagnosis if only clinical criteria are adopted.

References

1. Sekijima Y, Ueda M, Koike H, Misawa S, Ishii T, Ando Y. Diagnosis and management of transthyretin familial amyloid polyneuropathy in Japan: red-flag symptom clusters and treatment algorithm. *Orphanet J Rare Dis.* 2018;13(1):6-6.

2. Planté-Bordeneuve V, Said G. Familial amyloid polyneuropathy. *The Lancet Neurology.* 2011;10(12):1086-1097.

3. Çakar A, Durmuş-Tekçe H, Parman Y. Familial Amyloid Polyneuropathy. *Noro Psikiyatr Ars.* 2019;56(2):150-156.

4. Andrade C A peculiar form of peripheral neuropathy: Familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. *Brain.* 1952;75(3):408-427.

5. Parman Y, Adams D, Obici L, et al. Sixty years of transthyretin familial amyloid polyneuropathy (TTR-FAP) in Europe: where are we now? A European network approach to defining the epidemiology and management patterns for TTR-FAP. *Curr Opin Neurol.* 2016;29 Suppl 1(Suppl 1):S3-S13.

6. Ando Y, Nakamura M, Araki S. Transthyretin-Related Familial Amyloidotic Polyneuropathy. *Archives of Neurology.* 2005;62(7):1057-1062.

7. Lacerda P, Vitorino R, Costa P A paramiloidose em Portugal: reflexão sobre o paradigma da transplantação hepática motivada por um caso clínico. Boletim Epidemiológico Observações - Especial Doenças Raras 2015;(5): p. 41-3.

8. Andersson R. Familial amyloidosis with polyneuropathy. A clinical study based on patients living in northern Sweden. *Acta Med Scand Suppl.* 1976;590:1-64.

9. Planté-Bordeneuve V, Ferreira A, Lalu T, et al. Diagnostic pitfalls in sporadic transthyretin familial amyloid polyneuropathy (TTR-FAP). *Neurology.* 2007;69(7):693-698.

10. Sousa A, Coelho T, Barros J, Sequeiros J. Genetic epidemiology of familial amyloidotic polyneuropathy (FAP)-type I in Póvoa do Varzim and Vila do Conde (north of Portugal). *Am J Med Genet.* Dec 18 1995;60(6):512-521.

11. Adams D. Recent advances in the treatment of familial amyloid polyneuropathy. *Ther Adv Neurol Disord.* Mar 2013;6(2):129-139.

12. Adams D, Ando Y, Beirao JM, et al. Expert consensus recommendations to improve diagnosis of ATTR amyloidosis with polyneuropathy. *J Neurol.* Jan 6 2020.

13. Conceição I, González-Duarte A, Obici L, et al. "Red-flag" symptom clusters in transthyretin familial amyloid polyneuropathy. *J Peripher Nerv Syst.* 2016;21(1):5-9.

14. Ando Y, Coelho T, Berk JL, et al. Guideline of transthyretin-related hereditary amyloidosis for clinicians. *Orphanet J Rare Dis.* 2013;8:31-31.

15. Conceição I, Clínica e História Natural da Polineuropatia Amiloidótica, Familiar S, Sociedade Portuguesa de Neurologia, Suplemento 1, Volume 6, nº1, 2006; 86-91.

16. Plante-Bordeneuve V. Transthyretin familial amyloid polyneuropathy: an update. *Journal of Neurology.* 2018/04/01 2018;265(4):976-983.

17. Adams D, Suhr OB, Hund E, et al. First European consensus for diagnosis, management, and treatment of transthyretin familial amyloid polyneuropathy. *Curr Opin Neurol.* 2016;29 Suppl 1(Suppl 1):S14-S26.

18. Hornsten R, Pennlert J, Wiklund U, Lindqvist P, Jensen SM, Suhr OB. Heart complications in familial transthyretin amyloidosis: impact of age and gender. *Amyloid.* Jun 2010;17(2):63-68.

19. Eduardo F, Bezinelli L, Carvalho D, et al. Minor salivary gland biopsy for the diagnosis of familial amyloid polyneuropathy. *Neurological Sciences.* 2017;38(2):311-318.

20. Koike H, Hashimoto R, Tomita M, et al. Diagnosis of sporadic transthyretin Val30Met familial amyloid polyneuropathy: a practical analysis. *Amyloid.* 2011/06/01 2011;18(2):53-62.

21. Coelho T, Maurer MS, Suhr OB. THAOS - The Transthyretin Amyloidosis Outcomes Survey: initial report on clinical manifestations in patients with hereditary and wild-type transthyretin amyloidosis. *Curr Med Res Opin.* Jan 2013;29(1):63-76.

22. Furtado E. Transplantação Hepática na Polineuropatia amiloidótica Familiar. Sinapse, 2006;6:151-4. PdSPdN.

23. Holmgren G, Steen L, Ekstedt J, et al. Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). *Clinical Genetics.* 1991;40(3):242-246.

24. Yamashita T, Ando Y, Okamoto S, et al. Long-term survival after liver transplantation in patients with familial amyloid polyneuropathy. *Neurology.* 2012;78(9):637-643.

25. Carvalho A, Rocha A, Lobato L. Liver transplantation in transthyretin amyloidosis: issues and challenges. *Liver Transpl.* Mar 2015;21(3):282-292.

26. Conceição I, Damy T, Romero M, et al. Early diagnosis of ATTR amyloidosis through targeted follow-up of identified carriers of TTR gene mutations*. *Amyloid.* 2019/01/02 2019;26(1):3-9.

27. Cortese A, Vegezzi E, Lozza A, et al. Diagnostic challenges in hereditary transthyretin amyloidosis with polyneuropathy: avoiding misdiagnosis of a treatable hereditary neuropathy. *J Neurol Neurosurg Psychiatry.* May 2017;88(5):457-458.

28. Do Amaral B, Coelho T, Sousa A, Guimarães A. Usefulness of labial salivary gland biopsy in familial amyloid polyneuropathy Portuguese type. *Amyloid.* 2009;16(4):232-238.

29. Selikoff IJ, Robitzek EH. Gingival Biopsy for the Diagnosis of Generalized Amyloidosis. *Am J Pathol.* Nov 1947;23(6):1099-1111.

30. Foli A, Palladini G, Caporali R, et al. The role of minor salivary gland biopsy in the diagnosis of systemic amyloidosis: results of a prospective study in 62 patients. *Amyloid.* Jun 2011;18 Suppl 1:80-82.

31. Hachulla E, Grateau G. Diagnostic tools for amyloidosis. *Joint Bone Spine.* Dec 2002;69(6):538-545.

32. Lechapt-Zalcman E, Authier FJ, Creange A, Voisin MC, Gherardi RK. Labial salivary gland biopsy for diagnosis of amyloid polyneuropathy. *Muscle Nerve.* Jan 1999;22(1):105-107.

33. Mnatsakanova D, Živković SA. Iatrogenic amyloid polyneuropathy after domino liver transplantation. *World J Hepatol.* 2017;9(3):126-130.

34. P Coutinho, A Silva, J Lopes, el at. Forty years of experience with type I amyloid neuropathy. Review of 483 cases. *In: Glenner GG, Pinho e Costa P, Falcao de Freitas A, editors. Amyloid and amyloidosis. Amsterdam: Excerpta Medica; 1980; 88–98.*

35. Chuang E, Hori AM, Hesketh CD, Shorter J. Amyloid assembly and disassembly. *J Cell Sci.* Apr 13 2018;131(8).

36. Dias A, Santos D, Coelho T, et al. C1QA and C1QC modify age-at-onset in familial amyloid polyneuropathy patients. *Annals of Clinical and Translational Neurology.* 2019;6(4):748-754.

37. Fernandes A, Coelho T, Rodrigues A, et al. Clinicopathological correlations of sural nerve biopsies in TTR Val30Met familial amyloid polyneuropathy. *Brain Communications.* 2019;1(1).

38. Kim DH, Zeldenrust SR, Low PA, Dyck PJ. Quantitative sensation and autonomic test abnormalities in transthyretin amyloidosis polyneuropathy. *Muscle Nerve.* Sep 2009;40(3):363-370.

39. Haagsma EB, Van G, II, Bijzet J, Posthumus MD, Hazenberg BP. Familial amyloidotic polyneuropathy: long-term follow-up of abdominal fat tissue aspirate in patients with and without liver transplantation. *Amyloid.* Sep 2007;14(3):221-226.

40. Dardiotis E, Koutsou P, Papanicolaou EZ, et al. Epidemiological, clinical and genetic study of familial amyloidotic polyneuropathy in Cyprus. *Amyloid.* Mar 2009;16(1):32-37.

Tables and Figures

Table I – List of symptoms/signs

Adapted from Conceição, I., et al., *Early diagnosis of ATTR amyloidosis through targeted follow-up of identified carriers of TTR gene mutations.* Amyloid, 2019

Neuropathic symptoms	**Autonomic symptoms**	**Neuropathic signs**
Positive sensory symptoms	Sexual dysfunction	*Small fibre impairment*
Spontaneous pain	Impaired bladder function	Pinprick sensory loss
Burning pain	Constipation alternating with diarrhoea *(de novo)*	Thermal sensory loss
Sharp pain	Early satiety	Allodynia
Sunburn-like pain	Orthostatic dizziness/syncope	Hyperalgesia
Evoked pain	Unintentional weight loss	*Large fibre impairment*
Allodynia	**Cardiac symptoms**	Light touch, vibratory, proprioceptive sensory loss
Non-painful sensations	Dyspnoea on exercise and at rest	Absent deep tendon reflexes
Paraesthesia	Orthopnoea	Muscle waste/weakness
Restless leg syndrome	Syncope	Bilateral carpal tunnel syndrome
Negative sensory symptoms	Palpitations	Hyperalgesia
Sensory loss	Pulmonary oedema	
Ocular symptoms and signs		
Vitreous opacities associated with gradual vision loss		
Glaucoma		

Table II - Relation between age of events and gender

	All	Male	Female	p
N[1]	357	179 (50.1%)	178 (49.9%)	
Age at biopsy[2] (years)	38 (19-78) [31-50]	36 (22-78) [29-49]	39 (19-78) [32-52]	0.181
N	346	176 (50.9%)	170 (49.1%)	
Age at onset of symptoms (years)	36 (18-78) [19-60]	36 (20-78) [28-48]	37 (18-76) [30-49]	0.237
Time from onset of symptoms to biopsy (months)	14 (0-221) [6-26]	13 (0-158) [6-30]	14 (0-221) [7-24]	0.691
N	233	129 (55.4%)	104 (44.6%)	
Time from biopsy to start of treatment (months)	3 (0-108) [1-9]	3 (0-96) [1-9]	3 (0-108) [1-9]	0.903

[1] - values presented in the form of absolute frequency (number of cases) and relative frequencies (% of cases).

[2] - values presented in the form of median (minimum – maximum) and inter-quartile range (IQR) [25th percentile to 75th percentile].

Table III – Symptoms/signs at the time of the biopsy

Symptoms/signs[1]	**All**	**%**
Autonomic Symptoms	305	85.4
Constipation/Diarrhoea	197	55.2
Unintentional weight Loss	137	38.4
Early Satiety	106	29.7
Orthostatic Dizziness	95	26.6
Impaired Bladder Function	95	26.6
Sexual Dysfunction	87	24.4
Sensory Neuropathic Symptoms	299	83.8
Positive Sensory Symptoms	262	73.4
Spontaneous Pain	222	62.2
Non-Painful Sensations	81	22.7
Evoked Pain	55	15.4
Negative Sensory Symptoms	146	40.9
Sensory Neuropathic Signs	270	75.6
Small Fibre Signs	249	69.7
Large Fibre Signs	*155*	*43.4*
Absent deep tendon reflexes	65	18.2
Muscle waste/weakness	61	17.1
Ocular Symptoms/Signs	19	5.3
Cardiac Symptoms/Signs	18	5.0

[1] - Values presented in the form of absolute frequency (number of cases) and relative frequencies (% of cases).

Table IV - Labial salivary gland biopsy results by gender

	All		Male		Female	
N	357		179		178	
Negative[1]	109 (30.5%)		37 (20.7%)		72 (40.5%)	
Positive	248 (69.5%)	**1** 45 (12.6%)	142 (79.3%)	**1** 17 (9.5%)	106 (59.5%)	**1** 28 (15.7%)
		2 75 (21.0%)		**2** 52 (29.0%)		**2** 23 (12.9%)
		3 128 (35.9%)		**3** 73 (40.8%)		**3** 55 (30.9%)

[1] - values presented in the form of absolute frequency (number of cases) and relative frequencies (% of cases).

0 – Grade 0; 1 – Grade 1; 2 – Grade 2; 3 – Grade 3;

Table V - Relation between age of events and biopsy result

	All	Negative	Positive	p
N[1]	357	109 (30.5%)	248 (69.5%)	
Age at biopsy[2] (years)	39 (19-78) [31-50]	39 (19-78) [30-55]	37 (20-78) [31-48]	0.497
N	346	103 (29.8%)	243 (70.2%)	
Age at onset of symptoms (years)	36 (18-78) [19-60]	38 (18-76) [28+53]	35 (20-78) [30-50]	0.237
Time from onset of symptoms to biopsy (months)	14 (0-221) [6-26]	12 (0-156) [5-25]	14 (1-221) [7-28]	0.041
N	233	20 (8.6%)	213 (91.4%)	
Time from biopsy to start of treatment (months)	3 (0-108) [1-9]	24 (1-108) [6-40]	3 (0-88) [1-7]	<0.001

[1] - values presented in the form of absolute frequency (number of cases) and relative frequencies (% of cases).

[2] - values presented in the form of median (minimum – maximum) and inter-quartile range (IQR) [25th percentile to 75th percentile].

Table VI – Relation between symptoms/signs and biopsy result

Symptoms/signs	All[1]	Biopsy		X^2 (1)[2]	p
		Positive	Negative		
Autonomic Symptoms	305 (85.4%)	222 (61.8%)	83 (23.6%)	8.699	0.003
Constipation/Diarrhoea	197 (55.2%)	154 (42.9%)	43 (12.3%)	14.723	<0.001
Unintentional weight Loss	137 (38.4%)	119 (33.1%)	18 (5.3%)	31.933	<0.001
Early Satiety	106 (29.7%)	83 (23.1%)	23 (6.6%)	5.660	0.017
Orthostatic Dizziness	95 (26.6%)	77 (21.4%)	18 (5.2%)	7.257	0.007
Impaired Bladder Function	95 (26.6%)	72 (20.1%)	23 (6.5%)	2.513	0.113
Sexual Dysfunction	87 (24.4%)	78 (21.7%)	9 (2.7%)	22.256	<0.001
Sensory Neuropathic Symptoms	299 (83.8%)	224 (62.4%)	75 (21.4%)	22.439	<0.001
Positive Sensory Symptoms	262 (73.4%)	191 (53.2%)	71 (20.2%)	4.194	0.041
Spontaneous Pain	222 (62.2%)	170 (47.4%)	52 (14.8%)	11.965	0.001
Non-Painful Sensations	81 (22.7%)	54 (15.0%)	27 (7.7%)	0.615	0.433
Evoked Pain	55 (15.4%)	49 (13.6%)	6 (1.8%)	11.899	0.001
Negative Sensory Symptoms	146 (40.9%)	128 (35.7%)	18 (5.2%)	36.760	<0.001
Sensory Neuropathic Signs	270 (75.6%)	206 (57.4%)	64 (18.2%)	23.043	<0.001
Small Fibre Signs	249 (69.7%)	193 (5.8%)	56 (15.9%)	23.818	<0.001
Large Fibre Signs	155 (43.4%)	130 (36.2%)	25 (7.2%)	25.349	<0.001
Absent deep tendon reflexes	65 (18.2%)	61 (17,1%)	4 (1.1%)	22.267	<0.001
Muscle waste/weakness	61 (17.1%)	57 (16.0%)	4 (1.1%)	19.938	<0.001
Ocular Symptoms/Signs	19 (5.3%)	12 (3.3%)	7 (2%)	0.363	0.547
Cardiac Symptoms/Signs	18 (5.0%)	14 (3.9%)	4 (1.1%)	0.632	0.427

1 - values presented in the form of absolute frequency (number of cases) and relative frequencies (% of cases).

2 - Chi-square (degrees of freedom)

Table VII - Relation between labial salivary gland biopsy and treatment

		Biopsy Result		Total
		Positive	Negative	
Treatment	Yes	213	20	233
	No	6	72	78
Total		219	92	311

Table VIII - Labial salivary gland biopsy results by the time from biopsy to start of treatment

	Time from biopsy to start of treatment					
	All		≤ 12 Months		>12 Months	
N	311		187		124	
Negative[1]	92 (29.6%)	5	7 (3.7%)		85 (68.5%)	
Positive	219 (70.4%)	**1** 41 (13.2%)	180 (96.3%)	**1** 27 (14.4%)	39 (31.5%)	**1** 14 (11.3%)
		2 64 (20.6%)		**2** 52 (27.8%)		**2** 12 (9.7%)
		3 114 (36.6%)		**3** 101 (54.0%)		**3** 13 (10.5%)

[1] - values presented in the form of absolute frequency (number of cases) and relative frequencies (% of cases).

0 – Grade 0; 1 – Grade 1; 2 – Grade 2; 3 – Grade 3;

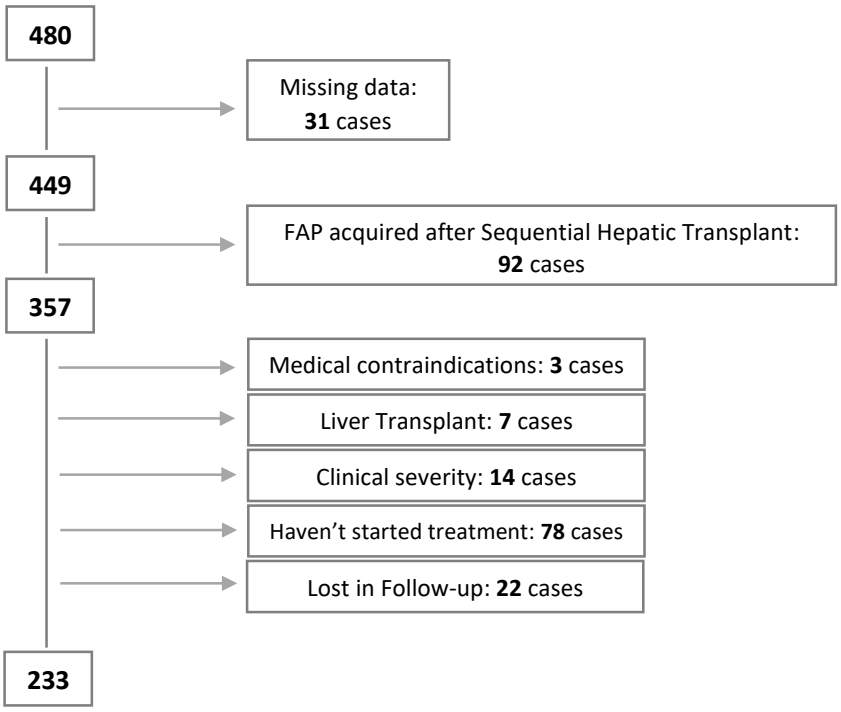


Figure 1 - Study enrollment flow chart. Abbreviations: FAP - Familial Amyloid Polyneuropathy.

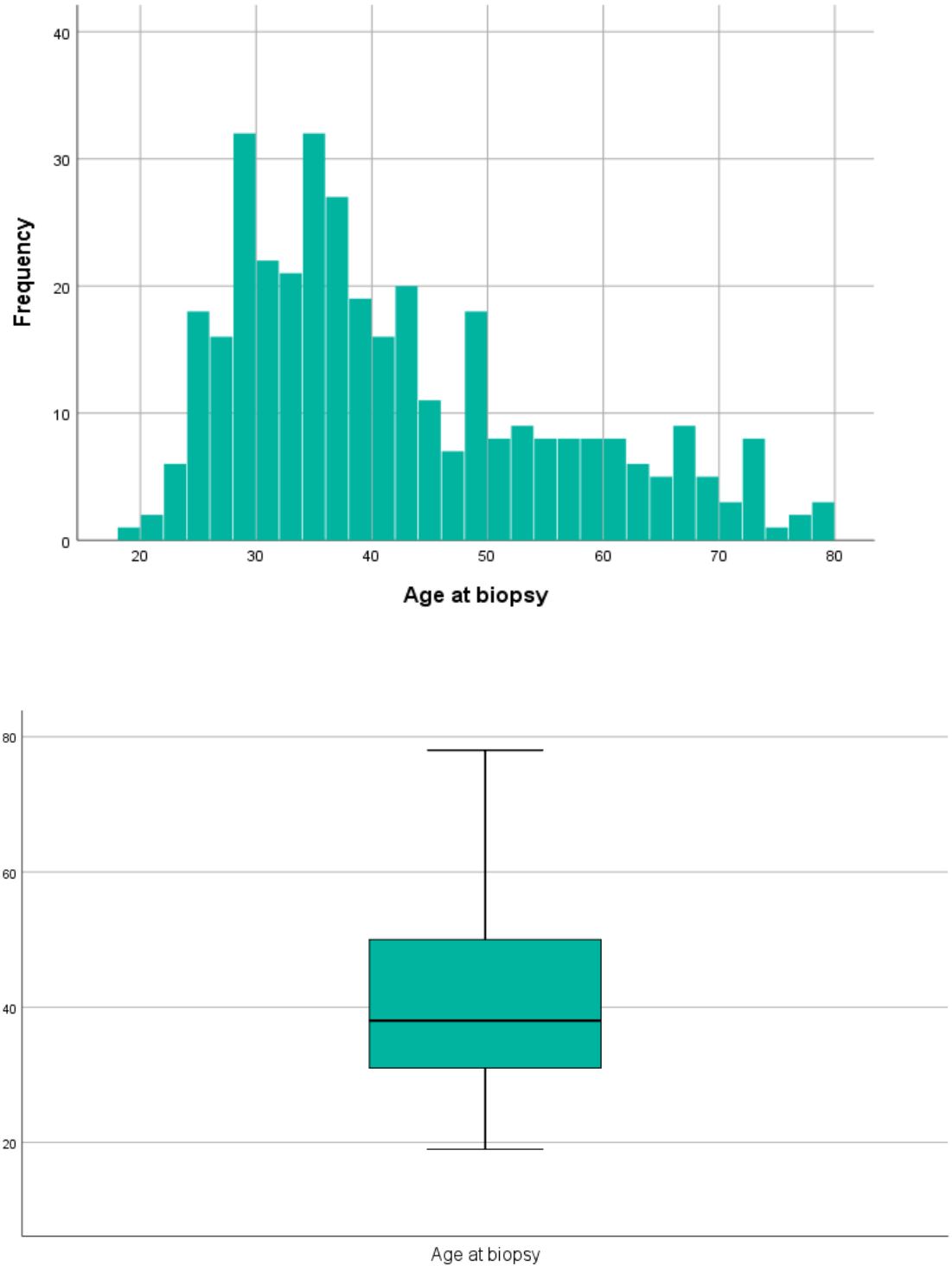


Figure 2 - Histogram and Box-plot of the patients distributed by age at biopsy

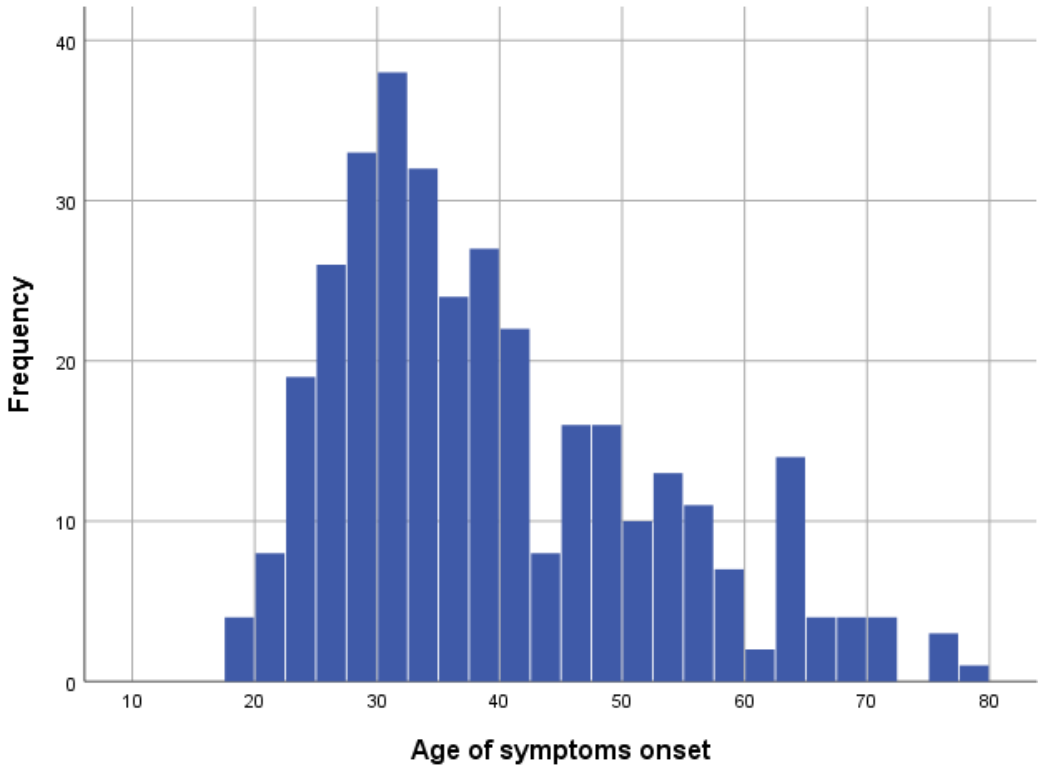


Figure 3 - Histogram of the patients distributed by age of symptoms onset

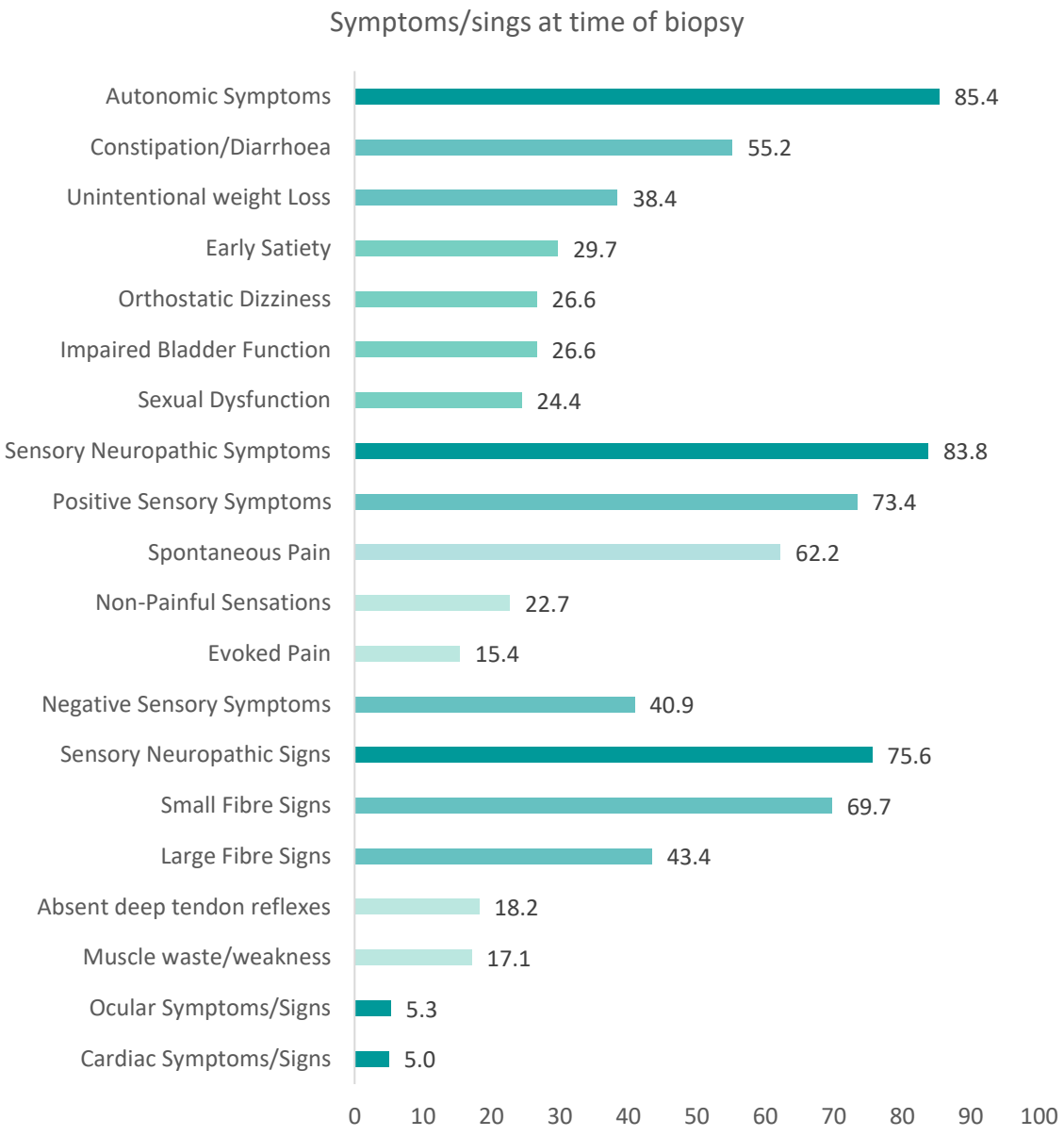


Figure 4 – Symptoms/signs at the time of the biopsy - numbers shown in percentages (%)

Figure 5 - Labial salivary gland biopsy grade results, in total and per gender - numbers shown in percentages (%)

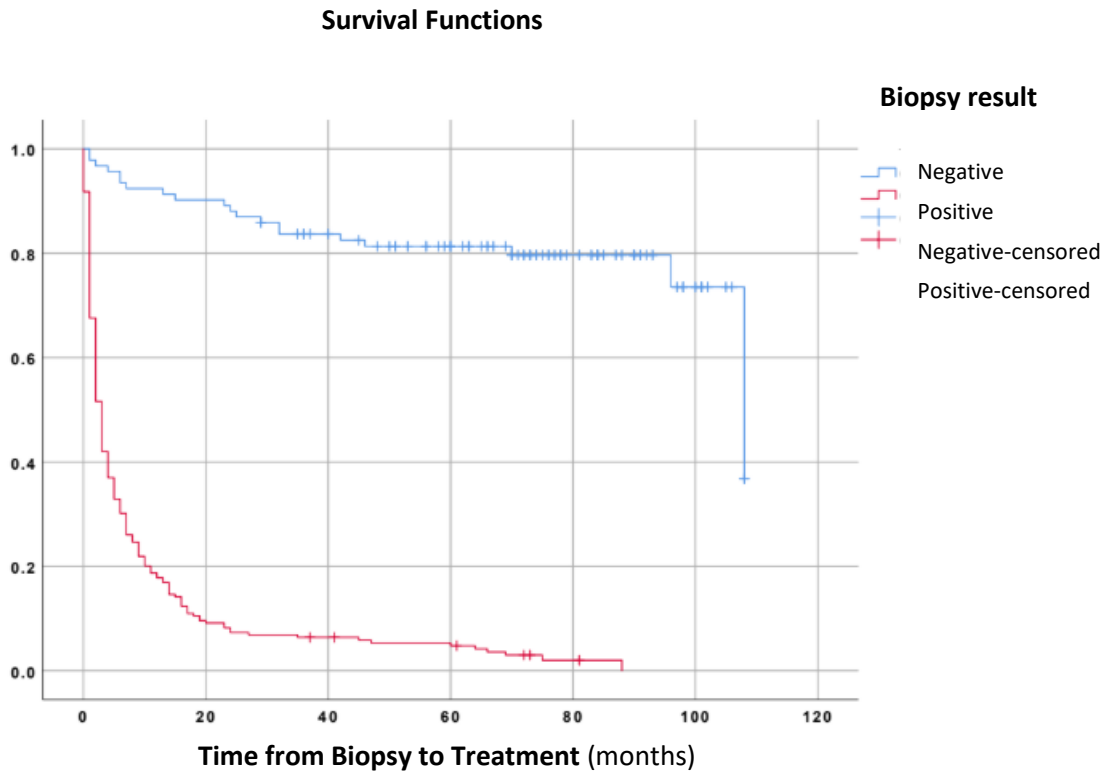


Figure 6 – Survival curve between positive and negative biopsy results